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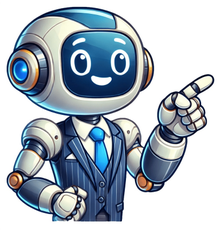

Publication link2024 3D DNA origami pincers that multitask on giant unilamellar vesiclesVesicle Prep Pro Publication in Science advances (2024) Authors: Zhan P., Yang J., Ding L., Jing X., Hipp K., Nussberger S., Yan H., Liu N.Proteins self-assemble to function in living cells. They may execute essential tasks in the form of monomers, complexes, or supramolecular cages via oligomerization, achieving a sophisticated balance between structural topology and functional dynamics. The modularity and programmability make DNA origami unique in mimicking these key features. Here, we demonstrate three-dimensional reconfigurable DNA origami pincers (DOPs) that multitask on giant unilamellar vesicles (GUVs). By programmably adjusting their pinching angle, the DOPs can dynamically control the degree of GUV remodeling. When oligomerized on the GUV to form origami cages, the DOP units interact with one another and undergo reorganization, resulting in the capture, compartmentalization, and detachment of lipid fragments. This oligomerization process is accompanied with membrane disruptions, enabling the passage of cargo across the membrane. We envisage that interfacing synthetic cells with engineered, multifunctional DNA nanostructures may help to confer customized cellular properties, unleashing the potential of both fields.Publication link2024 411P Phenotypical differences in 2 immortalized dystrophic cell lines from pediatric patients: a glimpse into pharmacological personalized therapyPatchliner Publication in Neuromuscular Disorders (2024) Authors: Cappellari O., Quarta R., Cristiano E., Boccanegra B., Cerchiara A., Marinelli M., Barile S., Mouly V., Lasorsa M., Imbrici P., De Luca A.Duchenne muscular dystrophy is a genetic x-linked neuromuscular disorder with no cure. It is due to heterogeneous mutations in the dystrophin gene. Those differences increase the hurdles to finding a universal therapy; the only available therapy is corticosteroids which delay pathology progression without correcting the primary defect, the absence of dystrophin. Since the heterogeneity of the mutations is responsible for different clinical phenotypes and challenges the already complex therapeutic scenario, it is of the utmost importance to characterize the differences underlying the most common mutations. Phenotype-genotype correlations have never been investigated at patient-derived muscle precursors level, which may represent a simplified system to advance in personalized therapy. We used two dystrophic immortalized satellite cell lines (granted from the biobank Myoline), AB1022 (HDMD1) with a stop codon mutation at exon 59 and AB1098 (HDMD2) with deletion of 48-52 exon. We also used a third immortalized cell line from a healthy control (HWT). Preliminary results showed a remarkable difference in the Oxygen Consumption Rate between HDMD2 and HDMD1 cells, assessed via mitostress assays. We observed a greater and significant reduction of all OCR parameters (basal respiration, ATP turnover/oligomycin-sensitive respiration, and maximal respiration) in HDMD1 during differentiation at 48 hours, 96 hours, and 17 days, compared to HDMD2, hence suggesting that the respiratory defect is strongly exacerbated by the differentiation process. Moreover, a first electrophysiological assessment indicates differences in calcium currents at myoblast level between the two cell lines recorded via automated patch-clamp (Patchliner). Molecular biology experiments are ongoing to assess variance in dystrophin mRNA quantification, myogenic cascade, and players involved in the mitochondrial defects. Overall, this characterization will shed light on the genotype-phenotype correlation at the cellular level, thus helping to identify molecular mechanisms underlying patients-specific mutations and new druggable targets.Publication link2024 A lumen-tunable triangular DNA nanopore for molecular sensing and cross-membrane transportOrbit mini and Vesicle Prep Pro Publication in Nature communications (2024) Authors: Liu X., Liu F., Chhabra H., Maffeo C., Chen Z., Huang Q., Aksimentiev A., Arai T.Synthetic membrane nanopores made of DNA are promising systems to sense and control molecular transport in biosensing, sequencing, and synthetic cells. Lumen-tunable nanopore like the natural ion channels and systematically increasing the lumen size have become long-standing desires in developing nanopores. Here, we design a triangular DNA nanopore with a large tunable lumen. It allows in-situ transition from expanded state to contracted state without changing its stable triangular shape, and vice versa, in which specific DNA bindings as stimuli mechanically pinch and release the three corners of the triangular frame. Transmission electron microscopy images and molecular dynamics simulations illustrate the stable architectures and the high shape retention. Single-channel current recordings and fluorescence influx studies demonstrate the low-noise repeatable readouts and the controllable cross-membrane macromolecular transport. We envision that the proposed DNA nanopores could offer powerful tools in molecular sensing, drug delivery, and the creation of synthetic cells.Publication link2024 A molecular mechanism for how pressure induces interdigitation of phospholipid bilayer membranesVesicle Prep Pro Publication in BBA - Biomembranes (2024) Authors: Goto M., Yoshida S., Habara S., Wilk-Kohlbrecher A., Kohlbrecher J., Tamai N., Matsuki H.The phase transition from the ripple gel phase to the interdigitated gel phase of bilayers of phosphatidylcholines (PCs) with two saturated long-chain fatty acids under high pressure was investigated by pressure-scanning microscopy, fluorometry, and dynamic light scattering (DLS) measurements. Microscopic observation for giantvesicles (GVs) of distearoyl-PC (DSPC) under high pressure showed that spherical GVs transforms significantly into warped and distorted spherical ones instantaneously at the pressure-induced interdigitation. The fluorescence intensities of amphiphilic probe Prodan and hydrophobic probe Laurdan in the dipalmitoyl-PC (DPPC) bilayer steeply decreased and increased, respectively, at the interdigitation, suggesting that the conformational change of the polar head group of DPPC molecule in the bilayer transiently occurred at the interdigitation. Further, it was found from the high-pressure DLS measurements that the size of the vesicle particles of the DPPC and DSPC transiently increases near the interdigitation pressure, whereas the chemically induced interdigitationby adding ethanol to the DSPC bilayer membrane under atmospheric pressure produce no such change in the particle size. Taking account of the critical packing parameter of the PC molecule, the above experimental resultswould lead us to the conclusion that the pressure-induced interdigitation is attributable to the increase in repulsive interaction between the polar head groups of the PC molecules resulting from the orientational change of the head group from a parallel alignment to a perpendicular one with respect to the bilayer surface by applyingpressure, namely the transient state: it occurs when the repulsive interaction exceeds a threshold value for the balance between the repulsive interaction and the attractive interaction among the hydrophobic acyl chains.Publication link2024 A non-B DNA binding peptidomimetic channel alters cellular functionsVesicle Prep Pro Publication in Nature communications (2024) Authors: Paul R., Dutta D., Mukhopadhyay T.K., Mller D., Lala B., Datta A., Schwalbe H., Dash J.DNA binding transcription factors possess the ability to interact with lipid membranes to construct ion-permeable pathways. Herein, we present a thiazole-based DNA binding peptide mimicTBP2, which forms transmembrane ion channels, impacting cellular ion concentration and consequently stabilizing G-quadruplex DNA structures.TBP2self-assembles into nanostructures, e.g., vesicles and nanofibers and facilitates the transportation of Na+and K+across lipid membranes with high conductance (~0.6 nS). Moreover,TBP2exhibits increased fluorescence when incorporated into the membrane or in cellular nuclei. MonomericTBP2can enter the lipid membrane and localize to the nuclei of cancer cells. The coordinated process of time-dependent membrane or nuclear localization ofTBP2, combined with elevated intracellular cation levels and direct G-quadruplex (G4) interaction, synergistically promotes formation and stability of G4 structures, triggering cancer cell death. This study introduces a platform to mimic and control intricate biological functions, leading to the discovery of innovative therapeutic approaches.Publication link2024 A novel 4-aminoquinoline chemotype with multistage antimalarial activity and lack of cross-resistance with PfCRT and PfMDR1 mutantsSURFE2R N1 Publication in PLOS Pathogens (2024) Authors: Ferreira L. T., Cassiano G. C., Alvarez L. C. S., Okombo J., Calit J., Fontinha D., Gil-Iturbe E., Coyle R., Andrade C. H., Sunnerhagen P., Bargieri D. Y., Prudncio M., Quick M., Cravo P. V., Lee M. C. S., Fidock D. A., Maranho Costa F. T.Artemisinin-based combination therapy (ACT) is the mainstay of effective treatment ofPlasmodium falciparummalaria. However, the long-term utility of ACTs is imperiled by widespread partial artemisinin resistance in Southeast Asia and its recent emergence in parts of East Africa. This underscores the need to identify chemotypes with new modes of action (MoAs) to circumvent resistance to ACTs. In this study, we characterized the asexual blood stage antiplasmodial activity and resistance mechanisms of LDT-623, a 4-aminoquinoline (4-AQ). We also detected LDT-623 activity against multiple stages (liver schizonts, stage IV-V gametocytes, and ookinetes) ofPlasmodiums life cycle, a feature unlike other 4-AQs such as chloroquine (CQ) and piperaquine (PPQ). Using heme fractionation profiling and drug uptake studies in PfCRT-containing proteoliposomes, we observed inhibition of hemozoin formation and PfCRT-mediated transport, which constitute characteristic features of 4-AQs MoA. We also found minimal cross-resistance to LDT-623 in a panel of mutantpfcrtorpfmdr1lines, but not the PfCRT F145I mutant that is highly resistant to PPQ resistance yet is very unfit. NoP. falciparumparasites were recovered in anin vitroresistance selection study, suggesting a high barrier for resistance to emerge. Finally, a competitive growth assay comprising >50 barcoded parasite lines with mutated resistance mediators or major drug targets found no evidence of cross-resistance. Our findings support further exploration of this promising 4-AQ.Publication link2024 A Study on the Effect of Quaternization of Polyene Antibiotics Structures on Their Activity, Toxicity, and Impact on Membrane ModelsVesicle Prep Pro Publication in Antibiotics (2024) Authors: Omelchuk O., Tevyashova A., Efimova S., Grammatikova N., Bychkova E., Zatonsky G., Dezhenkova L., Savin N., Solovieva S., Ostroumova O., Shchekotikhin A.,Polyene antibiotics have been used in antifungal therapy since the mid-twentieth century. They are highly valued for their broad spectrum of activity and the rarity of pathogen resistance to their action. However, their use in the treatment of systemic mycoses often results in serious side-effects. Recently, there has been a renewed interest in the development of new antifungal drugs based on polyenes, particularly due to the emergence of highly dangerous pathogenic strains of fungi, such asCandida auris, and the increased incidence of mucormycosis. Considerable understanding has been established regarding the structurebiological activity relationships of polyene antifungals. Yet, no previous studies have examined the effect of introducing quaternized fragments into their molecular structure. In this study, we present a series of amides of amphotericin B, nystatin, and natamycin bearing a quaternized group in the side chain, and discuss their biological properties: antifungal activity, cytotoxicity, and effects on lipid bilayers that mimic fungal and mammalian cell membranes. Our research findings suggest that the nature of the introduced quaternized residue plays a more significant role than merely the introduction of a constant positive charge. Among the tested polyenes, derivatives4b,5b, and6b, which contain a fragment ofN-methyl-4-(aminomethyl)pyridinium in their structure, are particularly noteworthy due to their biological activity.Publication link2024 A sustainable chemiosmotic strategy for driving solute transport in synthetic cellsSURFE2R N1 Pre-Print Publication in Research Square (2024) Authors: Poolman B., Ruiz M.P., Anshari Z., Gaastra B., Slotboom D.Cellular homeostasis requires sustained provision of metabolic energy in the form of ATP and electrochemical ion gradients. Primary and secondary active transporters are prominent consumers of cellular energy, and couple ATP hydrolysis and ion gradient dissipation, respectively, to translocation of molecules across biological membranes. Active transport is essential for the translocation of most charged and/or large hydrophilic molecules, both for nutrient uptake into and waste export from living cells. Endeavours to build synthetic cells crucially depend on simulating real cell behaviour by supplying stable and sustained energy sources and deploying them for membrane transport. Here, we provide synthetic cells with long-lasting metabolic energy supply in the form of an electrochemical proton gradient. Leveraging the L-malate decarboxylation pathway from Lactococcus lactis we generate a stable proton gradient and electrical potential in lipid vesicles by electrogenic L-malate/L-lactate exchange coupled to L-malate decarboxylation. By co-reconstitution of the pathway with the Escherichia coli transporters GltP and LacY, the synthetic cells maintain accumulation of L-glutamate and lactose over periods of hours, mimicking nutrient feeding in living cells. This study underscores the potential of harnessing a proton motive force via a simple metabolic network, involving electrogenic substrate/product exchange and substrate decarboxylation, paving the way for the development of more complex synthetic systems.Publication link2024 An automated planar patch-clamp approach to measure the membrane potential and resting membrane currents in a human cerebrovascular endothelial cell linePort-a-Patch Publication in Neuroscience methods (2024) Authors: Soda T., Negri S., Scarpellino G., Berra-Romani R., De Sarro G., Moccia F., Brunetti V.BackgroundThe conventional whole-cell patch-clamp recording technique is widely used to measure the resting membrane potential (VM) and to dissect the underlying membrane ionic conductances in isolated vascular endothelial cells.New methodHerein, we assessed whether the automated patch-clamp (APC) technology, which replaces the traditional patch-pipette with a planar substrate to permit researchers lacking formal training in electrophysiology to generate large amounts of data in a relatively short time, can be used to characterize the bioelectrical activity of vascular endothelial cells. We assessed whether the Port-a-Patch planar patch-clamp system, which is regarded as the smallest electrophysiological rig available on the market, can be used to measure the VMand resting membrane currents in the human cerebrovascular endothelial cell line, hCMEC/D3.Comparison with existing methodsWe demonstrated that the Port-a-Patch planar patch-clamp system provides the same values of the resting VMas those provided by the conventional patch-clamp technique. Furthermore, the APC technology provides preliminary data demonstrating that the resting VMof hCMEC/D3 cells is primarily contributed by Cl-and Na+, as demonstrated with the patch-clamp technique for many other endothelial cell types.ConclusionsThe Port-a-Patch planar patch-clamp system can be successfully used to measure the resting VMand the underlying membrane ionic conductances in hCMEC/D3 cells. We envisage that this easy-to-use APC system could also be extremely useful for the investigation of the membrane currents that can be activated by chemical, thermal, optical, and mechanical stimuli in this cell line as well as in other types of isolated vascular endothelial cells.Publication link2024 An experimental investigation of rundown of the L-type calcium currentSyncroPatch 384PE (a predecessor model of the SyncroPatch 384) Publication in Wellcome Open Res (2024) Authors: Agrawal A., Clerx M., Wang K., Gissinger E., Gavaghan D., Polonchuk L., Mirams G.BackgroundL-type calcium channels (LCCs) are multi-protein macro-molecular ion channel complexes that are involved in several critical functions in cardiac, skeletal, neuronal, smooth muscle, and endocrine cells. Like other ion channels, LCCs can be selectively over-expressed in a host cell line and studied using voltage-clamp patch-clamp experiments. However, L-type calcium current (ICaL) recordings commonly exhibit a reduction in current magnitude over time, commonly termed rundown. Previous studies have shown the effect of phosphorylation on rundown, here we provide evidence that accumulation of Ca2+ inside the cell also contributes towards ICaL rundown.MethodsWe generated experimental conditions that should promote the accumulation of sub-membrane Ca2+ in a CHO expression system, by increasing calcium import or decreasing export. These interventions took the form of: a decrease in inter-pulse duration between sweeps, block of the sodium-calcium exchanger, and increased temperature.ResultsOn average, we found that current reduced to 63% of its initial value within 325 seconds. This reduction of current with time was found to follow two main patterns: linear or saturating decay. Additionally, current magnitude in some cells increased before stabilising or decaying.ConclusionsThis study shows that the rundown of ICaL in patch-clamp experiments can be reduced by modifying the experimental conditions, and implies that reduced accumulation of Ca2+ inside the cell membrane reduces calcium-dependent inactivation of ICaL.Publication link2024 Analgesic effect of Botulinum toxin in neuropathic pain is sodium channel independentSyncroPatch 384i (a predecessor model of the SyncroPatch 384) Publication in Neuropharmacology (2024) Authors: Kesdoan A., Neureiter A., Gaebler A., Kalia A., Krner J., Lampert A.Botulinum neurotoxin type A BoNT/A is used off-label as a third line therapy for neuropathic pain. However, the mechanism of action remains unclear. In recent years, the role of voltage-gated sodium channels (Nav) in neuropathic pain became evident and it was suggested that block of sodium channels by BoNT/A would contribute to its analgesic effect.We assessed sodium channel function in the presence of BoNT/A in heterologously expressed Nav1.7, Nav1.3, and the neuronal cell line ND7/23 by high throughput automated and manual patch-clamp. We used both the full protein and the isolated catalytic light chain LC/A for acute or long-term extracellular or intracellular exposure. To assess the toxins effect in a human cellular system, we differentiated induced pluripotent stem cells (iPSC) into sensory neurons from a healthy control and a patient suffering from a hereditary neuropathic pain syndrome (inherited erythromelalgia) carrying the Nav1.7/p.Q875E-mutation and carried out multi electrode array measurements.Both BoNT/A and the isolated catalytic light chain LC/A showed limited effects in heterologous expression systems and the neuronal cell line ND7/23. Spontaneous activity in iPSC derived sensory neurons remained unaltered upon BoNT/A exposure both in neurons from the healthy control and the mutation carrying patient.BoNT/A may not specifically be beneficial in pain syndromes linked to sodium channel variants. The favorable effects of BoNT/A in neuropathic pain are likely based on mechanisms other than sodium channel blockage and new approaches to understand BoNT/As therapeutic effects are necessary.Publication link2024 Automated Patch Clamp for the Detection of Tetrodotoxin in Pufferfish SamplesPatchliner Publication in Marine Drugs (2024) Authors: Camps M., Revert J., Tud ., Alkassar M., Diogne J., Sureda FX.Tetrodotoxin (TTX) is a marine toxin responsible for many intoxications around the world. Its presence in some pufferfish species and, as recently reported, in shellfish, poses a serious health concern. Although TTX is not routinely monitored, there is a need for fast, sensitive, reliable, and simple methods for its detection and quantification. In this work, we describe the use of an automated patch clamp (APC) system with Neuro-2a cells for the determination of TTX contents in pufferfish samples. The cells showed an IC50of 6.4 nM for TTX and were not affected by the presence of muscle, skin, liver, and gonad tissues of aSphoeroides pachygasterspecimen (TTX-free) when analysed at 10 mg/mL. The LOD achieved with this technique was 0.05 mg TTX equiv./kg, which is far below the Japanese regulatory limit of 2 mg TTX equiv./kg. The APC system was applied to the analysis of extracts of aLagocephalus sceleratusspecimen, showing TTX contents that followed the trend of gonads > liver > skin > muscle. The APC system, providing an in vitro toxicological approach, offers the advantages of being sensitive, rapid, and reliable for the detection of TTX-like compounds in seafood.Publication link2024 Bioprinting of Synthetic Cell-like Lipid Vesicles to Augment the Functionality of Tissues after ManufacturingVesicle Prep Pro Publication in ACS Synthetic Biology (2024) Authors: Thaden O., Schneider N., Walther T., Spiller E., Taoum A., Gpfrich K., Duarte Campos D.Bioprinting is an automated bioassembly method that enables the formation of human tissue-like constructs to restore or replace damaged tissues. Regardless of the employed bioprinting method, cells undergo mechanical stress that can impact their survival and function postprinting. In this study, we investigate the use of a synthetic cell-like unit, giant unilamellar vesicles (GUVs), as adjuvants of the cellular function of human cells postprinting, or in future as the complete replacement of human cells. We analyzed the impact of two nozzle-based bioprinting methods (drop-on-demand and extrusion bioprinting) on the structure, stability, and function of GUVs. We showed that over 65% of the GUVs remain intact when printing at 0.5 bar, demonstrating the potential of using GUVs as a synthetic cell source. We further increased the stability of GUVs in a cell culture medium by introducing polyethylene glycol (PEG) into the GUV lipid membrane. The presence of PEG, however, diminished the structural properties of GUVs postprinting, and reduced the interaction of GUVs with human cells. Although the design of PEG-GUVs can still be modified in future studies for better cellGUV interactions, we demonstrated that GUVs are functional postprinting. Chlorin e6-PEG-GUVs loaded with a fluorescent dye were bioprinted, and they released the dye postprinting only upon illumination. This is a new strategy to deliver carriers, such as growth factors, drugs, nutrients, or gases, inside large bioprinted specimens on a millimeter to centimeter scale. Overall, we showed that printed GUVs can augment the functionality of manufactured human tissues.Publication link2024 Capillary trapping of various nanomaterials on additively manufactured scaffolds for 3D micro-/nanofabricationBuffer solutions Publication in Nature communications (2024) Authors: Lyu X., Zheng Z., Shiva A., Han M., Balda Dayan C., Zhang M., Sitti M.High-precision additive manufacturing technologies, such as two-photon polymerization, are mainly limited to photo-curable polymers and currently lacks the possibility to produce multimaterial components. Herein, we report a physically bottom-up assembly strategy that leverages capillary force to trap various nanomaterials and assemble them onto three-dimensional (3D) microscaffolds. This capillary-trapping strategy enables precise and uniform assembly of nanomaterials into versatile 3D microstructures with high uniformity and mass loading. Our approach applies to diverse materials irrespective of their physiochemical properties, including polymers, metals, metal oxides, and others. It can integrate at least four different material types into a single 3D microstructure in a sequential, layer-by-layer manner, opening immense possibilities for tailored functionalities on demand. Furthermore, the 3D microscaffolds are removable, facilitating the creation of pure material-based 3D microstructures. This universal 3D micro-/nanofabrication technique with various nanomaterials enables the creation of advanced miniature devices with potential applications in multifunctional microrobots and smart micromachines.Publication link2024 Chamber-specific contractile responses of atrial and ventricular hiPSC-cardiomyocytes to GPCR and ion channel targeting compounds: A microphysiological system for cardiac drug developmentFLEXcyte 96 Publication in Journal of Pharmacological and Toxicological Methods (2024) Authors: Lickiss B., Hunker J., Bhagwan J., Linder P., Thomas U., Lotay H., Broadbent S., Dragicevic E., Stoelzle-Feix S., Turner J., Gossmann M.Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs) have found utility for conductingin vitrodrug screening and disease modelling to gain crucial insights into pharmacology or disease phenotype. However, diseases such as atrial fibrillation, affecting >33M people worldwide, demonstrate the need for cardiac subtype-specific cells. Here, we sought to investigate the base characteristics and pharmacological differences between commercially available chamber-specific atrial or ventricular hiPSC-CMs seeded onto ultra-thin, flexible PDMS membranes to simultaneously measure contractility in a 96 multi-well format. We investigated the effects of GPCR agonists (acetylcholine and carbachol), a Ca2+channel agonist (S-Bay K8644), an HCN channel antagonist (ivabradine) and K+channel antagonists (4-AP and vernakalant). We observed differential effects between atrial and ventricular hiPSC-CMs on contractile properties including beat rate, beat duration, contractile force and evidence of arrhythmias at a range of concentrations.As an excerpt of the compound analysis, S-Bay K8644 treatment showed an induced concentration-dependent transient increase in beat duration of atrial hiPSC-CMs, whereas ventricular cells showed a physiological increase in beat rate over time. Carbachol treatment produced marked effects on atrial cells, such as increased beat duration alongside a decrease in beat rate over time, but only minimal effects on ventricular cardiomyocytes. In the context of this chamber-specific pharmacology, we not only add to contractile characterization of hiPSC-CMs but propose a multi-well platform for medium-throughput early compound screening.Overall, these insights illustrate the key pharmacological differences between chamber-specific cardiomyocytes and their application on a multi-well contractility platform to gain insights forin vitrocardiac liability studies and disease modelling.Publication link2024 Chemiosmotic nutrient transport in synthetic cells powered by electrogenic antiport coupled to decarboxylationSURFE2R N1 Publication in Nature communications (2024) Authors: Patio-Ruiz M.F.,Anshari Z.R., Gaastra B., Slotboom D.J., Poolman B.Cellular homeostasis depends on the supply of metabolic energy in the form of ATP and electrochemical ion gradients. The construction of synthetic cells requires a constant supply of energy to drive membrane transport and metabolism. Here, we provide synthetic cells with long-lasting metabolic energy in the form of an electrochemical proton gradient. Leveraging the L-malate decarboxylation pathway we generate a stable proton gradient and electrical potential in lipid vesicles by electrogenic L-malate/L-lactate exchange coupled to L-malate decarboxylation. By co-reconstitution with the transporters GltP and LacY, the synthetic cells maintain accumulation of L-glutamate and lactose over periods of hours, mimicking nutrient feeding in living cells. We couple the accumulation of lactose to a metabolic network for the generation of intermediates of the glycolytic and pentose phosphate pathways. This study underscores the potential of harnessing a proton motive force via a simple metabolic network, paving the way for the development of more complex synthetic systems.Publication link2024 Conformational Flexibility Driving Charge-Selective Substrate Translocation Across Bacterial TransporterOrbit 16 TC Publication in Chemical Science (2024) Authors: Vikraman D., Majumdar B.B., Sharavanakkumar S.K., Weichbrodt C., Fertig N., Winterhalter M., Mondal J., Kozhinjampara M.Bacterial membrane porins facilitate the translocation of small molecules while restricting large molecules, and this mechanism remains elusive at the molecular level. Here, we investigate the selective uptake of large cyclic sugars across an unusual passive membrane transporter CymA, comprising a charged zone and a constricting N terminus segment. Using a combination of electrical recordings, protein mutagenesis and molecular dynamics simulations, we establish substrate translocation across CymA governed by the electrostatic pore properties and conformational dynamics of the constriction segment. Notably, we show that the variation in pH of the environment resulted in reversible modulation of the substrate binding site in the pore, thereby regulating charge-selective transport of cationic, anionic and neutral cyclic sugars. The quantitative kinetics of cyclic sugar translocation across CymA obtained in electrical recordings at different pHs are comparable with molecular dynamics simulations that revealed transport pathway, energetics and favorable affinity sites in the pore for substrate binding. We further define the molecular basis of cyclic sugar translocation and establish that the constriction segment is flexible and can reside inside or outside the pore, regulating substrate translocation distinct from the ligand-gated transport mechanism. Our study contributes novel insights into energy-independent large molecular membrane transport for targeted drug design strategies.Publication link2024 Discovery of a novel natural compound, vitekwangin B, with ANO1 protein reduction properties and anticancer potentialPatchliner Publication in Frontiers in Pharmacology (2024) Authors: Yohan S., Sion L., Minuk K., Dongguk K., Baek J.S., Raju D., Armin S., SeonJu P., Xuan N.N., Thi Thanh H.P., Oh-Bin K., Wan N., Joohan W.Background:Prostate cancer and non-small cell lung cancer (NSCLC) present significant challenges in the development of effective therapeutic strategies. Hormone therapies for prostate cancer target androgen receptors and prostate-specific antigen markers. However, treatment options for prostatic small-cell neuroendocrine carcinoma are limited. NSCLC, on the other hand, is primarily treated with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors but exhibits resistance. This study explored a novel therapeutic approach by investigating the potential anticancer properties of vitekwangin B, a natural compound derived fromVitex trifolia.Methods:Vitekwangin B was chromatographically isolated from the fruits ofV. trifolia. ANO1 protein levels in prostate cancer and NSCLC cells were verified and evaluated again after vitekwangin B treatment.Results:Vitekwangin B did not inhibit anoctamin1 (ANO1) channel function but significantly reduced ANO1 protein levels. These results demonstrate that vitekwangin B effectively inhibited cancer cell viability and induced apoptosis in prostate cancer and NSCLC cells. Moreover, it exhibited minimal toxicity to liver cells and did not affect hERG channel activity, making it a promising candidate for further development as an anticancer drug.Conclusion:Vitekwangin B may offer a new direction for cancer therapy by targeting ANO1 protein, potentially improving treatment outcomes in patients with prostate cancer and NSCLC. Further research is needed to explore its full potential and overcome existing drug resistance challenges.Publication link2024 Discovery of a Novel Potent Tetrazole Antifungal Candidate with High Selectivity and Broad SpectrumSyncroPatch 384 Publication in J. Med. Chem. (2024) Authors: Ni T., Hao Y., Ding Z., Chi X., Xie F., Wang R., Bao J., Yan L., Li L., Wang T., Zhang D., Jiang Y.Thirty-one novel albaconazole derivatives were designed and synthesized based on our previous work. All compounds exhibited potentin vitroantifungal activities against seven pathogenic fungi. Among them, tetrazole compoundD2was the most potent antifungal with MIC values of 10 m in diameter) prior to extrusion into small unilamellar vesicles (SUVs, 100 nm) versus (2) direct acoustic loading into pre-formed SUVs. The GUV-first approach demonstrated better performance, achieving 60.04% 1.55% encapsulation efficiency (EE%) at 250 mW acoustic powera 5.93% enhancement over direct SUV loading (54.11% 0.72%). Structural analysis via TEM confirmed intact SUV morphology post-loading, while power-dependent EE% analysis showed a linear trend. This work bridges gaps in nanocarrier engineering by optimizing drug loading strategies, aiming to offer a potential drug carrier platform for drug delivery in biomedical treatment in future.Publication link2025 Identification of Lauric Acid as a Potent Sodium Channel NaV1.5 Blocker from Compound Chinese Medicine Wenxin KeliCardioExcyte 96 Publication in Drug Design Development and Therapy (2025) Authors: Xie W., Gao J., Liang Y., Huang C., Zhang B., Chen X., Yao X., Nan G., Wu H., Wang Y., Wu L., Wang T., Zhu Y.Purpose:The major cardiac voltage-gated sodium channel NaV1.5 (INa) is essential for cardiac action potential initiation and subsequent propagation. Compound Chinese medicine Wenxin Keli (WXKL) has been shown to suppress arrhythmias and heart failure. However, its active components have not been fully elucidated. This study focused on identifying the active inhibitor of INain WXKL and exploring their mode of action in electrophysiological conduction.Methods:A chemical fraction library was constructed from an aqueous extract of WXKL and screened using an automated patch-clamping system in cells stably expressing the NaV1.5 gene SCN5A. Candidate fractions with INa-inhibition activity were analyzed by HPLC-ESI-IT-TOF-MS and GC-MS to identify the ingredients. NaV1.5 blocker molecules identified by single-cell electrocardiogram were tested in hiPSC-derived cardiomyocytes. We evaluated the SCN5A inhibitory potential of Wenxin Keli effective monomer employing molecular docking and molecular dynamics simulation approaches.Results:A primary screen of the WXKL chemical library identified five fractions that significantly inhibited the NaV1.5 channel, with one of them rich in poly-saturated fatty acids. Molecular structural characterization revealed the presence of lauric acid, myristic acid, palmitic acid, and stearic acid in the active subfraction. Electrophysiological characterization demonstrated lauric acid (LA) as the most effective monomer for INa-inhibition with an IC50at 27.40 12.78 M. LA shifted the steady-state inactivation of INato more negative potentials and decreased the amplitude of extracellular field potential in hiPSC-derived cardiomyocytes. We demonstrate for the first time that naturally poly-saturated fatty acid, lauric acid, as a potential novel INablocker. Molecular docking and molecular dynamics simulation suggested that LA binds to the NaV1.5 protein, with a significant binding affinity forming interactions with functionally essential residues and blocks the inward flow of Na+. Mechanistically, lauric acid acts on the fast inactivation of NaV1.5 alter electrophysiology conduction of hiPSC-derived cardiomyocytes and contribute to the antiarrhythmic effect of WXKL.Conclusion:Lauric acid is a potent blocker for sodium channel NaV1.5 and alleviates arrhythmia via inhibiting INa.Publication link2025 Invention of an oral medication for cardiac Fabry disease caused by RNA mis-splicingSyncroPatch 384PE (a predecessor model of SyncroPatch 384) Publication in Science Advances (2025) Authors: Awaya T., Ajiro M., Kobayashi H., Sawada T., Gotanda K., Noji T., Takemoto N., Iida K., Saito M.K., Hagiwara M.Pathogenic RNA splicing variants have emerged as promising therapeutic targets due to their role in disease while preserving coding sequences. In this study, we developed RECTAS-2.0, a small molecule designed to correct RNA mis-splicing caused by theGLAc.639+919G>A mutation, which leads to the inclusion of a 57-nucleotide poison exon, resulting in later-onset Fabry disease, particularly prevalent in East Asia. RECTAS-2.0 restored normalGLAmRNA splicing and -galactosidase activity in patient-derived B-lymphoblastoid cell lines and induced pluripotent stem cellderived cardiomyocytes. Furthermore, oral administration of RECTAS-2.0 effectively corrected splicing in a transgenic mouse model, demonstrating its substantial splice-switching activity and safety for clinical application. RECTAS-2.0 demonstrated potential applicability to other genetic disorders that involve similar exon competition. These findings underscore the therapeutic potential of RECTAS-2.0 for Fabry disease and highlight its broader implications for RNA splicingtargeted therapies in genetic disorders.Publication link2025 Large and Stable Nanopores Formed by Complement Component 9 for Characterizing Single Folded ProteinsOrbit mini Publication on ACS Nano (2025) Authors: Chanakul W., Mukhopadhyay A., Awasthi S., Protopopova A., Ianiro A., Mayer M.Biological nanopores offer a promising approach for single-molecule analysis of nucleic acids, peptides, and proteins. The work presented here introduces a biological nanopore formed by the self-assembly of complement component 9 (C9). This exceptionally large and cylindrical protein pore is composed of 20 4 monomers of C9 resulting in a diameter of 10 4 nm and an effective pore length of 13 nm. These poly(C9) pores remain stable for up to 30 min without indications of gating, flickering, or clogging across a range of transmembrane voltages (150 to +150 mV) and ionic strengths (50 to 1000 mM). At physiologic pH, the ring-shaped distribution of negative and positive surface charges in the lumen of the pore enables capture of analyte proteins by electro-osmotic flow and leads to residence times of analyte proteins whose most probable values can exceed 300 s. We used poly(C9) nanopores to determine the volume and shape of unlabeled folded proteins with molecular weights between 9 and 230 kDa with unprecedented accuracy in the context of resistive pulse recordings. Finally, poly(C9) pores made it possible to distinguish between the open and closed conformations of adenylate kinase based on differences in current modulations within resistive pulses and the corresponding differences in approximations of their shape. Thus, poly(C9) nanopores enable highly sensitive and accurate characterization of a wide range of natively folded proteins on a single molecule level.Publication link2025 LYSO-Preps for organellar electrophysiology: A new tool for high throughput measurement of lysosomal TRPML1 channels using the SyncroPatch 384, SURFE2R N1 and SURFE2R 96SESURFE2R 9SE, SURFE2R N1, SyncroPatch 384 Whitepaper (2025) Authors: Murciano N., Masson B., Zerlotti R., Cole K., Barthmes M., Bazzone A., Seibertz F., Friis S., Becker N., Obergrussberger A., Debbiche R., Moutia M., Santinho A., Thiam A.R., George C., George M., Fertig N., Rapedius M.Lysosomes are essential for cellular homeostasis and their dysfunction is linked to a wide range of diseases. Given the immediate medical relevance, there is a current need for high throughput electrophysiology on lysosomes.Oria Bioscience have pioneered a novel approach for lysosomal isolation resulting in large batches of ready-to-use LYSO-Preps of high purity. We have successfully characterized LYSO-Preps overexpressing TRPML1 channels through pharmacology of activators, inhibitors and intraluminal pH sensitivity using the SyncroPatch 384, the SURFE2R N1 and the SURFE2R 96SE.Publication link2025 Lysosomal Ion Channels and Transporters: Recent Findings, Therapeutic Potential, and Technical ApproachesSyncroPatch 384, SURFE2R N1, SURFE2R 96SE Publication in Bioelectricity (2025) Authors: Kondratskyi A., Bazzone A., Rapedius M., Zerlotti R., Masson B., Sadanandan N.P., Parker J.L., Santinho A., Moutia M., Thiam A.R., Kemp A., Seibertz F., Murciano N., Friis S., Becker N., Obergrussberger A., Barthmes M., George C., George M., Dalrymple D., Gasnier B., Newstead S., Grimm C., Fertig N.In recent years, there has been a growing interest in lysosomal ion channels and transporters due to their critical role in maintaining lysosomal function and their involvement in a variety of diseases, particularly lysosomal storage diseases, cancer, and neurodegenerative disorders. Recent advancements in research techniques, including manual and automated patch clamp (APC) electrophysiology, solid-supported membrane-based electrophysiology (SSME), and fluorescence-based ion imaging, have further enhanced our ability to investigate lysosomal ion channels and transporters in both physiological and pathological conditions, spurring drug discovery efforts. Several pharmaceutical companies are now developing therapies aimed at modulating these channels and transporters to improve lysosomal function in disease. Small molecules targeting channels like transient receptor potential mucolipin (TRPML) 1 and TMEM175, as well as drugs modulating lysosomal pH, are currently in preclinical and clinical development. This review provides an overview of the role of lysosomal ion channels and transporters in health and disease, highlights the cutting-edge techniques used to study them, and discusses the therapeutic potential of targeting these channels and transporters in the treatment of various diseases. Furthermore, in addition to summarizing recent discoveries, we contribute novel functional data on cystinosin, TRPML1, and two-pore channel 2 (TPC2), utilizing both SSME and APC approaches.Publication link2025 Mechanisms of RCD-1 pore formation and membrane bendingVesicle Prep Pro Publication in Nature communications (2025) Authors: Ren K., Farrell J.D., Li Y., Guo X., Xie R., Liu X., Kang Q., Fan Q., Ye F., Ding J., Jiao F.Regulator of cell death-1 (RCD-1) governs the heteroallelic expression of RCD-1-1 and RCD-1-2, a pair of fungal gasdermin (GSDM)-like proteins, which prevent cytoplasmic mixing during allorecognition and safeguard against mycoparasitism, genome exploitation, and deleterious cytoplasmic elements (e.g., senescence plasmids) by effecting a form of cytolytic cell death. However, the underlying mechanisms by which RCD-1 acts on the cell membrane remain elusive. Here, we demonstrate that RCD-1 binds acidic lipid membranes, forms pores, and induces membrane bending. Using atomic force microscopy (AFM) and AlphaFold, we show that RCD-1-1 and RCD-1-2 form heterodimers that further self-assemble into ~14.5 nm-wide transmembrane pores (~10 heterodimers). Moreover, through AFM force spectroscopy and micropipette aspiration, we reveal that RCD-1 proteins bend membranes with low bending moduli. This combined action of pore formation and membrane deformation may constitute a conserved mechanism within the broader GSDM family.Publication link2025 Membrane Binding of Hydrophobic Ions: Application of New Kinetic TechniquesSURFE2R N1 Publication in Langmuir

the SyncroPatch 384SyncroPatch 384 application note: in collaboration with the Charles River and SB Drug DiscoveryWe have developed a method that allows fluoride-free, physiological solutions to be used with good success rates. We demonstrate this using the cardiac ion channels hERG expressed in HEK293 cells (SB Drug Discovery) and NaV1.5 expressed in CHO cells (Charles River).Case study PDFHigh throughput optogenetic stimulation for auditory neuroscienceFlyer PDFImmuno-oncology research AltaZFlyer PDFInvestigating TMEM175 with the SURFE2R 96SEApplication note PDFLiquid tumor cells Investigating cell lines derived from hematological malignancies via impedanceAtlaZ Application noteCancer remains one of the most prevalent and lethal diseases globally. Liquid tumor (hematological cancer) cell lines are vital in studying blood cancers and developing therapies. Immunotherapy, particularly chimeric antigen receptor (CAR) T-cell therapy, has shown great promise, improving outcomes in hematological malignancies. Since 2017, the FDA has approved six CAR T-cell therapies for blood cancers, including lymphomas, leukemia, and multiple myeloma.Advancing CAR T-cell therapy requires basic mechanistic research, bioengineering, and clinical trials to enhance T-cell biology and improve CAR T-cell proliferation and durability. CRISPR-Cas9 technology offers new opportunities for genome-wide screening to identify genes that can boost CAR T-cell resilience.This application note describes how liquid tumor cells can be monitored and how effects of cytolysing reagents or effector cells such as CAR T-cells can be quantified using an impedance-based assay employing the AtlaZ platform.Flyer PDFMolecular translocation Orbit miniApplication Note PDFNaV 1.7, nAChR Toxin block of NaV1.7 and nAChR receptors on the PatchlinerPatchliner application note: in collaboration with Alomone labsTraditional drug development for ion channels has predominantly focused on small molecules. While effective, this approach often encounters challenges, particularly with selectivity and safety. Biologics, including toxins and venom-derived peptides, have emerged as a promising alternative to address these limitations. Animal toxins have long been invaluable in ion channel research, offering insights into their structure, function, and gating mechanisms. Venoms are now recognized as a rich resource for innovative research tools and therapeutic leads. Peptide-based drugs, derived from these venoms, have gained attention for their unique advantages, such as larger interaction surfaces that confer greater specificity and selectivity compared to small molecules.Application Note PDFNaV, KV, GABAA, glycine The impact of astrocytes on the maturation of hiPSC-derived neuronsSyncroPatch 384 application note: in collaboration with the Fraunhofer Institute for Biomedical EngineeringStem cell-derived neurons have become important tools in neuroscience research and a number of hiPSC- derived neuronal types are currently available on the market. Culture conditions are critical for maturation of hiPSC-derived neurons and it was discovered early on that commonly used culture media, e.g. DMEM-basal, Neurobasal or serum, while promoting neuronal survival, could actually impair neurophysiological functions. In order to promote maturation of neurons, they can be co-cultured with astrocytes. In collaboration with the Fraunhofer Institute for Biomedical Engineering (IBMT) we explored co-culture of hiPSC-derived neurons (EBiSC-NEUR1) and astrocytes (ScienCell, #1800) by comparing the electrophysiological properties of the neurons using the SyncroPatch 384.Story PDFNovel small molecule modulation of PIEZO1 channelsStudent stories - Dr. Jacob Kinsella, University of LeedsFlyer PDFPatchControlHT Suite PatchlinerFlyer PDFPort-a-Patch at a glanceFlyer PDFQuantitative live-cell analytics with GxP data compliance AtlaZCase study PDFReplacing radiolabeling techniques with the SURFE2R N1Flyer PDFSERCA SURFE2R N1Case study PDFSpheroids Monitoring Spheroid Growth and Response to Treatment via ImpedancePodcastspotl]i[ght Podcast Katherine Henzler-Wildman (University of Wisconsin-Madison) & Nathan Thomas cGuest: Katherine Henzler-Wildman (University of Wisconsin-Madison) & Nathan Thomas (University of California - San Diego)On this episode of the podcast we spoke with Katie and Nathan about their work (specifically around developing a method to provide a fast, easy, general method for measuring transport stoichiometry via solid supported membrane electrophysiology) which will facilitate future mechanistic and functional studies of ion-coupled transporters. You can read the full publication titled: A solid-supported membrane electrophysiology assay for efficient characterization of ion-coupled transport here.Katie's Lab uses NMR as a primary technique to monitor protein structure and dynamics, taking advantage of the extensive resources available through NMRFAM. By comparing NMR data with biochemical and functional assays, insight into the mechanisms of secondary active transport, multidrug recognition, ion channel gating and ion selectivity is gained.Nathan is presentlt a Postdoctoral Researchers at Tezcan Lab - UCSD (University of California San Diego,) Department of Chemistry and Biochemistry.Podcastspotl]i[ght Podcast Kerstin Gpfrich (Max Planck Institute for Medical Research)Guest: Kerstin Gpfrich (Max Planck Institute for Medical Research)In this edition of the podcast - we speak to Kerstin Gpfrich Ph.D. who is currently attempting how to construct a cell with a bottom up approach and exploring how life could be different with DNA origami.Podcastspotl]i[ght Podcast Kerstin Gpfrich (Max Planck Institute for Medical Research)Guest: Kerstin Gpfrich (Max Planck Institute for Medical Research)In this edition of the podcast - we speak to Kerstin Gpfrich Ph.D. who is currently attempting how to construct a cell with a bottom up approach and exploring how life could be different with DNA origami.Podcastspotl]i[ght Podcast Nasreen Choudhury (Rush University)Guest: Nasreen Choudhury (Rush University)In this edition of the podcast - we spoke to Postdoctoral Research Fellow Nasreen Choudhury about her research of Alziehmer's disease and the role that ion channels play. Nasreen completed her PhD at the Indian Institute of Science where she published a paper about TREK1 channel, read it here. Nasreen went on to working at the University of Leicester where she published her investigation into Kv3 channels.When Nasreen finishes her postdoctoral stint at Rush University Medical Center, she plans to switch to an industry role where she can work with automated patch clamp platformsPodcastspotl]i[ght Podcast Nasreen Choudhury (Rush University)Guest: Nasreen Choudhury (Rush University)In this edition of the podcast - we spoke to Postdoctoral Research Fellow Nasreen Choudhury about her research of Alziehmer's disease and the role that ion channels play. Nasreen completed her PhD at the Indian Institute of Science where she published a paper about TREK1 channel, read it here. Nasreen went on to working at the University of Leicester where she published her investigation into Kv3 channels.When Nasreen finishes her postdoctoral stint at Rush University Medical Center, she plans to switch to an industry role where she can work with automated patch clamp platformsPodcastspotl]i[ght Podcast Shashank Pant (University Of Illinois Urbana-Champaign)Guest: Shashank Pant (University Of Illinois Urbana-Champaign)On this episode of the podcast we spoke with Shashank Pant (University of Illinois, Urbana-Champaign). We talked about how he started his journey as a researcher and how his curiosity of the scientific process continues to serve as a motivation to publish in high impact journals such as Nature (where he was recently part of an internationally collaboration with with Renae Ryan's Lab, University of Sydney).Here they looked at Glutamate, specifically providing insight into the mechanism by which glutamate transporters support their dual functionPodcastspotl]i[ght Podcast Shashank Pant (University Of Illinois Urbana-Champaign)Guest: Shashank Pant (University Of Illinois Urbana-Champaign)On this episode of the podcast we spoke with Shashank Pant (University of Illinois, Urbana-Champaign). We talked about how he started his journey as a researcher and how his curiosity of the scientific process continues to serve as a motivation to publish in high impact journals such as Nature (where he was recently part of an internationally collaboration with with Renae Ryan's Lab, University of Sydney).Here they looked at Glutamate, specifically providing insight into the mechanism by which glutamate transporters support their dual functionPodcastspotl]i[ght Podcast Trong Shen (University of Toronto)Guest: Trong Shen (University of Toronto)In this edition of the podcast - we speak to Tron Shen and his unique experiences working in an academic lab (Hinz Lab). Listen to his unique story, which encompases a stop in the world of finance.Podcastspotl]i[ght Podcast Wandi Zhu (Harvard)Guest: Wandi Zhu (Harvard)Bacterial and mammalian NaV channels provide insights into the molecular basis of channel gating and will facilitate organism-specific drug discovery.Podcastspotl]i[ght Podcast Wandi Zhu (Harvard)Guest: Wandi Zhu (Harvard)Bacterial and mammalian NaV channels provide insights into the molecular basis of channel gating and will facilitate organism-specific drug discovery.Flyer PDFSyncroPatch 384 NaV with adaptive voltage clampFlyer PDFSyncroPatch 384 NPC-384 ChipsCase study PDFTargeting TRP channels for the treatment of eye diseasesCase study PDFTargeting TRP channels for the treatment of eye diseasesFlyer PDFTRPV1 PatchlinerCase study PDFTwo years of the SyncroPatch 384 in the city of scienceStory PDFUsing the Patchliner to investigate Duchenne Muscular DystrophyStudent stories - Dr. Alessandro Cerchiara, University of BariFlyer PDFVirology research AtlaZPublication link2024 Wnt dose escalation during the exit from pluripotency identifies tranilast as a regulator of cardiac mesodermCardioExcyte 96 Publication in Developmental Cell (2024) Authors: Wu Z., Shen S., Mizikovsky D., Cao Y., Naval-Sanchez M., Tan S.Z., Alvarez Y., Sun Y., Chen X., Zhao Q., Kim D., Yang P., Hill T., Jones A., Fairlie D., Pbay A., Hewitt A., Tam P., White M., Nefzger C., Palpant N.Wnt signaling is a critical determinant of cell lineage development. This study used Wnt dose-dependent induction programs to gain insights into molecular regulation of stem cell differentiation. We performed single-cell RNA sequencing of hiPSCs responding to a dose escalation protocol with Wnt agonist CHIR-99021 during the exit from pluripotency to identify cell types and genetic activity driven by Wnt stimulation. Results of activated gene sets and cell types were used to build a multiple regression model that predicts the efficiency of cardiomyocyte differentiation. Cross-referencing Wnt-associated gene expression profiles to the Connectivity Map database, we identified the small-molecule drug, tranilast. We found that tranilast synergistically activates Wnt signaling to promote cardiac lineage differentiation, which we validate byinvitroanalysis of hiPSC differentiation andinvivoanalysis of developing quail embryos. Our study provides an integrated workflow that links experimental datasets, prediction models, and small-molecule databases to identify drug-like compounds that control cell differentiation.Publication link2024 Secretion of the fungal toxin candidalysin is dependent on conserved precursor peptide sequencesOrbit 16 (a predecessor model of the Orbit 16TC) Publication in Nature Microbiology (2024) Authors: Mller R., Knig A., Groth S., Zarnowski R., Visser C., Handrianz T., Maufrais C., Krger T., Himmel M., Lee S., Priest E., Yildirim D., Richardson J., Blango M., Bougnoux M.E., Kniemeyer O., dEnfert C., Brakhage A., Andes D., Trmper V., Nehls C., Kasper L., Mogavero S., Gutsmann T., Naglik J., Allert S., Hube B.The opportunistic fungal pathogenCandida albicansdamages host cells via its peptide toxin, candidalysin. Before secretion, candidalysin is embedded in a precursor protein, Ece1, which consists of a signal peptide, the precursor of candidalysin and seven non-candidalysin Ece1 peptides (NCEPs), and is found to be conserved in clinical isolates. Here we show that the Ece1 polyprotein does not resemble the usual precursor structure of peptide toxins.C. albicanscells are not susceptible to their own toxin, and single NCEPs adjacent to candidalysin are sufficient to prevent host cell toxicity. Using a series of Ece1 mutants, mass spectrometry and anti-candidalysin nanobodies, we show that NCEPs play a role in intracellular Ece1 folding and candidalysin secretion. Removal of single NCEPs or modifications of peptide sequences cause an unfolded protein response (UPR), which in turn inhibits hypha formation and pathogenicity in vitro. Our data indicate that the Ece1 precursor is not required to block premature pore-forming toxicity, but rather to prevent intracellular auto-aggregation of candidalysin sequences.Publication link2024 Reprogramming of DNA methylation patterns mediates perfluorooctane sulfonate-induced fetal cardiac dysplasiaCardioExcyte 96 Publication in Science of the Total Environment (2024) Authors: Qiu M., Chen J., Liu M., Shi Y., Nie Z., Dong G., Li X., Chen J., Ou Y., Zhuang J.Prenatal exposure to perfluorooctane sulfonate (PFOS) is associated with adverse health effects, including congenital heart disease, yet the underlying mechanisms remain elusive. Herein, we aimed to evaluate the embryotoxicity of PFOS using C57BL/6J mice to characterize fetal heart defects after PFOS exposure, with the induction of human embryonic stem cells (hESC) into cardiomyocytes (CMs) as a model of early-stage heart development. We also performed DNA methylation analysis to clarify potential underlying mechanisms and identify targets of PFOS. Our results revealed that PFOS caused septal defects and excessive ventricular trabeculation cardiomyopathy at 5mg/kg/day in embryonic mice and inhibited the proliferation and pluripotency of ESCs at concentrations >20M. Moreover, it decreased the beating rate and the population of CMs during cardiac differentiation. Decreases were observed in the abundances of NPPA+ trabecular and HEY2+ compact CMs. Additionally, DNA methyl transferases and ten-eleven translocation (TET) dioxygenases were regulated dynamically by PFOS, with TETs inhibitor treatment inducing significant decreases similar as PFOS. 850K DNA methylation analysis combined with expression analysis revealed several potential targets of PFOS, includingSORBS2,FHOD1,SLIT2,SLIT3,ADCY9, andHDAC9. In conclusion, PFOS may reprogram DNA methylation, especially demethylation, to induce cardiac toxicity, causing ventricular defects in vivo and abnormal cardiac differentiation in vitro.Application Note PDFHepatocytes Comprehensive impedance-based hepatotoxicity assay for metabolically active iPSC-derived hepatocytesCardioExcyte 96, AtlaZ Application Note: Cells were kindly provided by FUJIFILM CellularDynamics, FCDIHepatotoxicity and drug-induced liver injury (DILI) are leading reasons for drug failure to market, leading to approximately 18% of market withdrawals of drugs in the last decade.Additionally, only half of drugs with a potential to induce hepatotoxicity are actually identified during preclinical animal studies. This highlights the importance of generating moreadvanced cell-based models and experimental strategies to enhance the predictivity of these assays. Current existing in vitro models employed to predict DILI mostly focus onhepatocytes, though primary hepatocytes do not maintain their phenotype. iCell Hepatocytes 2.0 (FUJIFILM Cellular Dynamics, FCDI) are human iPSC-derived cells with awide variety of basic and functional characteristics which make them amenable to applications such as compound mediated ADME-T and DILI toxicity. In addition to displayingcharacteristic hepatocyte morphology, (i.e., polygonal shape, polynucleation and formation of bile canalicular channels), these cells also express liver cell markers, including albumin,A1AT, and HNF4a, and exhibit basic and induced P450 functions, as observed in primary human hepatocytes. iCell Hepatocytes 2.0 maintain morphology, marker expression,and metabolic function in culture over a longer time frame compared to primary human hepatocytes, rendering these cells useful for investigations of acute and chronic DILI responses in a 2D culture system using impedance. Combining these cells with the planar gold-film electrodes on the impedance systems reveal alterations in confluency, cell contact (morphological shape) and conductivity of adherent cells, thereby providing a measure of toxicity. Dose-dependent harmful effects of drugs could be evaluated over time in a functional 2D cell-based model without the need for 3D spheroid formation.Publication link2024 Nanobody-mediated neutralization of candidalysin prevents epithelial damage and inflammatory responses that drive vulvovaginal candidiasis pathogenesisOrbit 16 (a predecessor model of the Orbit 16TC) Publication in American Society for Microbiology (2024) Authors: Valentine M., Rudolph P., Dietschmann A., Tsavou A., Mogavero S., Lee S., Priest E., Zhurgenbayeva G., Jablonowski N., Timme S., Eggeling C., Allert S., Dolk E., Naglik J., Figge M., Gresnigt M., Hube B.Candida albicanscan cause mucosal infections in humans. This includes oropharyngeal candidiasis, which is commonly observed in human immunodeficiency virus infected patients, and vulvovaginal candidiasis (VVC), which is the most frequent manifestation of candidiasis. Epithelial cell invasion byC. albicanshyphae is accompanied by the secretion of candidalysin, a peptide toxin that causes epithelial cell cytotoxicity. During vaginal infections, candidalysin-driven tissue damage triggers epithelial signaling pathways, leading to hyperinflammatory responses and immunopathology, a hallmark of VVC. Therefore, we proposed blocking candidalysin activity using nanobodies to reduce epithelial damage and inflammation as a therapeutic strategy for VVC. Anti-candidalysin nanobodies were confirmed to localize around epithelial-invadingC. albicanshyphae, even within the invasion pocket where candidalysin is secreted. The nanobodies reduced candidalysin-induced damage to epithelial cells and downstream proinflammatory responses. Accordingly, the nanobodies also decreased neutrophil activation and recruitment.In silicomathematical modeling enabled the quantification of epithelial damage caused by candidalysin under various nanobody dosing strategies. Thus, nanobody-mediated neutralization of candidalysin offers a novel therapeutic approach to block immunopathogenic events during VVC and alleviate symptoms.Flyer PDFMitochondrial Membrane ResearchFlyer PDFSyncroPatch 384 DataControl 384Flyer PDFSyncroPatch 384 Optogenetic Stimulation ToolFlyer PDFPatchliner Single Channel RecordingsApplication Note PDFTMEM175, TPC2 High throughput organellar electrophysiology of TMEM175 and TPC2 from freshly isolated lysosomes recorded on the SyncroPatch 384SyncroPatch 384 application note: in collaboration with AxxamIntracellular ion channels are known to play an essential role in various signaling pathways for health and disease, considering that over 80% of transport processes occur insidethe cells. Among the variety of organellar channels and transporters the proton leak channel transmembrane protein 175 (TMEM175) and the lysosomal two-pore channel (TPC) have received increasing attention in the field given their potential roles in connecting lysosomal homeostasis with pathophysiological conditions such as Parkinsons disease and cancer. Consequently, the interest to explore intracellular ion channels as therapeutic targets has grown tremendously indicating a need for high-throughput electrophysiology including patch clamp. There has been some progress in alternative approaches such as solid supported membrane electrophysiology (SSME using the SURFE2R 96SE) recently, however, until now, HTS patch clamp has lacked the possibility to collect data from native lysosomes.PDF FlyerSyncroPatch 384 Advanced Temperature ControlPublication link2013 Functional TRPV1 expression in human corneal fibroblastsPort-a-Patch Publication in Experimental Eye Research (2013) Authors: Yang Y., Yang H., Wang Z., Mergler S., Wolosin JM., Reinach PS.Corneal wound healing in mice subsequent to an alkali burn results in dysregulated inflammation and opacification. Transient receptor potential vanilloid subtype 1 (TRPV1) channel activation in all tissue layers by endogenous ligands contributes to this sight compromising outcome since in TRPV1 knockout mice wound healing results instead in tissue transparency restoration. However, it is not known if primary human stromal fibroblasts exhibit such expression even though functional TRPV1 expression is evident in an immortalized human corneal epithelial cell line. In primary human corneal fibroblasts (HCF), TRPV1 gene expression and localization were identified based on the results of quantitative RT-PCR and immunocytochemistry, respectively. Western blot analysis identified a 100 kD protein corresponding to TRPV1 protein expression in a positive control. Single-cell fluorescence imaging detected in fura2-AM loaded cells Ca(2+) transients that rose 1.8-fold above the baseline induced by a selective TRPV1 agonist, capsaicin (CAP), which were blocked by a TRPV1 antagonist, capsazepine (CPZ) or exposure to a Ca(2+) free medium. The whole-cell mode of the planar patch-clamp technique identified TRPV1-induced currents that rose 1.76-fold between -60 and +130 mV. CAP-induced time dependent changes in the phosphorylation status of mitogen activated protein kinase (MAPK) signaling mediators that led to a 2.5-fold increase in IL-6 release after 24 h. This rise did not occur either in TRPV1 siRNA gene silenced cells or during exposure to SB203580 (10 M), a selective p38 MAPK inhibitor. Taken together, identification of functional TRPV1 expression in HCF suggests that in vivo its activation by injury contributes to corneal opacification and inflammation during wound healing. These undesirable effects may result in part from increases in IL-6 expression mediated by p-p38 MAPK signaling.Publication link2013 Cannabinoid receptor 1 suppresses transient receptor potential vanilloid 1-induced inflammatory responses to corneal injuryPort-a-Patch Publication in Cellular Signalling (2013) Authors: Yang Y., Yang H., Wang Z., Varadaraj K., Kumari SS., Mergler S., Okada Y., Saika S., Kingsley PJ., Marnett LJ., Reinach PS.Cannabinoid receptor type 1 (CB1)-induced suppression of transient receptor potential vanilloid type 1 (TRPV1) activation provides a therapeutic option to reduce inflammation and pain in different animal disease models through mechanisms involving dampening of TRPV1 activation and signaling events. As we found in both mouse corneal epithelium and human corneal epithelial cells (HCEC) that there is CB1 and TRPV1 expression colocalization based on overlap of coimmunostaining, we determined in mouse corneal wound healing models and in human corneal epithelial cells (HCEC) if they interact with one another to reduce TRPV1-induced inflammatory and scarring responses. Corneal epithelial debridement elicited in vivo a more rapid wound healing response in wildtype (WT) than in CB1(-/-) mice suggesting functional interaction between CB1 and TRPV1. CB1 activation by injury is tenable based on the identification in mouse corneas of 2-arachidonylglycerol (2-AG) with tandem LC-MS/MS, a selective endocannabinoid CB1 ligand. Suppression of corneal TRPV1 activation by CB1 is indicated since following alkali burning, CB1 activation with WIN55,212-2 (WIN) reduced immune cell stromal infiltration and scarring. Western blot analysis of coimmunoprecipitates identified protein-protein interaction between CB1 and TRPV1. Other immunocomplexes were also identified containing transforming growth factor kinase 1 (TAK1), TRPV1 and CB1. CB1 siRNA gene silencing prevented suppression by WIN of TRPV1-induced TAK1-JNK1 signaling. WIN reduced TRPV1-induced Ca(2+) transients in fura2-loaded HCEC whereas pertussis toxin (PTX) preincubation obviated suppression by WIN of such rises caused by capsaicin (CAP). Whole cell patch clamp analysis of HCEC showed that WIN blocked subsequent CAP-induced increases in nonselective outward currents. Taken together, CB1 activation by injury-induced release of endocannabinoids such as 2-AG downregulates TRPV1 mediated inflammation and corneal opacification. Such suppression occurs through protein-protein interaction between TRPV1 and CB1 leading to declines in TRPV1 phosphorylation status. CB1 activation of the GTP binding protein, G(i/o) contributes to CB1 mediated TRPV1 dephosphorylation leading to TRPV1 desensitization, declines in TRPV1-induced increases in currents and pro-inflammatory signaling events.Publication link2012 Altered calcium regulation by thermosensitive transient receptor potential channels in etoposide-resistant WERI-Rb1 retinoblastoma cellsPort-a-Patch Publication in Experimental Eye Research (2012) Authors: Mergler S, Cheng Y, Skosyrski S, Garreis F, Pietrzak P, Kociok N, Dwarakanath A, Reinach PS, Kakkassery V.Differences in transient receptor potential (TRP) and cannabinoid receptor type 1 (CB1) expression levels can serve as prognostic factors for retinoblastoma (RB) tumor progression. We hypothesized in RB tissue that such differences are also indicators of whether or not they are sensitive to etoposide. Accordingly, we compared in malignant etoposide-sensitive and etoposide-resistant WERI-Rb1 cells TRPV1, TRPM8 and TRPA1 subtype and CB1 gene expression pattern levels and accompanying functional activity using quantitative real-time RT-PCR, immunohistochemistry, immunofluorescence microscopy, calcium imaging as well as patch-clamp technology. Gene expression patterns were evaluated in enucleated human RB tissues (n = 4). Both etoposide-resistant and etoposide-sensitive WERI-Rb1 cells expressed all of the aforementioned channels based on responses to known activators and thermal challenges. However, TRPA1 was absent in the etoposide-resistant counterpart. Even though both types of RB cells express TRPV1 as well as TRPM8 and CB1, the capsaicin (50 M) (CAP)-induced Ca(2+) rise caused by TRPV1 activation was prompt and transient only in etoposide-resistant RB cells (n = 8). In this cell type, the inability of CB1 activation (10 M WIN) to suppress Ca(2+) responses to CAP (50 M; n = 4) may be attributable to the absence of TRPA1 gene expression. Therefore, using genetic approaches to upregulate TRPA1 expression could provide a means to induce etoposide sensitivity and suppress RB cell tumorigenesis.Publication link2015 Thyronamine induces TRPM8 channel activation in human conjunctival epithelial cellsPort-a-Patch Publication in Cellular Signalling (2015) Authors: Khajavi N, Reinach PS, Slavi N, Skrzypski M, Lucius A, Strau O, Khrle J, Mergler S.3-Iodothyronamine (T1AM), an endogenous thyroid hormone (TH) metabolite, induces numerous responses including a spontaneously reversible body temperature decline. As such an effect is associated in the eye with increases in basal tear flow and thermosensitive transient receptor potential melastatin 8 (TRPM8) channel activation, we determined in human conjunctival epithelial cells (IOBA-NHC) if T1AM also acts as a cooling agent to directly affect TRPM8 activation at a constant temperature. RT-PCR and quantitative real-time PCR (qPCR) along with immunocytochemistry probed for TRPM8 gene and protein expression whereas functional activity was evaluated by comparing the effects of T1AM with those of TRPM8 mediators on intracellular Ca2+([Ca2+]i) and whole-cell currents. TRPM8 gene and protein expression was evident and icilin (20M), a TRPM8 agonist, increased Ca2+influx as well as whole-cell currents whereas BCTC (10M), a TRPM8 antagonist, suppressed these effects. Similarly, either temperature lowering below 23C or T1AM (1M) induced Ca2+transients that were blocked by this antagonist. TRPM8 activation by both 1M T1AM and 20M icilin prevented capsaicin (CAP) (20M) from inducing increases in Ca2+influx through TRP vanilloid 1 (TRPV1) activation, whereas BCTC did not block this response. CAP (20M) induced a 2.5-fold increase in IL-6 release whereas during exposure to 20M capsazepine this rise was completely blocked. Similarly, T1AM (1M) prevented this response. Taken together, T1AM like icilin is a cooling agent since they both directly elicit TRPM8 activation at a constant temperature. Moreover, there is an inverse association between changes in TRPM8 and TRPV1 activity since these cooling agents blocked both CAP-induced TRPV1 activation and downstream rises in IL-6 release.Publication link2024 Transport mechanism of DgoT, a bacterial homolog of SLC17 organic anion transportersSURFE2R N1 Preprint Publication in bioRxiv (2024) Authors: Dmitrieva N., Gholami S., Alleva C., Carloni P., Alfonso-Prieto M., Fahlke C.The solute carrier 17 (SLC17) family contains anion transporters that accumulate neurotransmitters in secretory vesicles, remove carboxylated monosaccharides from lysosomes, or extrude organic anions from the kidneys and the liver. We combined experimental and computational approaches to describe the transport mechanisms of a model bacterial protein, the D-galactonate transporter DgoT, at atomic resolution. We found that protonation of D46 and E133 precedes galactonate binding and that substrate binding induces closure of the extracellular gate, with the conserved R47 coupling substrate binding to transmembrane helix movement. After isomerization to an inward-facing conformation, deprotonation of E133 and subsequent proton trans-fer from D46 to E133 opens the intracellular gate and permits galactonate dissociation. After release of the second proton, apo DgoT returns to the outward-facing conformation. Our results provide a framework to understand how various SLC17 transport functions with distinct transport stoichiometries can be attained through subtle variations in proton and substrate binding/unbinding.Publication link2024 Methoxychalcones as potential anticancer agents for colon cancer: Is membrane perturbing potency relevant?Vesicle Prep Pro Publication in Biochimica et Biophysica Acta (BBA) - General Subjects (2024) Authors: Palko-abuz A., Wesoowska O., Baszczyk M., Uryga A., Sobieszczaska B., Skonieczna M., Kostrzewa-Susow E., Janeczko T., roda-Pomianek K.Chalcones are naturally produced by many plants, and constitute precursors for the synthesis of flavons and flavanons. They were shown to possess antibacterial, antifungal, anti-cancer, and anti- inflammatory properties. The goal of the study was to assess the suitability of three synthetic methoxychalcones as potential anticancer agents. In a panel of colon cancer cell lines they were demonstrated to be cytotoxic, proapoptotic, causing cell cycle arrest, and increasing intracellular level of reactive oxygen species. Anticancer activity of the compounds was not diminished in the presence of stool extract containing microbial enzymes that could change the structure of chalcones. Moreover, methoxychalcones interacted strongly with model phosphatidylcholine membranes as detected by differential scanning calorimetry. Metohoxychalcones particularly affected the properties of lipid domains in giant unilamellar liposomes formed from raft-mimicking lipid composition. This may be of importance since many molecular targets for therapy of metastatic colon cancer are raft-associated receptors (e.g., receptor tyrosine kinases). The importance of membrane perturbing potency of methoxychalcones for their biological activity was additionally corroborated by the results obtained by molecular modelling.Publication link2024 Novel Cocrystal Structures of Peptide Antagonists Bound to the Human Melanocortin Receptor 4 Unveil Unexplored Grounds for Structure-Based Drug DesignSyncroPatch 384 PE (a predecessor model of the SyncroPatch 384) Publication in J. Med. Chem. (2024) Authors: Gimenez L.E., Martin C., Yu J., Hollanders C., Hernandez C.C., Wu Y., Yao D., Han G.W., Dahir N.S., Wu L., Van der Poorten O., Lamouroux A., Mannes M., Zhao S., Tourw D., Stevens R.C., Cone R.D., Ballet S.Melanocortin 4 receptor (MC4-R) antagonists are actively sought for treating cancer cachexia. We determined the structures of complexes withPG-934andSBL-MC-31. These peptides differ fromSHU9119by substituting His6with Pro6and inserting Gly10or Arg10. The structures revealed two subpockets at the TM7-TM1-TM2 domains, separated by N2857.36. Two peptide series based on the complexed peptides led to an antagonist activity and selectivity SAR study. Most ligands retained theSHU9119potency, but severalSBL-MC-31-derived peptides significantly enhanced MC4-R selectivity over MC1-R by 60- to 132-fold. We also investigated MC4-R coupling to the K+channel, Kir7.1. Some peptides activated the channel, whereas others induced channel closure independently of G protein coupling. In cell culture studies, channel activation correlated with increased feeding, while a peptide with Kir7.1 inhibitory activity reduced eating. These results highlight the potential for targeting the MC4-R:Kir7.1 complex for treating positive and restrictive eating disorders.Publication link2024 Prognostic Value of Multiplexed Assays of Variant Effect and Automated Patch-clamping for KCNH2-LQTS Risk StratificationSyncroPatch 384 PE (a predecessor model of the SyncroPatch 384) Pre-Print Publication in medRxiv (2024) Authors: Liu J., Huang J., Wang K., Li Y., Li C., Zhu Y., He X., Zhang Y., Zhao Y., Hu C., Xi Z., Tong M., Li M., Gong P., Hou Y.Background: Long QT syndrome (LQTS) is a lethal arrhythmia condition, frequently caused by rare loss-of-function variants in the cardiac potassium channel encoded by KCNH2. Variant-based risk stratification is complicated by heterogenous clinical data, incomplete penetrance, and low-throughput functional data. Objective: To test the utility of variant-specific features, including high-throughput functional data, to predict cardiac events among KCNH2 variant heterozygotes. Methods: We quantified cell-surface trafficking of 18,796 missense variants in KCNH2 and recorded potassium current densities for 506 KCNH2 variants. Next, we deeply phenotyped 1150 KCNH2 missense variant patients, including ECG features, cardiac event history (528 total cardiac events), and mortality. We then assessed variant functional, in silico, structural, and LQTS penetrance data to stratify event-free survival for cardiac events in the study cohort. Results: Traditional risk factors of QT interval adjusted for heart rate (Hazard Ratio 1.09 [1.07-1.12]) and sex (HR 0.60 [0.47-0.76]) were most significant for predicting events; however, variant-specific current density (HR 0.44 [0.26-0.70]) and estimates of LQTS penetrance (HR 1.93; [1.13-3.39]) were independently predictive of severe cardiac events when controlling for patient-specific features. Conclusion: We show that high-throughput functional data, and other variant-specific features, meaningfully contribute to both diagnosis and prognosis of a clinically actionable monogenic disease.Publication link2024 Cellular-level analyses of SCN5A mutations in left ventricular noncompaction cardiomyopathy suggest electrophysiological mechanisms for ventricular tachycardiaCardioExcyte 96 Publication in Biochemistry and Biophysics Reports (2024) Authors: Li Y., Liu S., Huang J., Xie Y., Hou A., Wei Y.Left ventricular noncompaction cardiomyopathy (LVNC) is a cardiovascular disease characterized by arrhythmia and heart failure. In this study, LVNC myocardial samples were collected from patients who underwent heart transplantation and were analyzed using exome sequencing. Approximately half of the LVNC patients carriedSCN5Avariants, which are associated with clinical symptoms ofventricular tachycardia. To investigate the electrophysiological functions of theseSCN5Avariants and the underlying mechanism by which they increase arrhythmia susceptibility in LVNC patients, functional evaluations were conducted in CHOK1 cells and human embryonic stem cell-derived cardiomyocytes (hESC-CMs) using patch-clamp ormicroelectrodearray (MEA) techniques. These findings demonstrated that theseSCN5Amutants exhibited gain-of-function properties, leading to increased channel activation and enhanced fast inactivation in CHOK1 cells. Additionally, these mutants enhanced the excitability andcontractilityof the cardiomyocyte population in hESC-CMs models. AllSCN5Avariants induced fibrillation-like arrhythmia and increased the heart rate in cardiomyocytes. However, the administration of Lidocaine, an antiarrhythmic drug that acts on sodium ion channels, was able to rescue or alleviate fibrillation-like arrhythmias and secondary beat phenomenon. Based on these findings, it is speculated thatSCN5Avariants may contribute to susceptibility to arrhythmia in LVNC patients. Furthermore, the construction of cardiomyocyte models withSCN5Avariants and their application in drug screening may facilitate the development of precise therapies for arrhythmia in the future.Publication link2024 A dynamically gated triangular DNA nanopore for molecular sensing and cross-membrane transportVesicle Prep Pro and Orbit mini Preprint Publication in Research Square (2024) Authors: Liu X., Liu F., Chhabra H., Maffeo C., Huang Q., Aksimentiev A., Arai T.Synthetic membrane nanopores made of DNA are promising systems to sense and control molecular transport in biosensing, sequencing, and synthetic cells. Dynamically gating cargo transport like the natural ion channels and systematically increasing the lumen size have become long-standing desires in developing nanopores. Here, we design a triangular DNA nanopore with a large dynamically-gated lumen. It can switch between expanded and contracted states without changing its stable triangular shape, whereby specific DNA bindings as stimuli mechanically pinch and release the three corners of the triangular frame. Transmission electron microscopy images and molecular dynamics simulations illustrated the large lumen up to 539 nm2, the stable architectures, and the high shape retention. Single channel current recordings and fluorescence influx studies demonstrated the low-noise repeatable readouts and the controllable cross-membrane macromolecular transport. We envision that the proposed DNA nanopores could offer powerful tools in molecular sensing, drug delivery, and the creation of synthetic cells.Publication link2024 5,6-diHETE lactone (EPA-L) mediates hypertensive microvascular dilation by activating the endothelial GPR-PLC-IP3 signaling pathwayPort-a-Patch Publication in Biochemical and Biophysical Research Communications (2024) Authors: Asulin M., Gorodetzer N., Fridman R., Ben-Shushan R., Cohen Z., Beyer A., Chuyun D., Gutterman D., Szuchman-Sapir A.Endothelial microvascular dysfunction affects multi-organ pathologic processes that contribute to increasedvascular toneand is at the base of impaired metabolic and cardiovascular diseases. Thevascular dilationimpaired bynitric oxide(NO) deficiency in such dysfunctional endothelium is often balanced by endothelial-derived hyperpolarizing factors (EDHFs), which play a critical role in managingvascular tone. Our latest research has uncovered a new group of lactoneoxylipinsproduced in the polyunsaturated fatty acids (PUFAs) CYP450epoxygenasepathway, significantly affectingvascular dilation. The lactone oxylipin, derived fromarachidonic acid(5,6-diHET lactone, AA-L), has been previously shown to facilitate vasodilation dependent on the endothelium in isolated human microvessels. The administration of the lactone oxylipin derived fromeicosapentaenoic acid(5,6-diHETE lactone, EPA-L) to hypertensive rats demonstrated a significant decrease in blood pressure and improvement in the relaxation of microvessels. However, the molecular signaling processes that underlie these observations were not fully understood. The current study delineates the molecular pathways through which EPA-L promotes endothelium-dependent vascular dilation. In microvessels from hypertensive individuals, it was found that EPA-L mediates endothelium-dependent vasodilation while thesignaling pathwaywas not dependent on NO.In vitro studieson human endothelial cells showed that the hyperpolarization mediated by EPA-L relies on G-protein-coupled receptor (GPR)-phospholipase C (PLC)-IP3 signaling that further activates calcium-dependentpotassium flux. The pathway was confirmed using a range of inhibitors and cells overexpressing GPR40, where a specific antagonist reduced the calcium levels and outward currents induced by EPA-L. The downstream AKT and endothelial NO synthase (eNOS) phosphorylations were non-significant. These findings show that the GPR-PLC-IP3 pathway is a key mediator in the EPA-L-triggered vasodilation of arterioles. Therefore, EPA-L is identified as a significant lactone-based PUFA metabolite that contributes to endothelial and vascular health.Publication link2024 Solid-phase synthesis as a tool to create exactly defined, branched polymer vectors for cell membrane targetingVesicle Prep Pro Publication in Macromolecules (2024) Authors: Elter J., Likov V., Moravec O., Vragovi M., Filipov M., tpnek P., cha P., Hrub M.Modern drug formulations often require, besides the active drug molecule, auxiliaries to enhance their pharmacological properties. Tailor-made, biocompatible polymers covalently connected to the drug molecule can fulfill this function by increasing its solubility, reducing its toxicity, and guiding it to a specific target. If targeting membrane-bound proteins, localization of the drug close to the cell membrane and its target is beneficial to increase drug efficiency and residence time. In this study, we present the synthesis of highly defined, branched polymeric structures with membrane

stimulationSyncroPatch 384 Publication in J Gen Physiol (2023) Authors: Murciano N., Rotordam M., Becker N., Ludlow M. , Parsonage G. , Darras A., Kaestner L. , Beech D., George M., Fertig N., Rapedius M., Brggemann A.PIEZO1 channels are mechanically activated cation channels that play a pivotal role in sensing mechanical forces in various cell types. Their dysfunction has been associated with numerous pathophysiological states, including generalized lymphatic dysplasia, varicose vein disease, and hereditary xerocytosis. Given their physiological relevance, investigating PIEZO1 is crucial for the pharmaceutical industry, which requires scalable techniques to allow for drug discovery. In this regard, several studies have used high-throughput automated patch clamp (APC) combined with Yoda1, a specific gating modifier of PIEZO1 channels, to explore the function and properties of PIEZO1 in heterologous expression systems, as well as in primary cells. However, a combination of solely mechanical stimulation (M-Stim) and high-throughput APC has not yet been available for the study of PIEZO1 channels. Here, we show that optimization of pipetting parameters of the SyncroPatch 384 coupled with multihole NPC-384 chips enables M-Stim of PIEZO1 channels in high-throughput electrophysiology. We used this approach to explore differences between the response of mouse and human PIEZO1 channels to mechanical and/or chemical stimuli. Our results suggest that applying solutions on top of the cells at elevated pipetting flows is crucial for activating PIEZO1 channels by M-Stim on the SyncroPatch 384. The possibility of comparing and combining mechanical and chemical stimulation in a high-throughput patch clamp assay facilitates investigations on PIEZO1 channels and thereby provides an important experimental tool for drug development.Publication link2023 A BODIPY-Based Molecular Rotor in Giant Unilamellar Vesicles: A Case Study by Polarization-Resolved TimeResolved Emission and Transient Absorption SpectroscopyVesicle Prep Pro Publication in ChemPhotoChem (2023) Authors: Jha K., Prabhakaran A., Spantzel L., Sia R., Prez I., Arellano-Reyes R., Elmanova A., Dasgupta A., Eggeling C., Brsch M., Guthmuller J., Presselt M., Keyes T., Dietzek-Ivani B.BODIPY and BODIPY-derived systems are widely applied as fluorophores and as probes for viscosity detection in solvents and biological media. Their orientational and rotational dynamics in biological media are thus of vital mechanistic importance and extensively investigated. In this contribution, polarization-resolved confocal microscopy is used to determine the orientation of an amphiphilic BODIPY-cholesterol derivative in homogeneous giant unilamellar vesicles (GUV) made from 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). The BODIPY-moiety of the molecule is placed near the polar headgroups, and the cholesterol moiety is embedded in the membrane along the acyl chain of the lipids. The rotational relaxation of fluorophore is conventionally investigated by time-resolved emission anisotropy (TEA); and this method is also used here. However, TEA depends on the emission of the fluorophore and may not be useful to probe rotational dynamics of the non-emissive triplet states. Thus, we employ femtosecond transient absorption anisotropy (TAA), that relies on the absorption of the molecule to complement the studies of the amphiphilic BODIPY in DCM and GUV. The photoinduced anisotropy of the BODIPY molecule in DCM decays tri-exponentially, the decay components (sub-5ps, 43ps and 440ps) of anisotropy are associated with the non-spherical shape of the BODIPY molecule. However, the anisotropy decay in homogenous GUVs follows a biexponential decay; which arises from the wobbling-in-a-cone motion of the non-spherical molecule in the high viscous lipid bilayer media. The observations for the BODIPY-chol molecule in the GUV environment by TAA will extend to the investigation of non-emissive molecules in cellular environment since GUV structure and size resembles the membrane of a biological cell.Publication link2023 Mechanistic Insight into the Early Stages of Toroidal Pore Formation by the Antimicrobial Peptide Smp24Port-a-Patch, Vesicle Prep Pro Publication in Pharmaceutics (2023) Authors: Bertelsen M., Lacey M., Nichol T., Miller, K.The antimicrobial peptide Smp24, originally derived from the venom ofScorpio maurus palmatus, is a promising candidate for further drug development. However, before doing so, greater insight into the mechanism of action is needed to construct a reliable structureactivity relationship. The aim of this study was to specifically investigate the critical early stages of peptide-induced membrane disruption. Single-channel current traces were obtained via planar patch-clamp electrophysiology, with multiple types of pore-forming events observed, unlike those expected from the traditional, more rigid mechanistic models. To better understand the molecular-level structures of the peptide-pore assemblies underlying these observed conductance events, molecular dynamics simulations were used to investigate the peptide structure and orientation both before and during pore formation. The transition of the peptides to transmembrane-like states within disordered toroidal pores occurred due to a peptide-induced bilayer-leaflet asymmetry, explaining why pore stabilization does not always follow pore nucleation in the experimental observations. To fully grasp the structureactivity relationship of antimicrobial peptides, a more nuanced view of the complex and dynamic mechanistic behaviour must be adoptedPublication link2023 Design, synthesis, and biological evaluation of a novel series of 1,2,4-oxadiazole inhibitors of SLACK potassium channels: Identification of in vitro tool VU0935685SyncroPatch 384 Publication in Bioorganic & Medicinal Chemistry (2023) Authors: Qunies A., Spitznagel B., Du Y., Weaver C., Emmitte K.Malignant migratingpartial seizureof infancy (MMPSI) is a devastating and pharmacoresistant form of infantile epilepsy. MMPSI has been linked to multiple gain-of-function (GOF) mutations in theKCNT1gene, which encodes for apotassium channeloften referred to asSLACK.SLACKchannels are sodium-activated potassium channels distributed throughout thecentral nervous system(CNS) and the periphery. The investigation described here aims to discover SLACK channel inhibitor tool compounds and profile theirpharmacokineticandpharmacodynamicproperties. A SLACK channel inhibitor VU0531245 (VU245) was identified via a high-throughput screen (HTS) campaign. Structure-activity relationship (SAR) studies were conducted in five distinct regions of the hit VU245. VU245 analogs were evaluated for their ability to affect SLACK channel activity using a thalliumflux assayin HEK-293 cells stably expressing wild-type (WT) human SLACK. Selected analogs were tested for metabolic stability in mouseliver microsomesand plasma-protein binding in mouse plasma. The same set of analogs was tested via thallium flux for activity versus human A934T SLACK and other structurally related potassium channels, including SLICK and Maxi-K. In addition, potencies for selected VU245 analogs were obtained using whole-cellelectrophysiology(EP) assays inCHO cellsstably expressing WT human SLACK through anautomated patch clampsystem. Results revealed that this scaffold tolerates structural changes in some regions, with some analogs demonstrating improved SLACK inhibitory activity, good selectivity against the other channels tested, and modest improvements inmetabolic clearance. Analog VU0935685 represents a new, structurally distinct small-molecule inhibitor of SLACK channels that can serve as anin vitrotool for studying this target.Publication link2024 Acute antiarrhythmic effects ofSGLT2 inhibitorsdapagliflozin lowers theexcitability ofatrial cardiomyocytesSyncroPatch 384 Publication in Basic Research in Cardiology (2024) Authors: Paasche A., Wiedmann F., Kraft M., Seibertz F., Herlt V., Blochberger PL., Jvorszky N., Beck M., Weirauch L., Seeger T., Blank A., Haefeli W.E., Arif R., Meyer A.L., Warnecke G., Karck M., Voigt N., Frey N., Schmidt C.In recent years, SGLT2 inhibitors have become an integral part of heart failure therapy, and several mechanisms contributing to cardiorenal protection have been identified. In this study, we place special emphasis on the atria and investigate acute electrophysiological effects of dapagliflozin to assess the antiarrhythmic potential of SGLT2 inhibitors. Direct electrophysiological effects of dapagliflozin were investigated in patch clamp experiments on isolated atrial cardiomyocytes. Acute treatment with elevated-dose dapagliflozin caused a significant reduction of the action potential inducibility, the amplitude and maximum upstroke velocity. The inhibitory effects were reproduced in human induced pluripotent stem cell-derived cardiomyocytes, and were more pronounced in atrial compared to ventricular cells. Hypothesizing that dapagliflozin directly affects the depolarization phase of atrial action potentials, we examined fast inward sodium currents in human atrial cardiomyocytes and found a significant decrease of peak sodium current densities by dapagliflozin, accompanied by a moderate inhibition of the transient outward potassium current. Translating these findings into a porcine large animal model, acute elevated-dose dapagliflozin treatment caused an atrial-dominant reduction of myocardial conduction velocity in vivo. This could be utilized for both, acute cardioversion of paroxysmal atrial fibrillation episodes and rhythm control of persistent atrial fibrillation. In this study, we show that dapagliflozin alters the excitability of atrial cardiomyocytes by direct inhibition of peak sodium currents. In vivo,dapagliflozin exerts antiarrhythmic effects, revealing a potential new additional role of SGLT2 inhibitors in the treatment of atrial arrhythmias.Publication link2024 Revisiting the urease production of MICP-relevant bacterium Sporosarcina pasteurii during cultivationCardioExcyte 96 Publication in Biocatalysis and Agricultural Biotechnology (2024) Authors: Lapierre FM. Huber R.The ureolytic bacterium Sporosarcina pasteurii is commonly used for Microbial Induced Calcium Carbonate Precipitation (MICP). This process has a variety of applications in the fields of construction, geotechnical engineering, and environmental remediation. However, the factors influencing urease activity of S. pasteurii during cultivation are not yet fully understood. Even though there is debate over whether higher urease activity is in fact beneficial for MICP applications, knowledge about urease production can help provide ureolytic biomass more cost- effectively, reducing overall production costs. This study revisits the effect of various cultivation conditions on growth and urease activity by using an automated high-throughput microbioreactor system combined with a novel system for high-throughput urease activity quantification. This experimental set-up allows for unprecedented data density and enables new insights into urease production of S. pasteurii. Several factors were tested, including oxygen limitation, the feeding of urea, choice of ammonium salt, the proportion of ammonium salt in the medium, and lowering the pH during cultivation; none of these had a notable impact on urease production. Only the addition of a broad spectrum of nutrients through regular feeding with highly concentrated yeast extract and glucose led to a 70 % increased specific urease activity compared to a batch culture. These results can improve the understanding of the regulatory mechanisms governing urease expression in S. pasteurii during cultivation, and allow to adapt the cultivation process accordingly. Additionally, the new system for automated high-throughput enzyme activity determination presented in this study could be applied to optimize bioprocesses involving other ion producing enzymes.Publication link2023 The Potential Mechanisms behind Loperamide-Induced Cardiac Arrhythmias Associated with Human Abuse and Extreme OverdoseSyncroPatch 384 Publication in Biomolecules (2023) Authors: Lu H., Damiano B., Kreir M., Rohrbacher J., van der Linde H., Saidov T., Teisman A., Gallacher D.Loperamide has been a safe and effective treatment for diarrhea for many years. However, many cases of cardiotoxicity with intentional abuse of loperamide ingestion have recently been reported. We evaluated loperamide in in vitro and in vivo cardiac safety models to understand the mechanisms for this cardiotoxicity. Loperamide slowed conduction (QRS-duration) starting at 0.3 M [~1200-fold () its human Free Therapeutic Plasma Concentration; FTPC] and reduced the QT-interval and caused cardiac arrhythmias starting at 3 M (~12,000 FTPC) in an isolated rabbit ventricular-wedge model. Loperamide also slowed conduction and elicited Type II/III A-V block in anesthetized guinea pigs at overdose exposures of 879 and 3802 FTPC. In ion-channel studies, loperamide inhibited hERG (IKr),INa,and ICacurrents with IC50values of 0.390 M, 0.526 M, and 4.091 M, respectively (i.e., >1560 FTPC). Additionally, in silico trials in human ventricular action potential models based on these IC50s confirmed that loperamide has large safety margins at therapeutic exposures (600 FTPC) and confirmed repolarization abnormalities in the case of extreme doses of loperamide. The studies confirmed the large safety margin for the therapeutic use of loperamide but revealed that at the extreme exposure levels observed in human overdose, loperamide can cause a combination of conduction slowing and alterations in repolarization time, resulting in cardiac proarrhythmia. Loperamides inhibition of the INachannel and hERG-mediated IKr are the most likely basis for this cardiac electrophysiological toxicity at overdose exposures. The cardiac toxic effects of loperamide at the overdoses could be aggravated by co-medication with other drug(s) causing ion channel inhibition.Publication link2023 A simple quantitative model of neuromodulation, Part I: Ion flow through neural ion channelsBuffer Solutions Publication in Journal of the Mechanics and Physics of Solids (2023) Authors: Werneck L., Han M., Yildiz E., Keip M., Sitti M., Ortiz M.We develop a simple model of ionic current through neuronal membranes as a function of membrane potential and extracellularion concentration. The model combines a simplified PoissonNernstPlanck (PNP) model of ion transport through individual ion channels with channelactivation functionscalibrated fromad hocin-house experimental data. The simplified PNP model is validated against bacterial gramicidin A ion channel data. The calibrated model accounts for the transport of calcium, sodium,potassium, and chloride and exhibits remarkable agreement with the experimentally measured currentvoltage curves for the differentiated human neural cells. All relevant data and code related to the ion flow models are available at Werneck et al. (2023)Publication link2023 Membrane Tension Inhibits Lipid Mixing by Increasing the Hemifusion Stalk EnergyVesicle Prep Pro Publication in ACS Nano (2023) Authors: Shendrick P., Golani G., Dharan R., Schwarz U., Sorkin R.Fusion of biological membranes is fundamental in various physiological events. The fusion process involves several intermediate stages with energy barriers that are tightly dependent on the mechanical and physical properties of the system, one of which is membrane tension. As previously established, the late stages of fusion, including hemifusion diaphragm and pore expansions, are favored by membrane tension. However, a current understanding of how the energy barrier of earlier fusion stages is affected by membrane tension is lacking. Here, we apply a newly developed experimental approach combining micropipette-aspirated giant unilamellar vesicles and optically trapped membrane-coated beads, revealing that membrane tension inhibits lipid mixing. We show that lipid mixing is 6 times slower under a tension of 0.12 mN/m compared with tension-free membranes. Furthermore, using continuum elastic theory, we calculate the dependence of the hemifusion stalk formation energy on membrane tension and intermembrane distance and find the increase in the corresponding energy barrier to be 1.6kBTin our setting, which can explain the increase in lipid mixing time delay. Finally, we show that tension can be a significant factor in the stalk energy if the pre-fusion intermembrane distance is on the order of several nanometers, while for membranes that are tightly docked, tension has a negligible effect.Publication link2023 Determination of single-molecule transport activity of OATP2B1 by measuring the number of transporter molecules using electrophysiological approachPort-a-Patch Publication in Journal of Pharmacological Sciences (2023) Authors: Yajima K., Akiyoshi T., Sakamoto K., Suzuki Y., Oka T., Imaoka A., Yamamura H., Kurokawa J., Ohtani H.Transporter-mediated clearance is determined by two factors, its single-molecule clearance, and expression level. However, no reliable method has been developed to evaluate them separately. Thisstudy aimed to develop a reliable method for evaluating the single-molecule activity of membrane transporters, such as organic anion transporting polypeptide (OATP) 2B1. HEK293 cells that co-expressed large conductance calcium-activated potassium (BK) channel and OATP2B1 were established and used for the following experiments. i) BK channel-mediated whole-cell conductance was measured using patch-clamp technique and divided by its unitary conductance to estimate the number of channels on plasma membrane (QI). ii) Using plasma membrane fraction, quantitative targeted absolute proteomics determined the stoichiometric ratio (r) of OATP2B1 to BK channel. iii) The uptake of estrone 3-sulfate was evaluated to calculate the Michaelis constant and uptake clearance (CL) per cell. Single-molecule clearance (CLint) was calculated by dividing CL by QIxp. QI and r values were estimated to be 916 and 2.16, respectively, yielding CLint of 5.23 fL/min/molecule. We successfully developed a novel method to reliably measure the single-molecule activity of a transporter, which could be used to evaluate the influences of factors such as genetic variations and post-translational modifications on the intrinsic activity of transportersPublication link2023 Synergy between Winter Flounder antimicrobial peptidesVesicle Prep Pro, Port-a-Patch Publication in Nature npj Antimicrobials and Resistance (2023) Authors: Clarke M., Hind C., Ferguson P., Manzo G., Mistry B., Yue B., Romanopulos J., Clifford M., Bui T., Drake A., Lorenz C., Sutton M., Mason J.Some antimicrobial peptides (AMPs) have potent bactericidal activity and are being considered as potential alternatives to classical antibiotics. In response to an infection, such AMPs are often produced in animals alongside other peptides with low or no perceivable antimicrobial activity, whose role is unclear. Here we show that six AMPs from the Winter Flounder (WF) act in synergy against a range of bacterial pathogens and provide mechanistic insights into how this increases the cooperativity of the dose-dependent bactericidal activity and potency that enable therapy. Only two WF AMPs have potent antimicrobial activity when used alone but we find a series of two-way combinations, involving peptides which otherwise have low or no activity, yield potent antimicrobial activity. Weakly active WF AMPs modulate the membrane interactions of the more potent WF AMPs and enable therapy in a model ofAcinetobacter baumanniiburn wound infection. The observed synergy and emergent behaviour may explain the evolutionary benefits of producing a family of related peptides and are attractive properties to consider when developing AMPs towards clinical applications.Publication link2024 Functional Analysis of Human GRIN2A Mutations Associated with Schizophrenia and Neurodevelopmental Disorders Reveals Distinct Pathological MechanismSyncroPatch 384 Publication in Scientific Reports (2024) Authors: Shepard N., Baez-Nieto D., Iqbal S., Kurgano E., Budnik N., Campbell A., Pan J., Sheng M., Farsi Z.Human genetic studies have revealed rare missense and protein-truncating variants inGRIN2A, encoding for the GluN2A subunit of the NMDA receptors, that confer significant risk for schizophrenia (SCZ). Mutations inGRIN2Aare also associated with epilepsy and developmental delay/intellectual disability (DD/ID). However, it remains enigmatic how alterations to the same protein can result in diverse clinical phenotypes. Here, we performed functional characterization of human GluN1/GluN2A heteromeric NMDA receptors that contain SCZ-linked GluN2A variants, and compared them to NMDA receptors with GluN2A variants associated with epilepsy or DD/ID. Our findings demonstrate that SCZ-associatedGRIN2Avariants were predominantly loss-of-function (LoF), whereas epilepsy and DD/ID-associated variants resulted in both gain- and loss-of-function phenotypes. We additionally show that M653I and S809R, LoFGRIN2Avariants associated with DD/ID, exert a dominant-negative effect when co-expressed with a wild-type GluN2A, whereas E58Ter and Y698C, SCZ-linked LoF variants, and A727T, an epilepsy-linked LoF variant, do not. These data offer a potential mechanism by which SCZ/epilepsy and DD/ID-linked variants can cause different effects on receptor function and therefore result in divergent pathological outcomes.Publication link2023 A Comparative Study on the Lysosomal Cation Channel TMEM175 Using Automated Whole-Cell Patch-Clamp, Lysosomal Patch-Clamp, and Solid Supported Membrane-Based Electrophysiology: Functional Characterization and High-Throughput Screening Assay DevelopmentSURFE2R 96SE, SURFE2R N1, SyncroPatch 384 Publication in International Journal of Molecular Sciences (2023) Authors: Bazzone A., Barthmes M., George C., Brinkwirth N., Zerlotti R., Prinz V., Cole K., Friis S., Dickson A., Rice S., Lim J., Toh M., Mohammadi M. Pau D., Stone D., Renger J., Fertig N.The lysosomal cation channel TMEM175 is a Parkinsons disease-related protein and a promising drug target. Unlike whole-cell automated patch-clamp (APC), lysosomal patch-clamp (LPC) facilitates physiological conditions, but is not yet suitable for high-throughput screening (HTS) applications. Here, we apply solid supported membrane-based electrophysiology (SSME), which enables both direct access to lysosomes and high-throughput electrophysiological recordings. In SSME, ion translocation mediated by TMEM175 is stimulated using a concentration gradient at a resting potential of 0 mV. The concentration-dependent K+response exhibited an I/c curve with two distinct slopes, indicating the existence of two conducting states. We measured H+fluxes with a permeability ratio of PH/PK= 48,500, which matches literature findings from patch-clamp studies, validating the SSME approach. Additionally, TMEM175 displayed a high pH dependence. Decreasing cytosolic pH inhibited both K+and H+conductivity of TMEM175. Conversely, lysosomal pH and pH gradients did not have major effects on TMEM175. Finally, we developed HTS assays for drug screening and evaluated tool compounds (4-AP, Zn as inhibitors; DCPIB, arachidonic acid, SC-79 as enhancers) using SSME and APC. Additionally, we recorded EC50data for eight blinded TMEM175 enhancers and compared the results across all three assay technologies, including LPC, discussing their advantages and disadvantages.Publication link2023 Pathogenic SCN2A variants are associated with familial and sporadic hemiplegic migraineSyncroPatch 384 Pre-print in Research Square (2023) Authors: Riant F., Thompson C., DeKeyser J., Abramova T., Gazal S., Moulin T.,Chaigne D., Kort L., Corpechot M., Tournier-Lasserve E., George A., Ducros A.Background:Familial hemiplegic migraine is a severe autosomal dominant subtype of migraine with aura characterized by transient motor weakness during attacks. Previously identified genesCACNA1A,ATP1A2,SCN1AandPRRT2account for less than 20% of cases with hemiplegic migraine referred for genetic diagnosis.Objectives and Methods:To identify a novel gene, we conducted a whole-genome linkage analysis combined with mini-exome sequencing in a four-generation pedigree with hemiplegic migraine. A candidate ion channel gene was analyzed for mutations in six other affected pedigrees comprising at least three available affected members, and in a large panel of unrelated probands with hemiplegic migraine referred for molecular diagnosis, all without mutations in the known genes. The functional consequences of the identified variants were determined.Results:In the discovery pedigree, we identified a heterozygous missense mutation (c.4438A>G, p.Lys1480Glu) in the neuronal voltage-gated sodium channel geneSCN2A, which cosegregated with the hemiplegic migraine phenotype. We detected another mutation (c.769T>A, p.Phe257Ile) cosegregating with hemiplegic migraine in a second family, in which two members also had infantile seizures. A third variant (c.3955C>G, p.Arg1319Gly) was found in a sporadic hemiplegic migraine case. All threeSCN2Avariants were absent in the genome aggregation database gnomAD. Heterologous expression in HEK293T cells coupled with automated patch clamp recording demonstrated abnormal voltage-dependent and kinetic properties of all threeSCN2Avariants.Conclusions:Dysfunction of the neuronal sodium channelSCN2Acan be associated with familial and sporadic hemiplegic migraine. Our finding expands the genetic landscape for migraine and contributes to the diverse genotype-phenotype spectrum associated withSCN2A.Publication link2023 A previously unrecognized superfamily of macro-conotoxins includes an inhibitor of the sensory neuron calcium channel Cav2.3Patchliner Publication in PLoS Biol (2023) Authors: Hackney C., Flrez Salcedo P., Mueller E., Lund Koch T., Kjelgaard L., Watkins M., Zachariassen L., Snderby Tuelung P., McArthur J., Adams D., Kristensen A., Olivera B., Finol-Urdaneta R., Safavi-Hemami H., Preben Morth J., Ellgaard L.Animal venom peptides represent valuable compounds for biomedical exploration. The venoms of marine cone snails constitute a particularly rich source of peptide toxins, known as conotoxins. Here, we identify the sequence of an unusually large conotoxin, Mu8.1, which defines a new class of conotoxins evolutionarily related to the well-known con-ikot-ikots and 2 additional conotoxin classes not previously described. The crystal structure of recombinant Mu8.1 displays a saposin-like fold and shows structural similarity with con-ikot-ikot. Functional studies demonstrate that Mu8.1 curtails calcium influx in defined classes of murine somatosensory dorsal root ganglion (DRG) neurons. When tested on a variety of recombinantly expressed voltage-gated ion channels, Mu8.1 displayed the highest potency against the R-type (Cav2.3) calcium channel. Ca2+signals from Mu8.1-sensitive DRG neurons were also inhibited by SNX-482, a known spider peptide modulator of Cav2.3 and voltage-gated K+(Kv4) channels. Our findings highlight the potential of Mu8.1 as a molecular tool to identify and study neuronal subclasses expressing Cav2.3. Importantly, this multidisciplinary study showcases the potential of uncovering novel structures and bioactivities within the largely unexplored group of macro-conotoxins.Publication link2023 Structure and function of a YeeE-YeeD complex for sophisticated thiosulfate uptakeSURFE2R N1 Publication in bioRxiv (2023) Authors: Ikei M., Miyazaki R., Monden K., Naito Y., Takeuchi A., Takahashi Y., Tanaka Y., Mori T., Ichikawa M., Tsukazaki T.Uptake of thiosulfate ions as an inorganic sulfur source from the environment is important for bacterial sulfur assimilation. Recently, a selective thiosulfate uptake pathway involving membrane protein YeeE (TsuA) was characterized. However, the precise function of YeeE and a putative cofactor in thethiosulfate ion uptake pathway remained unclear. Here, we assessed selective thiosulfate transport via YeeE in vitro and characterized YeeD (TsuB) as an adjacent and essential cofactor for YeeE-mediated thiosulfate uptake in vivo. We further showed that YeeD possesses thiosulfate decomposition activityand that a conserved cysteine in YeeD was modified in several forms in the presence of thiosulfate. Finally, the crystal structure of a YeeE-YeeD fusion protein at 2.6- resolution revealed their interactions. The association was evaluated by a binding assay using purified proteins. Based on these results, a model of the sophisticated uptake of thiosulfate ions by YeeE and YeeD is proposed.Publication link2023 De novo design of diverse small molecule binders and sensors using Shape Complementary PseudocyclesOrbit 16 TC Pre-Print Publication in BioRxiv (2023) Authors: An L., Said M., Tran L., Majumder S., Goreshnik I., Lee G.R., Juergens D., Dauparas J., Anishchenko I., Coventry B., Bera A.K., Kang A., Levine P.M., Alvarez V., Pillai A., Norn C., Feldman D., Zorine D., Hicks D.R., Li X., Sanchez M.G., Vafeados D.K., Salveson P.J., Vorobieva A.A., Baker D.A general method for designing proteins to bind and sense any small molecule of interest would be widely useful. Due to the small number of atoms to interact with, binding to small molecules with high affinity requires highly shape complementary pockets, and transducing binding events into signals is challenging. Here we describe an integrated deep learning and energy based approach for designing high shape complementarity binders to small molecules that are poised for downstream sensing applications. We employ deep learning generated psuedocycles with repeating structural units surrounding central pockets; depending on the geometry of the structural unit and repeat number, these pockets span wide ranges of sizes and shapes. For a small molecule target of interest, we extensively sample high shape complementarity pseudocycles to generate large numbers of customized potential binding pockets; the ligand binding poses and the interacting interfaces are then optimized for high affinity binding. We computationally design binders to four diverse molecules, including for the first time polar flexible molecules such as methotrexate and thyroxine, which are expressed at high levels and have nanomolar affinities straight out of the computer. Co-crystal structures are nearly identical to the design models. Taking advantage of the modular repeating structure of pseudocycles and central location of the binding pockets, we constructed low noise nanopore sensors and chemically induced dimerization systems by splitting the binders into domains which assemble into the original pseudocycle pocket upon target molecule addition.Publication link2023 Multi-site validation of a functional assay to adjudicate SCN5A Brugada Syndrome-associated variantsSyncroPatch 384 Pre-Print Publication in MedRxiv (2023) Authors: Ma J.G., ONeill M.J., Richardson E., Thomson K.L., Ingles J., Muhammad A., Solus Joseph F., Davogustto G., Anderson K.C., Shoemaker M.B., Stergachis A.B., Floyd B.J., Dunn K., Parikh V.N., Chubb H., Perrin M.J., Roden D.M., Vandenberg J.I., Ng C-A., Glazer A.M.Brugada Syndrome (BrS) is an inheritable arrhythmia condition that is associated with rare, loss-of-function variants in the cardiac sodium channel gene,SCN5A. Interpreting the pathogenicity ofSCN5Amissense variants is challenging and 79% ofSCN5Amissense variants in ClinVar are currently classified as Variants of Uncertain Significance (VUS). Anin vitro SCN5A-BrS automated patch clamp assay was generated for high-throughput functional studies of NaV1.5. The assay was independently studied at two separate research sites Vanderbilt University Medical Center and Victor Chang Cardiac Research Institute revealing strong correlations, including peakINadensity (R2=0.86). The assay was calibrated according to ClinGen Sequence Variant Interpretation recommendations using high-confidence variant controls (n=49). Normal and abnormal ranges of function were established based on the distribution of benign variant assay results. The assay accurately distinguished benign controls (24/25) from pathogenic controls (23/24). Odds of Pathogenicity values derived from the experimental results yielded 0.042 for normal function (BS3 criterion) and 24.0 for abnormal function (PS3 criterion), resulting in up to strong evidence for both ACMG criteria. The calibrated assay was then used to studySCN5AVUS observed in four families with BrS and other arrhythmia phenotypes associated withSCN5Aloss-of-function. The assay revealed loss-of-function for three of four variants, enabling reclassification to likely pathogenic. This validated APC assay provides clinical-grade functional evidence for the reclassification of current VUS and will aid futureSCN5A-BrS variant classification.Publication link2023 Sculpting conducting nanopore size and shape through de novo protein designOrbit 16 TC Pre-Print Publication in BioRxiv (2023) Authors: Berhanu S., Majumder S., Mntener T., Whitehouse J., Berner C., Bera A.K., Kang A., Liang B., Khan G.N., Sankaran B., Tamm L.K., Brockwell D.J., Hiller S., Radford S.E., Baker D., Vorobieva A.A.Transmembrane -barrels (TMBs) are widely used for single molecule DNA and RNA sequencing and have considerable potential for a broad range of sensing and sequencing applications. Current engineering approaches for nanopore sensors are limited to naturally occurring channels such as CsgG, which have evolved to carry out functions very different from sensing, and hence provide sub-optimal starting points. In contrast,de novoprotein design can in principle create an unlimited number of new nanopores with any desired properties. Here we describe a general approach to the design of transmembrane -barrel pores with different diameter and pore geometry. NMR and crystallographic characterization shows that the designs are stably folded with structures close to the design models. We report the first examples ofde novodesigned TMBs with 10, 12 and 14 stranded -barrels. The designs have distinct conductances that correlate with their pore diameter, ranging from 110 pS (0.5 nm pore diameter) to 430 pS (1.1 nm pore diameter), and can be converted into sensitive small-molecule sensors with high signal to noise ratio. The capability to generate on demand -barrel pores of defined geometry opens up fundamentally new opportunities for custom engineering of sequencing and sensing technologies.Publication link2023 Deep Learning-Assisted Single-Molecule Detection of Protein Post-translational Modifications with a Biological NanoporeOrbit mini Publication in ACS Nano (2023) Authors: Cao C.,MagalhaesP., Krapp L.F.,Bada J.F.J, Mayer S.F., Rukes V., Chiki A., Lashuel H.A., Dal Peraro M.Protein post-translational modifications (PTMs) play a crucial role in countless biological processes, profoundly modulating protein properties on both spatial and temporal scales. Protein PTMs have also emerged as reliable biomarkers for several diseases. However, only a handful of techniques are available to accurately measure their levels, capture their complexity at a single molecule level, and characterize their multifaceted roles in health and disease. Nanopore sensing provides high sensitivity for the detection of low-abundance proteins, holding the potential to impact single-molecule proteomics and PTM detection, in particular. Here, we demonstrate the ability of a biological nanopore, the pore-forming toxin aerolysin, to detect and distinguish -synuclein-derived peptides bearing single or multiple PTMs, namely, phosphorylation, nitration, and oxidation occurring at different positions and in various combinations. The characteristic current signatures of the -synuclein peptide and its PTM variants could be confidently identified by using a deep learning model for signal processing. We further demonstrate that this framework can quantify -synuclein peptides at picomolar concentrations and detect the C-terminal peptides generated by digestion of full-length -synuclein. Collectively, our work highlights the advantage of using nanopores as a tool for simultaneous detection of multiple PTMs and facilitates their use in biomarker discovery and diagnostics.Application Note PDFCardiotox., cardio-oncology Chronic cardiotoxicity study on H9C2 cells using HESI stem cell working group reference compoundsAtlaZ application noteLong-term exposure to cancer-related therapeutics has been linked to alterations of cardiac function in patients. The Stem Cell Working Group as part of the Health and EnvironmentalScience Institute (HESI) currently endeavors to gain further insight into chronic cardiotoxicity. The objective of the HESI study is to optimize non-clinical safety assessment strategiesof chronic cardiotoxicity by testing prolonged exposure of compounds on different cell-based assay systems using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), by investigating e.g. contractility or contractile forcePublication link2023 Functional Characterization of the Lysosomal Peptide/Histidine Transporter PHT1 (SLC15A4) by Solid Supported Membrane Electrophysiology (SSME)SURFE2R N1 Publication in Preprints.org (2023) Authors: Pujol-Gimnez J., Hediger M.A.Peptide/Histidine Transporter PHT1 (SLC15A4) is expressed in lysosomal membranes of immune cells where it plays an important role in metabolic and inflammatory signaling. PHT1 is an H+-coupled/histidine symporter that can transport a broad range of oligopeptides, including a variety of bacterial-derived peptides. Moreover, it enables the scaffolding of various metabolic signaling molecules and interacts with key regulatory elements of the immune response. Therefore, it is not surprising that PHT1 is associated with the development of autoimmune diseases, such as systemic lupus erythematosus (SLE). Unfortunately, the pharmacological development of PHT1 has been hampered by the lack of appropriate transport assays. With the aim to address this shortcoming, a novel transport assay based on solid supported membrane-based electrophysiology (SSME) is presented. Key findings of the present SSME studies include the first recordings of electrophysiological properties, a pH dependence analysis, an assessment of PHT1 substrate selectivity, as well as the transport kinetics of the identified substrates. In contrast to previous works, PHT1 is studied its native lysosomal environment. Moreover, observed substrate selectivity is validated by molecular docking. Overall, this new SSME-based assay is expected to contribute to unlock the pharmacological potential of PHT1 and to deepen the understanding of its functional properties.Publication link2023 Peptide toxins that target vertebrate voltage-gated sodium channels underly the painful stings of harvester antsSyncroPatch 384 publication in JBC (2023) Authors: Robinson SD., Deuis JR., Niu P., Touchard A., Mueller A., Schendel V., Brinkwirth N., King GF., Vetter I., Schmidt JO.Harvester ants (genusPogonomyrmex) are renowned for their stings which cause intense, long-lasting pain and other neurotoxic symptoms in vertebrates. Here we show that harvester ant venoms are relatively simple and composed largely of peptide toxins. One class of peptides is primarily responsible for the long-lasting local pain of envenomation via activation of peripheral sensory neurons. These hydrophobic, cysteine-free peptides potently modulate mammalian voltage-gated sodium (NaV) channels, reducing the voltage threshold for activation and inhibiting channel inactivation. These toxins appear to have evolved specifically to deter vertebrates.Publication link2023 Epileptic Encephalopathy GABRB Structural Variants Share Common Gating and Trafficking DefectsSyncroPatch 384 Publication in Biomolecules (2023) Authors: Hernandez C.C., Hu N., Shen W., Macdonald R.L.Variants in theGABRBgene, which encodes the subunit of the GABAAreceptor, have been implicated in various epileptic encephalopathies and related neurodevelopmental disorders such as Dravet syndrome and Angelman syndrome. These conditions are often associated with early-onset seizures, developmental regression, and cognitive impairments. The severity and specific features of these encephalopathies can differ based on the nature of the genetic variant and its impact on GABAAreceptor function. These variants can lead to dysfunction in GABAAreceptor-mediated inhibition, resulting in an imbalance between neuronal excitation and inhibition that contributes to the development of seizures. Here, 13de novoEE-associatedGABRBvariants, occurring as missense mutations, were analyzed to determine their impact on protein stability and flexibility, channel function, and receptor biogenesis. Our results showed that all mutations studied significantly impact the protein structure, altering protein stability, flexibility, and function to varying degrees. Variants mapped to the GABA-binding domain, coupling zone, and pore domain significantly impact the protein structure, modifying the +/ interface of the receptor and altering channel activation and receptor trafficking. Our study proposes that the extent of loss or gain of GABAAreceptor function can be elucidated by identifying the specific structural domain impacted by mutation and assessing the variability in receptor structural dynamics. This paves the way for future studies to explore and uncover links between the incidence of a variant in the receptor topology and the severity of the related disease.Case study PDFSB Drug Discovery and Nanion: Accelerating Growth for SuccessApplication protocolContractility Analysis of iCell Cardiomyocytes2 : Evaluating iCell Cardiomyocytes2 in co-culture with iCell Cardiac Fibroblasts on the FLEXcyte 96 SystemCardioExcyte 96, FLEXcyte 96, collaboration with FUJIFILM Cellular Dynamics, Inc. and InnoVitroPublication link2023 Exome sequencing of ATP1A3-negative cases of alternating hemiplegia of childhood reveals SCN2A as a novel causative geneSyncroPatch 384 Publication in Eur. J. Hum. Gen Basic Research in Cardiology (2023) Authors: Panagiotakaki E., Tiziano F.D., Mikati M.A., Vijfhuizen L.S., Nicole S., Lesca G., Abiusi E., Novelli A., Di Pietro L., I.B.AHC Consortium, IAHCRC Consortium, Harder A.V.E., Walley N.M., De Grandis E., Poulat A-L., Des Portes V., Lpine A., Nassogne M-C., Arzimanoglou A., Vavassori R., Koenderink J., Thompson C.H., George Jr. A.L., Gurrieri F., van den Maagdenberg A.M.J.M., Heinzen E.L.Alternating hemiplegia of childhood (AHC) is a rare neurodevelopment disorder that is typically characterized by debilitating episodic attacks of hemiplegia, seizures, and intellectual disability. Over 85% of individuals with AHC have a de novo missense variant inATP1A3encoding the catalytic 3 subunit of neuronal Na+/K+ATPases. The remainder of the patients are genetically unexplained. Here, we used next-generation sequencing to search for the genetic cause of 26ATP1A3-negative index patients with a clinical presentation of AHC or an AHC-like phenotype. Three patients had affected siblings. Using targeted sequencing of exonic, intronic, and flanking regions ofATP1A3in 22 of the 26 index patients, we found no ultra-rare variants. Using exome sequencing, we identified the likely genetic diagnosis in 9 probands (35%) in five genes, includingRHOBTB2(n=

confirmed that Hsp70 has strong specificity toward membranes composed of negatively charged phosphatidylserine (PS), compared to neutral phosphatidylcholine membranes. Using differential scanning calorimetry, we have shown for the first time that Hsp70 affects the thermotropic behavior of saturated PS and leads to the interdigitation that controls membrane thickness and rigidity. Hsp70-PS interaction depended on the lipid phase state; the protein stabilized ordered domains enriched with high-melting PS, increasing their area, probably due to formation of quasi-interdigitated phase. Moreover, the ability of Hsp70 to form ion-permeable pores in PS membranes may also be determined by the bilayer thickness. These observations contribute to a better understanding of Hsp70-PS interaction and biological functions of membrane-bound Hsp70 in cancer cells.Publication link2023 Biophysical characterization of chloride intracellular channel 6 (CLIC6)SyncroPatch 384 Publication in JBC (2023) Authors: Loyo-Celis V., Patel D., Sanghvi S., Kaur K., Ponnalagu D., Zheng Y., Bindra S., Bhachu HR., Deschenes I., Gururaja Rao S., Singh H.Chloride intracellular channels (CLICs) are a family of proteins that exist in soluble and transmembrane forms. The newest discovered member of the family CLIC6 is implicated in breast, ovarian, lung gastric, and pancreatic cancers and is also known to interact with dopamine-(D(2)-like) receptors. The soluble structure of the channel has been resolved, but the exact physiological role of CLIC6, biophysical characterization, and the membrane structure remain unknown. Here, we aimed to characterize the biophysical properties of this channel using a patch-clamp approach. To determine the biophysical properties of CLIC6, we expressed CLIC6 in HEK-293cells. On ectopic expression, CLIC6 localizes to the plasma membrane of HEK-293cells. We established the biophysical properties of CLIC6 by using electrophysiological approaches. Using various anions and potassium (K+) solutions, we determined that CLIC6 is more permeable to chloride-(Cl) as compared to bromide-(Br), fluoride-(F), and K+ions. In the whole-cell configuration, the CLIC6 currents were inhibited after the addition of 10M of IAA-94 (CLIC-specific blocker). CLIC6 was also found to be regulated by pH and redox potential. We demonstrate that the histidine residue at 648 (H648) in the C terminus and cysteine residue in the N terminus (C487) are directly involved in the pH-induced conformational change and redox regulation of CLIC6, respectively. Using qRT-PCR, we identified that CLIC6 is most abundant in the lung and brain, and we recorded the CLIC6 current in mouse lung epithelial cells. Overall, we have determined the biophysical properties of CLIC6 and established it as a Clchannel.Publication link2023 Gating Of the Channel Pore of Ionotropic Glutamate Receptors with Bacterial Substrate Binding Proteins: Functional Coupling of the Ectoine Binding Protein Ehub to Glur0Port-a-Patch Publication in Journal of Biotechnology and Biomedicine. (2023) Authors: Bernhard M., Laube BMammalian neuronal tetrameric ionotropic glutamate receptors (iGluRs) are thought to have originally arisen from the fusion of a bacterial substrate binding protein (SBP) with an inverted potassium channel. This hypothesis is based on structural and sequential similarities between the ligand binding and channel domains of iGluR subunits with SBPs and potassium channels. Ligand binding occurs at the interface between two lobed domains in both ligand binding domains (LBDs) and leads to closure of the shell-like structure, which is considered to be a key element in the transition from ligand recognition to ion channel gating in iGluRs. Here we report the functional coupling of the ectoine-binding protein EhuB to the channel pore of the GluR0 receptor. Fusion of an unmodified EhuB-binding protein to the transmembrane domain of GluR0 did not result in activation of the channel pore. Only by stabilizing the inserted EhuB-binding domain with a dimerization interface the resulting chimera was activated by ectoine, resembling the activation properties of other iGluRs. These results demonstrate the functional compatibility of SBPs to the gate of the channel pore of an iGluR and highlight the role of LBD dimerization in the functional evolution of iGluRs. Based on the high specificity and affinity of SBPs for an incredible variety of substrates, our results demonstrate the competence of SBP/ion channel chimeras for the development of new Biosensors for specific recognition of analytes by functionally linking a bacterial binding protein to the channel pore of an iGluR.Publication link2023 SARS-CoV-2 spike protein S1 activates Cx43 hemichannels and disturbs intracellular Ca2+ dynamicsPatchliner Publication in Biological Research (2023) Authors: Prieto-Villalobos J., Lucero C.M., Rovegno M., Gmez G.I., Retamal M.A. Orellana J.A.BackgroundSevere acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the ongoing coronavirus disease 2019 (COVID-19). An aspect of high uncertainty is whether the SARS-CoV-2 per se or the systemic inflammation induced by viral infection directly affects cellular function and survival in different tissues. It has been postulated that tissue dysfunction and damage observed in COVID-19 patients may rely on the direct effects of SARS-CoV-2 viral proteins. Previous evidence indicates that the human immunodeficiency virus and its envelope protein gp120 increase the activity of connexin 43 (Cx43) hemichannels with negative repercussions for cellular function and survival. Here, we evaluated whether the spike protein S1 of SARS-CoV-2 could impact the activity of Cx43 hemichannels.ResultsWe found that spike S1 time and dose-dependently increased the activity of Cx43 hemichannels in HeLa-Cx43 cells, as measured by dye uptake experiments. These responses were potentiated when the angiotensin-converting enzyme 2 (ACE2) was expressed in HeLa-Cx43 cells. Patch clamp experiments revealed that spike S1 increased unitary current events with conductances compatible with Cx43 hemichannels. In addition, Cx43 hemichannel opening evoked by spike S1 triggered the release of ATP and increased the [Ca2+]idynamics elicited by ATP.ConclusionsWe hypothesize that Cx43 hemichannels could represent potential pharmacological targets for developing therapies to counteract SARS-CoV-2 infection and their long-term consequences.Publication link2023 Analysis of the effect of the scorpion toxin AaH-II on action potential generation in the axon initial segmentSyncroPatch 384 Pre-Publication in BioRxiv (2023) Authors: Abbas F., Blmer L.A., Millet H., Montnach J., De Waard M., Canepari M.The toxin AaH-II, from the scorpionAndroctonus australis Hectorvenom, is a 64 amino acid peptide that targets voltage-gated Na+channels (VGNCs) and slows their inactivation. While at macroscopic cellular level AaH-II prolongs the action potential (AP), a functional analysis of the effect of the toxin in the axon initial segment (AIS), where VGNCs are highly expressed, was never performed so far. Here, we report an original analysis of the effect of AaH-II on the AP generation in the AIS of neocortical layer-5 pyramidal neurons from mouse brain slices. After determining that AaH-II does not discriminate between Nav1.2 and Nav1.6, i.e. between the two VGNC isoforms expressed in this neuron, we established that 7 nM was the smallest toxin concentration producing a minimal detectable deformation of the somatic AP after local delivery of the toxin. Using membrane potential imaging, we found that, at this minimal concentration, AaH-II substantially widened the AP in the AIS. Using ultrafast Na+imaging, we found that local application of 7 nM AaH-II caused a large increase in the slower component of the Na+influx in the AIS. Finally, using ultrafast Ca2+imaging, we observed that 7 nM AaH-II produces a spurious slow Ca2+influx via Ca2+-permeable VGNCs. Molecules targeting VGNCs, including peptides, are proposed as potential therapeutic tools. Thus, the present analysis in the AIS can be considered a general proof-of-principle on how high-resolution imaging techniques can disclose drug effects that cannot be observed when tested at the macroscopic level.Case study PDF32-well mode Advancing cardiac research: 32-wells at a timePublication link2024 The Grdos Channel and Piezo1 Revisited: Comparison between Reticulocytes and Mature Red Blood CellsPatchliner and SyncroPatch 384 Publication in Int. J. Mol. Sci. (2024) Authors: Petkova-Kirova P., Murciano N., Iacono G., Jansen J., Simionato G., Qiao M., Van der Zwaan C., Rotordam M.G., John T., Hertz L., Hoogendijk A.J., Becker N., Wagner C., von Lindern M., Egee S., Van den Akker E., Kaestner L.The Grdos channel (KCNN4) and Piezo1 are the best-known ion channels in the red blood cell (RBC) membrane. Nevertheless, the quantitative electrophysiological behavior of RBCs and its heterogeneity are still not completely understood. Here, we use state-of-the-art biochemical methods to probe for the abundance of the channels in RBCs. Furthermore, we utilize automated patch clamp, based on planar chips, to compare the activity of the two channels in reticulocytes and mature RBCs. In addition to this characterization, we performed membrane potential measurements to demonstrate the effect of channel activity and interplay on the RBC properties. Both the Grdos channel and Piezo1, albeit their average copy number of activatable channels per cell is in the single-digit range, can be detected through transcriptome analysis of reticulocytes. Proteomics analysis of reticulocytes and mature RBCs could only detect Piezo1 but not the Grdos channel. Furthermore, they can be reliably measured in the whole-cell configuration of the patch clamp method. While for the Grdos channel, the activity in terms of ion currents is higher in reticulocytes compared to mature RBCs, for Piezo1, the tendency is the opposite. While the interplay between Piezo1 and Grdos channel cannot be followed using the patch clamp measurements, it could be proved based on membrane potential measurements in populations of intact RBCs. We discuss the Grdos channel and Piezo1 abundance, interdependencies and interactions in the context of their proposed physiological and pathophysiological functions, which are the passing of small constrictions, e.g., in the spleen, and their active participation in blood clot formation and thrombosis.Publication link2023 Structure-based development and preclinical evaluation of the SARS-CoV-2 3C-like protease inhibitor simnotrelvirPatchliner Publication in Nature Communications (2023) Authors: Jiang X., Su H., Shang W., Zhou F., Zhang Y., Zhao W, Zhang Q., Xie H., Jiang L., Nie T., Yang F., Xiong M., Huang X., Li M., Chen P., Peng S., Xiao G., Jiang H., Tang R., Zhang L., Shen J., Xu Y.The persistent pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its variants accentuates the great demand for developing effective therapeutic agents. Here, we report the development of an orally bioavailable SARS-CoV-2 3C-like protease (3CLpro) inhibitor, namely simnotrelvir, and its preclinical evaluation, which lay the foundation for clinical trials studies as well as the conditional approval of simnotrelvir in combination with ritonavir for the treatment of COVID-19. The structure-based optimization of boceprevir, an approved HCV protease inhibitor, leads to identification of simnotrelvir that covalently inhibits SARS-CoV-2 3CLprowith an enthalpy-driven thermodynamic binding signature. Multiple enzymatic assays reveal that simnotrelvir is a potent pan-CoV3CLproinhibitor but has high selectivity. It effectively blocks replications of SARS-CoV-2 variants in cell-based assays and exhibits good pharmacokinetic and safety profiles in male and female rats and monkeys, leading to robust oral efficacy in a male mouse model of SARS-CoV-2 Delta infection in which it not only significantly reduces lung viral loads but also eliminates the virus from brains. The discovery of simnotrelvir thereby highlights the utility of structure-based development of marked protease inhibitors for providing a small molecule therapeutic effectively combatting human coronaviruses.Publication link2023 Unravelling Novel SCN5A Mutations Linked to Brugada Syndrome: Functional, Structural, and Genetic InsightsPatchliner Publication in Int. J. Mol. Sci. (2023) Authors: Frosio A., Micaglio E., Polsinelli I., Calamaio S., Melgari D., Prevostini R., Ghiroldi A., Binda A., Carrera P., Villa M., Mastrocinque F., Presi S., Salerno R., Boccellino A., Anastasia L., Ciconte G.,Ricagno S., Pappone C., Rivolta I.Brugada Syndrome (BrS) is a rare inherited cardiac arrhythmia causing potentially fatal ventricular tachycardia or fibrillation, mainly occurring during rest or sleep in young individuals without heart structural issues. It increases the risk of sudden cardiac death, and its characteristic feature is an abnormal ST segment elevation on the ECG. While BrS has diverse genetic origins, a subset of cases can be conducted to mutations in theSCN5Agene, which encodes for the Nav1.5 sodium channel. Our study focused on three novelSCN5Amutations (p.A344S, p.N347K, and p.D349N) found in unrelated BrS families. Using patch clamp experiments, we found that these mutations disrupted sodium currents: p.A344S reduced current density, while p.N347K and p.D349N completely abolished it, leading to altered voltage dependence and inactivation kinetics when co-expressed with normal channels. We also explored the effects of mexiletine treatment, which can modulate ion channel function. Interestingly, the p.N347K and p.D349N mutations responded well to the treatment, rescuing the current density, while p.A344S showed a limited response. Structural analysis revealed these mutations were positioned in key regions of the channel, impacting its stability and function. This research deepens our understanding of BrS by uncovering the complex relationship between genetic mutations, ion channel behavior, and potential therapeutic interventions.Publication link2023 Evidence of Cytolysin A nanopore incorporation in mammalian cells assessed by a graphical user interfacePatchliner Publication in Nanoscale (2023) Authors: Lucas F.L.R., Finol-Urdaneta R.K., Van Thillo T., McArthur J.R., van der Heide N.J., Maglia G., Dedecker P., Strauss O., Wloka C.Technologies capable of assessing cellular metabolites with high precision and temporal resolution are currently limited. Recent developments in the field of nanopore sensor sallow the non-stochastic quantification of metabolites, where a nanopore is acting as an electrical transducer for selective substrate binding proteins (SBPs). Here we show that incorporation of the pore-forming toxin Cytolysin A (ClyA) into the plasma membrane of Chinese hamster ovary cells (CHO-K1) results in the appearance of single-channel conductance amenable to multiplexed automated patch-clamp (APC) electrophysiology. In CHO-K1 cells, SBPs modify the ionic current flowing though ClyA nanopores, thus demonstrating its potential for metabolite sensing of living cells. Moreover, we developed a graphical user interface for the analysis of the complex signals resulting from multiplexed APC recordings. This system lays the foundation to bridge the gap between recent advances in the nanopore field (e.g., proteomic and transcriptomic) and potential cellular applications.Publication link2023 Formation of Giant Unilamellar Vesicles Assisted byFluorinated NanoparticlesVesicle Prep Pro Publication in Advanced Science (2023) Authors: Waeterschoot J., Gossel W., Zeinabad H.A., Lammertyn J., Koos E., Casadevall i Solvas X.In the quest to produce artificial cells, one key challenge that remains to besolved is the recreation of a complex cellular membrane. Among the existingmodels, giant unilamellar vesicles (GUVs) are particularly interesting due totheir intrinsic compartmentalisation ability and their resemblance in size andshape to eukaryotic cells. Many techniques have been developed to produceGUVs all having inherent advantages and disadvantages. Here, the authorsshow that fluorinated silica nanoparticles (FNPs) used to form Pickeringemulsions in a fluorinated oil can destabilise lipid nanosystems to templatethe formation of GUVs. This technique enables GUV production across abroad spectrum of buffer conditions, while preventing the leakage of theencapsulated components into the oil phase. Furthermore, a simplecentrifugation process is sufficient for the release of the emulsion-trappedGUVs, bypassing the need to use emulsion-destabilising chemicals. Withfluorescent FNPs and transmission electron microscopy, the authors confirmthat FNPs are efficiently removed, producing contaminant-free GUVs. Furtherexperiments assessing the lateral diffusion of lipids and unilamellarity of theGUVs demonstrate that they are comparable to GUVs produced viaelectroformation. Finally, the ability of incorporating transmembrane proteinsis demonstrated, highlighting the potential of this method for the productionof GUVs for artificial cell applications.Publication link2023 Encoding extracellular modification of artificial cell membranes using engineered self-translocating proteinsOrbit mini Pre-Print Publication in BioRxiv (2023) Authors: Harjung A., Fracassi A., Devaraj N.A common method of generating artificial cells is to encapsulate protein expression systems within lipid vesicles. However, to communicate with the external environment, protein translocation across lipid membranes must take place. In living cells, protein transport across membranes is achieved with the aid of complex translocase systems which are difficult to reconstitute into artificial cells. Thus, there is need for simple mechanisms by which proteins can be encoded and expressed inside synthetic compartments yet still be externally displayed. Here we present a genetically encodable membrane functionalization system based on mutants of pore-forming proteins. We show that the membrane translocating loop of -hemolysin can be engineered to translocate functional peptides up to 52 amino acids across lipid membranes. Engineered hemolysins can be used for genetically programming artificial cells to display interacting peptide pairs, enabling their assembly into artificial tissue-like structures capable of signal transduction.Application Note PDFCAR T, Cancer CAR T cell-mediated killing of cancer cellsAtlaZ application noteThe US Food and Drug Administration has approved a number of chimeric antigen receptor (CAR) T-cell therapies. Due to the nature of CAR T cells as living drugs, they display a unique toxicity profile. As CAR T-cell therapy is extending towards multiple diseases and being broadly employed in hematology and oncology, being able to reliably predict treatment efficacy and a quantification of responses are of high relevance. Furthermore, for continued breakthroughs, novel CAR designs are needed. This includes different antigenbinding domains such as antigen-ligand binding partners and variable lymphocyte receptors (1). Now, after amazing advances for treating block cancers, CAR T cell therapy is showing promise for solid tumors.In general, identifying T cells that kill cancer cells in vivo is critical to the development of successful cell therapies. The label-free AtlaZ immune cell killing assay can be used to measure rate of killing at Effector:Target (E:T) ratios to predict in vivo activity. In order to gain a deeper understanding of cancer cells, real-time and continuous monitoring is necessary to access kinetic and phenotypic information. Such monitoring captures also unique toxicity profiles of CAR T cells.Publication link2023 A Small Multidrug Resistance Transporter in Pseudomonas aeruginosa Confers Substrate-Specific Resistance or SusceptibilitySURFE2R N1 Pre-Print Publication in BioRxiv (2023) Authors: Wegrzynowicz A.K., Heelan W.J., Demas S.P., McLean M.S., Peters J.M., Henzler-Wildman K.A.Voltage-sensing domains control the activation of voltage-gated ion channels, with a few exceptions. One such exception is the sperm-specific Na+/H+exchanger SLC9C1, which is the only known transporter to be regulated by voltage-sensing domains. After hyperpolarization of sperm flagella, SLC9C1 becomes active, causing pH alkalinization and CatSper Ca2+channel activation, which drives chemotaxis. SLC9C1 activation is further regulated by cAMP, which is produced by soluble adenyl cyclase (sAC). SLC9C1 is therefore an essential component of the pHsACcAMP signalling pathway in metazoa, required for sperm motility and fertilization. Despite its importance, the molecular basis of SLC9C1 voltage activation is unclear. Here we report cryo-electron microscopy (cryo-EM) structures of sea urchin SLC9C1 in detergent and nanodiscs. We show that the voltage-sensing domains are positioned in an unusual configuration, sandwiching each side of the SLC9C1 homodimer. The S4 segment is very long, 90 in length, and connects the voltage-sensing domains to the cytoplasmic cyclic-nucleotide-binding domains. The S4 segment is in the up configurationthe inactive state of SLC9C1. Consistently, although a negatively charged cavity is accessible for Na+to bind to the ion-transporting domains of SLC9C1, an intracellular helix connected to S4 restricts their movement. On the basis of the differences in the cryo-EM structure of SLC9C1 in the presence of cAMP, we propose that, upon hyperpolarization, the S4 segment moves down, removing this constriction and enabling Na+/H+exchange.Publication link2023 Target-based discovery of a broad spectrum flukicidePatchliner Publication Pre-print in bioRxiv (2023) Authors: Sprague D.J., Park S-K., Gramberg S., Bauer L., Rohr C.M., Chulkov E.G., Smith E., Scampavia L., Spicer T.P., Haeberlein S., Marchant J.S.Diseases caused by parasitic flatworms impart a considerable healthcare burden worldwide. Many of these diseases for example, the parasitic blood fluke infection, schistosomiasis are treated with the drug praziquantel (PZQ). However, PZQ is ineffective against disease caused by liver flukes from the genusFasciola. This is due to a single amino acid change within the target of PZQ, a transient receptor potential ion channel (TRPMPZQ), inFasciolaspecies. Here we identify benzamidoquinazolinone analogs that are active againstFasciolaTRPMPZQ. Structure-activity studies define an optimized ligand (BZQ) that caused protracted paralysis and damage to the protective tegument of these liver flukes. BZQ also retained activity againstSchistosoma mansonicomparable to PZQ and was active against TRPMPZQorthologs in all profiled species of parasitic fluke. This broad spectrum activity was manifest as BZQ adopts a pose within the binding pocket of TRPMPZQdependent on a ubiquitously conserved residue. BZQ therefore acts as a universal activator of trematode TRPMPZQand a first-in-class, broad spectrum flukicide.Publication link2023 Enhancing the performance of a mutant pyrrolysyl-tRNA synthetase to create a highly versatile eukaryotic cell-free protein synthesis toolOrbit 16 (a predecessor model of the Orbit 16 TC) Publication in Scientific Reports (2023) Authors: Schlohauer J., Zemella A., Dondapati S., Thoring L., Meyer M., Stefan Kubick S.Modification of proteins with a broad range of chemical functionalities enables the investigation of protein structure and activity by manipulating polypeptides at single amino acid resolution. Indeed, various functional groups including bulky non-canonical amino acids like strained cyclooctenes could be introduced by the unique features of the binding pocket of the double mutant pyrrolysyl-tRNA synthetase (Y306A, Y384F), but the instable nature of the enzyme limits its application in vivo. Here, we constructed a cell-free protein production system, which increased the overall enzyme stability by combining different reaction compartments. Moreover, a co-expression approach in a one-pot reaction allowed straightforward site-specific fluorescent labeling of the functional complex membrane protein cystic fibrosis transmembrane conductance regulator. Our work provides a versatile platform for introducing various non-canonical amino acids into difficult-to-express proteins for structural and fluorescence based investigation of proteins activity.Publication link2023 Pharmacological characterization of SAGE-718, a novel positive allosteric modulator of N-methyl-d-aspartate receptorsSyncroPatch 384 Publication in British Journal of Pharmacology (2023) Authors: Beckley J., Aman T., Ackley M., Kazdoba T., Lewis M., Smith A., Farley B., Dai J., Deats W., Hoffmann E., Robichaud A., Doherty J., Quirk M.Background and PurposeSelect neuroactive steroids tune neural activity by modulating excitatory and inhibitory neurotransmission, including the endogenous cholesterol metabolite 24(S)-hydroxycholesterol (24(S)-HC), which is an N-methyl-d-aspartate (NMDA) receptor positive allosteric modulator (PAM). NMDA receptor PAMs are potentially an effective pharmacotherapeutic strategy to treat conditions associated with NMDA receptor hypofunction.Experimental ApproachUsing in vitro and in vivo electrophysiological recording experiments and behavioural approaches, we evaluated the effect of SAGE-718, a novel neuroactive steroid NMDA receptor PAM currently in clinical development for the treatment of cognitive impairment, on NMDA receptor function and endpoints that are altered by NMDA receptor hypoactivity and assessed its safety profile.Key ResultsSAGE-718 potentiated GluN1/GluN2A-D NMDA receptors with equipotency and increased NMDA receptor excitatory postsynaptic potential (EPSP) amplitude without affecting decay kinetics in striatal medium spiny neurons. SAGE-718 increased the rate of unblock of the NMDA receptor open channel blocker ketamine on GluN1/GluN2A in vitro and accelerated the rate of return on the ketamine-evoked increase in gamma frequency band power, as measured with electroencephalogram (EEG), suggesting that PAM activity is driven by increased channel open probability. SAGE-718 ameliorated deficits due to NMDA receptor hypofunction, including social deficits induced by subchronic administration of phencyclidine, and behavioural and electrophysiological deficits from cholesterol and 24(S)-HC depletion caused by 7-dehydrocholesterol reductase inhibition. Finally, SAGE-718 did not produce epileptiform activity in a seizure model or neurodegeneration following chronic dosing.Conclusions and ImplicationsThese findings provide strong evidence that SAGE-718 is a neuroactive steroid NMDA receptor PAM with a mechanism that is well suited as a treatment for conditions associated with NMDA receptor hypofunction.Publication link2023 PI-(3,5)P2-mediated oligomerization of the endosomal sodium/proton exchanger NHE9SURFE2R N1 Pre-Print Publication in BioRxiv (2023) Authors: Kokane S., Meier P.F., Gulati A., Matsuoka R., Pipatpolkai T., Delemotte L., Drew D.Na+/H+exchangers (NHE) are found in all cells to regulate intracellular pH, sodium levels and cell volume. The NHE isoform 9 (SLC9A9) fine-tunes endosomal pH, and its activity is linked to glioblastoma, epilepsy, autism spectrum and attention-deficit-hyperactivity disorders. Here, we report cryo-EM structures ofhorseNHE9 and a cysteine-variant at 3.6 and 3.1 resolution, respectively. We show how lysine residues, from a previously unresolved TM2-TM3 -hairpin loop domain, are positioned above the dimerization interface and interact with the endosomal-specific PI-(3,5)P2lipid, together with residues located on dimer domain helices. Thermal-shift assays, solid-state membrane (SSM) electrophysiology and MD simulations, corroborates that NHE9 can specifically bind PI-(3,5)P2, and that its addition stabilizes the homodimer and enhances NHE9 activity. We have further determined the cryo-EM structure ofE. coliNhaA, confirming the expected coordination of cardiolipin at the dimerization interface, solidifying the concept that Na+/H+exchanger dimerization and transporter activity can be regulated by specific lipids. Taken together, we propose that the activity of NHE9 is regulated by the PI-(3,5)P2lipid upon reaching endosomes, which we refer to as an lipid-activation-upon-arrival model.Publication link2023 ParSE-seq: A Calibrated Multiplexed Assay to Facilitate the Clinical Classification of Putative Splice-altering VariantsSyncroPatch 384 Pre-Publication in MedRxiv (2023) Authors: ONeill M.J., Yang T., Laudeman J., Calandranis M., Solus J., Roden D.M., Glazer A.M.Background:Interpreting the clinical significance of putative splice-altering variants outside 2-base pair canonical splice sites remains difficult without functional studies.Methods:We developed Parallel Splice Effect Sequencing (ParSE-seq), a multiplexed minigene-based assay, to test variant effects on RNA splicing quantified by high-throughput sequencing. We studied variants in SCN5A, an arrhythmia-associated gene which encodes the major cardiac voltage-gated sodium channel. We used the computational tool SpliceAI to prioritize exonic and intronic candidate splice variants, and ClinVar to select benign and pathogenic control variants. We generated a pool of 284 barcoded minigene plasmids, transfected them into Human Embryonic Kidney (HEK293) cells and induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), sequenced the resulting pools of splicing products, and calibrated the assay to the American College of Medical Genetics and Genomics scheme. Variants were interpreted using the calibrated functional data, and experimental data were compared to SpliceAI predictions. We further studied some splice-altering missense variants by cDNA-based automated patch clamping (APC) in HEK cells and assessed splicing and sodium channel function in CRISPR-edited iPSC-CMs.Results:ParSE-seq revealed the splicing effect of 224SCN5Avariants in iPSC-CMs and 244 variants in HEK293 cells. The scores between the cell types were highly correlated (R2=0.84). In iPSCs, the assay had concordant scores for 21/22 benign/likely benign and 24/25 pathogenic/likely pathogenic control variants from ClinVar. 43/112 exonic variants and 35/70 intronic variants with determinate scores disrupted splicing. 11 of 42 variants of uncertain significance were reclassified, and 29 of 34 variants with conflicting interpretations were reclassified using the functional data. SpliceAI computational predictions correlated well with experimental data (AUC = 0.96). We identified 20 uniqueSCN5Amissense variants that disrupted splicing, and 2 clinically observed splice-altering missense variants of uncertain significance had normal function when tested with the cDNA-based APC assay. A splice-altering intronic variant detected by ParSE-seq, c.18915C>G, also disrupted splicing and sodium current when introduced into iPSC-CMs at the endogenous locus by CRISPR editing.Publication Link2023 Cell-Free Synthesis and Electrophysiological Analysis of Multipass Voltage-Gated Ion Channels Tethered in Microsomal MembranesOrbit 16TC Publication in Advances in Biochemical Engineering/Biotechnology (2023) Authors: Pandey Y., Dondapati S., Wstenhagen D., Kubick S.Cell-free protein synthesis (CFPS) has emerged as a powerful tool for the rapid synthesis and analysis of various structurally and functionally distinct proteins. These include difficult-to-express membrane proteins such as large multipass ion channel receptors. Owing to their membrane localization, eukaryotic CFPS supplemented with endoplasmic reticulum (ER)-derived microsomal vesicles has proven to be an efficient system for the synthesis of functional membrane proteins. Here we demonstrate the applicability of the eukaryotic cell-free systems based on lysates from the mammalian Chinese Hamster Ovary (CHO) and insectSpodoptera frugiperda(Sf21)cells. We demonstrate the efficiency of the systems in thede novocell-free synthesis of the human cardiac ion channels: ether-a-go-go potassium channel (hERG) KV11.1 and the voltage-gated sodium channel hNaV1.5.Read more in the publication here.Publication link2023 Nicotine-mediated effects in neuronal and mouse models of synucleinopathyPatchliner Publication in Front. Neurosci. (2023) Authors: Fares M.B., Alijevic O., Johne S., Overk C., Hashimoto M., Kondylis A., Adame A., Dulize R., Peric D., Nury C., Battey J., Guedj E., Sierro N., Mc Hugh D., Rockenstein E., Kim C. Rissman R.A., Hoeng J., Peitsch M.C., Masliah E., Mathis C.Introduction:Alpha-synuclein (-Syn) aggregation, transmission, and contribution to neurotoxicity represent central mechanisms underlying Parkinsons disease. The plant alkaloid nicotine was reported to attenuate -Syn aggregation in different models, but its precise mode of action remains unclear.Methods:In this study, we investigated the effect of 2-week chronic nicotine treatment on -Syn aggregation, neuroinflammation, neurodegeneration, and motor deficits in D-line -Syn transgenic mice. We also established a novel humanized neuronal model of -Syn aggregation and toxicity based on treatment of dopaminergic neurons derived from human induced pluripotent stem cells (iPSC) with -Syn preformed fibrils (PFF) and applied this model to investigate the effects of nicotine and other compounds and their modes of action.Results and discussion:Overall, our results showed that nicotine attenuated -Syn-provoked neuropathology in both models. Moreover, when investigating the role of nicotinic acetylcholine receptor (nAChR) signaling in nicotines neuroprotective effects in iPSC-derived dopaminergic neurons, we observed that while 4-specific antagonists reduced the nicotine-induced calcium response, 4 agonists (e.g., AZD1446 and anatabine) mediated similar neuroprotective responses against -Syn PFF-provoked neurodegeneration. Our results show that nicotine attenuates -Syn-provoked neuropathologyin vivoand in a humanized neuronal model of synucleinopathy and that activation of 42 nicotinic receptors might mediate these neuroprotective effects.Publication link2023 Photophysics of Conjugated Oligoelectrolytes Relevant to Two-Photon Fluorescence-Lifetime Imaging MicroscopyVesicle Prep Pro Publication in Advanced Functional Materials (2023) Authors: Zhu J-Y., Mikhailovsky A., Wei S.C.J., Moreland A., Limwongyut J., Guarrotxena N., Bazan G.C.Conjugated oligoelectrolytes (COEs) comprise a class of cell-membrane intercalating molecules that serve as effective optical reporters. However, little is known about the photophysical properties of COEs in biological environments such as buffers, cell membranes, and intracellular organelles, which is critical to optimize performance. Herein, how COE self-assembly depends on the dielectric environment (polarity and ion content) is explored based on the representative molecule 6-ring phenylenevinylene (PV) conjugated oligoelectrolyte (COE-S6), and its optical properties within mammalian cells are subsequently studied. Two-photon fluorescence lifetime imaging microscopy (FLIM), confocal laser scanning microscopy, and optical properties in solutions are brought together to obtain information about the location, accumulation, and characteristics of the local surroundings. FLIM imaging lifetime phasor plots, decays, and fluorescence spectra on stained mammalian cells provide evidence of successful COE-S6 internalization via endocytosis. The fluorescence lifetime of COE-S6 is identical when in A549 mammalian cells and in giant unilamellar vesicle model membranes, thereby providing a correlation between living system and artificial constructs.Publication link2023 Plasticity of the binding pocket in peptide transporters underpins promiscuous substrate recognitionSURFE2R N1 Publication in Cell Reports (2023) Authors: Kotov V. Killer M. Jungnickel K.E.J., Lei J., Finocchio G., Steinke J., Bartels K., Strauss J., Dupeux F., Humm A-S., Cornaciu I., Mrquez J.A., Pardon E., Steyaert J., Lw C.Proton-dependent oligopeptide transporters (POTs) are promiscuous transporters of the major facilitator superfamily that constitute the main route of entry for a wide range of dietary peptides and orally administrated peptidomimetic drugs. Given their clinical and pathophysiological relevance, several POT homologs have been studied extensively at the structural and molecular level. However, the molecular basis of recognition and transport of diverse peptide substrates has remained elusive. We present 14 X-ray structures of the bacterial POT DtpB in complex with chemically diverse di- and tripeptides, providing novel insights into the plasticity of the conserved central binding cavity. We analyzed binding affinities for more than 80 peptides and monitored uptake by a fluorescence-based transport assay. To probe whether all 8400 natural di- and tripeptides can bind to DtpB, we employed state-of-the-art molecular docking and machine learning and conclude that peptides with compact hydrophobic residues are the best DtpB binders.Publication Link2023 Epilepsy-associated SCN2A (NaV1.2) Variants Exhibit Diverse and Complex Functional PropertiesSyncroPatch 768PE (a predecessor model of the SyncroPatch 384) Publication in J. Gen. Physiol. (2023) Authors: Thompson C.H., Potet F., Abramova T.V., DeKeyser J.M., Ghabra N.F., Vanoye C.G., Millichap J.,George, Jr. A.L.Pathogenic variants in voltage-gated sodium (NaV) channel genes includingSCN2A, encoding NaV1.2, are discovered frequently in neurodevelopmental disorders with or without epilepsy.SCN2Ais also a high-confidence risk gene for autism spectrum disorder (ASD) and nonsyndromic intellectual disability (ID). Previous work to determine the functional consequences ofSCN2Avariants yielded a paradigm in which predominantly gain-of-function variants cause neonatal-onset epilepsy, whereas loss-of-function variants are associated with ASD and ID. However, this framework was derived from a limited number of studies conducted under heterogeneous experimental conditions, whereas most disease-associatedSCN2Avariants have not been functionally annotated. We determined the functional properties ofSCN2Avariants using automated patch-clamp recording to demonstrate the validity of this method and to examine whether a binary classification of variant dysfunction is evident in a larger cohort studied under uniform conditions. We studied 28 disease-associated variants and 4 common variants using two alternatively spliced isoforms of NaV1.2 expressed in HEK293T cells. Automated patch-clamp recording provided a valid high throughput method to ascertain detailed functional properties of NaV1.2 variants with concordant findings for variants that were previously studied using manual patch clamp. Many epilepsy-associated variants in our study exhibited complex patterns of gain- and loss-of-functions that are difficult to classify by a simple binary scheme. The higher throughput achievable with automated patch clamp enables study of variants with greater standardization of recording conditions, freedom from operator bias, and enhanced experimental rigor. This approach offers an enhanced ability to discern relationships between channel dysfunction and neurodevelopmental disorders.Publication link2023 Transport of metformin metabolites by guanidinium exporters of the Small Multidrug Resistance familySURFE2R N1 Pre-Print Publication in BioRxiv (2023) Authors: Lucero R.M., Demirer K., Yeh T.J., Stockbridge R.B.Proteins from the Small Multidrug Resistance (SMR) family are frequently associated with horizontally transferred multidrug resistance gene arrays found in bacteria from wastewater and the human-adjacent biosphere. Recent studies suggest that a subset of SMR transporters might participate in metabolism of the common pharmaceutical metformin by bacterial consortia. Here, we show that both genomic and plasmid-associated transporters of the SMRGdxfunctional subtype export byproducts of microbial metformin metabolism, with particularly high export efficiency for guanylurea. We use solid supported membrane electrophysiology to evaluate the transport kinetics for guanylurea and native substrate guanidinium by four representative SMRGdxhomologues. Using an internal reference to normalize independent electrophysiology experiments, we show that transport rates are comparable for genomic and plasmid-associated SMRGdxhomologues, and using a proteoliposome-based transport assay, we show that 2 proton:1 substrate transport stoichiometry is maintained. Additional characterization of guanidinium and guanylurea export properties focuses on the structurally characterized homologue, Gdx-Clo, for which we examined the pH dependence and thermodynamics of substrate binding and solved an x-ray crystal structure with guanylurea bound. Together, these experiments contribute in two main ways. By providing the first detailed kinetic examination of the structurally characterized SMRGdxhomologue Gdx-Clo, they provide a functional framework that will inform future mechanistic studies of this model transport protein. Second, this study casts light on a potential role for SMRGdxtransporters in microbial handling of metformin and its microbial metabolic byproducts, providing insight into how native transport physiologies are co-opted to contend with new selective pressures.Publication link2023 Discovery of novel triazoles containing benzyloxy phenyl isoxazole side chain with potent and broad-spectrum antifungal activitySyncroPatch 384 Publication in Biomolecules (2023) Authors: Chi X., Xie F., Li L., Hao Y., Wu H., Li X., Xia G., Yan L., Zhang D., Jiang Y., Ni T.As a continuation study, 29 novel triazoles containing benzyloxy phenyl isoxazole side chain were designed and synthesized based on our previous work. The majority of the compounds exhibited high potency in vitro antifungal activities against eight pathogenic fungi. The most active compounds 13, 20 and 27 displayed outstanding antifungal activity with MIC values ranging from 1000-fold more potent than brevenal. Consistent with effects observed in native tissues, Nav1.2 and Nav1.4 channels were PbTx-3- and brevenal-sensitive, whereas Nav1.5 and Nav1.7 appeared resistant. Interestingly, the incorporation of brevenal in the intracellular solution caused Nav channels to become less sensitive to PbTx-3 actions. Furthermore, we generated a computational model of PbTx-2 bound to the lipid-exposed side of the interface between domains I and IV of Nav1.2. Our results are consistent with competitive antagonism between brevetoxins and brevenal, setting a basis for future mutational analyses of Nav channels interaction with brevetoxins and brevenal. Our findings provide valuable insights into the functional modulation of Nav channels by brevetoxins and brevenal, which may have implications for the development of new Nav channel modulators with potential therapeutic applications.Read more about the publ

of the eight TKIs determined by measuring their effects on cell viability, contractility, and respiration is largely consistent with that derived from database or literature, indicating that human cardiomyocytes are a good cellular model for studying cardiotoxicity. When transcriptomes are measured for selected TKI treatments with different levels of toxicity in human cardiomyocytes, the data are classified into 7 clusters with mainly single-drug clusters. Drug-specific effects on the transcriptome dominate over dose-, time- or toxicity-dependent effects. Two clusters with three TKIs (afatinib, ponatinib, and sorafenib) have the top enriched pathway as the endoplasmic reticulum stress (ERS). All three TKIs induce ERS in rat primary cardiomyocytes and ponatinib activates the IRE1-XBP1s axis downstream of ERS in the hearts of rats underwent a 7-day course of drug treatment. To look for potential triggers of ERS, we find that the three TKIs induce transient reactive oxygen species followed by lipid peroxidation. Inhibiting either PERK or IRE1 downstream of ERS blocks TKI-induced cardiac damages, represented by the induction of cardiac fetal and pro-inflammatory genes without causing more cell death.ConclusionsOur data contain rich information about phenotypic and transcriptional responses of human cardiomyocytes to eight TKIs, uncovering potential molecular mechanisms in modulating cardiotoxicity. ER stress is activated by multiple TKIs and leads to cardiotoxicity through promoting expression of pro-inflammatory factors and cardiac fetal genes. ER stress-induced inflammation is a promising therapeutic target to mitigate ponatinib- and sorafenib-induced cardiotoxicity.Read more in the publication here.Publication link2023 Evaluation of Nanopore Sensor Design Using Electrical and Optical AnalysesOrbit mini Publication in ACS Nano (2023) Authors: Mayse L., Imran A., Wang Y., Ahmad M., Oot R., Wilkens S., Movileanu L.Nanopores are currently utilized as powerful tools for single-molecule protein sensing. The reporting signal typically requires protein analytes to enter the nanopore interior, yet a class of these sensors has emerged that allows targeted detection free in solution. This tactic eliminates the spatial limitation of nanopore confinement. However, probing proteins outside the nanopore implies numerous challenges associated with transducing the physical interactions in the aqueous phase into a reliable electrical signature. Hence, it necessitates extensive engineering and tedious optimization routes. These obstacles have prevented the widespread adoption of these sensors. Here, we provide an experimental strategy by developing and validating single-polypeptide-chain nanopores amenable to single-molecule and bulk-phase protein detection approaches. We utilize protein engineering, as well as nanopore and nanodisc technologies, to create nanopore sensors that can be integrated with an optical platform in addition to traditional electrical recordings. Using the optical modality over an ensemble of detectors accelerates these sensors optimization process for a specific task. It also provides insights into how the construction of these single-molecule nanopore sensors influences their performance. These outcomes form a basis for evaluating engineered nanopores beyond the fundamental limits of the resistive-pulse technique.Read more in the publication here.Publication link2023 Cryo-EM analysis of scorpion toxin binding to Ryanodine Receptors reveals subconductance that is abolished by PKA phosphorylationOrbit mini Publication in Science Advances (2023) Authors: Haji-Ghassemi O., Seby Chen Y., Woll K., Gurrola G., Valdivia C., Cai W., Li S., Valdivia H., Van Petegem F.Calcins are peptides from scorpion venom with the unique ability to cross cell membranes, gaining access to intracellular targets. Ryanodine Receptors (RyR) are intracellular ion channels that control release of Ca2+ from the endoplasmic and sarcoplasmic reticulum. Calcins target RyRs and induce long-lived subconductance states, whereby single-channel currents are decreased. We used cryoelectron microscopy to reveal the binding and structural effects of imperacalcin, showing that it opens the channel pore and causes large asymmetry throughout the cytosolic assembly of the tetrameric RyR. This also creates multiple extended ion conduction pathways beyond the transmembrane region, resulting in subconductance. Phosphorylation of imperacalcin by protein kinase A prevents its binding to RyR through direct steric hindrance, showing how posttranslational modifications made by the host organism can determine the fate of a natural toxin. The structure provides a direct template for developing calcin analogs that result in full channel block, with potential to treat RyR-related disorderRead more in the publication here.Publication link2023 Structural mechanisms of TRPM7 activation and inhibitionOrbit mini Publication in Nature Communications (2023) Authors: Nadezhdin K., Correia L., Narangoda C., Patel D., Neuberger A., Gudermann T., Kurnikova M., Chubanov V., Sobolevsky A.The transient receptor potential channel TRPM7 is a master regulator of the organismal balance of divalent cations that plays an essential role in embryonic development, immune responses, cell mobility, proliferation, and differentiation. TRPM7 is implicated in neuronal and cardiovascular disorders, tumor progression and has emerged as a new drug target. Here we use cryo-EM, functional analysis, and molecular dynamics simulations to uncover two distinct structural mechanisms of TRPM7 activation by a gain-of-function mutation and by the agonist naltriben, which show different conformational dynamics and domain involvement. We identify a binding site for highly potent and selective inhibitors and show that they act by stabilizing the TRPM7 closed state. The discovered structural mechanisms provide foundations for understanding the molecular basis of TRPM7 channelopathies and drug development.Read more in the publication here.Publication link2023 Activation energy for pore opening in lipid membranes under an electric fieldOrbit mini Publication in PNAS (2023) Authors: Lafarge E., Muller P., Schroder A., Zaitseva E., Behrends J., Marques C.The standard model of pore formation was introduced more than fifty years ago, and it has been since, despite some refinements, the cornerstone for interpreting experiments related to pores in membranes. A central prediction of the model concerning pore opening under an electric field is that the activation barrier for pore formation is lowered proportionally to the square of the electric potential. However, this has only been scarcely and inconclusively confronted to experiments. In this paper, we study the electropermeability of model lipid membranes composed of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) containing different fractions of POPC-OOH, the hydroperoxidized form of POPC, in the range 0 to 100 mol %. By measuring ion currents across a 50-m-diameter black lipid membrane (BLM) with picoampere and millisecond resolution, we detect hydroperoxidation-induced changes to the intrinsic bilayer electropermeability and to the probability of opening angstrom-size or larger pores. Our results over the full range of lipid compositions show that the energy barrier to pore formation is lowered linearly by the absolute value of the electric field, in contradiction with the predictions of the standard model.Product Sheet PDFSyncroPatch 384 Interactive BrochurePublication link2023 Omentin-1 drives cardiomyocyte cell cycle arrest and metabolic maturation by interacting with BMP7CardioExcyte 96 Publication in Cell & Mol. Life Sci. (2023) Authors: Yang H., Song S., Li J., Li Y., Feng J., Sun Q., Qiu X., Chen Z., Bai X., Liu X., Lian H., Liu L., Bai Y., Zhang G., Nie Y.Mammalian cardiomyocytes (CMs) undergo maturation during postnatal heart development to meet the increased demands of growth. Here, we found that omentin-1, an adipokine, facilitates CM cell cycle arrest and metabolic maturation. Deletion ofomentin-1causes mouse heart enlargement and dysfunction in adulthood and CM maturation retardation in juveniles, including delayed cell cycle arrest and reduced fatty acid oxidation. Through RNA sequencing, molecular docking analysis, and proximity ligation assays, we found that omentin-1 regulates CM maturation by interacting directly with bone morphogenetic protein 7 (BMP7). Omentin-1 prevents BMP7 from binding to activin type II receptor B (ActRIIB), subsequently decreasing the downstream pathways mothers against DPP homolog 1 (SMAD1)/Yes-associated protein (YAP) and p38 mitogen-activated protein kinase (p38 MAPK). In addition, omentin-1 is required and sufficient for the maturation of human embryonic stem cell-derived CMs. Together, our findings reveal that omentin-1 is a pro-maturation factor for CMs that is essential for postnatal heart development and cardiac function maintenance.Flyer PDFInvestigating PIEZO1 using the SyncroPatch 384Publication link2023 Biological fractionation of lithium isotopes by cellular Na+/H+ exchangers unravels fundamental transport mechanismsOrbit mini Publication in iScience (2023) Authors: Poet M., Vigier N., Bouret Y., Jarretou G., Gautier R., Bendahhou S., Balter V., Montanes M., Thibon F., Counillon L.Lithium (Li) has a wide range of uses in science, medicine, and industry, but its isotopy is underexplored, except in nuclear science and in geoscience.6Li and7Liisotopic ratioexhibits the second largest variation on earths surface and constitutes a widely used tool for reconstructing past oceans and climates. As large variations have been measured in mammalian organs, plants or marine species, and as6Li elicits stronger effects than natural Li (95%7Li), a central issue is the identification and quantification of biological influence of Li isotopes distribution. We show that membrane ion channels and Na+-Li+/H+exchangers (NHEs) fractionate Li isotopes. This systematic6Li enrichment is driven by membrane potential for channels, and byintracellular pHfor NHEs, where it displays cooperativity, a hallmarkof dimeric transport. Evidencing that transport proteins discriminate betweenisotopes differing by one neutron opens new avenues for transport mechanisms, Li physiology, and paleoenvironments.Publication link2023 Metamorphic proteins at the basis of human autophagy initiation and lipid transferVesicle Prep Pro Publication in Mol. Cell (2023) Authors: Nguyen A., Lugarini F., David C., Hosnani P., Alagz ., Friedrich A., Schltermann D., Knotkova B., Patel A., Parfentev I., Urlaub H., Meinecke M., Stork B., Faesen A.C.Autophagy is a conserved intracellular degradation pathway that generatesde novodouble-membraneautophagosomesto target a wide range of material for lysosomal degradation. In multicellular organisms, autophagy initiation requires the timely assembly of a contact site between the ER and the nascent autophagosome. Here, we report theinvitroreconstitution of a full-length seven-subunit human autophagy initiation supercomplex built on a core complex of ATG13-101 and ATG9. Assembly of this core complex requires the rare ability of ATG13 and ATG101 to switch between distinct folds. The slow spontaneous metamorphic conversion is rate limiting for the self-assembly of the supercomplex. The interaction of the core complex with ATG2-WIPI4 enhances tethering ofmembrane vesiclesand accelerateslipidtransfer of ATG2 by both ATG9 and ATG13-101. Our work uncovers the molecular basis of the contact site and its assembly mechanisms imposed by the metamorphosis of ATG13-101 to regulate autophagosome biogenesis in space and time.Publication Link2023 DNA Origami Signaling Units Transduce Chemical and Mechanical Signals in Synthetic CellsVesicle Prep Pro Publication in Advanced Functional Materials (2023) Authors: Jahnke K., Illig M., Scheffold M., Tran M., Mersdorf U., Gpfrich K.Transmembrane proteins transmit chemical signals as well as mechanical cues. The latter is often achieved by coupling to the cytoskeleton. The incorporation of fully engineerable membrane-spanning structures for the transduction of chemical and, in particular, mechanical signals is therefore a critical aim for bottom-up synthetic biology. Here, a membrane-spanning DNA origami signaling units (DOSUs) is designed and mechanically coupled to DNA cytoskeletons encapsulated within giant unilamellar vesicles (GUVs). The incorporation of the DOSUs into the GUV membranes is verified and clustering upon external stimulation is achieved. Dye-influx assays reveal that clustering increases the insertion efficiency. The transmembrane-spanning DOSUs act as pores to allow for the transport of single-stranded DNA into the GUVs. This is employed to trigger the reconfiguration of DNA cytoskeletons within GUVs. In addition to chemical signaling, mechanical coupling of the DOSUs to the internal DNA cytoskeletons is induced. With chemical cues from the environment, clustering of the DOSUs is induced, which triggers a symmetry break in the organization of the DNA cytoskeleton inside of the GUV. DNA-based transmembrane structures are engineered that transduce signals without transporting the signaling molecule itselfproviding a route toward signal processing and adaptive syntheticcells.Read more in the publication here.Publication Link2023 Molecular orientation and optimization of membrane dyes based on conjugated oligoelectrolytesVesicle Prep Pro Publication in Cell Reports Physical Science (2023) Authors: Zhu J., Bazan G.Conjugated oligoelectrolytes (COEs) are amphiphilic, fluorogenic molecules that spontaneously associate withlipid bilayer membranesand are gaining attention as molecular reporters, particularly for exosome detection by flowcytometry. Questions nonetheless remain on how to best design COEs for optimal performance and on the geometry of lipid bilayer intercalation. In response, we designed a series of oligo-phenylenevinylene COEs with varying lengths and numbers of charged groups to address these uncertainties. Examination of the organization within lipid bilayers throughpolarized fluorescencemicroscopy shows that theoptical transitionmoments are perpendicular to the bilayer plane, with the conjugated segment flanked byhydrophobicphospholipidtails. COEs initially form a disorganized layer on the vesicle periphery, reflectingelectrostaticassociation before intercalation. Uptake experiments show that longer dimensions and increased numbers of charges allow for a higher degree of cellular association. Both shorter core length and increased number of charges accelerate the rate needed to achieve emission saturation.Read more in the publication here.Publication Link2023 Calcium-induced compaction and clustering of vesicles tracked with molecular resolutionVesicle Prep Pro Publication in Biophys. J (2023) Authors: Saldanha O., Schiller L., Hauser K.Theory and simulations predict the complex nature of calcium interaction with the lipid membrane. By maintaining the calcium concentrations at physiological conditions, herein we demonstrate experimentally the effect of Ca2+ in a minimalistic cell-like model. For this purpose, giant unilamellar vesicles (GUVs) with a neutral lipid DOPC are generated, and the ion-lipid interaction is observed with attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy providing molecular resolution. Firstly, Ca2+ encapsulated within the vesicle binds to the phosphate head groups of the inner leaflets and triggers vesicle compaction. This is tracked by changes in vibrational modes of the lipid groups. As the calcium concentration within the GUV increases, IR intensities change indicating vesicle dehydration and lateral compression of the membrane. Secondly, by inducing a calcium gradient across the membrane up to a ratio of 1:20, interaction between several vesicles occurs as Ca2+ can bind to the outer leaflets leading to vesicle clustering. It is observed that larger calcium gradients induce stronger interactions. These findings with an exemplary biomimetic model reveal that divalent calcium ions not only cause local changes to the lipid packing but also have macroscopic implications to initiate vesicle-vesicle interaction.Read more in the publication here.Publication Link2023 Modulators of cellular cholesterol homeostasis as antiproliferative and model membranes perturbing agentsVesicle Prep Pro Publication in BBA - Biomembranes (2023) Authors: Baszczyk M., Kozio A., Palko-abuz A., roda-Pomianek K., Wesoowska O.Cholesterol is an important component ofmammalian cellmembranes affecting their fluidity and permeability. Together withsphingomyelin, cholesterol forms microdomains, calledlipid rafts. They play important role insignal transductionforming platforms for interaction of signal proteins. Altered levels of cholesterol are known to be strongly associated with the development of various pathologies (e.g., cancer, atherosclerosis and cardiovascular diseases). In the present work, the group of compounds that share the property of affecting cellularhomeostasisof cholesterol was studied. It contained antipsychotic and antidepressant drugs, as well as the inhibitors ofcholesterol biosynthesis,simvastatin, betulin, and its derivatives. All compounds were demonstrated to be cytotoxic to colon cancer cells but not to non-cancerous cells. Moreover, the most active compounds decreased the level of free cellular cholesterol. The interaction of drugs with raft-mimickingmodel membraneswas visualized. All compounds reduced the size oflipiddomains, however, only some affected their number and shape. Membrane interactions of betulin and its novel derivatives were characterized in detail.Molecular modelingindicated that high dipole moment and significantlipophilicitywere characteristic for the most potent antiproliferative agents. The importance of membrane interactions of cholesterol homeostasis-affecting compounds, especially betulin derivatives, for their anticancer potency was suggested.Publication Link2023 Systematic comparison of unilamellar vesicles reveals that archaeal core lipid membranes are more permeable than bacterial membranesVesicle Prep Pro Publication in PLoS Biol (2023) Authors: apiska U., Glover G., Kahveci Z., Irwin N., Milner D., Tourte M., Albers S., Santoro A., Richards T., Pagliara S.One of the deepest branches in the tree of life separates the Archaea from the Bacteria. These prokaryotic groups have distinct cellular systems including fundamentally different phospholipid membrane bilayers. This dichotomy has been termed the lipid divide and possibly bestows different biophysical and biochemical characteristics on each cell type. Classic experiments suggest that bacterial membranes (formed from lipids extracted fromEscherichia coli, for example) show permeability to key metabolites comparable to archaeal membranes (formed from lipids extracted fromHalobacterium salinarum), yet systematic analyses based on direct measurements of membrane permeability are absent. Here, we develop a new approach for assessing the membrane permeability of approximately 10 m unilamellar vesicles, consisting of an aqueous medium enclosed by a single lipid bilayer. Comparing the permeability of 18 metabolites demonstrates that diether glycerol-1-phosphate lipids with methyl branches, often the most abundant membrane lipids of sampled archaea, are permeable to a wide range of compounds useful for core metabolic networks, including amino acids, sugars, and nucleobases. Permeability is significantly lower in diester glycerol-3-phosphate lipids without methyl branches, the common building block of bacterial membranes. To identify the membrane characteristics that determine permeability, we use this experimental platform to test a variety of lipid forms bearing a diversity of intermediate characteristics. We found that increased membrane permeability is dependent on both the methyl branches on the lipid tails and the ether bond between the tails and the head group, both of which are present on the archaeal phospholipids. These permeability differences must have had profound effects on the cell physiology and proteome evolution of early prokaryotic forms. To explore this further, we compare the abundance and distribution of transmembrane transporter-encoding protein families present on genomes sampled from across the prokaryotic tree of life. These data demonstrate that archaea tend to have a reduced repertoire of transporter gene families, consistent with increased membrane permeation. These results demonstrate that the lipid divide demarcates a clear difference in permeability function with implications for understanding some of the earliest transitions in cell origins and evolution.Read more in the publication here.Publication Link2023 The Specific Effect of Grapefruit Seed, Sea-Buckthorn Leaves, and Chaga Extracts on the Properties of Model Lipid MembranesVesicle Prep Pro Publication in Cell Tiss. Biol. (2023). Authors: Efimova S, Zakharova A., Chernyshova D., Ostroumova O.The effect of extracts of grapefruit seeds (EGSs), sea-buckthorn leaves (EBLs), and chaga (ECs) on model lipid membranes has been investigated. It has been shown that the threshold concentrations of EGSs and ECs that induced destabilization of phosphatidylglycerol-enriched bilayers are 1.31.4 times lower than for phosphatidylcholine-containing membranes. It has been established that EGSs and EBLs reduce the boundary potential of membranes formed from a mixture of phosphatadylcholine and cholesterol (the changes reach 45 and 40 mV at concentrations of 60 and 800 g/mL, respectively). ECs did not produce pronounced potential-modifying effect. It was shown that changes in the boundary potential in the presence of EBLs were due to the presence of flavonols, quercetin, and myricetin in its composition. Using the method of differential scanning calorimetry, it was also found that quercetin and myricetin were able to influence the thermotropic behavior of membrane lipids and, consequently, their packing density. The potentiation of the pore-forming activity of the antifungal polyene macrolide nystatin and antibacterial lipopeptide polymyxin B was shown with introduction of an EBLs. These data indicate a possible synergism of the antimicrobial action of the tested antibiotics and EBLs, which can be used to generate combined broad-spectrum antimicrobial agents.Read more in the publication here.Publication Link2023 Transmembrane signaling by a synthetic receptor in artificial cellsVesicle Prep Pro Publication in Nat. Commun (2023) Authors: Bretschneider Sgaard A., Btker Pedersen A., Borup Lvschall K., Monge P., Hammer Jakobsen J., Dabbarova L., Friis Nielsen L., Stevanovic S., Walther R., Zelikin A.Signal transduction across biological membranes is among the most important evolutionary achievements. Herein, for the design of artificial cells, we engineer fully synthetic receptors with the capacity of transmembrane signaling, using tools of chemistry. Our receptors exhibit similarity with their natural counterparts in having an exofacial ligand for signal capture, being membrane anchored, and featuring a releasable messenger molecule that performs enzyme activation as a downstream signaling event. The main difference from natural receptors is the mechanism of signal transduction, which is achieved using a self-immolative linker. The receptor scaffold is modular and can readily be re-designed to respond to diverse activation signals including biological or chemical stimuli. We demonstrate an artificial signaling cascade that achieves transmembrane enzyme activation, a hallmark of natural signaling receptors. Results of this work are relevant for engineering responsive artificial cells and interfacing them and/or biological counterparts in co-cultures.Read more in the publication here.Publication link2023 CNS4 causes subtype-specific changes in agonist efficacy and reversal potential of permeant cations in NMDA receptorsPort-a-Patch Publication in Pharmacol. Res. Perspect. (2023) Boehringer S.C., Johnston T.V., Kwapisz L.C., VandeVord P.J., Costa B.M.The NMDA subtype of glutamate receptor serves as an attractive drug target for the treatment of disorders evolving from hyper- or hypoglutamatergic conditions. Compounds that optimize the function of NMDA receptors are of great clinical sig -nificance. Here, we present the pharmacological characterization of a biased allosteric modulator, CNS4. Results indicate that CNS4 sensitizes ambient levels of agonists and reduces higher- concentration glycine & glutamate efficacy in 1/2AB receptors, but minimally alters these parameters in diheteromeric 1/2A or 1/2B receptors. Glycine efficacy is increased in both 1/2C and 1/2D, while glutamate efficacy is decreased in 1/2C and unaltered in 1/2D. CNS4 does not affect the activity of competitive antago-nist binding at glycine (DCKA) and glutamate (DL- AP5) sites; however, it decreases memantine potency in 1/2A receptors but not in 1/2D receptors. Currentvoltage (I- V) relationship studies indicate that CNS4 potentiates 1/2A inward currents, a phe -nomenon that was reversed in the absence of permeable Na+ ions. In 1/2D recep-tors, CNS4 blocks inward currents based on extracellular Ca2+ concentration. Further, CNS4 positively modulates glutamate potency on E781A_1/2A mutant receptors, indicating its role at the distal end of the 1/2A agonist binding domain interface. Together, these findings reveal that CNS4 sensitizes ambient agonists and allosteri-cally modulates agonist efficacy by altering Na+ permeability based on the GluN2 subunit composition. Overall, the pharmacology of CNS4 aligns with the need for drug candidates to treat hypoglutamatergic neuropsychiatric conditions such as loss function GRIN disorders and anti-NMDA receptor encephalitis.Publication link2023 Electrophysiological Techniques on the Study of Endolysosomal Ion ChannelsPort-a-Patch Publication in Handbook of Experimental Pharmacology (2023) Authors: Cheng-Chan ChenEndolysosomal ion channels are a group of ion channel proteins that are functionally expressed on the membrane of endolysosomal vesicles. The electrophysiological properties of these ion channels in the intracellular organelle membrane cannot be observed using conventional electrophysiological techniques. This section compiles the different electrophysiological techniques utilized in recent years to study endolysosomalion channels and describes their methodological characteristics, emphasizing the most widely used technique for whole endolysosome recordingsto date. This includes the use of different pharmacological tools and genetic tools for the application of patch-clamping techniques for specific stages of endolysosomes, allowing the recording of ion channel activity in different organelles, such as recycling endosomes, early endosomes, late endosomes, and lysosomes. These electrophysiological techniques are not only cutting-edge technologies that help to investigate the biophysical properties of known and unknown intracellular ion channels but also help us to investigate the physiopathological role of these ion channels in the distribution of dynamic vesicles and to identify new therapeutic targets for precision medicine and drug screening.Read more about the article here.Publication Link2023 Voltage-gated sodium channels, potential targets of Tripterygium wilfordii Hook. f. to exert activity and produce toxicityPort-a-Patch Publication in J. Ethnopharmacol. (2023). Authors: Xu Y., Li W., Wen R., Sun J., Liu X., Zhao S., Zhang J., Liu Y., Zhao M.Ethnopharmacology relevanceTripterygium wilfordii Hook. f. has been widely used in clinical practice due to its good anti-inflammatory and analgesic activities. However, its application is limited by potential toxicity and side effects.Aim of the studyThe study aimed to identify the mechanisms responsible for the pharmacological activity and cardiotoxicity of the main monomers of Tripterygium wilfordii.Materials and methodsDatabase analysis predicted that ion channels may be potential targets of Tripterygium wilfordii. The regulatory effects of monomers (triptolide, celastrol, demethylzeylasteral, and wilforgine) on protein Nav1.5 and Nav1.7 were predicted and detected by Autodock and patch clamping. Then, we used the formalin-induced pain model and evaluated heart rate and myocardial zymograms to investigate the analgesic activity and cardiotoxicity of each monomer in vivo.ResultsAll four monomers were able to bind to Nav1.7 and Nav1.5 with different binding energies and subsequently inhibited the peak currents of both Nav1.7 and Nav1.5. The monomers all exhibited analgesic effects on formalin-induced pain; therefore, we hypothesized that Nav1.7 is one of the key analgesic targets. Demethylzeylasteral reduced heart rate and increased the level of creatine kinase-MB, thus suggesting a potential cardiac risk; data suggested that the inhibitory effect on Nav1.5 might be an important factor underlying its cardiotoxicity.ConclusionOur findings provide an important theoretical basis for the further screening of active monomers with higher levels of activity and lower levels of toxicity.Publication Link2023 Adeno-Associated virus 8 delivers an immunomodulatory peptide to mouse liver more efficiently than to rat liverPort-a-Patch publicaation in PLoS ONE (2023) Authors: Wang Y., Hurley A., De Giorgi M., Tanner M., Hu R., Pennington M., Lagor W., Beeton C.Targeting the Kv1.3 potassium channel has proven effective in reducing obesity and the severity of animal models of autoimmune disease. Stichodactyla toxin (ShK), isolated from the sea anemoneStichodactyla helianthus, is a potent blocker of Kv1.3. Several of its analogs are some of the most potent and selective blockers of this channel. However, like most biologics, ShK and its analogs require injections for their delivery, and repeated injections reduce patient compliance during the treatment of chronic diseases. We hypothesized that inducing the expression of an ShK analog by hepatocytes would remove the requirement for frequent injections and lead to a sustained level of Kv1.3 blocker in the circulation. To this goal, we tested the ability of Adeno-Associated Virus (AAV)8 vectors to target hepatocytes for expressing the ShK analog, ShK-235 (AAV-ShK-235) in rodents. We designed AAV8 vectors expressing the target transgene, ShK-235, or Enhanced Green fluorescent protein (EGFP). Transduction of mouse livers led to the production of sufficient levels of functional ShK-235 in the serum from AAV-ShK-235 single-injected mice to block Kv1.3 channels. However, AAV-ShK-235 therapy was not effective in reducing high-fat diet-induced obesity in mice. In addition, injection of even high doses of AAV8-ShK-235 to rats resulted in a very low liver transduction efficiency and failed to reduce inflammation in a well-established rat model of delayed-type hypersensitivity. In conclusion, the AAV8-based delivery of ShK-235 was highly effective in inducing the secretion of functional Kv1.3-blocking peptide in mouse, but not rat, hepatocytes yet did not reduce obesity in mice fed a high-fat diet.Read more about the article here.Publication Link2023 Enriched cell-free and cell-based native membrane derived vesicles (nMV) enabling rapid in-vitro electrophysiological analysis of the voltage-gated sodium channel 1.5Port-a-Patch and Orbit 16 Publication in BBA - Biomembranes (2023) Authors: Pandey Y, Dondapati S.K., Kubick S.Here, we demonstrate the utility of native membrane derived vesicles (nMVs) as tools for expeditious electrophysiological analysis of membrane proteins. We used a cell-free (CF) and a cell-based (CB) approach for preparing protein-enriched nMVs. We utilized the Chinese HamsterOvary (CHO) lysate-based cell-free protein synthesis (CFPS) system to enrich ER-derived microsomes in the lysate with the primary human cardiac voltage-gated sodium channel 1.5 (hNaV1.5; SCN5A) in 3h. Subsequently, CB-nMVs were isolated from fractions of nitrogen-cavitated CHO cells overexpressing the hNaV1.5. In an integrative approach, nMVs were micro-transplanted into Xenopus laevisoocytes. CB-nMVs expressed native lidocaine-sensitive hNaV1.5 currents within 24h; CF-nMVs did not elicit any response. Both the CB- and CF-nMV preparations evoked single-channel activity on theplanar lipid bilayerwhile retaining sensitivity to lidocaine application. Our findings suggest a high usability of the quick-synthesis CF-nMVs and maintenance-free CB-nMVs as ready-to-use tools for in-vitro analysis of electrogenic membrane proteins and large, voltage-gated ion channels.Application noteTMEM175 TMEM 175 activity measurement in lysosomes on Nanions SURFE2R N1SURFE2R N1 application note:TMEM175 is a lysosomal cation leak channel, which impairment has been linked to Parkinsons disease and other neurodegenerative disorders; thereby making it an interesting drug target. The presented recordings illustrate SURFE2Rs potential as means for target validation and compound screening against TMEM175 channels residing in lysosomal membranes.Publication link2023 Predicting hERG repolarization power at 37C from recordings at room temperatureSyncroPatch 384 Publication in Clinical and Translational Medicine (2023) Authors: Oliveira-Mendes B.B.R., Alameh M., Montnach J., Ollivier B., Gibaud S., Feliciangeli S., Lesage F., Charpentier F., Loussouarn G., De Waard M., Bar I.Loss-of-function and gain-of-function mutations in theKCNH2 gene cause long and short-QT syndromes (LQTS or SQTS), respectively, predisposing to life-threatening cardiac arrhythmias.KCNH2encodes the voltage-gated K+channel hERG that generates the delayed rectifier K+current IKr controlling the action potential (AP) duration.Prolonged or shortened ventricular AP durations are visualized as abnormal QT interval duration on the electrocardiogram. The occurrence and severity ofKCNH2-related arrhythmias are determined by the variant functional impact. SequencingKCNH2has provided a plethora of variants associated or not with pathological cardiac phenotypes and indexed in the ClinVar NCBI database. Discriminating pathogenic variants from benign ones would clarify the genetic background of patients and relatives, and stratify the risk of adverse events. In the face of a wide spectrum of hERG functional defects, we looked for a way to summarize the net loss or gain of function in a unique index. We defined the repolarization power as the time integral of the K+current (IhERG) developed during an AP clamp.Publication link2023 G-Quadruplex-Filtered Selective Ion-to-Ion Current Amplification for Non-Invasive Ion Monitoring in Real TimeOrbit mini Publication in Advanced Materials (2023) Authors: Yoo H., Lee H-R, Kang S-B, Lee J., Park K., Yoo H., Kim J., Chung T.D., Lee K-M., Lim H-H., Son C.Y., Sun J-Y, Oh S.S.Living cells efflux intracellular ions for maintaining cellular life, so intravital measurements of specific ion signals are of significant importance for studying cellular functions and pharmacokinetics. In this work, de novo synthesis of artificial K+-selective membrane and its integration with polyelectrolyte hydrogel-based open-junction ionic diode (OJID) is demonstrated, achieving a real-time K+-selective ion-to-ion current amplification in complex bioenvironments. By mimicking biological K+channels and nerve impulse transmitters, in-line K+-binding G-quartets are introduced across freestanding lipid bilayers by G-specific hexylation of monolithic G-quadruplex, and the pre-filtered K+flow is directly converted to amplified ionic currents by the OJID with a fast response time at 100ms intervals. By the synergistic combination of charge repulsion, sieving, and ion recognition, the synthetic membrane allows K+transport exclusively without water leakage; it is 250 and 17 more permeable toward K+than monovalent anion, Cl, and polyatomic cation,N-methyl-d-glucamine+, respectively. The molecular recognition-mediated ion channeling provides a 500% larger signal for K+as compared to Li+(0.6 smaller than K+) despite the same valence. Using the miniaturized device, non-invasive, direct, and real-time K+efflux monitoring from living cell spheroids is achieved with minimal crosstalk, specifically in identifying osmotic shock-induced necrosis and drug-antidote dynamics.Publication link2023 Amphipathic peptidephospholipid nanofibers: Kinetics of fiber formation and molecular transfer between assembliesVesicle Prep ProPublicationin Biophysical Chemistry (2023) Authors: Shimizu C., Ikeda K., Nakao H., Nakano M.Understanding the kinetics of nano-assembly formation is important to elucidate thebiological processesinvolved and develop novelnanomaterialswith biological functions. In the present study, we report the kinetic mechanisms ofnanofiberformation from a mixture ofphospholipidsand the amphipathic peptide 18A[A11C], carrying cysteine substitution of theapolipoproteinA-I-derived peptide 18A at residue 11. 18A[A11C] with acetylated N-terminus and amidated C-terminus can associate withphosphatidylcholineto formfibrousaggregates at neutral pH and lipid-to-peptide molar ratio of 1, although the reaction pathways of self-assembly remain unclear. Here, the peptide was added to giant 1-palmitoyl-2-oleoylphosphatidylcholinevesicles to monitornanofiberformation underfluorescence microscopy. The peptide initially solubilized thelipid vesiclesinto particles smaller than the resolution of optical microscope, andfibrousaggregates appeared subsequently.Transmission electron microscopyanddynamic light scatteringanalyses revealed that the vesicle-solubilized particles were spherical or circular, measuring 1020nm in diameter. The rate of nanofiber formation of 18A with 1,2-dipalmitoyl phosphatidylcholine from the particles was proportional to the square of lipidpeptide concentration in the system, suggesting that the association of particles, accompanied by conformational changes, was the rate-limiting step. Moreover, molecules in the nanofibers could be transferred between aggregates faster than those in thelipidvesicles. These findings provide useful information for the development and control of nano-assembling structures using peptides andphospholipids.Publication link2023 D-galactose causes sinoatrial node dysfunction: from phenotype to mechanismCardioExcyte 96 Publication in Aging (2023) Authors: Zhang H., Chen C., Liu Y., Chen W., Qi J., Xu Y., Ren Lu., Yang G., Min D., Liu Z., Cai X., Hao M., Xu G., Hou P.With the population aging, age-related sinoatrial node dysfunction (SND) has been on the rise. Sinoatrial node (SAN) degeneration is an important factor for the age-related SND development. However, there is no suitable animal modeling method in this field. Here, we investigated whether D-galactose could induce SAN degeneration and explored the associated mechanism.In vivo, twelve C57BL/6 mice were divided into Control and D-galactose group to receive corresponding treatments. Senescence was confirmed by analyzing the hair and weight; cardiac function was evaluated through echocardiography, cerebral blood flux and serum-BNP; the SAN function was evaluated by electrocardiogram; fibrotic change was evaluated by Masson's trichrome staining and oxidative stress was assessed through DHE staining and serum indicators. Mechanism was verified through immunofluorescence-staining and Western blotting.In vitro, mouse-atrial-myocytes were treated with D-galactose, and edaravone was utilized as the ROS scavenger. Senescence, oxidative stress, proliferation ability and mechanism were verified through various methods, and intuitive evidence was obtained through electrophysiological assay. Finally, we concluded that D-galactose can be used to induce age-related SND, in which oxidative stress plays a key role, causing PITX2 ectopic expression and downregulates SHOX2 expression, then through the downstream GATA4/NKX2-5 axis, results in pacing-related ion channels dysfunction, and hence SND development.Publication link2023 PTX3 from vascular endothelial cells contributes to trastuzumab-induced cardiac complicationsCardioExcyte 96 Publication in Cardivasc. Res. (2023) Authors: Xu Z., Gao Z., Fu H., Zeng Y., Jin Y., Xu B., Zhang Y., Pan Z., Chen X., Zhang X., Wang X., Yan H., Yang X., Yang B., He Q., Luo P.AimsTrastuzumab, the first humanized monoclonal antibody that targets human epidermal growth factor receptor 2 (ERBB2/HER2), is currently used as a first-line treatment for HER2 (+) tumours. However, trastuzumab increases the risk of cardiac complications without affecting myocardial structure, suggesting a distinct mechanism of cardiotoxicity.Methods and resultsWe used medium from trastuzumab-treated human umbilical vein endothelial cells (HUVECs) to treat CCC-HEH-2 cells, the human embryonic cardiac tissue-derived cell lines, and human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to assess the crosstalk between vascular endothelial cells (VECs) and cardiomyocytes. Protein mass spectrometry analysis was used to identify the key factors from VECs that regulate the function of cardiomyocytes. We applied RNA-sequencing to clarify the mechanism, by which PTX3 causes cardiac dysfunction. We used an anti-human/rat HER2 (neu) monoclonal antibody to generate a rat model that was used to evaluate the effects of trastuzumab on cardiac structure and function and the rescue effects of lapatinib on trastuzumab-induced cardiac side effects. Medium from trastuzumab-treated HUVECs apparently impaired the contractility of CCC-HEH-2 cells and iPSC-CMs. PTX3 from VECs caused defective cardiomyocyte contractility and cardiac dysfunction in mice, phenocopying trastuzumab treatment. PTX3 affected calcium homoeostasis in cardiomyocytes, which led to defective contractile properties. EGFR/STAT3 signalling in VECs contributed to the increased expression and release of PTX3. Notably, lapatinib, a dual inhibitor of EGFR/HER2, could rescue the cardiac complications caused by trastuzumab by blocking the release of PTX3.ConclusionWe identified a distinct mode of cardiotoxicity, wherein the activation of EGFR/STAT3 signalling by trastuzumab in VECs promotes PTX3 excretion, which contributes to the impaired contractility of cardiomyocytes by inhibiting cellular calcium signalling. We confirmed that lapatinib could be a feasible preventive agent against trastuzumab-induced cardiac complications and provided the rationale for the combined application of lapatinib and trastuzumab in cancer therapy.Publication Link2023 Symport and antiport mechanisms of human glutamate transportersSURFE2R N1 Publication in Nature Communications (2023) Qiu B., Boudker O.Excitatory amino acid transporters (EAATs) uptake glutamate into glial cells and neurons. EAATs achieve million-fold transmitter gradients by symporting it with three sodium ions and a proton, and countertransporting a potassium ion via an elevator mechanism. Despite the availability of structures, the symport and antiport mechanisms still need to be clarified. We report high-resolution cryo-EM structures of human EAAT3 bound to the neurotransmitter glutamate with symported ions, potassium ions, sodium ions alone, or without ligands. We show that an evolutionarily conserved occluded translocation intermediate has a dramatically higher affinity for the neurotransmitter and the countertransported potassium ion than outward- or inward-facing transporters and plays a crucial role in ion coupling. We propose a comprehensive ion coupling mechanism involving a choreographed interplay between bound solutes, conformations of conserved amino acid motifs, and movements of the gating hairpin and the

most of the organs and tissues, and demonstrated high molecular stabilityin vitroandin vivo. The most prominent accumulation occurred in the epiphyseal plates of trabecular bones. The high stability and bioavailability, low normal-tissue uptake of [64Cu]Cu-NOTA-HsTX1[R14A], and accumulation in regions of up-regulated Kv channels bothin vitroandin vivodemonstrate that HsTX1[R14A] represents a valuable lead for conditions treatable by blockade of the voltage-gated potassium channel Kv1.3. The pharmacokinetics shows that both intravenous and subcutaneous applications are viable routes for the delivery of this potent peptide.Publication Link2022 Reclassification of a likely pathogenic Dutch founder variant in KCNH2; implications of reduced penetranceSyncroPatch 384PE (a predecessor model of SyncroPatch 384) Publication in Human Molecular Genetics (2022) Authors: Copier J.S., Bootsma M., Ng C.A., Wilde A.A.M., Bertels R.A., Bikker H., Christiaans I., van der Crabben S. N., Hol J.A., Koopmann T.T., Knijnenburg J., Lommerse A.A.J., van der Smagt J.J., Bezzina C.R., Vandenberg J.I., Verkerk A.O, Barge-Schaapveld D.Q.C.M, Lodder E.M.Due to challenges with historical data and the diversity of assay formats, in silico models for safety-related endpoints are often based on discretized data instead of the data on a natural continuous scale. Models for discretized endpoints have limitations in usage and interpretation that can impact compound design. Here, we present a consistent data inference approach, exemplified on two data sets of Ether--go-go-Related Gene (hERG) K+ inhibition data, for doseresponse and screening experiments that are generally applicable for in vitro assays. hERG inhibition has been associated with severe cardiac effects and is one of the more prominent safety targets assessed in drug development, using a wide array of in vitro and in silico screening methods. In this study, the IC50for hERG inhibition is estimated from diverse historical proprietary data. The IC50derived from a two-point proprietary screening data set demonstrated high correlation (R = 0.98, MAE = 0.08) with IC50sderived from six-point doseresponse curves. Similar IC50estimation accuracy was obtained on a public thallium flux assay data set (R = 0.90, MAE = 0.2). The IC50data were used to develop a robust quantitative model. The models MAE (0.47) and R2(0.46) were on par with literature statistics and approached assay reproducibility. Using a continuous model has high value for pharmaceutical projects, as it enables rank ordering of compounds and evaluation of compounds against project-specific inhibition thresholds. This data inference approach can be widely applicable to assays with quantitative readouts and has the potential to impact experimental design and improve model performance, interpretation, and acceptance across many standard safety endpoints.Publication Link2023 Toward Quantitative Models in Safety Assessment: A Case Study to Show Impact of DoseResponse Inference on hERG Inhibition ModelsSyncroPatch 384 Publication in Int. J. Mol. Sci. (2023). Authors: Melnikov F., Anger L.T., Hasselgren C.Due to challenges with historical data and the diversity of assay formats, in silico models for safety-related endpoints are often based on discretized data instead of the data on a natural continuous scale. Models for discretized endpoints have limitations in usage and interpretation that can impact compound design. Here, we present a consistent data inference approach, exemplified on two data sets of Ether--go-go-Related Gene (hERG) K+ inhibition data, for doseresponse and screening experiments that are generally applicable for in vitro assays. hERG inhibition has been associated with severe cardiac effects and is one of the more prominent safety targets assessed in drug development, using a wide array of in vitro and in silico screening methods. In this study, the IC50 for hERG inhibition is estimated from diverse historical proprietary data.Publication Link2022 Discovery of Novel Orally Bioavailable Triazoles with Potent and Broad-Spectrum Antifungal Activity In Vitro and In VivoSyncroPatch 384i (a predecessor model of the SyncroPatch 384) Publication in Journal of Medicinal Chemistry (2022) Authors: Ni T., Xie F., Hao Y., Li L., Zhu S., Wu H., Chi X., Yan L., Jiang Y., Zhang D.In our continuing efforts to discover novel triazoles with improved antifungal activity in vitro and in vivo, a series of 41 novel compounds containing 1,2,3-triazole side chains were designed and synthesized via a click reaction based on our previous work. Most of the compounds showed moderate to excellent broad-spectrum antifungal activity in vitro. Among them, the most promising compound 9A16 displayed excellent antifungal and anti-drug-resistant fungal ability (MIC80 = 0.01568 g/mL). In addition, compound 9A16 showed powerful in vivo efficacy on mice systematically infected with Candida albicans SC5314, Cryptococcus neoformans H99, fluconazole-resistant C. albicans 100, and Aspergillus fumigatus 7544. Moreover, compared to fluconazole, compound 9A16 showed better in vitro anti-biofilm activity and was more difficult to induce drug resistance in a 1 month induction of resistance assay in C. albicans. With favorable pharmacokinetics, an acceptable safety profile, and high potency in vitro and in vivo, compound 9A16 is currently under preclinical investigation.Publication link2022 Improved PIEZO1 agonism through 4-benzoic acid modification of Yoda1SyncroPatch 384 Publication in Br. J. Pharmacol. (2022) Authors: Parsonage G., Cuthbertson K., Endesh N., Murciano N., Hyman A.J., Revill C.H., Povstyan O.V., Chuntharpursat-Bon E., Debant M., Ludlow M.J., Futers T.S., Lichtenstein L., Kinsella J.A., Bartoli F., Rotordam M.G., Becker N., Brggemann A., Foster R., Beech D.J.4-Benzoic acid modification of Yoda1 improves PIEZO1 agonist activity at PIEZO1 channels. We suggest naming this new modulator Yoda2. It should be a useful tool compound in physiological assays and facilitate efforts to identify a binding site. Such compounds may have therapeutic potential, for example, in diseases linked genetically to PIEZO1 such as lymphatic dysplasia.2023- Solid-Supported Membrane (SSM)-Based Electrophysiology Assays Using Surface Electrogenic Event Reader Technology (SURFE2R) in Early Drug DiscoverySURFE2R-technology (custom-built system) Publication in Current Protocols (2023) Authors: Pommereau A., Licher T., Brenz F.This article presents detailed descriptions of procedures and troubleshooting tips for solid-supported membrane (SSM)-based electrophysiology assays (SURFE2R) to measure electrogenic solute carrier transporter proteins (SLCs) and assess the effects of compounds that modulate their activity. SURFE2R allows the use of the standard 96-well format, making it an ideal platform for tertiary assays in a drug-discovery campaign. The assays are performed with cell-line-derived membrane fractions or proteoliposomes containing the transporter of interest. Three main protocols are described for the isolation of membrane fractions from cell culture and the generation of proteoliposomes containing the transporter of interest. Additionally, detailed protocols for SURFE2R single concentration and dose-response experiments are included to measure the potencies of test compounds in stimulating or inhibiting transporter function (EC50 or IC50 values, respectively) and kinetic functional assays to calculate apparent affinity (kM) and maximal velocity (Vmax) of substrate uptake.Find the article here.2023 Monitoring the Reversibility of GPCR Signaling by Combining Photochromic Ligands with Label-free Impedance AnalysisCardioExcyte 96 technology (custom-built system) Publication in Angewandte Chemie (2023) Authors: Wirth U., Erl J., Azzam S., Hring C., Skiba M., Singh R., Hochmuth K., Keller M., Wegener J., Knig B.G protein-coupled cell surface receptors (GPCR) trigger complex intracellular signaling cascades upon agonist binding. Classic pharmacological assays provide information about binding affinities, activation or blockade at different stages of the signaling cascade, but real time dynamics and reversibility of these processes remain often disguised. We show that combining photochromic NPY receptor ligands, which can be toggled in their receptor activation ability by irradiation with light of different wavelengths, with whole cell label-free impedance assays allows observing the cell response to receptor activation and its reversibility over time. The concept demonstrated on NPY receptors may be well applicable to many other GPCRs providing a deeper insight into the time course of intracellular signaling processes.Publication link2023 Multi-Stimuli-Responsive and Mechano-Actuated Biomimetic Membrane Nanopores Self-Assembled from DNAOrbit mini Publication in Advanced Materials (2023) Authors: Xing Y., Dorey A., Howorka S.In bioinspired design, biological templates are mimicked in structure and function by highly controllable synthetic means. Of interest are static barrel-like nanopores that enable molecular transport across membranes for use in biosensing, sequencing, and biotechnology. However, biological ion channels offer additional functions such as dynamic changes of the entire pore shape between open and closed states, and triggering of dynamic processes with biochemical and physical stimuli. To better capture this complexity, this report presents multi-stimuli and mechano-responsive biomimetic nanopores which are created with DNA nanotechnology. The nanopores switch between open and closed states, whereby specific binding of DNA and protein molecules as stimuli locks the pores in the open state. Furthermore, the physical stimulus of high transmembrane voltage switches the pores into a closed state. In addition, the pore diameters are larger and more tunable than those of natural templates. These multi-stimuli-responsive and mechanically actuated nanopores mimic several aspects of complex biological channels yet offer easier control over pore size, shape and stimulus response. The designer pores are expected to be applied in biosensing and synthetic biology.2023 Human Induced Pluripotent Stem Cell Derived Nociceptors suitable for Automated Patch Clamp High Throughput Pain Drug DiscoveryApplication Note PDFNav, Cav, Kv High throughput APC recordings from RealDRGTM iPSC sensory neurons for analgesia drug discoveryPatchliner and SyncroPatch 384 application note: RealDRGs kindly provided by Anatomic Inc.Conference poster2023- Pharmacology of transient receptor potential cation (TRP) channels using different activation stimuliPoster presented at the 7th RSC-BMCS / SCI Symposium on Ion Channels as Therapeutic Targets2023- Identification of novel TMEM175 modulators using high-throughput automated patch-clamp and solid supported membrane- (SSM-) based electrophysiology platformsPublication link2023 Automated Patch-Clamp and Induced Pluripotent Stem Cell-Derived Cardiomyocytes: A Synergistic Approach in the Study of Brugada SyndromePatchliner and SyncroPatch 384 Publication (review) in Int. J. Mol. Sci (2023) Authors: Melgari D., Calamaio S., Frosio A., Prevostini R., Anastasia L., Pappone C., Rivolta I.The development of high-throughput automated patch-clamp technology is a recent breakthrough in the field of Brugada syndrome research. Brugada syndrome is a heart disorder marked by abnormal electrocardiographic readings and an elevated risk of sudden cardiac death due to arrhythmias. Various experimental models, developed either in animals, cell lines, human tissue or computational simulation, play a crucial role in advancing our understanding of this condition, and developing effective treatments. In the perspective of the pathophysiological role of ion channels and their pharmacology, automated patch-clamp involves a robotic system that enables the simultaneous recording of electrical activity from multiple single cells at once, greatly improving the speed and efficiency of data collection. By combining this approach with the use of patient-derived cardiomyocytes, researchers are gaining a more comprehensive view of the underlying mechanisms of heart disease. This has led to the development of more effective treatments for those affected by cardiovascular conditions.Publication link2023 Energetics and kinetics of membrane permeation of photoresists for bioprintingVesicle Prep Pro Pre-Print Publication in BioRxiv (2023) Authors: Diedrich L., Brosz M., Abele T., Steinke S., Grter F., Gpfrich K. Aponte-Santamara C.Three-dimensional (3D) bioprinting is a promising technology which typically uses bioinks to pattern cells and their scaffolds. The selection of cytocompatible inks is critical for the printing success. In laserbased 3D bioprinting, photoresist molecules are used as bioinks. We propose that cytotoxicity can be a consequence of the interaction of photoresists with lipid membranes and their permeation into the cell. Here, molecular dynamics simulations and in vitro assays address this issue, retrieving partition coefficients, free energies, and permeabilities for eight commonly-used photoresists in model lipid bilayers. Crossing the hydrophobic center of the membrane constitutes the rate limiting step during permeation. In addition, three photoresists feature a preferential localization site at the acyl chain headgroup interface. Photoresist permeabilities range over eight orders of magnitude, with some molecules being membrane-permeable on bioprinting timescales. Moreover, permeation correlates well with the oil-water partition coefficients and is severely hampered by the lipid ordering imposed by the lipid saturation. Overall, the mechanism of interaction of photoresists with model lipid bilayers is provided here, helping to classify them according to their residence in the membrane and permeation through it. This is useful information to guide the selection of cytocompatible photoresists for 3D bioprinting.Publication link2023 Cryo-EM reveals an unprecedented binding site for NaV1.7 inhibitors enabling rational design of potent hybrid inhibitorsSyncroPatch 384 Publication in eLife (2023) Authors: Kschonsak M., Jao C.C., Arthur C.P., Rohou A.L., Bergeron P., Ortwine D.F., McKerrall S.J., Hackos D.H., Deng L., Chen J., Li T., Dragovich P.S., Volgraf M., Wright M.R., Payandeh J., Ciferri C., Tellis J.C.The voltage-gated sodium (NaV) channel NaV1.7 has been identified as a potential novel analgesic target due to its involvement in human pain syndromes. However, clinically available NaVchannel-blocking drugs are not selective among the nine NaVchannel subtypes, NaV1.1NaV1.9. Moreover, the two currently known classes of NaV1.7 subtype-selective inhibitors (aryl- and acylsulfonamides) have undesirable characteristics that may limit their development. To this point understanding of the structureactivity relationships of the acylsulfonamide class of NaV1.7 inhibitors, exemplified by the clinical development candidateGDC-0310, has been based solely on a single co-crystal structure of an arylsulfonamide inhibitor bound to voltage-sensing domain 4 (VSD4). To advance inhibitor design targeting the NaV1.7 channel, we pursued high-resolution ligand-bound NaV1.7-VSD4 structures using cryogenic electron microscopy (cryo-EM). Here, we report thatGDC-0310engages the NaV1.7-VSD4 through an unexpected binding mode orthogonal to the arylsulfonamide inhibitor class binding pose, which identifies a previously unknown ligand binding site in NaVchannels. This finding enabled the design of a novel hybrid inhibitor series that bridges the aryl- and acylsulfonamide binding pockets and allows for the generation of molecules with substantially differentiated structures and properties. Overall, our study highlights the power of cryo-EM methods to pursue challenging drug targets using iterative and high-resolution structure-guided inhibitor design. This work also underscores an important role of the membrane bilayer in the optimization of selective NaVchannel modulators targeting VSD4.Publication link2023 Protein 14-3-3 Influences the Response of the Cardiac Sodium Channel Nav1.5 to Antiarrhythmic DrugsSyncroPatch 384 Publication in J. Pharmacol. Exp. Ther. (2023) Authors: Zheng Y., Deschnes I.The cardiac sodium channel Nav1.5 is a key contributor to the cardiac action potential, and dysregulations in Nav1.5 can lead to cardiac arrhythmias. Nav1.5 is a target of numerous antiarrhythmic drugs (AADs). Previous studies identified the protein 14-3-3 as a regulator of Nav1.5 biophysical coupling. Inhibition of 14-3-3 can remove the Nav1.5 functional coupling and has been shown to inhibit the dominant-negative effect of Brugada syndrome mutations. However, it is unknown whether the coupling regulation is involved with AADs modulation of Nav1.5. Indeed, AADs could reveal important structural and functional information about Nav1.5 coupling. Here, we investigated the modulation of Nav1.5 by four classic AADs, quinidine, lidocaine, mexiletine, and flecainide, in the presence of 14-3-3 inhibition. The experiments were carried out by high-throughput patch-clamp experiments in an HEK293 Nav1.5 stable cell line. We found that 14-3-3 inhibition can enhance acute block by quinidine, whereas the block by other drugs was not affected. We also saw changes in the use- and dose-dependency of quinidine, lidocaine, and mexiletine when inhibiting 14-3-3. Inhibiting 14-3-3 also shifted the channel activation toward hyperpolarized voltages in the presence of the four drugs studied and slowed the recovery of inactivation in the presence of quinidine. Our results demonstrated that the protein 14-3-3 and Nav1.5 coupling could impact the effects of AADs. Therefore, 14-3-3 and Nav1.5 coupling are new mechanisms to consider in the development of drugs targeting Nav1.5.Publication link2023 ANXA11 biomolecular condensates facilitate protein-lipid phase coupling on lysosomal membranesVesicle Prep Pro Pre-Print Publication in BioRxiv (2023) Authors: Nixon-Abell J., Ruggeri F.S., Qamar S., Herling T.W., Czekalska M.A., Shen Yi., Wang G., King C., Fernandopulle M.S., Sneideris T., Watson J.L., Pillai V.V.S., Meadows W., Henderson J.W., Chambers J.E., Wagstaff J.L., Williams S.H., Coyle H., Lu Y., Zhang S., Marciniak S.J., Freund S.M.V., Derivery E., Ward M.E., Vendruscolo M., Knowles T.P.J. St George-Hyslop P.Phase transitions of cellular proteins and lipids play a key role in governing the organisation and coordination of intracellular biology. The frequent juxtaposition of proteinaceous biomolecular condensates to cellular membranes raises the intriguing prospect that phase transitions in proteins and lipids could be co-regulated. Here we investigate this possibility in the ribonucleoprotein (RNP) granule-ANXA11-lysosome ensemble, where ANXA11 tethers RNP granule condensates to lysosomal membranes to enable their co-trafficking. We show that changes to the protein phase state within this system, driven by the low complexity ANXA11 N-terminus, induce a coupled phase state change in the lipids of the underlying membrane. We identify the ANXA11 interacting proteins ALG2 and CALC as potent regulators of ANXA11-based phase coupling and demonstrate their influence on the nanomechanical properties of the ANXA11-lysosome ensemble and its capacity to engage RNP granules. The phenomenon of protein-lipid phase coupling we observe within this system offers an important template to understand the numerous other examples across the cell whereby biomolecular condensates closely juxtapose cell membranes.Publication link2023- Functional characterization of SGLT1 using SSM-based electrophysiology: Kinetics of sugar binding and translocationSURFE2R- N1 Publication in Front. Physiol. (2023) Authors: Bazzone A., Zerlotti R., Barthmes M., Fertig N.Beside the ongoing efforts to determine structural information, detailed functional studies on transporters are essential to entirely understand the underlying transport mechanisms. We recently found that solid supported membrane-based electrophysiology (SSME) enables the measurement of both sugar binding and transport in the Na+/sugar cotransporter SGLT1 (Bazzone et al, 2022a). Here, we continued with a detailed kinetic characterization of SGLT1 using SSME, determining KMand KDappfor different sugars, kobsvalues for sugar-induced conformational transitions and the effects of Na+, Li+, H+and Clon sugar binding and transport. We found that the sugar-induced pre-steady-state (PSS) charge translocation varies with the bound ion (Na+, Li+, H+or Cl), but not with the sugar species, indicating that the conformational state upon sugar binding depends on the ion. Rate constants for the sugar-induced conformational transitions upon binding to the Na+-bound carrier range from 208s1for D-glucose to 95s1for 3-OMG. In the absence of Na+, rate constants are decreased, but all sugars bind to the empty carrier. From the steady-state transport current, we found a sequence for sugar specificity (Vmax/KM): D-glucose > MDG > D-galactose > 3-OMG > D-xylose. While KMdiffers 160-fold across tested substrates and plays a major role in substrate specificity, Vmaxonly varies by a factor of 1.9. Interestingly, D-glucose has the lowest Vmaxacross all tested substrates, indicating a rate limiting step in the sugar translocation pathway following the fast sugar-induced electrogenic conformational transition. SGLT1 specificity for D-glucose is achieved by optimizing two ratios: the sugar affinity of the empty carrier for D-glucose is similarly low as for all tested sugars (KD,Kapp= 210mM). Affinity for D-glucose increases 14-fold (KD,Naapp= 15mM) in the presence of sodium as a result of cooperativity. Apparent affinity for D-glucose during transport increases 8-fold (KM= 1.9mM) compared to KD,Naappdue to optimized kinetics. In contrast, KMand KDappvalues for 3-OMG and D-xylose are of similar magnitude. Based on our findings we propose an 11-state kinetic model, introducing a random binding order and intermediate states corresponding to the electrogenic transitions detectedviaSSME upon substrate binding.Read more in the paper and find out more about other Solute Carriers in the article collection here.Publication link2023 Nav1.2 and BK channel interaction shapes the action potential in the axon initial segmentSyncroPatch 384 Publication in J. Physiol. (2023) Authors: Filipis L., Blmer L.A., Montnach J., Loussouarn G., De Waard M., Canepari M.In neocortical layer-5 pyramidal neurons, the action potential (AP) is generated in the axon initial segment (AIS) when the membrane potential (Vm) reaches the threshold for activation of the voltage-gated Na+channels (VGNCs) Nav1.2 and Nav1.6. Yet, whereas these VGNCs are known to differ in spatial distribution along the AIS and in biophysical properties, our understanding of the functional differences between the two channels remains elusive. Here, using ultrafast Na+,Vmand Ca2+imaging in combination with partial block of Nav1.2 by the peptide G1G4-huwentoxin-IV, we demonstrate an exclusive role of Nav1.2 in shaping the generating AP. Precisely, we show that selective block of 30% of Nav1.2 widens the AP in the distal part of the AIS and we demonstrate that this effect is due to a loss of activation of BK Ca2+-activated K+channels (CAKCs). Indeed, Ca2+influx via Nav1.2 activates BK CAKCs, determining the amplitude and the early phase of repolarization of the AP in the AIS. By using control experiments using 4,9-anhydrotetrodotoxin, a moderately selective inhibitor of Nav1.6, we concluded that the Ca2+influx shaping the early phase of the AP is exclusive of Nav1.2. Hence, we mimicked this result with a neuron model in which the role of the different ion channels tested reproduced the experimental evidence. The exclusive role of Nav1.2 reported here is important for understanding the physiology and pathology of neuronal excitability.2022 The suitability of high throughput automated patch clamp for physiological applicationsSyncroPatch 384 Publication in The Journal of Physiology (2022). Authors: Obergrussberger A., Rinke-Wei I., Goetze T.A., Rapedius M., Brinkwirth N., Becker N., Rotordam M.G., Hutchison L., Madau P., Pau D., Dalrymple D., Braun N., Friis S., Pless S.A., Fertig N.Although automated patch clamp (APC) devices have been around for many years and have become an integral part of many aspects of drug discovery, high throughput instruments with G seal data quality are relatively new. Experiments where a large number of compounds are screened against ion channels are ideally suited to high throughput APC, particularly when the amount of compound available is low. Here we evaluate different APC approaches using a variety of ion channels and screening settings. We have performed a screen of 1,920 compounds on GluN1/GluN2A NMDA receptors for negative allosteric modulation using both the SyncroPatch 384 and FLIPRTM. Additionally, we tested the effect of 36 arthropod venoms on NaV1.9 using a single 384-well plate on the SyncroPatch 384. As an example for mutant screening, a range of acid-sensing ion channel variants were tested and the success rate increased through FACS prior to APC experiments. G seal data quality makes the 384- format accessible to recording of primary and stem cell-derived cells on the SyncroPatch 384. We show recordings in voltage and current clamp modes of stem cell-derived cardiomyocytes. In addition, the option of intracellular solution exchange enabled investigations into the effects of intracellular Ca2+ and cAMP on TRPC5 and HCN2 currents, respectively. Together, this highlights the broad applicability and versatility of APC platforms and also outlines some limitations of the approach.Publication link2023 Beyond CBD: Inhibitory effects of lesser studied phytocannabinoids on human voltage-gated sodium channelsPatchliner Publication in Front. Physiol. (2023) Authors: Milligan C.J., Anderson L.L., McGregor I.S., Arnold J.C., Petrou S.Introduction:Cannabis contains cannabidiol (CBD), the main non-psychoactive phytocannabinoid, but also many other phytocannabinoids that have therapeutic potential in the treatment of epilepsy. Indeed, the phytocannabinoids cannabigerolic acid (CBGA), cannabidivarinic acid (CBDVA), cannabichromenic acid (CBCA) and cannabichromene (CBC) have recently been shown to have anti-convulsant effects in a mouse model of Dravet syndrome (DS), an intractable form of epilepsy. Recent studies demonstrate that CBD inhibits voltage-gated sodium channel function, however, whether these other anti-convulsant phytocannabinoids affect these classic epilepsy drug-targets is unknown. Voltage-gated sodium (NaV) channels play a pivotal role in initiation and propagation of the neuronal action potential and NaV1.1, NaV1.2, NaV1.6 and NaV1.7 are associated with the intractable epilepsies and pain conditions.Methods:In this study, using automated-planar patch-clamp technology, we assessed the profile of the phytocannabinoids CBGA, CBDVA, cannabigerol (CBG), CBCA and CBC against these human voltage-gated sodium channels subtypes expressed in mammalian cells and compared the effects to CBD.Results:CBD and CBGA inhibited peak current amplitude in the low micromolar range in a concentration-dependent manner, while CBG, CBCA and CBC revealed only modest inhibition for this subset of sodium channels. CBDVA inhibited NaV1.6 peak currents in the low micromolar range in a concentration-dependent fashion, while only exhibiting modest inhibitory effects on NaV1.1, NaV1.2, and NaV1.7 channels. CBD and CBGA non-selectively inhibited all channel subtypes examined, whereas CBDVA was selective for NaV1.6. In addition, to better understand the mechanism of this inhibition, we examined the biophysical properties of these channels in the presence of each cannabinoid. CBD reduced NaV1.1 and NaV1.7 channel availability by modulating the voltage-dependence of steady-state fast inactivation (SSFI, V0.5inact), and for NaV1.7 channel conductance was reduced. CBGA also reduced NaV1.1 and NaV1.7 channel availability by shifting the voltage-dependence of activation (V0.5act) to a more depolarized potential, and for NaV1.7 SSFI was shifted to a more hyperpolarized potential. CBDVA reduced channel availability by modifying conductance, SSFI and recovery from SSFI for all four channels, except for NaV1.2, where V0.5inact was unaffected.Discussion:Collectively, these data advance our understanding of the molecular actions of lesser studied phytocannabinoids on voltage-gated sodium channel proteins.Webinar07.03.2013 | Webinar: Top gear ion channel screeningAuthors: Nanion ExpertsWebinar24.10.2013 | Webinar: SyncroPatch 384PE the PatchEngine. Superior Ion Channel Drug ScreeningAuthors: Nanion ExpertsPodcastspotl]i[ght Podcast Trong Shen (University of Toronto)Guest: Trong Shen (University of Toronto)In this edition of the podcast - we speak to Tron Shen and his unique experiences working in an academic lab (Hinz Lab). Listen to his unique story, which encompases a stop in the world of finance.Podcastspotl]i[ght Podcast Wandi Zhu (Harvard)Guest: Wandi Zhu (Harvard)Bacterial and mammalian NaV channels provide insights into the molecular basis of channel gating and will facilitate organism-specific drug discovery.Podcastspotl]i[ght Podcast Nasreen Choudhury (Rush University)Guest: Nasreen Choudhury (Rush University)In this edition of the podcast - we spoke to Postdoctoral Research Fellow Nasreen Choudhury about her research of Alziehmer's disease and the role that ion channels play. Nasreen completed her PhD at the Indian Institute of Science where she published a paper about TREK1 channel, read it here. Nasreen went on to working at the University of Leicester where she published her investigation into Kv3 channels.When Nasreen finishes her postdoctoral stint at Rush University Medical Center, she plans to switch to an industry role where she can work with automated patch clamp platformsPodcastspotl]i[ght Podcast Shashank Pant (University Of Illinois Urbana-Champaign)Guest: Shashank Pant (University Of Illinois Urbana-Champaign)On this episode of the podcast we spoke with Shashank Pant (University of Illinois, Urbana-Champaign). We talked about how he started his journey as a researcher and how his curiosity of the scientific process continues to serve as a motivation to publish in high impact journals such as Nature (where he was recently part of an internationally collaboration with with Renae Ryan's Lab, University of Sydney).Here they looked at Glutamate, specifically providing insight into the mechanism by which glutamate transporters support their dual functionPodcastspotl]i[ght Podcast Katherine Henzler-Wildman (University of Wisconsin-Madison) & Nathan Thomas cGuest: Katherine Henzler-Wildman (University of Wisconsin-Madison) & Nathan Thomas (University of California - San Diego)On this episode of the podcast we spoke with Katie and Nathan about their work (specifically around developing a method to provide a fast, easy, general method for measuring transport stoichiometry via solid supported membrane electrophysiology) which will facilitate future mechanistic and functional studies of ion-coupled transporters. You can read the full publication titled: A solid-supported membrane electrophysiology assay for efficient characterization of ion-coupled transport here.Katie's Lab uses NMR as a primary technique to monitor protein structure and dynamics, taking advantage of the extensive resources available through NMRFAM. By comparing NMR data with biochemical and functional assays, insight into the mechanisms of secondary active transport, multidrug recognition, ion channel gating and ion selectivity is gained.Nathan is presentlt a Postdoctoral Researchers at Tezcan Lab - UCSD (University of California San Diego,) Department of Chemistry and Biochemistry.Podcastspotl]i[ght Podcast Kerstin Gpfrich (Max Planck Institute for Medical Research)Guest: Kerstin Gpfrich (Max Planck Institute for Medical Research)In this edition of the podcast - we speak to Kerstin Gpfrich Ph.D. who is currently attempting how to construct a cell with a bottom up approach and exploring how life could be different with DNA origami.Podcastspotl]i[ght Podcast Dr. Filip Van Petegem (University of British Columbia)Guest: Dr. Filip Van Petegem (University of British Columbia)On this episode of the podcast we spoke with Dr. Filip Van Petegem about his journey starting as a structural biologist, to the work his lab is currently focused on. Van Petegem Lab studies the structure and function of ion channels, membrane protein responsible for electrical signaling in excitable cells.Podcastspotl]i[ght Podcast Dr. Yohei Ohashi (Medical Research Council - UK)Guest: Dr. Yohei Ohashi (Medical Research Council - UK)On this episode of the podcast we spoke with Dr. Yohei Ohashi about his unique journey from the University of Kyoto where he was working on his PhD specifically within plant molecular biology. Dr. Yohei Ohashi is a multidisciplinary researcher with over 15 years of experience in basic/translational biology.Podcastspotl]i[ght Podcast Dr. Carlos Pardo-Pastor (Kings College London)Guest: Dr. Carlos Pardo-Pastor (King's College London)Dr Carlos Pardo-Pastor (King's College London) is a Research Fellow in the Randall Centre for Cell & Molecular Biophysics. He started his scientific journey studying Human Biology, an MSc in Biomedical Research and then completed his PhD in Biomedicine at Universitat Pompeu Fabra.On this episode of the podcast we spoke with Carlos about his most recent publication (Piezo2 Channel), you can read the full publication here. He highlights the importance of funding in science, his personal experiences in applying for grants, and some helpful tips for staying motivated as a researcher and connecting with peers in the field.Podcastspotl]i[ght Podcast Dr. Fernanda C Cardoso (University of Queensland)Guest: Dr. Fernanda C Cardoso (University of Queensland)Dr. Fernanda C Cardoso joined the Institute for Molecular Bioscience at University of Queensland to develop therapies to treat complex neurological diseases. In this episode, she tells us about her passion from a young age to study biology and arachnids. Find out more about Fernanda here.Here she describes the benefits of working with an Automated Patch Clamp set-up (in comparison to the more traditional manual patch clamp set-up). Her work focuses on Sodium and Calcium Channels, specifically dealing with disorders associated with chronic pain.Podcastspotl]i[ght Podcast Catherine Webley (University College London)Guest: Catherine Webley (University College London)In this edition of the podcast - we speak to Catherine Webley, Master of Chemistry, who completed a placement in the academic research lab in the Solar Centre at King Abdullah University of Science and Technology under the supervision of Prof. Iain McCulloch. She describes her 'love affair' with how the natural world works and the impact that the recent Nobel Prize winners have had on her.Podcastspotl]i[ght Podcast Claudia Weidling (University of Copenhagen)Guest: Claudia Weidling (University of Copenhagen)In this edition of the podcast - we speak to Claudia Weidling Ph.D. who tells us about her recent publication in Nature, her experience being a Ph.D student during a pandemic and what advice she would offer to prospective Ph.D students.Podcastspotl]i[ght Podcast Beatrice Badone (University of Milano-Bicocca)Guest: Beatrice Badone (University of Milano-Bicocca)Beatrice Badone, a Ph.D. student at the University of Milano-Bicocca. Beatrice works with Cardiomyocytes and explains how drug screening can be improved and can be made more accessible and ethical for scientists.Podcastspotl]i[ght Podcast Benjamin Lane (University of Leeds)Guest: Benjamin Lane (University of Leeds)Benjamin is a Wellcome trust PhD student, who is working under the supervision of Dr Christos Pliotas and Dr Stephen Muench. He recently joined their groups to work on his project combining Cryo-EM and PELDOR with electrophysiology, to structurally and functionally characterise novel mechanosensitive ion channels.Publication link2018 Structures of monomeric and oligomeric forms of the Toxoplasma gondii perforin-like protein 1Vesicle Prep Pro Publication in Science Advances (2018) Authors: Ni T., Williams S.I., Rezelj S., Anderluh G., Harlos K., Stansfeld P.J., Gilbert R.J.C.Toxoplasma and Plasmodium are the parasitic agents of toxoplasmosis and malaria, respectively, and use perforin-like proteins (PLPs) to invade host organisms and complete their life cycles. The Toxoplasma gondii PLP1 (TgPLP1) is required for efficient exit from parasitophorous vacuoles in which proliferation occurs. We report structures of the membrane attack complex/perforin (MACPF) and Apicomplexan PLP C-terminal -pleated sheet (APC) domains of TgPLP1. The MACPF domain forms hexameric assemblies, with ring and helix geometries, and the APC domain has a novel -prism fold joined to the MACPF domain by a short linker. Molecular dynamics simulations suggest that the helical MACPF oligomer preserves a biologically important interface, whereas the APC domain binds preferentially through a hydrophobic loop to membrane phosphatidylethanolamine, enhanced by the additional presence of inositol phosphate lipids. This mode of membrane binding is supported by site-directed mutagenesis data from a liposome-based assay. Together, these structural and biophysical findings provide insights into the molecular mechanism of membrane targeting by TgPLP1.Podcastspotl]i[ght Podcast Professor Lars Kaestner (Saarland University)Guest: Professor Lars Kaestner (Saarland University)In this edition of the podcast - we speak to Prof. Lars Kaestner and his work with Red Blood Cells; specifically looking at advances in diagnostic tools for Neuroacanthocytosis.Publication link2022 Unraveling the host-selective toxic interaction of cassiicolin with lipid membranes and its cytotoxicityVesicle Prep Pro Publication in Phytopathology (2022) Authors: Ngo K.X., Quo N.B., Nguyen P.D.N., Furusho H., Miyata M., Shimonaka T., Chau N.N.B.C., Vinh N.P., Nghia N.A., Mohammed T.O., Ichikawa T., Kodera N., Konno H., Fukuma T., Ando T.Corynespora cassiicola is the pathogen that causes Corynespora leaf fall (CLF) disease.Cassiicolin (Cas), a toxin produced by C. cassiicola, is responsible for CLF disease in rubbertrees (Hevea brasiliensis). Currently, the molecular mechanism of the cytotoxicity of Cas andits host selectivity have not been fully elucidated. To gain insight into these issues, we analyzedthe binding of Cas1 and Cas2 to membranes consisting of different plant lipids and theirmembrane-disruption activities. Our real-time observations with high-speed atomic forcemicroscopy (HS-AFM) and confocal microscopy reveal that the binding and disruptionactivities of Cas1 and Cas2 are strongly dependent on the types of membrane lipids. Themixtures of DPPC with DPPA, MGDG, DGDG, and stigmasterol are more susceptible tomembrane damage caused by Cas1 and Cas2 than DPPC alone or its mixtures with sitosterol,DGTS-d9, and DGTS. This difference derives from the stronger binding of the toxins tomembranes with the former lipid composition. Cytotoxicity tests on rubber leaves of RRIV 1,RRIV 4, and PB 255 clones suggest that the toxins cause necrosis of rubber leaves, except forthe strong resistance of PB 255 against Cas2. Cryo-SEM analyses of necrotic leaf tissuesexposed to Cas1 confirm that cytoplasmic membranes are vulnerable to the toxin. Thus, thehost selectivity of Cas toxin in CLF disease is attained by the lipid-dependent binding activityof Cas to the membrane, and the cytotoxicity of Cas arises from its ability to disrupt membranes.Product Sheet PDFVesicle Prep Pro Product SheetPublication link2022 The Small Heat Shock Protein, HSPB1, Interacts with and Modulates the Physical Structure of MembranesVesicle Prep ProPublicationin International Journal of Molecular Science (2022) Authors: Csoboz B., Gombos I., Kta Z., Dukic B., Klement E., Varga-Zsros V., Lipinszki Z., Pli T., Lszl Vgh L., Trk Z.Small heat shock proteins (sHSPs) have been demonstrated to interact with lipids and modulate the physical state of membranes across species. Through these interactions, sHSPs contribute to the maintenance of membrane integrity. HSPB1 is a major sHSP in mammals, but its lipid interaction profile has so far been unexplored. In this study, we characterized the interaction between HSPB1 and phospholipids. HSPB1 not only associated with membranes via membrane-forming lipids, but also showed a strong affinity towards highly fluid membranes. It participated in the modulation of the physical properties of the interacting membranes by altering rotational and lateral lipid mobility. In addition, the in vivo expression of HSPB1 greatly affected the phase behavior of the plasma membrane under membrane fluidizing stress conditions. In light of our current findings, we propose a new function for HSPB1 as a membrane chaperone.Publication link2022 The Staphylococcus aureus CidA and LrgA Proteins Are Functional Holins Involved in the Transport of By-Products of Carbohydrate MetabolismVesicle Prep ProPublicationin mBio (2022) Authors: Endres J. L., Chaudhari S. S., Zhang X., Prahlad J., Wang S-Q., Foley L. A., Luca S., Bose J. L., Thomas V. C., Bayles K. W.The Staphylococcus aureus cidABC and lrgAB operons encode members of a well-conserved family of proteins thought to be involved in programmed cell death (PCD). Based on the structural similarities that CidA and LrgA share with bacteriophage holins, we have hypothesized that these proteins function by forming pores within the cytoplasmic membrane. To test this, we utilized a "lysis cassette" system that demonstrated the abilities of the cidA and lrgA genes to support bacteriophage endolysin-induced cell lysis. Typical of holins, CidA- and LrgA-induced lysis was dependent on the coexpression of endolysin, consistent with the proposed holin-like functions of these proteins. In addition, the CidA and LrgA proteins were shown to localize to the surface of membrane vesicles and cause leakage of small molecules, providing direct evidence of their hole-forming potential. Consistent with recent reports demonstrating a role for the lrgAB homologues in other bacterial and plant species in the transport of by-products of carbohydrate metabolism, we also show that lrgAB is

results suggest membrane phase behavior, and the distribution of membrane elasticizing amphiphiles, impacts the ability of a membrane to stretch.Publication link2021 Highly Basic Clusters in the Herpes Simplex Virus 1 Nuclear Egress Complex Drive Membrane Budding by Inducing Lipid OrderingVesicle Prep ProPublicationin mBio (2021) Authors: Thorsen M.K., Lai A., Lee M.W., Hoogerheide D.P., Wong G.C.L., Freed J.H., Heldweina E.EDuring replication of herpesviruses, capsids escape from the nucleus into the cytoplasm by budding at the inner nuclear membrane. This unusual process is mediated by the viral nuclear egress complex (NEC) that deforms the membrane around the capsid by oligomerizing into a hexagonal, membrane-bound scaffold. Here, we found that highly basic membrane-proximal regions (MPRs) of the NEC alter lipid order by inserting into the lipid headgroups and promote negative Gaussian curvature. We also find that the electrostatic interactions between the MPRs and the membranes are essential for membrane deformation. One of the MPRs is phosphorylated by a viral kinase during infection, and the corresponding phosphomimicking mutations block capsid nuclear egress. We show that the same phosphomimicking mutations disrupt the NEC-membrane interactions and inhibit NEC-mediated budding in vitro, providing a biophysical explanation for the in vivo phenomenon. Our data suggest that the NEC generates negative membrane curvature by both lipid ordering and protein scaffolding and that phosphorylation acts as an off switch that inhibits the membrane-budding activity of the NEC to prevent capsid-less budding.Publication link2021 Dynamic remodeling of giant unilamellar vesicles induced by monoglyceride nano-micelles: Insights into supramolecular organizationVesicle Prep ProPublicationin Applied Materials Today (2021) Authors: Yoon B.K., Kim M.C., Jackman J.A., Cho N-JThere is broad interest in developing nanostructured assemblies composed of fatty acids and monoglycerides to inhibit membrane-enveloped pathogens and modulate immune cell behavior. Herein, we investigated the interactions of micellar nanostructures composed of a biologically active monoglyceride, glycerol monolaurate (GML), or its ether-bonded equivalent, 1-O-dodecyl-rac-glycerol (DDG), with cell-membrane-mimicking giant unilamellar vesicles (GUVs). Our findings revealed that GML nanostructures induced fission or fusion depending on the GML concentration and corresponding degree of supramolecular organization, while DDG nanostructures only caused aggregation-like disruption of the GUV outer surface. In specific conditions, the GML nanostructures also triggered pearling instability, which led to dynamic membrane remodeling behavior and the pattern of GML interactions was consistent across simplified and complex membrane compositions. Notably, the spectrum of membrane morphological changes induced by GML nanostructures, including fission, fusion, and pearling behaviors, is appreciably wider than the fission behavior exhibited by fatty acid nanostructures in past studies. Collectively, these findings demonstrate how controlling the supramolecular organization of monoglycerides within nanostructured assemblies can be useful to modulate the type and degree of membrane interactions relevant to biophysical and nanomedicine applications.Publication link2021 Electroformation of Giant Unilamellar Vesicles and A Case Study of the Specific Host-Toxin Interaction of Lipid Membranes with CassiicolinVesicle Prep Pro Protocol in Bio-101 (2021) Authors: Ngo KX., Weichbrodt C., Quoc NBAn alternating electric field is applied to induce swelling of thin lipid films and generation of giant unilamellar vesicles (GUVs) on an indium tin oxide (ITO)-coated glass surface. The process is, hereafter, referred to as the electroformation of GUVs. Several important parameters such as lipid manipulation, temperature, osmolarity and ionic strength of the solutions involved, and the electric field (current (DC, AC), amplitude, frequency) should be optimal for the successful electroformation of GUVs. In our case study,GUVs composed of lipid mixtures available in plant cells provide many benefits for studying the lipid-dependent pathogenicity of cassiicolin (Cas) toxins and thereby deciphering the host-selective toxin interaction of Cas toxins with the specific lipid membranes of plant cells. GUVs gently maintained in the solution furnish perfectly suspended and intact lipidmembranes similar to cytoplasmic membranes enabling us to examine the selective binding of GFP-Cas1 and GFP-Cas2 to the specific lipid membranes. In this protocol, we briefly explain the principle of electroformation method and provide the experimental conditions and the manipulation for successfully making GUVs composed of plant lipids (DPPC, DPPC/DPPA, DPPC/MGDG, DPPC/DGDG, DPPC/stigmasterol, DPPC/sitosterol, DPPC/DGTS-d9, and DPPC/DGTS).Publication link2021 Binding of DNA origami to lipids: maximizing yield and switching via strand displacementVesicle Prep ProPublicationin Nucleic Acids Research (2021) Authors: Daljit Singh J. K., Darley E., Ridone P., Gaston J. P., Abbas A., Wickham S. F J., Baker M. A BLiposomes are widely used as synthetic analogues of cell membranes and for drug delivery. Lipid-binding DNA nanostructures can modify the shape, porosity and reactivity of liposomes, mediated by cholesterol modifications. DNA nanostructures can also be designed to switch conformations by DNA strand displacement. However, the optimal conditions to facilitate stable, high-yield DNAlipid binding while allowing controlled switching by strand displacement are not known. Here, we characterized the effect of cholesterol arrangement, DNA structure, buffer and lipid composition on DNAlipid binding and strand displacement. We observed that binding was inhibited below pH 4, and above 200 mM NaCl or 40 mM MgCl2, was independent of lipid type, and increased with membrane cholesterol content. For simple motifs, binding yield was slightly higher for double-stranded DNA than single-stranded DNA. For larger DNA origami tiles, four to eight cholesterol modifications were optimal, while edge positions and longer spacers increased yield of lipid binding. Strand displacement achieved controlled removal of DNA tiles from membranes, but was inhibited by overhang domains, which are used to prevent cholesterol aggregation. These findings provide design guidelines for integrating strand displacement switching with lipid-binding DNA nanostructures. This paves the way for achieving dynamic control of membrane morphology, enabling broader applications in nanomedicine and biophysics.Publication link2021 Design of biologically active binary protein 2D materialsVesicle Prep Pro Publication in Nature (2021) Authors: Ben-Sasson A.J., Watson J.L., Sheffler W., Camp Johnson M., Bittleston A., Somasundaram L., Decarreau J., Jiao F., Chen J., Mela I., Drabek A.A., Jarrett S.M., Blacklow S.C., Kaminski C.F., Hura G.L., De Yoreo J.J., Kollman J.M., Ruohola-Baker H., Derivery E., Baker D.Ordered two-dimensional arrays such as S-layers and designed analogues have intrigued bioengineers, but with the exception of a single lattice formed with flexible linkers, they are constituted from just one protein component. Materials composed of two components have considerable potential advantages for modulating assembly dynamics and incorporating more complex functionality. Here we describe a computational method to generate co-assembling binary layers by designing rigid interfaces between pairs of dihedral protein building blocks, and use it to design a p6m lattice. The designed array components are soluble at millimolar concentrations, but when combined at nanomolar concentrations, they rapidly assemble into nearly crystalline micrometre-scale arrays nearly identical to the computational design model in vitro and in cells without the need for a two-dimensional support. Because the material is designed from the ground up, the components can be readily functionalized and their symmetry reconfigured, enabling formation of ligand arrays with distinguishable surfaces, which we demonstrate can drive extensive receptor clustering, downstream protein recruitment and signalling. Using atomic force microscopy on supported bilayers and quantitative microscopy on living cells, we show that arrays assembled on membranes have component stoichiometry and structure similar to arrays formed in vitro, and that our material can therefore impose order onto fundamentally disordered substrates such as cell membranes. In contrast to previously characterized cell surface receptor binding assemblies such as antibodies and nanocages, which are rapidly endocytosed, we find that large arrays assembled at the cell surface suppress endocytosis in a tunable manner, with potential therapeutic relevance for extending receptor engagement and immune evasion. Our work provides a foundation for a synthetic cell biology in which multi-protein macroscale materials are designed to modulate cell responses and reshape synthetic and living systems.Publication link2021 Alamethicin Channels as a Signal Transduction Element in an Immobilized Single Giant Unilamellar VesicleVesicle Prep Pro Publication in Sensors and Materials(2021) Authors: Fukuda H., Nitta M., Sakamoto M., Shoji A., Sugawara M.A single giant unilamellar vesicle (GUV) functionalized with an anti-bovine serum albumin (BSA) antibody was immobilized on an avidin slip, and alamethicin channels were embedded as a signal transduction element for creating a channel-based molecular sensing system. The GUV sensor based on the membrane-bound anti-BSA antibody receptor exhibited alamethicin activities that reflected the binding of BSA (an analyte) at the membrane/solution interface. The normalized integrated current at 60 mV was able to be used as a measure of the amount of BSA in a solution. The quantification of BSA at pg/mL level was demonstrated.Publication link2021 Binding of DNA origami to lipids: maximising yield and switching via strand-displacementVesicle Prep Pro Publication in Nucleic Acids Research (2021) Authors: Singh J.KD., Darley E.,Ridone P., Gaston J. P., Abbas A., Wickham S.FJ., Baker M.AB.iposomes are widely used as synthetic analogues of cell membranes and for drug delivery. Lipid-binding DNA nanostructures can modify the shape, porosity and reactivity of liposomes, mediated by cholesterol modifications. DNA nanostructures can also be designed to switch conformations by DNA strand displacement. However, the optimal conditions to facilitate stable, high-yield DNAlipid binding while allowing controlled switching by strand displacement are not known. Here, we characterized the effect of cholesterol arrangement, DNA structure, buffer and lipid composition on DNAlipid binding and strand displacement. We observed that binding was inhibited below pH 4, and above 200 mM NaCl or 40 mM MgCl2, was independent of lipid type, and increased with membrane cholesterol content. For simple motifs, binding yield was slightly higher for double-stranded DNA than single-stranded DNA. For larger DNA origami tiles, four to eight cholesterol modifications were optimal, while edge positions and longer spacers increased yield of lipid binding. Strand displacement achieved controlled removal of DNA tiles from membranes, but was inhibited by overhang domains, which are used to prevent cholesterol aggregation. These findings provide design guidelines for integrating strand displacement switching with lipid-binding DNA nanostructures. This paves the way for achieving dynamic control of membrane morphology, enabling broader applications in nanomedicine and biophysics.Publication link2020 The mechanisms of action of water-soluble aminohexanoic and malonic adducts of fullerene C60 with hexamethonium on model lipid membranesVesicle Prep Pro Publication found in Biochimica et Biophysica Acta (BBA) - Biomembranes (2020) Authors: Efimova S.S., Khaleneva D.A., Litasova E.V., Piotrovskiy L.B., Ostroumova O.S.In an attempt to understand the possibility of applications of the fullerene-based systems for transporting various polar compounds like hexamethonium through the bloodbrain barrier, we studied the influence of a series of derivatives of fullerene C60 in the form of salts with hexamethonium bis-anion, namely the adducts of fullerenols with 6-aminohexanoic acid (IEM-2197), and two bis-adduct malonic acid derivatives of fullerene with aminobenzoic acid found in two hemispheres (IEM-2143) and in equatorial positions (IEM-2144), on model membranes. We showed that IEM-2197 induced the disintegration of the bilayers composed of DOPC at the concentrations more than 2 mg/ml. IEM-2144 and IEM-2143-induced ion-permeable pores at concentrations of 0.3 and 0.02 mg/ml, respectively; herewith, IEM-2143 was characterized by the greater efficiency than IEM-2144. IEM-2197 did not significantly affect the phase behavior of DPPC, while the melting temperature significantly decreased with addition of IEM-2144 and IEM-2143. The increase in the half-width of the main transition peaks by more than 2.0 C in the presence of IEM-2144 and IEM-2143 was observed, along with the pronounced peak deconvolution. We proposed that the immersion of IEM-2144 and IEM-2143 into the polar region of the DOPC or DPPC bilayers led to an increase in the relative mobility of tails and formation of ion-permeable defects. IEM-2197 demonstrated the more pronounced effects on the melting and ion permeability of PG- and PS-containing bilayers compared to PC-enriched membranes. These results indicated that IEM-2197 preferentially interacts with the negatively charged lipids compared to neutral species.Publication link2021 A microfluidic platform for sequential assembly and separation of synthetic cell modelsVesicle Prep Pro Pre-Print Publication in ACS Synthetic Biology (2021) Authors: Tivony R., Fletcher M., Al Nahas K., Keyser U. F.Cell-sized vesicles like giant unilamellar vesicles (GUVs) are established as a promising biomimetic model for studying cellular phenomena in isolation. However, the presence of residual components and by-products, generated during vesicles preparation and manipulation, severely limits the utility of GUVs in applications like synthetic cells. Therefore, with the rapidly growing field of synthetic biology, there is an emergent demand for techniques that can continuously purify cell-like vesicles from diverse residues, while GUVs are being simultaneously synthesized and manipulated. We developed a microfluidic platform capable of purifying GUVs through stream bifurcation, where a stream of vesicles suspension is partitioned into three fractions - purified GUVs, residual components, and a washing solution. Using our purification approach, we showed that giant vesicles can be separated from various residues that range in size and chemical composition with a very high efficiency (e = 0.99), based on size and deformability of the filtered objects. In addition, by incorporating the purification module with a microfluidic-based GUV-formation method, octanol-assisted liposome assembly (OLA), we established an integrated production-purification microfluidic unit that sequentially produces, manipulates, and purifies GUVs. We demonstrate the applicability of the integrated device to synthetic biology through sequentially fusing SUVs with freshly prepared GUVs and separating the fused GUVs from extraneous SUVs and oil droplets at the same time.Publication link2020 Soluble cyanobacterial carotenoprotein as a robust antioxidant nanocarrier and delivery moduleVesicle Prep Pro Publication in Antioxidants (Basel) (2020) Authors: Maksimov E.G., Zamaraev A.V., Parshina E.Y., Slonimskiy Y.B., Slastnikova T.A., Abdrakhmanov A.A., Babaev P.A., Efimova, S.S., Ostroumova O.S., Stepanov A.V., Ryabova, A.V., Friedrich T., Sluchanko N.N.To counteract oxidative stress, antioxidants including carotenoids are highly promising, yet their exploitation is drastically limited by the poor bioavailability and fast photodestruction, whereas current delivery systems are far from being efficient. Here we demonstrate that the recently discovered nanometer-sized water-soluble carotenoprotein from Anabaena (termed CTDH) transiently interacts with liposomes to efficiently extract carotenoids via carotenoid-mediated homodimerization, yielding violet-purple protein samples amenable to lyophilization and long-term storage. We characterize spectroscopic properties of the pigment-protein complexes and thermodynamics of liposome-protein carotenoid transfer and demonstrate the highly efficient delivery of echinenone form CTDH into liposomes. Most importantly, we show carotenoid delivery to membranes of mammalian cells, which provides protection from reactive oxygen species. The described carotenoprotein may be considered as part of modular systems for the targeted antioxidant delivery. Significance statement:Carotenoids are excellent natural antioxidants but their delivery to vulnerable cells is challenging due to their hydrophobic nature and susceptibility to degradation. Thus, systems securing antioxidant stability and facilitating targeted delivery are of great interest for the design of medical agents. In this work, we have demonstrated that soluble cyanobacterial carotenoprotein can deliver echinenone into membranes of liposomes and mammalian cells with almost 70 % efficiency, which alleviates the induced oxidative stress. Our findings warrant the robustness of the protein-based carotenoid delivery for studies of carotenoid activities and effects on cell models.Publication link2020 The Disordering Effect of Plant Metabolites on Model Lipid Membranes of Various ThicknessVesicle Prep ProPublication in Cell and Tissue Biology (2020) Authors: Efimova S.S., Ostroumova O.S.This study was focused on the effect of plant metabolites (phloretin, capsaicin, digitonin, diosgeninThis study was focused on the effect of plant metabolites (phloretin, capsaicin, digitonin, diosgeninand betulin) on the model lipid membranes. The methods of assessing the permeability of lipid bilayers basedon measuring the leakage of a fluorescent marker (calcein) from liposomes and differential scanning microcalorimetryof vesicle suspension were used. It was found that the release rate of calcein from 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (POPC) liposomes with test compounds added to the suspension at aratio with lipid 1 : 50 decreased in the order capsaicin > phloretin >> betulin diosgenin digitonin. In thecase of cholesterol- and ergosterol-containing POPC liposomes, the activity decreased as diosgenin digitonin> betulin > capsaicin > phloretin. It was demonstrated that phloretin and capsaicin significantly reducethe melting temperature (Tm) and to increase the half-width of the main peak on the endotherm (T1/2) ofdipalmitoylphosphocholine (DPPC), distearoylphosphocholine (DSPC) and diarachidoylphosphocholine(DAPC). These results show that the incorporation of these small molecules between the polar heads ofphosphocholine. It was found that the increase in the length of saturated chains of membrane-forming lipids(from 16 to 20 hydrocarbon units), the absolute values of Tm and T1/2 decreased in the presence of phloretinand increased with capsaicin. This may be the result of differences in the localization of phloretin and capsaicinin the membrane. Steroid saponins exhibited a weak effect on the thermotropic behavior of phosphocholines.The absolute values of Tm and T1/2 decreased in the order DPPC, DSPC, and DAPC and increasedin the order betulin, diosgenin, and digitonin. Steroid saponins are characterized by a more pronouncedeffect on the thermotropic behavior of the sterolphospholipid mixture. The findings are consistent with theassumption of a high affinity of the tested saponins for sterol-containing membranes.Publication link2020 Possible Mechanisms of Toxicity of Local Aminoamide Anesthetics: Lipid-Mediated Action of RopivacaineVesicle Prep Pro Publication in Cell and Tissue Biology (2020) Authors: Zakharova A. A., Efimova S.S., Koryachkin V.A., Zabolotskii D.V., Ostroumova O.S.This work is devoted to the identification of molecular mechanisms of action of local anesthetic ropivacaine and other aminoamides (mepivacaine and bupivacaine) on the membrane physicochemical properties and formation and functioning of various ion channels in model lipid bilayers. The boundary membrane potential and its components, permeability for fluorescent markers, and the temperature and cooperativity of the melting of membrane lipid, as well as the mosaic organization of the bilayer, were studied. It was found that ropivacaine, as well as mepivacaine and bupivacaine, changed the surface charge of the bilayer and increased the membrane boundary potential. It was demonstrated that the permeability of lipid vesicles for calcein increased with the introduction of aminoamides, while the temperature and cooperativity of the melting of saturated phosphocholines decreased. The effect of anesthetics on the packing density of lipids in the membrane correlated with the hydrophobicity of their molecules. A comparison of the effects of aminoamides allowed three mechanisms of anesthetics action on the functioning of ion channels to be determined: increasing the surface potential of the membrane, decreasing the packing density of lipids in the membrane, and blocking ion channels.Publication link2020 Romo1-Derived Antimicrobial Peptide Is a New Antimicrobial Agent against Multidrug-Resistant Bacteria in a Murine Model of SepsisVesicle Prep ProPublication in mBio (2020) Authors: Lee H.-R., You D.-G., Kim H.K., Sohn J.W.,Kim M.J., Park J.K., Lee G.Y., Yoo Y.D.To overcome increasing bacterial resistance to conventional antibiotics, many antimicrobial peptides (AMPs) derived from host defense proteins have been developed. However, there are considerable obstacles to their application to systemic infections because of their low bioavailability. In the present study, we developed an AMP derived from Romo1 (AMPR-11) that exhibits a broad spectrum of antimicrobial activity. AMPR-11 showed remarkable efficacy against sepsis-causing bacteria, including multidrug-resistant strains, with low toxicity in a murine model of sepsis after intravenous administration. It seems that AMPR-11 disrupts bacterial membranes by interacting with cardiolipin and lipid A. From the results of this study, we suggest that AMPR-11 is a new class of agent for overcoming low efficacy in the intravenous application of AMPs and is a promising candidate to overcome multidrug resistance.Publication link2020 Membrane characteristics tune activities of endosomal and autophagic human VPS34 complexesVesicle Prep ProPublication in eLife (2020) Authors: Ohashi Y., Tremel S., Masson G.R., McGinney L., Boulanger J., Rostislavleva K., Johnson C.M., Niewczas I., Clark J., Williams R.J.The lipid kinase VPS34 orchestrates diverse processes, including autophagy, endocytic sorting, phagocytosis, anabolic responses and cell division. VPS34 forms various complexes that help adapt it to specific pathways, with complexes I and II being the most prominent ones. We found that physicochemical properties of membranes strongly modulate VPS34 activity. Greater unsaturation of both substrate and non-substrate lipids, negative charge and curvature activate VPS34 complexes, adapting them to their cellular compartments. Hydrogen/deuterium exchange mass spectrometry (HDX-MS) of complexes I and II on membranes elucidated structural determinants that enable them to bind membranes. Among these are the Barkor/ATG14L autophagosome targeting sequence (BATS), which makes autophagy-specific complex I more active than the endocytic complex II, and the Beclin1 BARA domain. Interestingly, even though Beclin1 BARA is common to both complexes, its membrane-interacting loops are critical for complex II, but have only a minor role for complex I.Publication link2020 PolymerLipid Hybrid Vesicles and Their Interaction with HepG2 CellsVesicle Prep ProPublication in Small (2020) Authors: Brodszkij E., Westensee I.N., Bertelsen M., Gal N., Boesen T.,Stdler B.Polymerlipid hybrid vesicles are an emerging type of nanoassemblies that show potential as artificial organelles among others. Phospholipids and poly(cholesteryl methacrylate)blockpoly(methionine methacryloyloxyethyl ester (METMA)random 2carboxyethyl acrylate (CEA)) labeled with a Frster resonance energy transfer (FRET) reporter pair are used for the assembly of small and giant hybrid vesicles with homogenous distribution of both building blocks in the membrane as confirmed by the FRET effect. These hybrid vesicles have no inherent cytotoxicity when incubated with HepG2 cells up to 1.1 1011 hybrid vesicles per mL, and they are internalized by the cells. In contrast to the fluorescent signal originating from the block copolymer, the fluorescent signal coming from the lipids is barely detectable in cells incubated with hybrid vesicles for 6 h followed by 24 h in cell media, suggesting that the two building blocks have a different intracellular fate. These findings provide important insight into the design criteria of artificial organelles with potential structural integrity.Publication link2020 Hybrid giant lipid vesicles incorporating a PMMA-based copolymerVesicle Prep Pro Publication found in Biochimica et Biophysica Acta (BBA) - General Subjects (2020) Authors: Miele Y., Mingotaud A.F., Caruso E., Malacarne M. C., Izzo L., Lonetti B., Rossi F.Background - In recent years, there has been a growing interest in the formation of copolymer-lipid hybrid self-assemblies, which allow combining and improving the main features of pure lipid-based and copolymer-based systems known for their potential applications in the biomedical field. As the most common method used to obtain giant vesicles is electroformation, most systems so far used low Tg polymers for their flexibility at room temperature.Publication link2020 Identification of a Membrane Binding Peptide in the Envelope Protein of MHV CoroNaVirusVesicle Prep ProPublication in Viruses (2020) Authors: Alsaadi E.A.J., Neuman B.W., Jones I.M.CoroNaViruses (CoVs) are enveloped, positive sense, single strand RNA viruses that cause respiratory, intestinal and neurological diseases in mammals and birds. Following replication, CoVs assemble on intracellular membranes including the endoplasmic reticulum Golgi intermediate compartment (ERGIC) where the envelope protein (E) functions in virus assembly and release. In consequence, E potentially contains membrane-modifying peptides. To search for such peptides, the E coding sequence of Mouse Hepatitis Virus (MHV) was inspected for its amino acid conservation, proximity to the membrane and/or predicted amphipathic helices. Peptides identified in silico were synthesized and tested for membrane-modifying activity in the presence of giant unilamellar vesicles (GUVs) consisting of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), sphingomyelin and cholesterol. To confirm the presence of membrane binding peptides identified in the context of a full-length E protein, the wild type and a number of mutants in the putative membrane binding peptide were expressed in Lenti-X-293T mammalian and insect cells, and the distribution of E antigen within the expressing cell was assessed. Our data identify a role for the post-transmembrane region of MHV E in membrane binding.Publication link2020 Exploring the Relationship between BODIPY Structure and Spectroscopic Properties to Design Fluorophores for BioimagingVesicle Prep Pro Publication in Chemistry a European Journal (2020) Authors: Donnelly J.L., Offenbartl-Stiegert D., Marn-Beloqui J.M., Rizzello L., Battaglia G., Clarke T.M., Howorka S., Wilden J.D.Designing chromophores for biological applications requires a fundamental understanding of how the chemical structure of a chromophore influences its photophysical properties. We here describe the synthesis of a library of BODIPY dyes, exploring diversity at various positions around the BODIPY core. The results show that the nature and position of substituents have a dramatic effect on the spectroscopic properties. Substituting in a heavy atom or adjusting the size and orientation of a conjugated system provides a means of altering the spectroscopic profiles with high precision. The insight from the structureactivity relationship was applied to devise a new BODIPY dye with rationally designed photochemical properties including absorption towards the nearinfrared region. The dye also exhibited switchon fluorescence to enable visualisation of cells with high signaltonoise ratio without washingout of unbound dye. The BODIPYbased probe is noncytotoxic and compatible with staining procedures including cell fixation and immunofluorescence microscopy.Publication link2020 Facile Mixing of Phospholipids Promotes Self-Assembly of Low-Molecular-Weight Biodegradable Block Co-Polymers into Functional Vesicular ArchitecturesVesicle Prep ProPublication in Polymers(2020) Authors: Khan A., Ho J.C.S., Roy S., Bo Liedberg B., Nallani M.In this work, we have used low-molecular-weight (PEG12-b-PCL6, PEG12-b-PCL9 or PEG16-b-PLA38; MW, 1.25-3.45 kDa) biodegradable block co-polymers to construct nano- and micron-scaled hybrid (polymer/lipid) vesicles, by solvent dispersion and electroformation methods, respectively. The hybrid vesicles exhibit physical properties (size, bilayer thickness and small molecule encapsulation) of a vesicular boundary, confirmed by cryogenic transmission electron microscopy, calcein leakage assay and dynamic light scattering. Importantly, we find that these low MW polymers, on their own, do not self-assemble into polymersomes at nano and micron scales. Using giant unilamellar vesicles (GUVs) model, their surface topographies are homogeneous, independent of cholesterol, suggesting more energetically favorable mixing of lipid and polymer. Despite this mixed topography with a bilayer thickness similar to that of a lipid bilayer, variation in surface topology is demonstrated using the interfacial sensitive phospholipase A2 (sPLA2). The biodegradable hybrid vesicles are less sensitive to the phospholipase digestion, reminiscent of PEGylated vesicles, and the degree of sensitivity is polymer-dependent, implying that the nano-scale surface topology can further be tuned by its chemical composition. Our results reveal and emphasize the role of phospholipids in promoting low MW polymers for spontaneous vesicular self-assembly, generating a functional hybrid lipid-polymer interface.Publication link2020 Destructing the Plasma Membrane with Activatable Vesicular DNA NanoporesVesicle Prep Pro Publication in ACS Appl. Mater. Interfaces (2020) Authors: Chen L, Liang S., Chen Y., Wu M., Zhang Y.Pore-forming proteins are an agent for attack or defense in various organisms, and its cytolytic activity has medical potential in cancer therapy. Despite recent advances in mimicking these proteins by amphipathic DNA nanopores, it remains inefficient to incorporate them into lipid bilayers. Here, we present the development of vesicular DNA nanopores that can controllably open a lipid membrane. Different from previously reported DNA nanopores that randomly insert into the planar bilayers, we design on-command fusogenic liposome-incorporated transmembrane DNA nanopores (FLIPs) that bypass the direct insertion process. By steric deshielding of fusogenic liposomal supports under low pH conditions, the embedded FLIPs are transferred and perforate lipid bilayers. We find that FLIPs depolarize the plasma membrane and thereby induce pyroptosis-like cell death. We further demonstrate the use of FLIPs to inhibit tumor growth in murine tumor models, which provides a new route to cancer nanotherapy.Publication link2020 Effect of DMSO on the Mechanical and Structural Properties of Model and Biological MembranesVesicle Prep ProPublication in theBiophysical Journal (2020) Authors: Gironi B., Kahveci Z., McGill B., Lechner B.D., Pagliara S., Metz J., Morresi A., Palombo F., Sassi P., Petrov P.G.Dimethyl sulfoxide (DMSO) is widely used in a number of biological and biotechnological applications, mainly because of its effects on the cell plasma membrane, but the molecular origins of this action are yet to be fully clarified. In this work, we used two- and three-component synthetic membranes (liposomes) and the plasma membrane of human erythrocytes to investigate the effect of DMSO when added to the membrane-solvating environment. Fourier transform infrared spectroscopy and thermal fluctuation spectroscopy revealed significant differences in the response of the two types of liposome systems to DMSO in terms of the bilayer thermotropic behavior, available free volume of the bilayer, its excess surface area, and bending elasticity. DMSO also alters the mechanical properties of the erythrocyte membrane in a concentration-dependent manner and is capable of increasing membrane permeability to ATP at even relatively low concentrations (3% v/v and above). Taken in its entirety, these results show that DMSO is likely to have a differential effect on heterogeneous biological membranes, depending on their local composition and structure, and could affect membrane-hosted biological functions.Publication link2020 Design and Assembly of Membrane-Spanning DNA NanoporesVesicle Prep Pro Publication in Nanopore Technology (2020) Authors: Gpfrich K., Ohmann A., Keyser U.F.Versatile lipid membrane-inserting nanopores have been made by functionalizing DNA nanostructures with hydrophobic tags. Here, we outline design and considerations to obtain DNA nanopores with the desired dimensions and conductance properties. We further provide guidance on their reconstitution into lipid membranes.Publication link2020 Design of Protein Logic Gate System Operating on Lipid MembranesVesicle Prep Pro Publication in ACS Synth. Biol. (2020) Authors: Omersa N., Aden S., Kisovec M., Podobnik M., Anderluh G.Lipid membranes are becoming increasingly popular in synthetic biology due to their biophysical properties and crucial role in communication between different compartments. Several alluring proteinmembrane sensors have already been developed, whereas protein logic gates designs on membrane-embedded proteins are very limited. Here we demonstrate the construction of a two-level proteinmembrane logic gate with an OR-AND logic. The system consists of an engineered pH-dependent pore-forming protein listeriolysin O and its DARPin-based inhibitor, conjugated to a lipid vesicle membrane. The gate responds to low pH and removal of the inhibitor from the membrane either by switching to a reducing environment, protease cleavage, or any other signal depending on the conjugation chemistry used for inhibitor attachment to the membrane. This unique protein logic gate vesicle system advances generic sensing and actuator platforms used in synthetic biology and could be utilized in drug delivery.Publication link2020 Cholesterol and desmosterol incorporation into ram sperm membrane before cryopreservation: Effects on membrane biophysical properties and sperm qualityVesicle Prep ProPublication in Biochimica et Biophysica Acta (BBA) - Biomembranes (2020) Authors: de las Mercedes Carro M., Pealva D.A., Antollini S.S., Hozbora F.A., Buschiazzo J.Ram sperm are particularly sensitive to freeze-thawing mainly due to their lipid composition, limiting their use in artificial insemination programs. We evaluated the extent of cholesterol and desmosterol incorporation into ram sperm through incubation with increasing concentrations of methyl--cyclodextrin (MCD)-sterol complexes, and its effect on membrane biophysical properties, membrane lateral organization and cryopreservation outcome. Sterols were effectively incorporated into the sperm membrane at 10 and 25 mM MCD-sterols, similarly increasing membrane lipid order at physiological temperature and during temperature decrease. Differential ordering effect of sterols in ternary-mixture model membranes revealed a reduced tendency of desmosterol of segregating into ordered domains. Live cell imaging of fluorescent cholesterol showed sterol incorporation and evidenced the presence of sperm sub-populations compatible with different sterol contents and a high concentration of sterol rich-ordered domains mainly at the acrosome plasma membrane. Lateral organization of the plasma membrane, assessed by identification of GM1-related rafts, was preserved after sterol incorporation except when high levels of sterols (25 mM MCD-desmosterol) were incorporated. Ram sperm incubation with 10 mM MCD-sterols prior to cryopreservation in a cholesterol-free extender improved sperm quality parameters after cooling and freezing. While treatment with 10 mM MCD-cholesterol increased sperm motility, membrane integrity and tolerance to osmotic stress after thawing, incorporation of desmosterol increased the ability of ram sperm to overcome osmotic stress. Our research provides evidence on the effective incorporation and biophysical behavior of cholesterol and desmosterol in ram sperm membranes and on their consequences in improving functional parameters of sperm after temperature decrease and freezing.Publication link2020 Coating and Stabilization of Liposomes by Clathrin-Inspired DNA Self-AssemblyVesicle Prep Pro Publication found in ACS Nano (2020) Authors: Baumann, K.N., Piantanida L., Garca-Nafra J., Sobota D., Votchovsky K.,Knowles T.P.J., Hernandez-Ainsa S.The self-assembly of the protein clathrin on biological membranes facilitates essential processes of endocytosis and has provided a source of inspiration for materials design by the highly ordered structural appearance. By mimicking the architecture of the protein building blocks and clathrin self-assemblies to coat liposomes with biomaterials,advanced hybrid carriers can be derived. Here, we present a method for fabricating DNA-coated liposomes by hydrophobically anchoring and subsequently connecting DNA-based triskelion structures on the liposome surface inspired by the assembly of the protein clathrin. Dynamic light scattering, -potential, confocal microscopy, and cryo-electron microscopy measurements independently demonstrate successful DNA coating. Nanomechanical measurements conducted with atomic force microscopy show that the DNA coating enhances the mechanical stability of the liposomes relative to uncoated ones. Furthermore, we provide the possibility to reverse the coating process by triggering the disassembly of the DNA coats through a toehold-mediated displacement reaction. Our results describe a straightforward, versatile, and reversible approach for coating and stabilizing lipid vesicles through the assembly of rationally designed DNA structures. This method has potential for further development toward the ordered arrangement of tailored functionalities on the surface of liposomes and for applications as hybrid nanocarriers.Publication link2020 Atg3 promotes Atg8 lipidation via altering lipid diffusion and rearrangementVesicle Prep ProPublication in Protein Science (2020) Authors: Wang S., Li Y., Ma C.Atg3catalyzed transferring of Atg8 to phosphatidylethanolamine (PE) in the phagophore membrane is essential for autophagy. Previous studies have demonstrated that this process requires Atg3 to interact with the phagophore membrane via its Nterminal amphipathic helix. In this study, by using combined biochemical and biophysical approaches, our data showed that in addition to binding to the membranes, Atg3 attenuates lipid diffusion and enriches lipid molecules with smaller headgroup. Our data suggest that Atg3 promotes Atg8 lipidation via altering lipid diffusion and rearrangement.Publication link2020 Characterization of lipid composition and diffusivity in OLA generated vesiclesVesicle Prep Pro Publication in Biochimica et Biophysica Acta (BBA) - Biomembranes (2020) Authors: Schaich M., Sobota D., Sleath H., Cama J., Keyser U.F.Giant Unilamellar Vesicles (GUVs) are a versatile tool in many branches of science, including biophysics and synthetic biology. Octanol-Assisted Liposome Assembly (OLA), a recently developed microfluidic technique enables the production and testing of GUVs within a single device under highly controlled experimental conditions. It is therefore gaining significant interest as a platform for use in drug discovery, the production of artificial cells and more generally for controlled studies of the properties of lipid membranes. In this work, we expand the capabilities of the OLA technique by forming GUVs of tunable binary lipid mixtures of DOPC, DOPG and DOPE. Using fluorescence recovery after photobleaching we investigated the lateral diffusion coefficients of lipids in OLA liposomes and found the expected values in the range of 1 m2/s for the lipid systems tested. We studied the OLA derived GUVs under a range of conditions and compared the results with electroformed vesicles. Overall, we found the lateral diffusion coefficients of lipids in vesicles obtained with OLA to be quantitatively similar to those in vesicles obtained via traditional electroformation. Our results provide a quantitative biophysical validation of the quality of OLA derived GUVs, which will facilitate the wider use of this versatile platform.Publication link2019 The Mechanisms of Action of Triindolylmethane Derivatives on Lipid MembranesVesicle Prep Pro Publication in Acta Naturae (2019) Authors: Efimova, S.S. Tertychnaya T.E., Lavrenov S.N., Ostroumova, O.S.The effects of new synthetic antibacterial agents tris(1-pentyl-1H-indol-3-yl)methylium chloride (LCTA-1975) and (1-(4-(dimethylamino)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1H-indol-3-yl)bis(1-propyl- 1H-indol-3-yl)methylium chloride (LCTA-2701 on model lipid membranes were studied. The ability of the tested agents to form ion-conductive transmembrane pores, influence the electrical stability of lipid bilayers and the phase transition of membrane lipids, and cause the deformation and fusion of lipid vesicles was investigated. It was established that both compounds exert a strong detergent effect on model membranes. The results of differential scanning microcalorimetry and measuring of the threshold transmembrane voltage that caused membrane breakdown before and after adsorption of LCTA-1975 and LCTA-2701 indicated that both agents cause disordering of membrane lipids. Synergism of the uncoupling action of antibiotics and the alkaloid capsaicin on model lipid membranes was shown. The threshold concentration of the antibiotic that caused an increase in the ion permeability of the lipid bilayer depended on the membrane lipid composition. It was lower by an order of magnitude in the case of negatively charged lipid bilayers than for the uncharged membranes. This can be explained by the positive charge of the tested agents. At the same time, LCTA-2701 was characterized by greater efficiency than LCTA-1975. In addition to its detergent action, LCTA-2701 can induce ion-permeable transmembrane pores: step-like current fluctuations corresponding to the opening and closing of individual ion channels were observed. The difference in the mechanisms of action might be related to the structural features of the antibiotic molecules: in the LCTA-1975 molecule, all three substituents at the nitrogen atoms of the indole rings are identical and represent n-alkyl (pentyl) groups, while LCTA-2701 contains a maleimide group, along with two alkyl substituents (n-propyl). The obtained results might be relevant to our understanding of the mechanism of action of new antibacterial agents, explaining the difference in the selectivity of action of the tested agents on the target microorganisms and their toxicity to human cells. Model lipid membranes should be used in further studies of the trends in the antibacterial agents.Publication link2020 Actuation of synthetic cells with proton gradients generated by light-harvesting E. coliVesicle Prep Pro prePublication in Research Square (2020) Authors: Jahnke K., Ritzmann N., F

resorting to high speed optical modulators. Second, we devised a flexible data sampling method directly leading to higher image contrast by over 2-fold and digital images with 100 megapixels (10 24010 240) per frame at 0.25Hz. This generates sub-diffraction limited pixels (60nm per pixels over the FOV of 512m) which increases the degrees of freedom to extract signals computationally. The unique combined optical and digital control recorded fine fluorescence recovery after localized photobleaching (r ~10 m) within fluorescent giant unilamellar vesicles and micro-vascular dynamics after laser-induced injury during thrombus formation in vivo. These new improvements expand the quantitative biological-imaging capacity of any polygon scanning microscope system.Publication link2018 Hybrid vesicles from lipids and block copolymers: Phase behavior from the micro- to the nano-scaleVesicle Prep Pro Publication in Colloids and Surfaces B: Biointerfaces (2018) Authors: Magnani C., Montis C., Mangiapia G., Mingotaud A.-F., Mingotaud C., Roux C., Joseph P., Berti D., Lonetti B.In recent years, there has been a growing interest in the formation of copolymers-lipids hybrid self-assemblies, which allow combining and improving the main features of pure lipids-based and copolymer-based systems known for their potential applications in the biomedical field. In this contribution we investigate the self-assembly behavior of dipalmitoylphosphatidylcholine (DPPC) mixed with poly(butadiene-b-ethyleneoxide) (PBD-PEO), both at the micro- and at the nano-length scale. Epifluorescence microscopy and Laser Scanning Confocal microscopy are employed to characterize the morphology of micron-sized hybrid vesicles. The presence of fluid-like inhomogeneities in their membrane has been evidenced in all the investigated range of compositions. Furthermore, a microfluidic set-up characterizes the mechanical properties of the prepared assemblies by measuring their deformation upon flow: hybrids with low lipid content behave like pure polymer vesicles, whereas objects mainly composed of lipids show more variability from one vesicle to the other. Finally, the structure of the nanosized assemblies is characterized through a combination of Dynamic Light Scattering, Small Angle Neutron Scattering and Transmission Electron Microscopy. A vesicles-to-wormlike transition has been evidenced due to the intimate mixing of DPPC and PBD-PEO at the nanoscale. Combining experimental results at the micron and at the nanoscale improves the fundamental understanding on the phase behavior of copolymer-lipid hybrid assemblies, which is a necessary prerequisite to tailor efficient copolymer-lipid hybrid devices.Publication link2018 Development of an on-chip antibiotic permeability assay with single molecule detection capabilityVesicle Prep Pro Publication in IEEE Transactions on NanoBioscience (2018) Authors: Guzel F.D., Citak F.Electrophysiology is the method of choice to characterize membrane channels. In this study, we demonstrate a patch pipette based simple miniaturization that allows performing conductance measurements on a planar lipid bilayer in a microfluidic channel. Membrane proteins were reconstituted into Giant Unilamellar Vesicles (GUVs) by electroswelling, and GUVs with a single channel insertion were patched at the tip of pipette. We applied this approach to investigate the interactions of porins from E. coli with single antibiotics, and this will potentially provide information on the permeability rates. The results of this study suggest that this approach can be extended to the integration of several pipettes into the microfluidic channel from different positions, allowing the multiplexed recordings and also reducing the substrate consumption below L volumes.Publication link2018 Differential interactions of bacterial lipopolysaccharides with lipid membranes: implications for TRPA1-mediated chemosensationVesicle Prep Pro Publication in Scientific Reports (2018) Authors: Startek J.B., Talavera K., Voets T., Alpizar Y.A.Bacterial lipopolysaccharides (LPS) activate the TRPA1 cation channels in sensory neurons, leading to acute pain and inflammation in mice and to aversive behaviors in fruit flies. However, the precise mechanisms underlying this effect remain elusive. Here we assessed the hypothesis that TRPA1 is activated by mechanical perturbations induced upon LPS insertion in the plasma membrane. We asked whether the effects of different LPS on TRPA1 relate to their ability to induce mechanical alterations in artificial and cellular membranes. We found that LPS from E. coli, but not from S. minnesota, activates TRPA1. We then assessed the effects of these LPS on lipid membranes using dyes whose fluorescence properties change upon alteration of the local lipid environment. E. coli LPS was more effective than S. minnesota LPS in shifting Laurdans emission spectrum towards lower wavelengths, increasing the fluorescence anisotropy of diphenylhexatriene and reducing the fluorescence intensity of merocyanine 540. These data indicate that E. coli LPS induces stronger changes in the local lipid environment than S. minnesota LPS, paralleling its distinct ability to activate TRPA1. Our findings indicate that LPS activate TRPA1 by producing mechanical perturbations in the plasma membrane and suggest that TRPA1-mediated chemosensation may result from primary mechanosensory mechanisms.Publication link2018 Cargo induces retromer-mediated membrane remodeling on membranesVesicle Prep Pro Publication in ascb Molecular Biology of the Cell (2018) Authors: Purushothaman L.K., Ungermann C.Endosomes serve as a central sorting station of lipids and proteins that arrive via vesicular carrier from the plasma membrane and the Golgi complex. At the endosome, retromer complexes sort selected receptors and membrane proteins into tubules or vesicles that bud off the endosome. The mature endosome finally fuses with the lysosome. Retromer complexes consist of a cargo selection complex (CSC) and a membrane remodeling part (SNX-BAR or Snx3 in yeast), and different assemblies of retromer mediate recycling of different cargoes. Due to this complexity, the exact order of events that results in carrier formation is not yet understood. Here, we reconstituted this process on giant unilamellar vesicles together with purified retromer complexes from yeast and selected cargoes. Our data reveal that the membrane remodeling activity of both Snx3 and the SNX-BAR complex is strongly reduced at low concentrations, which can be reactivated by CSC. At even lower concentrations, these complexes still associate with membranes, but only remodel membranes in the presence of their specific cargoes. Our data thus favor a simple model, where cargo functions as a specific trigger of retromer-mediated sorting on endosomes.Publication link2018 Cholesterol Enriched Archaeosomes as a Molecular System for Studying Interactions of Cholesterol-Dependent Cytolysins with MembranesVesicle Prep Pro Publication in The Journal of Membrane Biology (2018) Authors: Rezelj S., Kozorog M, vigelj T., Ulrih N.P., nidari N., Podobnik M., Anderluh G.Archaeosomes are vesicles made of lipids from archaea. They possess many unique features in comparison to other lipid systems, with their high stability being the most prominent one, making them a promising system for biotechnological applications. Here, we report a preparation protocol of large unilamellar vesicles, giant unilamellar vesicles (GUVs), and nanodiscs from archaeal lipids with incorporated cholesterol. Incorporation of cholesterol led to additional increase in thermal stability of vesicles. Surface plasmon resonance, sedimentation assays, intrinsic tryptophan fluorescence measurements, calcein release experiments, and GUVs experiments showed that members of cholesterol-dependent cytolysins, listeriolysin O (LLO), and perfringolysin O (PFO), bind to cholesterol-rich archaeosomes and thereby retain their pore-forming activity. Interestingly, we observed specific binding of LLO, but not PFO, to archaeosomes even in the absence of cholesterol. This suggests a new capacity of LLO to bind to carbohydrate headgroups of archaeal lipids. Furthermore, we were able to express LLO inside GUVs by cell-free expression. GUVs made from archaeal lipids were highly stable, which could be beneficial for synthetic biology applications. In summary, our results describe novel model membrane systems for studying membrane interactions of proteins and their potential use in biotechnology.Publication link2018 A synthetic enzyme built from DNA flips 10e7 lipids per second in biological membranesVesicle Prep Pro Publication in the Nature Communications (2018) Authors: Ohmann A, Li C-Y, Maffeo C, Al Nahas K, Baumann K.N, Gpfrich K, Yoo J, Keyser U.F, & Aksimentiev A.Mimicking enzyme function and increasing performance of naturally evolved proteins is one of the most challenging and intriguing aims of nanoscience. Here, we employ DNA nanotechnology to design a synthetic enzyme that substantially outperforms its biological archetypes. Consisting of only eight strands, our DNA nanostructure spontaneously inserts into biological membranes by forming a toroidal pore that connects the membranes inner and outer leaflets. The membrane insertion catalyzes spontaneous transport of lipid molecules between the bilayer leaflets, rapidly equilibrating the lipid composition. Through a combination of microscopic simulations and fluorescence microscopy we find the lipid transport rate catalyzed by the DNA nanostructure exceeds 107molecules per second, which is three orders of magnitude higher than the rate of lipid transport catalyzed by biological enzymes. Furthermore, we show that our DNA-based enzyme can control the composition of human cell membranes, which opens new avenues for applications of membrane-interacting DNA systems in medicine.Publication link2018 Acyl chain asymmetry and polyunsaturation of brain phospholipids facilitate membrane vesiculation without leakageVesicle Prep Pro Publication in eLife (2018) Authors: Manni M.M., Tiberti M.L., Pagnotta S., Barelli H., Gautier R., Antonny B.Phospholipid membranes form cellular barriers but need to be flexible enough to divide by fission. Phospholipids generally contain a saturated fatty acid (FA) at position sn1 whereas the sn2-FA is saturated, monounsaturated or polyunsaturated. Our understanding of the impact of phospholipid unsaturation on membrane flexibility and fission is fragmentary. Here, we provide a comprehensive view of the effects of the FA profile of phospholipids on membrane vesiculation by dynamin and endophilin. Coupled to simulations, this analysis indicates that: (i) phospholipids with two polyunsaturated FAs make membranes prone to vesiculation but highly permeable; (ii) asymmetric sn1-saturated-sn2-polyunsaturated phospholipids provide a tradeoff between efficient membrane vesiculation and low membrane permeability; (iii) When incorporated into phospholipids, docosahexaenoic acid (DHA; omega-3) makes membranes more deformable than arachidonic acid (omega-6). These results suggest an explanation for the abundance of sn1-saturated-sn2-DHA phospholipids in synaptic membranes and for the importance of the omega-6/omega-3 ratio on neuronal functions.Publication link2017 Lipid phase separation in the presence of hydrocarbons in giant unilamellar vesiclesVesicle Prep Pro Publication in AIMS Biophysics (2017) Authors: Bartelds R., Barnoud J., Boersma A.J., Marrink S.J., Poolman B.Hydrophobic hydrocarbons are absorbed by cell membranes. The effects of hydrocarbons on biological membranes have been studied extensively, but less is known how these compounds affect lipid phase separation. Here, we show that pyrene and pyrene-like hydrocarbons can dissipate lipid domains in phase separating giant unilamellar vesicles at room temperature. In contrast, related aromatic compounds left the phase separation intact, even at high concentration. We hypothesize that this behavior is because pyrene and related compounds lack preference for either the liquid-ordered (Lo) or liquid-disordered (Ld) phase, while larger molecules prefer Lo, and smaller, less hydrophobic molecules prefer Ld. In addition, our data suggest that localization in the bilayer (depth) and the shape of the molecules might contribute to the effects of the aromatic compounds. Localization and shape of pyrene and related compounds are similar to cholesterol and therefore these molecules could behave as such.Publication link2018 A direct role for hepatitis B virus X protein in inducing mitochondrial membrane permeabilizationVesicle Prep Pro Publication in Journal of Viral Hepatitis (2018) Authors: Lee HR., Cho Y.Y., Lee G.Y., You Dg., Yoo Y.D., Kim Y.J.Hepatitis B virus X protein (HBx) acts as a multifunctional protein that regulates intracellular signalling pathways during HBV infection. It has mainly been studied in terms of its interaction with cellular proteins. Here, we show that HBx induces membrane permeabilization independently of the mitochondrial permeability transition pore complex. We generated mitochondrial outer membranemimic liposomes to observe the direct effects of HBx on membranes. We found that HBx induced membrane permeabilization, and the region comprising the transmembrane domain and the mitochondrialtargeting sequence was sufficient for this process. Membrane permeabilization was inhibited by nonselective channel blockers or by N(nnonyl)deoxynojirimycin (NNDNJ), a viroporin inhibitor. Moreover, NNDNJ inhibited HBxinduced mitochondrial depolarization in Huh7 cells. Based on the results of this study, we can postulate that the HBx protein itself is sufficient to induce mitochondrial membrane permeabilization. Our finding provides important information for a strategy of HBx targeting during HBV treatmentPublication link2017 Dipole Modifiers Regulate Lipid Lateral Heterogeneity in Model MembranesVesicle Prep Pro Publication in Acta Naturae (2017) Authors: Efimova S.S., Ostroumova O.S.In this study we report on experimental observations of giant unilamellar liposomes composed of ternary mixtures of cholesterol (Chol), phospholipids with relatively low Tmelt (DOPC, POPC, or DPoPC) and high Tmelt (sphingomyelin (SM), or tetramyristoyl cardiolipin (TMCL)) and their phase behaviors in the presence and absence of dipole modifiers. It was shown that the ratios of liposomes exhibiting noticeable phase separation decrease in the series POPC, DOPC, DPoPC regardless of any high-Tmelt lipid. Substitution of SM for TMCL led to increased lipid phase segregation. Taking into account the fact that the first and second cases corresponded to a reduction in the thickness of the lipid domains enriched in low- and high-Tmelt lipids, respectively, our findings indicate that the phase behavior depends on thickness mismatch between the ordered and disordered domains. The dipole modifiers, flavonoids and styrylpyridinium dyes, reduced the phase segregation of membranes composed of SM, Chol, and POPC (or DOPC). The other ternary lipid mixtures tested were not affected by the addition of dipole modifiers. It is suggested that dipole modifiers address the hydrophobic mismatch through fluidization of the ordered and disordered domains. The ability of a modifier to partition into the membrane and fluidize the domains was dictated by the hydrophobicity of modifier molecules, their geometric shape, and the packing density of domain-forming lipids. Phloretin, RH 421, and RH 237 proved the most potent among all the modifiers examined.Publication link2017 Ion- and water-binding sites inside an occluded hourglass pore of a trimeric intracellular cation (TRIC) channelVesicle Prep Pro Publication in BioMed Central Biology (2017) Authors: Ou X., Guo J., Wang L., Yang H., Liu X., Sun J., Liu Z.Background:Trimeric intracellular cation (TRIC) channels are crucial for Ca2+handling in eukaryotes and are involved in K+uptake in prokaryotes. Recent studies on the representative members of eukaryotic and prokaryotic TRIC channels demonstrated that they form homotrimeric units with the ion-conducting pores contained within each individual monomer.Results:Here we report detailed insights into the ion- and water-binding sites inside the pore of a TRIC channel fromSulfolobus solfataricus(SsTRIC). Like the mammalian TRIC channels,SsTRIC is permeable to both K+and Na+with a slight preference for K+, and is nearly impermeable to Ca2+, Mg2+, or Cl. In the 2.2- resolution K+-bound structure ofSsTRIC, ion/water densities have been well resolved inside the pore. At the central region, a filter-like structure is shaped by the kinks on the second and fifth transmembrane helices and two nearby phenylalanine residues. Below the filter, the cytoplasmic vestibule is occluded by a plug-like motif attached to an array of pore-lining charged residues.Conclusions:The asymmetric filter-like structure at the pore center ofSsTRIC might serve as the basis for the channel to bind and select monovalent cations. A Velcro-like plug-pore interacting model has been proposed and suggests a unified framework accounting for the gating mechanisms of prokaryotic and eukaryotic TRIC channels.Publication link2013 Lipid-Bilayer-Spanning DNA Nanopores with a Bifunctional Porphyrin AnchorVesicle Prep Pro Publication in Angewandte Chemie International Edition (2013) Authors: Burns J.R., Gpfrich K., Wood J.W.,Thacker V.V.,Stulz E., Keyser U.F., Howorka S.An artificial membrane nanopore assembled from DNA oligonucleotides carries porphyrin tags, which anchor the nanostructure into the lipid bilayer. The porphyrin moieties also act as fluorescent dyes to aid the microscopic visualization of the DNA nanopore.Publication link2014 Nanoscale phase behavior on flat and curved membranesVesicle Prep Pro Publication in Nanotechnology (2014) Authors: Andersen T., Bahadori A., Ott D., Kyrsting A., Reihani S.N.S., Bendix P.M.The diverse physical properties of membranes play a critical role in many membrane associated biological processes. Proteins responsible for membrane transport can be affected by the lateral membrane order and lateral segregation of proteins is often controlled by the preference of certain membrane anchors for membrane phases having a physically ordered state. The dynamic properties of coexisting membrane phases are often studied by investigating their thermal behavior. Optical trapping of gold nanoparticles is a useful tool to generate local phase transitions in membranes. The high local temperatures surrounding an irradiated gold nanoparticle can be used to melt a part of a giant unilamellar lipid vesicle (GUV) which is then imaged using phase sensitive fluorophores embedded within the bilayer. By local melting of GUVs we reveal how a protein-free, one component lipid bilayer can mediate passive transport of fluorescent molecules by localized and transient pore formation. Also, we show how tubular membrane curvatures can be generated by optical pulling from the melted region on the GUV. This will allow us to measure the effect of membrane curvature on the phase transition temperature.Publication link2012 Hydrophobic gating of mechanosensitive channel of large conductance evidenced by single-subunit resolutionVesicle Prep Pro Publication in Proceedings of the National Academy of Sciences of the United States of America (2012) Authors: Birkner JP, Poolman B, Koer A.Mechanosensitive (MS) ion channels are membrane proteins that detect and respond to membrane tension in all branches of life. In bacteria, MS channels prevent cells from lysing upon sudden hypoosmotic shock by opening and releasing solutes and water. Despite the importance of MS channels and ongoing efforts to explain their functioning, the molecular mechanism of MS channel gating remains elusive and controversial. Here we report a method that allows single-subunit resolution for manipulating and monitoring mechanosensitive channel of large conductance fromEscherichia coli. We gradually changed the hydrophobicity of the pore constriction in this homopentameric protein by modifying a critical pore residue one subunit at a time. Our experimental results suggest that both channel opening and closing are initiated by the transmembrane 1 helix of a single subunit and that the participation of each of the five identical subunits in the structural transitions between the closed and open states is asymmetrical. Such a minimal change in the pore environment seems ideal for a fast and energy-efficient response to changes in the membrane tension.Publication link2013 Effect of flavonoids on the phase separation in giant unilamellar vesicles formed from binary lipid mixturesVesicle Prep Pro Publication in Chemistry and Physics of Lipids (2013) Authors: Ostroumova O.S., Chulkov E.G., Stepanenko O.V., Schagina L.V.Confocal fluorescence microscopy have been employed to investigate phase separation in giant unilamellar vesicles prepared from binary mixtures of unsaturated dioleoylphosphocholine with saturated phosphocholines or brain sphingomyelin in the absence and presence of the flavonoids, biochanin A, phloretin, and myricetin. It has been demonstrated that biochanin A and phloretin make uncolored domains more circular or eliminate visible phase separation in liposomes while myricetin remains the irregular shape of fluorescence probe-excluding domains. Influence of the flavonoids on the endotherms of liposome suspension composed of dioleoylphosphocholine and dimyristoylphosphocholine was investigated by the differential scanning calorimetry. Calorimetry data do not contradict to confocal imaging results.Poster PDF2015 Organellar Transporters and Ion Channels How to access their electrophysiology by using the SURFE2R technology and Planar Patch ClampSURFE2R N1, SyncroPatch 96 (a predecessor model of the SyncroPatch 384) and Port-a-Patch poster, GRC - Organellar Channels and Transporters 2015Poster PDF2018 Expression and pharmacology of GluA2-containing AMPA receptors in cell lines and stem cell-derived neuronsPort-a-Patch, Patchliner and SyncroPatch 384PE (a predecessor model of the SyncroPatch 384) poster, Europhysiology Meeting 2018Poster PDF2015 The backstage pass to study your favorite TRP channelPort-a-Patch, Patchliner and SyncroPatch 384PE (a predecessor model of the SyncroPatch 384) poster, TRP Meeting 2015Poster PDF2021 Activation and inhibition of assay-ready TRPA1 and TRPV cells: an automated patch clamp studyPort-a-Patch, Patchliner and SyncroPatch 384i (a predecessor model of SyncroPatch 384), Biophysical Society Meeting 2021Webinar12.09.2018 | Webinar: CiPA study: Bridging ion channel and myocyte dataAuthors: Dr. Sonja Stlzle-FeixCiPA myocyte phase II validation study results: cross-site comparison using the CardioExcyte 96;HTS Phase I study: an update on progress of the CiPA Ion Channel Work Stream using the SyncroPatch 384PE and PatchlinerGet up-to-date with the CiPA progress of the Myocyte and Ion Channel Work Goups: Since 2005 the S7B and E14 guidances from ICH and FDA have been in place to assess a potential drug candidate's ability to cause long QT syndrome. To refine these guidelines, the FDA proposed the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, where the assessment of drug effects on cardiac repolarization was one subject of investigation. Within the myocyte validation study, effects of pharmaceutical compounds on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were assessed and this article will focus on the evaluation of the proarrhythmic potential of 23 blinded drugs in four hiPSC-CM cell lines.Experiments were performed on the CardioExcyte 96 at different sites. A combined readout of contractility (via impedance) and electrophysiology endpoints (field potentials) was performed.Our data demonstrates that hERG blockers such as dofetilide and further high risk categorized compounds prolong the field potential duration. Arrhythmia were detected in both impedance as well as field potential recordings. Intermediate risk compounds induced arrhythmia in almost all cases at the highest dose. In the case of low risk compounds, either a decrease in FPDmax was observed, or not a significant change from pre-addition control values.With exceptions, hiPSC-CMs are sensitive and exhibit at least 10% delayed or shortened repolarization from pre-addition values and arrhythmia after drug application and thus can provide predictive cardiac electrophysiology data. The baseline electrophysiological parameters vary between iPS cells from different sources, therefore positive and negative control recordings are recommended.Webinar12.09.2018 | Webinar: CiPA study: Bridging ion channel and myocyte dataAuthors: Tim StrassmaierGet up-to-date with the CiPA progress of the Myocyte and Ion Channel Work Goups: Since 2005 the S7B and E14 guidances from ICH and FDA have been in place to assess a potential drug candidate's ability to cause long QT syndrome. To refine these guidelines, the FDA proposed the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, where the assessment of drug effects on cardiac repolarization was one subject of investigation. Within the myocyte validation study, effects of pharmaceutical compounds on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were assessed and this article will focus on the evaluation of the proarrhythmic potential of 23 blinded drugs in four hiPSC-CM cell lines.Experiments were performed on the CardioExcyte 96 at different sites. A combined readout of contractility (via impedance) and electrophysiology endpoints (field potentials) was performed.Our data demonstrates that hERG blockers such as dofetilide and further high risk categorized compounds prolong the field potential duration. Arrhythmia were detected in both impedance as well as field potential recordings. Intermediate risk compounds induced arrhythmia in almost all cases at the highest dose. In the case of low risk compounds, either a decrease in FPDmax was observed, or not a significant change from pre-addition control values.With exceptions, hiPSC-CMs are sensitive and exhibit at least 10% delayed or shortened repolarization from pre-addition values and arrhythmia after drug application and thus can provide predictive cardiac electrophysiology data. The baseline electrophysiological parameters vary between iPS cells from different sources, therefore positive and negative control recordings are recommended.Poster PDF2015 Complementary automated patch clamp, extracellular field potential and impedance recordings of iPSCs: safety screening tool box for the futureWebinar2018 HTS Phase I study: an update on progress of the CiPA Ion Channel Work Stream using the SyncroPatch 384PE and PatchlinerPresenter: Tim Strassmaier, Nanion Technologies Inc. USA, Source: Webinar: "CiPA study: Bridging ion channel and myocyte data", September 12, 2018Title: CiPA myocyte phase II validation study results: cross-site comparison using the CardioExcyte 96The CiPA HTS Ion Channel Working Group finalized its phase I study in 2017. Amongst other external sites, Nanion Technologies in Germany, USA and Japan participated with the Patchliner and the SyncroPatch 384PE in this study. A comparative view of the ion channel targets and a cross-platform and cross-site comparison will be presented. Furthermore, results from the myocyte Work Stream using arrhythmogenic compounds will be compared and confirmed with patch clamp data derived from the HTS Work Stream.Please note: The original webinar presentation contained 8 slides with data of an upcoming publication. Due to confidentiality reasons, the relevant slides were cut out of the movie.Poster PDF2018 Combining electrophysiology and contractility recordings for more complete assessment of hiPSC-CMsSyncroPatch 384PE (a predecessor model of the SyncroPatch 384), Patchliner and CardioExcyte 96 poster, Europhysiology Meeting 2018Webinar28.07.2015 | Webinar: High Throughput and High Fidelity: Automated Patch Clamp in Screening and ResearchAuthors: Alfred L. George; Dr. Carlos Vanoye,User meeting video14.10.2020 | Webinar: Development and validation of ASIC1a ligand-gated ion channel drug discovery assays on automated patch clamp platforms.Authors: Marc Rogers, This is an on-demand webinar from Nan]i[on and Friends 2020.Whilst voltage-gated ion channels formed the bulk of academic and industrial effort in developing and utilising APC assays for ion channel drug discovery, recent years have seen increasing interest in ligand-gated receptors. These targets offer specific challenges for APC systems in terms of lower channel expression, rapid application and wash-off of ligands, and loss of responsiveness due to short- and long-term desensitisation. In this presentation I will outline successful development of pipette- and tip-based APC assay formats for the rapidly-activating ASIC1A channel on the Patchliner and SyncroPatch384i.User meeting video15.10.2020 | Webinar: Benchmarking best practices and calibration standards for HTS hERG recordings for improved proarrhythmic assessmentDr. Alison Obergrussberger (Nanion Technologies), This is an on-demand webinar from Nan]i[on and Friends 2020.The use of automated patch clamp (APC) electrophysiology in cardiac safety screening has increased over the years, and APC is now an established and accepted technique in most, if not all, safety testing laboratories. Since the introduction of the ICH S7B non-clinical guidance in November 2005 which requires all new drugs to be tested for activity on the IKr current carried by hERG expressed in recombinant cell lines using the patch-clamp technique, very few drugs have been withdrawn from the market due to pro-arrhythmic complications. APC has become the major workhorse in safety testing laboratories and is now considered to be the gold standard. Furthermore, with the introduction of the comprehensive in vitro pro-arrhythmia assay (CiPA) which recommends expanding electrophysiological recordings to include other cardiac ion channels, APC will continue to play a major role in cardiac safety testing. Recently, a large study comparing the results of a set of standard compounds tested on different instruments at different sites has been published[1] which highlights the need for standardized protocols for reliable results, for example, for hERG recordings.We have undertaken a study to identify key parameters that can affect IC50 values of compounds acting on hERG using the medium and high throughput APC systems, Patchliner, SyncroPatch 384PE and SyncroPatch 384i. Effects of experimental parameters such as voltage protocol, incubation time, labware, compound storage time and replicate number on IC50 values of a set of CiPA compounds will be presented and recommendations for best practices for hERG measurements using APC is provided. Furthermore, as outlined in the 2020 Best Practice Consideration for In vitro Studies, The concentration of compound to which the cells were exposed should be verified by applying a validated analytical method to the solution collected from the cell chamber[2] in patch clamp studies. Nanion has implemented a new procedure that enables sample collection from used wells from the NPC-384 chips and this will be described.Poster PDF2022 Characterization of ion channel currents endogenously expressed in Neuro2A cells using automated patch clampPoster PDF2022 The role of LRRC8 in the hypotonic stress response of human keratinocytesPoster PDF2020 Kinetic and pharmacological properties of P2X3 and P2X2/3 receptorsSyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) and Patchliner poster, 64th Annual Meeting of the Biophysical SocietyPoster PDF2021 Development and validation of ASIC1a ligand-gated ion channel drug discovery assays on automated patch clamp platformsPoster PDF2020 Reliable Identification of hERG Liability in Drug Discovery by Automated Patch ClampSyncroPatch 384i (a predecessor model of SyncroPatch 384) and Patchliner poster, 64th Annual Meeting of the Biophysical SocietyPoster PDF2018 Investigating pain pathways by inhibition of voltage-gated sodium channelsSyncroPatch 384PE (a predecessor model of the SyncroPatch 384) and Patchliner poster, FENS Meeting 2018Poster PDF2017 Cardiomyocytes in Voltage Clamp and Current Clamp by Automated Patch ClampSyncroPatch 384PE (a predecessor model of the SyncroPatch 384) and Patchliner poster, BPS Meeting 2017Poster PDF2021 Reliable identification of cardiac liability in drug discovery using automated patch clamp: Considerations and best practices for high throughput recordings of NaV 1.5Patchliner and SyncroPatch 384i (a predecessor model of SyncroPatch 384), Physiology 202Application Note PDFVRAC The role of LRRC8 in the hypotonic stress response of human keratinocytesSyncroPatch 384 and Patchliner application note: The human skin is constantly exposed to various stress factors such as temperature changes, mechanical stress, different humidity levels, air pollution or radiation. These factors can have a tremendous impact on the skin and can contribute to barrier disruption and inflammation, dry and fragile skin as well as premature ageing. Recent advances in different research areas point to an important role of LRRC8 volume regulated anion channels (VRACs) in a plethora of different physiological processes. The function of LRRC8 has been characterized in human keratinocytes and in the native human epidermis and the LRRC8 ion channel has been proposed to be a novel molecular target to modulate keratinocyte differentiation in a recent patent.LRRC8A (also named SWELL1) has been identified as the first essential component of VRACs in various cell types. LRRC8A is composed of four transmembrane domains and a C-terminal domain containing up to 17 leucine-rich repeats. Together with four additional LRRC8 family members (LRRC8B-E) it assembles into hetero-hexameric complexes. Interestingly, the LRRC8 subunit composition differs between cell types and influences VRAC properties such as inactivation kinetics, voltage-dependence and selectivity of the transported osmolyte. The generation of LRRC8A-/- knockout HaCaT keratinocytes have provided evidence for the essential function of LRRC8A in hypotonic stress response of human keratinocytes.In this Application Note we show electrophysiological data from WT and LRRC8A-/- knockout HaCaT keratinocytes which corroborate the essential function of LRRC8A in keratinocytes.Poster PDF2016 Next level toxicity screening: From single channel to overall cell behaviorOrbit mini,CardioExcyte 96 and SyncroPatch 384PE(a predecessor model of the SyncroPatch 384) poster,Meeting of the French Society of Toxinology (SFET) 2015Poster PDF2018 Optogenetic technologies enable high throughput ion channel drug discovery and toxicity screeningSyncroPatch 384PE (a predecessor model of the SyncroPatch 384) and CardioExcyte 96 poster, Biophysics Annual Meeting 2018Presentation PDF2018 Innovations for cell monitoring in safety and toxicity assaysPresenter: Dr. Elena Dragicevic, Senior Scientist / Sales Manager, Nanion Technologies; Source: SOT, Ncardia & Nanion hosted session, March 12. 2018Application Note PDFTRPC5 Internal perfusion of Ca2+ to activate hTRPC5 on Nanions SyncroPatch 384PESyncroPatch 384PE (a predecessor model of the SyncroPatch 384) application note Cells were kindly provided by Dr. Joel Ostrow at Case Western Reserve University.The TWIK-related K+channel (TREK-1) is a two pore domain (K2P) K+channel encoded by the KCNK2 gene. The protein is a homodimer, each subunit comprised of transmembrane (TM) helical regions (M1-M4), two pore domains (P1 & P2), an extracellular region with 2 helices, and intracellular N and C termini. The pore helices and pore loops form the K+selectivity filter. First discovered in 1996, it plays an important physiological role in background K+conductance and thus plays a major role in regulating resting membrane potential. TREK-1 widely is expressed throughout the CNS and spinal cord, particularly in the cortex, hippocampus, thalamus, hypothalamus, cerebellum and basal ganglia. Additionally, TREK-1 is expressed in high levels in small and medium sized dorsal root ganglion (DRG) neurons. It is also expressed in other regions such as lung, heart, kidney, skeletal muscle and human myometrium where it is up-regulated during pregnancy5 and may play a role in maintaining a negative membrane potential prior to labor. TREK-1 is modulated by a variety of different physical and chemical stimuli including mechanical (stretch), temperature, intracellular acidosis, poly-unsaturated fatty acids and phospoholipids. It has also been shown to be opened by volatile anaesthetics and is likely to be an important target for these agents. TREK-1 has been proposed to play a pivotal role in cerebral ischemia, epilepsy, depression, pain perception and temperature sensing and is an interesting therapeutic target. Human TREK-1 expressed in HEK cells were recorded on the SyncroPatch 384PE with good success rates. hTREK-1 was activated by BL-1249 and blocked by THA.Application Note PDFTRPA1 High Throughput Activation and Block of hTRPA1 on Nanions SyncroPatch 384PESyncroPatch 384PE (a predecessor model of the SyncroPatch 384) application note:Cells were kindly provided by Millipore.Transient receptor potential (TRP) channels have become important potential targets in drug discovery for the treatment of, for example, pain, respiratory diseases such as asthma, cancer and immune disorders, multiple kidney diseases and skeletal disorders1 . The transient receptor potential ankyrin 1 (TRPA1), a member of the TRP family of cation channels, plays a predominant role in the sensation of noxious cold2 and inflammatory pain3 . The channel is activated by a range of environmental irritants causing pain, pungent compounds found in foods such as garlic, mustard and cinnamon, as well as metabolites produced during oxidative stress4 . Consistent with its proposed function in nociception, TRPA1 has been shown to be expressed in sensory neurons of the dorsal root ganglion (DRG) and trigeminal ganglion, both of which transmit painful responses2. Thus, within drug development, much attention is paid to the TRPA1 channel. Preclinical data and data from a recent human genetic study5 highlight TRPA1 antagonists as a promising new approach for the treatment of acute and chronic pain. Indeed, a TRPA1 antagonist has shown positive results in a proof-ofconcept study for diabetic neuropathic pain6 . The most challenging aspects involved in the screening of the TRPA1 channel are the channels mechanosensitivity, fast desensitization and activity dependence on intracellular calcium. Here, we present high quality data with reliable pharmacology on hTRPA1 expressing HEK cells collected on the SyncroPatch 384PE. Data is presented showing activation of the TRPA1 channel by SCMA and inhibition by A-967079.Application Note PDFTASK-1 Activation and Inhibition of TASK-1 on Nanions SyncroPatch 384PESyncroPatch 384PE (a predecessor model of SyncroPatch 384) application note: Cells were kindly provided by SB Drug Discovery.The resting membrane potential of excitable cells isdetermined by leak conductances predominantly mediated by KCNK and two-pore-domain potassium channels (K2P). K2P channels are characterized by the presence of two pore forming regions and four trans-membrane spanning (4TMS) regions in each channel subunit and form functional dimers. These channels are essential for the production of background leak type potassium currents that act to regulate resting membrane potential and levels of cellular excitability. The TWIKrelated acid-sensitive K+ channel 1 (K2P3.1 or TASK-1) is a member of the K2P channel family and is encoded by the KCNK3 gene. TASK-1 is ubiquitously expressed throughout the CNS but also in other tissues such as in the heart, adrenal gland, lung, pancreas,kidney, intestine and prostate. TASK-1 has been implicated in atrial fibrillation (AF) pathophysiology and was suggested as an atrial-selective antiarrhythmic drug target. TASK-1 is activated by extracellular acidosis and inhibited by anandamide and by local anesthetics including bupivicaine. Volatile general anestethics such as halothan and xenon stimulate TASK-1.Application Note PDFTMEM16A (ANO1) Internal perfusion of Ca2+ to activate TMEM16A/ANO1 on the SyncroPatch 384PESyncroPatch 384PE (a predecessor model of SyncroPatch 384) application note Cells were kindly provided by SB Drug Discovery.TMEM16A/Anoctamin1 is a Ca2+-activated Clchannel (CACC) which has a broad functional spectrum in processes including trans-epithelial ion transport, olfaction, photo-transduction, smooth muscle contraction, nociception, cell proliferation and control of neuronal excitability. TMEM16A has been implicated to play a role in a number of health disorders and may be an important therapeutic target in cystic fibrosis, asthma, pain and some human cancers. TMEM16A is activated by elevated cytosolic calcium concentrations. In conventional patch clamp experiments, exchange of the intracellular solution to include calcium in order to initiate channel activity is challenging and typically performed using inside-out patches or by comparing the effect of internal calcium between different cells in the whole cell configuration. Furthermore, current run-down or desensitization are common problems associated with recording this ion channel. Here, we present data from HEK293 cells expressing hTMEM16A in whole cell and perforated patch mode using fluoride-free internal solution on the SyncroPatch 384PE. The data show that intracellular solution can be exchanged in a very robust manner to investigate calcium sensitivity, voltage dependence and pharmacology of the channel.Application Note PDFP2X3 Activation and inhibition of P2X3 channels recorded on the SyncroPatch 384PESyncroPatch 384PE (a predecessor model of SyncroPatch 384) application note: Cells were engineered and kindly provided by Axxam S.p.A., Milan.P2X receptors are ligand-gated ion channels that open in response to extracellular ATP. They are permeable to small monovalent cations, some having significant divalent or anion permeability. P2X receptors are found on many cell types including smooth muscle cells, sensory neurones, epithelia, bone and leukocytes. A role for P2X receptors has been suggested in transmission of thermal stimuli, chemosensory signalling, taste and pain. To date, 7 P2X receptor genes have been cloned and studied in heterologous expression systems. Functional receptors are trimeric, which can be homomeric or heteromeric. The P2X2and P2X3receptors can function either as homomers or as P2X2/3heteromers. When expressed together, a mixture of P2X2and P2X3homomers as well as P2X2/3heteromers are likely to exist, which may be distinguished through their biophysical and pharmacological properties. Both P2X3homomers and P2X2/3heteromeric receptors have been implicated in nociception and pain signalling and may be important therapeutic targets for analgesic drugs. Additionally, the P2X3and P2X2/3receptor antagonistMK- 7264 (gefapixant), has recently progressed to Phase III trials for refractory or unexplained chronic cough.Here, we present data collected on the SyncroPatch 384PE showing activation and inhibition of P2X3currents expressed in CHO cells with rapid and brief application of ligand (Ligand Puff). ATP or -methylene ATP (-MeATP) activated P2X3receptors with an EC50value similar to values found in the literature. P2X3receptors could be repetitively activated by ATP and blocked by A-317491 with an IC50value in good agreement with the literature.Application Note PDFQualification of Patch Ready Cells on a SyncroPatch 384PESyncroPatch 384P

the alpha-1C subunit, encoded by the CACNA1C gene on the human chromosome 12p133. Mutations in the L-type Ca2+channels have been associated with inherited arrhythmic disorders such as Timothy, Brugada and early repolarization syndromes. In addition, in the light of the CiPA initiative, the L-type channel is likely to become an important target for cardiac safety testing. Here we present high quality data with reliable pharmacology on CaV1.2 expressing CHO cells at a high throughput collected on the SyncroPatch 384PE. Current-voltage plots and concentration response curves for the compounds nifedipine and verapamil are shown. The IC50values for these compounds are within the expected range and success rates of >70% for completed experiments were recorded. Importantly, CaV1.2 recorded on the Syncro- Patch 384PE exhibited stable peak amplitudes during the course of the experiment and displayed little or no rundown.Application Note PDFCaV1.2 Stable Recordings and High Throughput Pharmacology of Assay Ready CaV1.2 Cells on the SyncroPatch 384SyncroPatch 384 application note: Cells were kindly provided by NMI TT Pharmaservices, Steinbeis-InnovationszentrumZellkulturtechnik and acCELLerateThe CaV1.2 channel is a voltage-gated calcium channel (VGCC) expressed in a variety of mammalian tissues and is essential for multiple processes including CNS function, cardiac and smooth muscle contraction and neuroendocrine regulation. VGCC are composed of 4 subunits, which include an alpha 1 subunit which forms the Ca2+-selective pore, contains the voltage sensor and many of the binding sites for regulatory modulators and drugs and accessory subunits 2, and which are involved in anchorage, trafficking and regulatory functions. The CaV1.2 channel contains the alpha-1C subunit, encoded by the CACNA1C gene on the human chromosome 12p13. Mutations in the L-type Ca2+channels have been associated with inherited arrhythmic disorders such as Timothy, Brugada and early repolarization syndromes. In addition, the L-type Ca2+channel is an important target for cardiac safety testing, especially in the light of the CiPA initiative. Here we present high quality data with reliable pharmacology on Assay Ready CaV1.2 Cells at a high throughput collected on the SyncroPatch 384. Current-voltage plots and concentration response curves for the compounds nifedipine and verapamil are shown. The IC50values for these compounds are within the expected range and success rates of >75% for completed experiments were recorded. Importantly, CaV1.2 recorded on the Syncro- Patch 384 exhibited stable peak amplitude during the course of the experiment and displayed low rundown.Application Note PDFAssay Ready TRP-Channel Expressing Cells a Flexible Tool to Screen for New Drug CandidatesSyncroPatch 384i (a predecessor model of SyncroPatch 384) application note: Patch Ready Cells provided by acCELLerateLooking for new targets in pain and cancer therapy, the transient receptor potential chan-nels (TRP-channels) gained a lot of interest during the last decade. The ligand-gated calcium channels play an important role in the perception of pain and temperature and are often dysregulated in tumor tissues. They have become appealing targets for Drug Discovery. Re-combinant cell lines which stably express different TRP-channels have been successfully used for lead identification and compound profiling.Looking for new targets in pain and cancer therapy, the transient receptor potential chan-nels (TRP-channels) gained a lot of interest during the last decade. The ligand-gated calcium channels play an important role in the perception of pain and temperature and are often dysregulated in tumor tissues. They have become appealing targets for Drug Discovery. Re-combinant cell lines which stably express different TRP-channels have been successfully used for lead identification and compound profiling.Assay ready cryopreserved aliquots prepared from these cell lines can be used instantly after thawing without prior cultivation. Here, we demonstrate that Assay Ready Cells prepared from TRP-channel expressing cell lines resemble the pharmacology of cells from continuous culture in different end-point assays. The cells were successfully qualified for plate-based fluorescent calcium-flux assays and for recording of activated ion channel currents using automated patch clamping.Application Note PDFCaV1.2 High Throughput Pharmacology of CaV1.2 Channels on Nanions SyncroPatch 384PESyncroPatch 384PE(a predecessor model of the SyncroPatch 384) application note: Cells were kindly provided by SB Drug Discovery.The CaV1.2 channel is a voltage-gated calciumchannel (VGCC) expressed in a variety of mammalian tissues and is essential for multiple processes including CNS function, cardiac and smooth muscle contraction and neuroendocrine regulation. VGCC are composed of 4 subunits, which include an alpha 1 subunit which forms theCa2+-selective pore, contains the voltage sensor and many of the binding sites for regulatory modulators and drugs and accessory subunits 2, and which are involved inanchorage, trafficking and regulatory functions. TheCaV1.2 channel contains the alpha-1C subunit, encoded by the CACNA1C gene on the human chromosome12p13. Mutations in the L-type Ca2+channels have been associated with inherited arrhythmic disorders such as Timothy, Brugada and early repolarization syndromes. In addition, in the light of the CiPA initiative, the L-typechannel is likely to become an important target for cardiac safety testing.Here we present high quality data with reliablepharmacology on CaV1.2 expressing HEK cells at a high throughput collected on the SyncroPatch 384PE. Current-voltage plots and concentration response curves for the compounds nifedipineand verapamil are shown. The IC50values for thesecompounds are within the expected range andsuccess rates of >75% for completed experiments wererecorded. Importantly, CaV1.2 recorded on the SyncroPatch384PE exhibited stable peak amplitude during the course of the experiment and displayed little or no rundown.Application Note PDFAMPA receptor (GluA2) Activation, potentiation and inhibition of AMPA receptors on the SyncroPatch 384PESyncroPatch 384PE (a predecessor model of SyncroPatch 384) application note: Cells were kindly provided by SB Drug Discovery.AMPA receptors are cation-permeable ionotropic glutamate receptors of the non-NMDA receptor subfamily. To date four subunits, GluA1-4, have been identified which are of similar size (approx. 900 kDa) and share 68-73% amino acid sequence identity. The functional receptor exists as a tetramer, either as homomers or heteromers (GluA1 and GluA4). The vast majority of excitatory fast synaptic transmission in the mammalian central nervous system is mediated by AMPA receptors of differing subunit combinations. It is well known that glutamate is a neurotoxin and it is proposed that overactivation of ionotropic glutamate receptors may underlie many neurodegenerative disorders such as ischemic stroke, epilepsy, Parkinsons and dementia, amongst others. Here we present data collected on the SyncroPatch 384PE showing recordings of GluA2-mediated currents. Glutamate activated GluA2 receptors with an EC50similar to those reported in the literature. CNQX inhibited and LY404187 enhanced GluA2-mediated responses.Application Note PDFASIC3 Activation and Inhibition of human ASIC3 Channels on Nanions SyncroPatch 384PESyncroPatch 384PE(a predecessor model of SyncroPatch 384) application note: Cells were kindly provided by Millipore.Acid-sensing ion channels (ASICs) are ligand-gated ionchannels activated by protons. They are members ofthe sodium-selective cation channels belonging to theepithelial sodium channel/degenerin (ENaC/DEG)family. ASICs are highly sensitive to extracellular acidosisand are permeable to cations, predominantly Na+.So far, 6 different ASIC subunits have been identifiedencoded by 4 genes. They are found expressed throughoutthe CNS and PNS and have a proposed role innociception and pain, and other neurologicaldiseases such as ischaemia and inflammation. The ASIC3channel was first identified in the late 1990s. It wasfound to be localized to primary afferent nociceptivefibers innervating the skin, muscles, joints and viscera, inagreement with a role in pain perception. Furthermore,ASIC3 is expressed in higher amounts in nociceptiveneurons innervating muscle (~ 50%) compared to skin(~ 10%), which indicates that ASIC3 may play animportant role in detecting muscle acidosis.Here we present high quality data at a high throughputcollected on the SyncroPatch 384PE showingactivation and inhibition of ASIC3 expressedin HEK cells. The pH which elicited a halfmaximalresponse was in good agreement with theliterature. The IC50for block of the ASIC3 currentby amiloride, a known blocker of ASIC and ENaCchannels, was also in good agreement with theliterature. Success rates of over 80% for completedexperiments were recorded.Application Note PDFNaV1.5 NaV1.5-KPQ late INa current properties and pharmacology on the SyncroPatch 384iSyncroPatch 384i (a predecessor model of the SyncroPatch 384) application note Cells were kindly provided by Metrion Biosciences.The cardiac late Na current (late INa) generates persistent currents throughout the plateau phase of the cardiac action potential. Several mutations in the SCN5A gene cause a form of hereditary long QT syndrome (LQT3)1-3. The KPQ mutation deletes residues Lys 1505, Pro 1506 and Gln 1507, resulting in a sustained, non-inactivating current during long (over 50 ms) depolarizations1,2. This sustained current causes prolongation of the action potential which can result in fatal ventricular arrhythmias such as Torsade de Pointes (TdP)1.One aim of the ComprehensiveIn VitroPro-arrhythmia Assay (CiPA) initiative is to improve drug safety testing in pre-clinical development by evaluating the pro-arrhythmic risk of a compound4,5. Validation studies confirm that testing the effect of compounds on an increased number of human cardiac ion channel currents including INa(NaV1.5 peak and late current) as well as IKr(hERG) leads to improved prediction of their clinical risk. Late INacan be recorded in WT NaV1.5 channels using the toxin ATX-II or veratridine, or using a cell line with LQT3 mutations in NaV1.5 without the need for pharmacological enhancement. The latter might also reduce the risk of cross-reactions between late-current enhancers and test compounds.Here we present data collected on the Syncro- Patch 384i showing the peak and late INacurrent recorded from WT and NaV1.5-KPQ cell lines. Peak current could be reliably recorded from both cell types. In WT cells, late INa was negligible in the absence of ATX-II, whereas the late INafrom NaV1.5-KPQ cells could be reliably recorded. Peak current from WT, and peak and late INafrom NaV1.5-KPQ was inhibited by ranolazine and mexiletine and IC50values agreed well with the literature6.Application Note PDFAChR 11 Reproducible activation and pharmacology of 11 nAChR on the SyncroPatch 384SyncroPatch 384 application note: Cells were obtained from CLSNicotinic acetylcholine receptors (nAChR) are acetylcholine- (ACh) and nicotine-gated cation permeable ion channels, which mediate fast synaptic transmission at central synapses and neuromuscular junctions. Neuromuscular nAChR form heteromeric proteins composed of four subunits: , , (or) and . Depending on the developmental stage, the AChR subunit stoichiometry changes from 11 (embryonic) to 11 (adult). Several inherited and acquired diseases are associated with nAChR dysfunction, most of which lead to impaired neuromuscular transmission and muscle weakness. The acquired autoimmune disease myasthenia gravis (MG) is caused by autoantibodies targeting muscle nAChRs that disrupts nerve-muscle communication resulting in muscle weakness and fatigue. Inherited diseases called congenital myasthenic syndromes (CMS) are associated with several abnormalities affecting ACh-release, acetylcholinesterase activity, nAChR function and/or nAChR number. Treatment has been limited to nonselective, chronic immunosuppressive therapies which have longterm toxicities. More selective and targeted therapies are now under development. Here we present data collected on the SyncroPatch 384 showing activation and block of nAChR11 expressed in human TE671 cells with rapid application of ligand or co-application with blockers. We found that ACh activates nAChR11 receptors with an EC50value similar to those reported in the literature. We recorded highly reproducible currents in response to ACh and obtained IC50values for mecamylamine and -conotoxin GI that were in good agreement with the literature.Application Note PDFCardiac Ion Channels Simultaneous Assessment of CiPA Stipulated Ion Channels on the SyncroPatch 384PESyncroPatch 384PE(a predecessor model of the SyncroPatch 384) application note Cells were kindly provided by Charles River.The cardiac action potential is defined by multiple voltage-dependent ion channels. A drug candidates capacity to interact with the ion channels involved in the depolarization or repolarization phases of the cardiac action potential is important for drug safety assessment. Until now, safety testing has focussed on interaction with the hERG channel and QT prolongation which can lead to potentially fatal torsades de pointes (TdP). Although this approach has been largely successful in preventing new drugs reaching the market with unexpected potential to cause TdP, it is also possible that potentially valuable therapeutics have failed due to this early screening. A new paradigm, the Comprehensive In-vitro Proarrhythmia Assay (CiPA), was introduced in 2013 to provide a more complete assessment of proarrythmic risk. An assessment of a multitude of cardiac ion channels, in addition to hERG, should provide a more accurate prediction of the proarrythmic risk of a compound compared with testing on hERG alone. Here we show recordings from HEK or CHO cells expressing the CiPA stipulated ion channels; NaV1.5, CaV1.2, hERG, KV7.1, Kir2.1 or KV4.3, activated within one single experiment on the SyncroPatch 384PE.Application Note PDFNaV1.5 High Throughput Pharmacology of NaV1.5 Channels on Nanions SyncroPatch 384PESyncroPatch 384i (a predecessor model of the SyncroPatch 384) application note Cells were kindly provided by Millipore.The NaV1.5 channel, encoded by the SCN5A gene, is a voltage-gated sodium (NaV) channel found in skeletal muscle and heart. It is TTX insensitive with an IC50in the micromolar range. NaV1.5 is responsible for the upstroke of the cardiac action potential in both ventricular and atrial myocytes and is therefore critical for generation and propagation of the cardiac action potential in human heart. Block of this channel can lead to prolongation of the QRS interval of the electrocardiogram (ECG) and can have profound effects on the rate of cardiac deploarization and conduction velocity, thus causing potentially dangerous cardiac arrythmias. Furthermore, effects of NaV1.5 inactivation can modify cardiac repolarization. Given the importance of this channel in maintaining cardiac function, it has become an important target in compound safety screening. Local anaesthetics, such as lidocaine, have been shown to exhibit state- and use-dependence when acting on the cardiac sodium channel. The IC50was shown to be approximately 30 times lower at depolarized holding potentials where inactivation was almost complete. For this reason, it is important to test potency of compounds at different holding potentials. Here we present high quality data with reliable pharmacology on hNaV1.5 expressing HEK293 cells at a high throughput collected on the SyncroPatch384PE. Current-voltage plots and concentration response curves for four NaVchannel blockers are shown, including lidocaine at different holding potentials.Webinar11.03.2021 | Webinar: automated patch clamp webinar high throughput functional evaluation of melanocortin and a CiPA based evaluation of proarrhythmic riskAuthors: Ciara Hernandez, Yuri KuryshevPresentation PDF2020 High Throughput screening of missense variants in KCNH2Presenter: Prof. Dr. Jamie Vandenberg, co-deputy director and head of cardiac electrophysiology, The Victor Chang Cardiac Research Institute, Australia ; Source: The 64th Annual Meeting of the Biophysical Society, San Diego, CA (USA) Feb 15-19. 2020Abstract: Mutations in the KCNH2 gene are a well-established cause of sudden cardiac death, resulting from disturbed electrical signalling, in otherwise healthy young people. Yet, the majority of missense variants identified in KCNH2 are likely to be benign. To differentiate between benign and pathogenic variants in KCNH2 we have developed a high throughput functional assay using the syncropatch 384PE automated patch clamp system. The Victor Chang Cardiac Research Institute is an independent, not-for-profit, medical research facility that is dedicated to finding cures for cardiovascular disease. Renowned for the quality of its breakthroughs, the Victor Chang Cardiac Research Institute is one of the most respected heart research facilities in the world.Webinar04.06.2020 | Webinar: Decrypting variants of unknown significance in the channelopathiesAuthors: Rodolfo Haedo, Jen Q. Pan; Al GeorgeWebinar07.06.2016 | External Webinar: A new analysis capability to improve assessment of cardiac liability in high throughput electrophysiologyAuthors: Ana Teixeira ; Matthew Bridgelend-TaylorWebinar2018 Biophysical and Pharmacological Characterization of Voltage-Gated Sodium Channels Involved in Pain PathwaysAuthors: Markus RapediusWebinar08.05.2018 | Webinar: HTS Sodium Ion Channel Assays on the SyncroPatch 384PEAuthors: Markus Rapedius; Elisa BalliniUser meeting video16.10.2020 | Webinar: Emerging Role of LRRC8 Volume-Regulated Anion Channels in the SkinAuthors: Torsten Fauth; Oliver Rauh; Giustina RotordamWebinar03.11.2016 | External Webinar: Accelerating Ion Channel Characterization and New Drug Candidate IdentificationAuthors: Alfred George; Matt FullerUser meeting video12.10.2020 | Webinar: The Sodium Channel Network Aachen meets the SyncroPatchAuthors: Angelika LampertUser meeting video15.10.2020 | Webinar: Turning Cells into ReagentsAuthors: Oliver Wehmeier; Tim StrassmaierUser meeting video12.10.2020 | Webinar: From Manual to Automated Planar Patch-Clamp Electrophysiology: Accelerating Drug Discovery Research in Academic SettingsAuthors: Fernanda LaezzaUser meeting video12.10.2020 | Webinar: PatchAnalyticsAuthors: Lei A. WangTutorial video2021 Unattended Recordings and the Method LauncherAuthors: Nanion ExpertsTutorial videoBasic principles of external solution exchange and compound additionAuthors: Nanion ExpertsProduct video2019 SyncroPatch 384i Product VideoAuthors: Nanion ExpertsProduct video2020 What is the SyncroPatch 384PE and what are the benefits of the SyncroPatch 384PE?Authors: Victor Chang Cardiac Research InstitutePoster PDF2022 Mechanical and Pharmacological Activation of Piezo1 Channels Characterized by High Throughput ElectrophysiologyProduct Sheet PDFSyncroPatch 384 Product SheetPoster PDF2020 Unattended Screening Workflow in a 384-well Automated Patch Clamp SystemPoster PDF2022 High performance automated patch-clamp characterisation of mammalian atrial cardiomyocytesPoster PDF2017 Investigation of the Ion Channels hTMEM16A/Ano1 and TRPC5 and their Modulation by Intracellular CalciumSyncroPatch 384PE (a predecessor model of the SyncroPatch 384) poster, BPS Meeting 2017Poster PDF2017 Pharmacological Characterization of the NMDA A-B-C by Automated Patch ClampSyncroPatch 384PE (a predecessor model of SyncroPatch 384) poster, BPS Meeting 2017Poster PDF2022 High-throughput electrophysiology reveals subtype specific temperature dependence of sodium channel activation and inactivationSyncroPatch 384 poster, BPS 2022Poster PDF2022 Cardiosafety Testing Based on CiPA at Scale with SyncroPatch 384 and Genedata ScreenerSyncroPatch 384 poster, SLAS 2022Poster PDF2017 Activation of CFTR channels in absence of internal fluoride using a highly parallel automated patch clamp systemSyncroPatch 384PE (a predecessor model of the SyncroPatch 384) poster, BPS Meeting 2017Poster PDF2022 Identification of cardiac liability in drug discovery using automated patch clamp: Experimental and technical considerations for high throughput recordings of NaV1.5 and hERGSyncroPatch 384 poster, BPS 2022Flyer PDFSyncroPatch 384 New Consumables: NPC-384TPoster PDF2015 High Throughput Automated Patch Clamp of Ion Channels Important in Cardiac Safety and Drug DiscoverySyncroPatch 384PE (a predecessor model of SyncroPatch 384) poster, Chantest Meeting 2015Flyer PDFSyncroPatch 384 Fluoride-Free RecordingsFlyer PDFSyncroPatch 384 IV Analysis ToolPublication link2022- Genetically-encoded BRET probes shed light on ligand biasinduced variable ion selectivity in TRPV1 and P2X5/7SyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publication in PNAS (2022) Authors: Chappe Y., Pierredon S., Joushomme A., Molle P., Garenne A., Canovi A., Barbeau S., Poulletier De Gannes F., Hurtier A., Lagroye I., Ducret T., Quignard J., Compan V., Percherancier Y.Whether ion channels experience ligand-dependent dynamic ion selectivity remains of critical importance since this could support ion channel functional bias. Tracking selective ion permeability through ion channels, however, remains challenging even with patch-clamp electrophysiology. In this study, we have developed highly sensitive bioluminescence resonance energy transfer (BRET) probes providing dynamic measurements of Ca2+ and K+ concentrations and ionic strength in the nanoenvironment of Transient Receptor Potential Vanilloid-1 Channel (TRPV1) and P2X channel pores in real time and in live cells during drug challenges. Our results indicate that AMG517, BCTC, and AMG21629, three well-known TRPV1 inhibitors, more potently inhibit the capsaicin (CAPS)-induced Ca2+ influx than the CAPS-induced K+ efflux through TRPV1. Even more strikingly, we found that AMG517, when injected alone, is a partial agonist of the K+ efflux through TRPV1 and triggers TRPV1-dependent cell membrane hyperpolarization. In a further effort to exemplify ligand bias in other families of cationic channels, using the same BRET-based strategy, we also detected concentration- and time-dependent ligand biases in P2X7 and P2X5 cationic selectivity when activated by benzoyl-adenosine triphosphate (Bz-ATP). These custom-engineered BRET-based probes now open up avenues for adding value to ion-channel drug discovery platforms by taking ligand bias into account.Publication link2022- The discovery of (1R, 3R)-1-(3-chloro-5-fluorophenyl)-3-(hydroxymethyl)-1,2,3,4-tetrahydroisoquinoline-6-carbonitrile, a potent and selective agonist of human transient receptor potential cation channel subfamily m member 5 (TRPM5) and evaluation ofSyncroPatch 384 Publication in Bioorganic & Medicinal Chemistry (2022) Authors: Davies M.R., Martinec M., Walls R., Schwarz R., Mirams G.R., Wang K., Steiner G., Surinach A., Flores C., Lave T., Singer T., Polonchuk L.This publication details the discovery of a series of selective transient receptor potential cation channel subfamily M member 5 (TRPM5) agonists culminating with the identification of the lead compound (1R, 3R)-1-(3-chloro-5-fluorophenyl)-3-(hydroxymethyl)-1,2,3,4-tetrahydroisoquinoline-6-carbonitrile (39). We describe herein our biological rationale for agonism of the target, the examination of the then current literature tool molecules, and finally the process of our discovery starting with a high throughput screening hit through lead development. We also detail the selectivity of the lead compound 39 versus related family members TRPA1, TRPV1, TRPV4, TRPM4 and TRPM8, the drug metabolism and pharmacokinetics (DMPK) profile and in vivo efficacy in a mouse model of gastrointestinal motility.Publication link2022 subunits of GABAA receptors form proton-gated chloride channels: Insights into the molecular basisSyncroPatch 384 Publication in Communications Biology (2022) Authors: Garifulina A., Friesacher T., Stadler1 M., Zangerl-Plessl E., Ernst M., Stary-Weinzinger A., Willam A., Hering S.Gamma-aminobutyric acid type A receptors (GABAARs) are ligand gated channels mediating inhibition in the central nervous system. Here, we identify a so far undescribed function of -subunit homomers as proton-gated anion channels. Mutation of a single H267A in 3 subunits completely abolishes channel activation by protons. In molecular dynamic simulations of the 3 crystal structure protonation of H267 increased the formation of hydrogen bonds between H267 and E270 of the adjacent subunit leading to a pore stabilising ring formation and accumulation of Cl- within the transmembrane pore. Conversion of these residues in proton insensitive 1 subunits transfers proton-dependent gating, thus highlighting the role of this interaction in proton sensitivity. Activation of chloride and bicarbonate currents at physiological pH changes (pH50 is in the range 6- 6.3) and kinetic studies suggest a physiological role in neuronal and non-neuronal tissues that express beta subunits, and thus as potential novel drug target.Publication link2022- A link between agrin signaling and CaV3.2 at the neuromuscular-junction in spinal muscular atrophySyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publication in Scientific Reports(2022) Authors: Delers P., Sapaly D., Salman B., De Waard S., De Waard M., Lefebvre S.SMN protein deficiency causes motoneuron disease spinal muscular atrophy (SMA). SMN-based therapies improve patient motor symptoms to variable degrees. An early hallmark of SMA is the perturbation of the neuromuscular junction (NMJ), a synapse between a motoneuron and muscle cell. NMJ formation depends on acetylcholine receptor (AChR) clustering triggered by agrin and its co-receptors lipoprotein receptor-related protein 4 (LRP4) and transmembrane muscle-specific kinase (MuSK) signalling pathway. We have previously shown that flunarizine improves NMJs in SMA model mice, but the mechanisms remain elusive. We show here that flunarizine promotes AChR clustering in cell-autonomous, dose- and agrin-dependent manners in C2C12 myotubes. This is associated with an increase in protein levels of LRP4, integrin-beta-1 and alpha-dystroglycan, three agrin co-receptors. Furthermore, flunarizine enhances MuSK interaction with integrin-beta-1 and phosphotyrosines. Moreover, the drug acts on the expression and splicing of Agrn and Cacna1h genes in a muscle-specific manner. We reveal that the Cacna1h encoded protein CaV3.2 closely associates in vitro with the agrin co-receptor LRP4. In vivo, it is enriched nearby NMJs during neonatal development and the drug increases this immunolabelling in SMA muscles. Thus, flunarizine modulates key players of the NMJ and identifies CaV3.2 as a new protein involved in the NMJ biology.Publication link2022 Translating the Measurement of Herg Kinetics and Drug Block for Cipa to a High Throughput PlatformSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) pre-print Publication in SSRN (2022) Authors: Windley M.J., Farra J., Vandenberg J.I., Hill A.P.The Comprehensive in vitro Proarrhythmic Assay (CiPA) has promoted use of in silico models of drugeffects on cardiac repolarization to improve proarrhythmic risk prediction. These models contain apharmacodynamic component describing drug binding to hERG channels that required in vitro datafor kinetics of block, in addition to potency, to constrain them. To date, development and validationhas been undertaken using data from manual patch-clamp. To enable the application of this approachat scale this requires the development of a high-throughput, automated patch-clamp (APC)implementation. Here, we present a comprehensive analysis of the implementation of the Milnes, or CiPA dynamic protocol, on an APC platform, including automated quality control and data analysis.Kinetics and potency of block were assessed for bepridil, cisapride, terfenadine and verapamilwith data retention/QC pass rate of 21.8%. The variability in IC50 and kinetics between manual andAPC was comparable to that seen between sites/platforms in previous APC studies of potency. Whilstthe experimental success is less than observed in screens of potency alone, it is still significantlygreater than manual patch. With appropriate consideration of protocol design, including sweeplength, number of repetitions, and leak correction, this protocol can be applied on APC to acquire datacomparable to manual patch clamp.Publication link2022 Veratridine Can Bind to a Site at the Mouth of the Channel Pore at Human Cardiac Sodium Channel NaV1.5SyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) pre-print Publication in Preprints (2022) Authors: Gulsevin A., Glazer A. M., Shields T., Kroncke B. M., Roden D. M., Meiler J.The cardiac sodium ion channel (NaV1.5) is a protein with four domains (DI-DIV), each with six transmembrane segments. Its opening and subsequent inactivation results in the brief rapid influx of Na+ ions resulting in the depolarization of cardiomyocytes. The neurotoxin veratridine (VTD) inhibits NaV1.5 inactivation resulting in longer channel opening times, and potentially fatal action potential prolongation. VTD is predicted to bind at the channel pore, but alternative binding sites have not been ruled out. To determine the binding site of VTD on NaV1.5, we performed docking calculations and high-throughput electrophysiology experiments. The docking calculations identified two distinct binding regions. The first site was in the pore, close to the binding site of NaV1.4 and NaV1.5 blocking drugs in experimental structures. The second site was at the mouth of the pore at the cytosolic side, partly solvent-exposed. Mutations at this site (L409, E417, and I1466) had large effects on VTD binding, while residues deeper in the pore had no effect, consistent with VTD binding at the mouth site. Overall, our results suggest a VTD binding site close to the cytoplasmic mouth of the channel pore. Binding at this alternative site might indicate an allosteric inactivation mechanism for VTD at NaV1.5.Publication link2022 There is no F in APC: Using physiological fluoride-free solutions for high throughput automated patch clamp experimentsSyncroPatch 384 Publication in Frontiers in Molecular Neuroscience (2022) Authors: Rapedius M., Obergrussberger A., Humphries E.S.A., Scholz S., Rinke-Weiss I., Goetze T.A., Brinkwirth N., Rotordam M.G., Strassmaier T., Randolph A., Friis S., Liutkute A., Seibertz F., Voigt N., Fertig N.Fluoride has been used in the internal recording solution for manual and automated patch clamp experiments for decades because it helps to improve the seal resistance and promotes longer lasting recordings. In manual patch clamp, fluoride has been used to record voltage-gated Na (NaV) channels where seal resistance and access resistance are critical for good voltage control. In automated patch clamp, suction is applied from underneath the patch clamp chip to attract a cell to the hole and obtain a good seal. Since the patch clamp aperture cannot be moved to improve the seal like the patch clamp pipette in manual patch clamp, automated patch clamp manufacturers use internal fluoride to improve the success rate for obtaining G seals. However, internal fluoride can affect voltage-dependence of activation and inactivation, as well as affecting internal second messenger systems and therefore, it is desirable to have the option to perform experiments using physiological, fluoride-free internal solution. We have developed an approach for high throughput fluoride-free recordings on a 384-well based automated patch clamp system with success rates >40% for G seals. We demonstrate this method using hERG expressed in HEK cells, as well as NaV1.5, NaV1.7, and KCa3.1 expressed in CHO cells. We describe the advantages and disadvantages of using fluoride and provide examples of where fluoride can be used, where caution should be exerted and where fluoride-free solutions provide an advantage over fluoride-containing solutions.Publication link2022 Transient receptor potential channel vanilloid type 2 in red cells of cannabis consumerSyncroPatch 384 Publication in American Journal of Hematology (2022) Authors: FlormannD.,QiaoM.,MurcianoN.,IaconoG.,DarrasA.,HofS.,RecktenwaldS.M.,RotordamM.G.,BeckerN., GeiselJ.,WagnerC.,von LindernM.,van den AkkerE.,KaestnerL.The presence of the Transient Receptor Potential channel Vanilloid type 2 (TRPV2) in red blood cells (RBCs) was recently discovered. TRPV2 is a non-selective cation channel that is reported to be mechanosensitive having numerous properties in common with Piezo1. TRPV2 channels can be activated by 9-tetrahydrocannabinol (9-THC) and the changes induced in RBC by application of 9-THC can be attributed to TRPV2 channel activity. A previous study shown that addition of 30 M 9-THC led to a large fraction of super hydrated RBCs in a healthy donor consuming marijuana in contrast to significantly milder response from no smoker healthy donors. For further investigations three marijuana consumers with very similar smoking habits were scouted, and blood was collected. Whether this heightened sensitivity of the smokers (MS) vs. no-smokers (NS) RBCs is caused by hypersensitizing of TRPV2 was tackled by comparing MS and NS RBCs in functional patch-clamp measurements using the high - throughput automated patch clamp platform SyncroPatch 384. Whole-cell currents elicited by THC application did not show a significant different thus indicates a similar cellular conductance for MS and NS RBCs and therefore rather a lack of difference in TRPV2 expression level. We demonstrate that APC technology is a suitable tool permits for studying TRPV2 channels in RBCs.Publication link2022 Pharmacological Dissection of the Crosstalk between NaV and CaV Channels in GH3b6 CellsSyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publication in International Journal of Molecular Sciences (2022) Authors: Rthor L., Park J., Montnach J., Nicolas S., Khoury J., Le Seach E., Mabrouk K., De Pomyers H., Tricoire-Leignel H., Mattei C., Henrion D., Fajloun Z., De Waard M., Legendre C., Legros C.Thanks to the crosstalk between Na+ and Ca2+ channels, Na+ and Ca2+ homeostasis interplay in so-called excitable cells enables the generation of action potential in response to electrical stimulation. Here, we investigated the impact of persistent activation of voltage-gated Na+ (NaV) channels by neurotoxins, such as veratridine (VTD), on intracellular Ca2+ concentration ([Ca2+]i) in a model of excitable cells, the rat pituitary GH3b6 cells, in order to identify the molecular actors involved in Na+-Ca2+ homeostasis crosstalk. By combining RT-qPCR, immunoblotting, immunocytochemistry, and patch-clamp techniques, we showed that GH3b6 cells predominantly express the NaV1.3 channel subtype, which likely endorses their voltage-activated Na+ currents. Notably, these Na+ currents were blocked by ICA-121431 and activated by the -scorpion toxin Tf2, two selective NaV1.3 channel ligands. Using Fura-2, we showed that VTD induced a [Ca2+]i increase. This effect was suppressed by the selective NaV channel blocker tetrodotoxin, as well by the selective L-type CaV channel (LTCC) blocker nifedipine. We also evidenced that crobenetine, a NaV channel blocker, abolished VTD-induced [Ca2+]i elevation, while it had no effects on LTCC. Altogether, our findings highlight a crosstalk between NaV and LTCC in GH3b6 cells, providing a new insight into the mode of action of neurotoxins.Publication link2022 Small-molecule inhibitors of Slack potassium channels as potential therapeutics for childhood epilepsiesSyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publication inPharmaceutical Patent Analyst(2022) Authors: Qunies A.M., Emmitte K.A.Slack channels are sodium-activated potassium channels that are encoded by theKCNT1gene. SeveralKCNT1gain of function mutations have been linked to malignant migrating partial seizures of infancy. Quinidine is an anti-arrhythmic drug that functions as a moderately potent inhibitor of Slack channels; however, quinidine use is limited by its poor selectivity, safetyand pharmacokinetic profile. Slack channels represent an interesting target for developing novel therapeutics for the treatment of malignant migrating partial seizures of infancy and other childhood epilepsies; thus, ongoing efforts are directed toward the discovery of small-molecules that inhibit Slack currents. This review summarizes patent applications published in 20202021 that describe the discovery of novel small-molecule Slack inhibitors.Publication link2022 Optimization of hERG and Pharmacokinetic Properties for Basic Dihydro-8H-purin-8-one Inhibitors of DNA-PKSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication inACS Medicinal Chemistry Letters (2022) Authors: Goldberg F.W., Ting A.K.T., Beattie D., Lamont G.M., Fallan C., Finlay M.R.V., Williamson B., Schimpl M., Harmer A.R., Adeyemi O.B., Nordell P., Cronin A.S., Vazquez-Chantada M., Barratt D., Ramos-Montoya A., Cadogan E.B., Davies B.R.The DNA-PK complex is activated by double-strand DNA breaks and regulates the non-homologous end-joining repair pathway; thus, targeting DNA-PK by inhibiting the DNA-PK catalytic subunit (DNA-PKcs) is potentially a useful therapeutic approach for oncology. A previously reported series of neutral DNA-PKcs inhibitors were modified to incorporate a basic group, with the rationale that increasing the volume of distribution while maintaining good metabolic stability should increase the half-life. However, adding a basic group introduced hERG activity, and basic compounds with modest hERG activity (IC50= 1015 M) prolonged QTc (time from the start of the Q wave to the end of the T wave, corrected by heart rate) in an anaesthetized guinea pig cardiovascular model. Further optimization was necessary, including modulation of pKa, to identify compound18, which combines low hERG activity (IC50= 75 M) with excellent kinome selectivity and favorable pharmacokinetic properties.Publication link2022 Palladium-Catalyzed -Arylation of Cyclic -Dicarbonyl Compounds for the Synthesis of CaV1.3 InhibitorsSyncroPatch 768PE (a predecessor model of the SyncroPatch 384 instrument)Publication in ACS Omega (2022) Authors: Yun J., Jeong D., Xie Z., Lee S., Kim J., Surmeier,D.J., Silverman R.B., Kang S.Cyclic -aryl -dicarbonyl derivatives are important scaffolds in medicinal chemistry. Palladium-catalyzed coupling reactions of haloarenes were conducted with diverse five- to seven-membered cyclic -dicarbonyl derivatives including barbiturate, pyrazolidine-3,5-dione, and 1,4-diazepane-5,7-dione. The coupling reactions of various para- or meta-substituted aryl halides occurred efficiently when Pd(t-Bu3P)2, Xphos, and Cs2CO3were used under 1,4-dioxane reflux conditions. Although the couplings of ortho-substituted aryl halides with pyrazolidine-3,5-dione and 1,4-diazepane-5,7-dione were moderate, the coupling with barbiturate was limited. Using the optimized reaction conditions, we synthesized several 5-aryl barbiturates as new scaffolds of CaV1.3 Ca2+channel inhibitors. Among the synthesized molecules,14ewas the most potent CaV1.3 inhibitor with an IC50of 1.42 M.Publication link2022 Novel Fluorescence-Based High-Throughput FLIPR Assay Utilizing Membrane-Tethered Genetic Calcium Sensors to Identify TType Calcium Channel ModulatorsSyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publication in ACS Pharmacology & Translational Science (2022) Authors: Zhang Y.L., Moran S.P., Allen A., Baez-Nieto D., Xu Q., Wang L.A., Martenis W.E., Sacher J.R., Gale J.P, Wewer M., Wagner F.F., Pan J.Q.T-type voltage-gated Ca2+channels have been implicated in many human disorders, and there has been increasing interest in developing highly selective and potent T-type Ca2+channel modulators for potential clinical use. However, the unique biophysical properties of T-type Ca2+channels are not conducive for developing high-throughput screening (HTS) assays to identify modulators, particularly potentiators. To illustrate, T-type Ca2+channels are largely inactivated and unable to open to allow Ca2+influx at 25 mV, the typical resting membrane potential of the cell lines commonly used in cellular screening assays. To address this issue, we developed cell lines that express Kir2.3 channels to hyperpolarize the membrane potential to 70 mV, thus allowing T-type channels to return to their resting state where they can be subsequently activated by membrane depolarization in the presence of extracellular KCl. Furthermore, to simplify the HTS assay and to reduce reagent cost, we stably expressed a membrane-tethered genetic calcium sensor, GCaMP6s-CAAX, that displays superior signal to the background compared to the untethered GCaMP6s or the synthetic Ca2+sensor Fluo-4AM. Here, we describe a novel GCaMP6s-CAAX-based calcium assay utilizing a high-throughput fluorometric imaging plate reader (Molecular Devices, Sunnyvale, CA) format that can identify both activators and inhibitors of T-type Ca2+channels. Lastly, we demonstrate the utility of this novel fluorescence-based assay to evaluate the activities of two distinct G-protein-coupled receptors, thus expanding the use of GCaMP6s-CAAX to a wide range of applications relevant for developing cellular assays

A.P,Kozek K.A.,Vanags L.R.,Mitchell D.,Kroncke B.M.,Vandenberg J.I.High throughput genomics has greatly facilitated identification of genetic variants. However, determining which variants contribute to disease causation is challenging with more than half of all missense variants now classified as variants of uncertain significance (VUS). A VUS leaves patients and their clinicians unable to utilize the variant information in clinical decision-making. In long QT syndrome type 2, KCNH2 channel function is directly associated with disease presentation. Therefore, functional phenotyping of KCNH2 variants can provide direct evidence to aid variant classification. Here, we investigated the expression of all codon variants in exon 2 of KCNH2 using a massively parallel trafficking assay and for a subset of 458 single nucleotide variants compared the results with peak tail current density and gating using automated patch clamp electrophysiology. Trafficking could correctly classify loss of peak tail current density variants with an AUC reaching 0.94 compared to AUCs of 0.75 to 0.8 for in silico variant classifiers. We suggest massively parallel trafficking assays can provide prospective and accurate functional assessment for all missense variants in KCNH2 and most likely many other ion channels and membrane proteins.Publication link2022 A modern automated patch-clamp approach for high throughput electrophysiology recordings in native cardiomyocytesSyncroPatch 384 Publication in Communications Biology (2022) Authors: Seibertz F., Rapedius M., Fakuade F., Tomsits P., Liutkute A., Cyganek L., Becker N., Majumder R., Clau S.,Fertig N., Voigt N.Crucial conventional patch-clamp approaches to investigate cellular electrophysiology suffer from low-throughput and require considerable experimenter expertise. Automated patch-clamp APC) approaches are more experimenter independent and offer high-throughput, but by design are predominantly limited to assays containing small, homogenous cells. In order to enable high-throughput APC assays on larger cells such as native cardiomyocytes isolated from mammalian hearts, we employed a fixed-well APC plate format. A broad range of We identified 35 LoF variants (10% of wild type [WT] peak current) and 15 partial LoF variants (10%-50% of WT peak current) that we assessed for dominant negative effects. SCN5A variants were studied in HEK293T cells, alone or in heterozygous coexpression with WT SCN5A using automated patch clamp. To assess the clinical risk, we compared the prevalence of dominant negative vs putative haploinsufficient (frameshift, splice, or nonsense) variants in a BrS consortium and the Genome Aggregation Database population database. detailed electrophysiological parameters including action potential, L-type calcium current and basal inward rectifier current were reliably acquired from isolated swine atrial and ventricular cardiomyocytes using APC. Effective pharmacological modulation also indicated that this technique is applicable for drug screening using native cardiomyocyte material. Furthermore, sequential acquisition of multiple parameters from a single cell was successful in a high throughput format, substantially increasing data richness and quantity per experimental run. When appropriately expanded, these protocols will provide a foundation for effective mechanistic and phenotyping studies of human cardiac electrophysiology. Utilizing scarce biopsy samples, regular high throughput characterization of primary cardiomyocytes using APC will facilitate drug development initiatives and personalized treatment strategies for a multitude of cardiac diseases.Publication link2022 A High-Throughput Screening Assay to Identify Drugs that Can Treat Long QT Syndrome Caused by Trafficking-Deficient KV11.1 (hERG) VariantsSyncroPatch 384 Publication in Molecular Pharmacology (2022) Authors: Egly C.L., Blackwell D.J., Schmeckpeper J., Delisle B.P., Weaver C.D., Knollmann B.C.Loss-of-function (LOF) variants in the KV11.1 potassium channel cause long QT syndrome (LQTS). Most variants disrupt intracellular channel transport (trafficking) to the cell membrane. Since some channel inhibitors improve trafficking of KV11.1 variants, a high-throughput screening (HTS) assay to detect trafficking enhancement would be valuable to the identification of drug candidates. The thallium (Tl+) flux assay technique, widely used for drug screening, was optimized using human embryonic kidney (HEK-293) cells expressing a trafficking-deficient KV11.1 variant in 384-well plates. Assay quality was assessed using Z prime (Z) scores comparing vehicle to E-4031, a drug that increases KV11.1 membrane trafficking. The optimized assay was validated by immunoblot, electrophysiology experiments, and a pilot drug screen. The combination of: 1) truncating the trafficking-deficient variant KV11.1-G601S (KV11.1-G601S-G965*X) with the addition of 2) KV11.1 channel activator (VU0405601) and 3) cesium (Cs+) to the Tl+flux assay buffer resulted in an outstanding Z of 0.83. To validate the optimized trafficking assay, we carried out a pilot screen that identified three drugs (ibutilide, azaperone, and azelastine) that increase KV11.1 trafficking. The new assay exhibited 100% sensitivity and specificity. Immunoblot and voltage-clamp experiments confirmed that all three drugs identified by the new assay improved membrane trafficking of two additional LQTS KV11.1 variants. We report two new ways to increase target-specific activity in trafficking assaysgenetic modification and channel activationthat yielded a novel HTS assay for identifying drugs that improve membrane expression of pathogenic KV11.1 variants.Publication link2022 A massively parallel assay accurately discriminates between functionally normal and abnormal variants in a hotspot domain of KCNH2SyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication inThe American Journal of Human Genetics (2022) Authors: Ng CA., Ullah R., Farr J., Hill A.P., Kozek K.A.,Vanags L.R., Mitchell D.W., Kroncke B.M., Vandenberg J.I.Many genes, including KCNH2, contain hotspot domains associated with a high density of variants associated with disease. This has led to the suggestion that variant location can be used as evidence supporting classification of clinical variants. However, it is not known what proportion of all potential variants in hotspot domains cause loss of function. Here, we have used a massively parallel trafficking assay to characterize all single-nucleotide variants in exon 2 of KCNH2, a known hotspot for variants that cause long QT syndrome type 2 and an increased risk of sudden cardiac death. Forty-two percent of KCNH2 exon 2 variants caused at least 50% reduction in protein trafficking, and 65% of these trafficking-defective variants exerted a dominant-negative effect when co-expressed with a WT KCNH2 allele as assessed using a calibrated patch-clamp electrophysiology assay. The massively parallel trafficking assay was more accurate (AUC of 0.94) than bioinformatic prediction tools (REVEL and CardioBoost, AUC of 0.81) in discriminating between functionally normal and abnormal variants. Interestingly, over half of variants in exon 2 were found to be functionally normal, suggesting a nuanced interpretation of variants in this hotspot domain is necessary. Our massively parallel trafficking assay can provide this information prospectively.Publication link2021 The Schizophrenia Variant V1282F in SCN2A Causes Functional Impairment of NaV1.2SyncroPatch Publicationin Journal of Schizophrenia Research (2021) Authors: Kohlnhofer B., Liu Y., Woodruff G., Lovenberg T., Kohlnhofer B., Liu Y., Woodruff G., BoNaVenture P., and Harrington AW.Neuropsychiatric disorders such as schizophrenia are challenging to treat due to the biological complexity of the disease and the lack of knowledge of the underlying pathophysiology. Whole exome and genome sequencing studies have identified disease-linked rare variants in patients with large effect size. Here, we functionally characterize the schizophrenia linked variant V1282F inSCN2A, encoding the sodium channel NaV1.2. This variant was introduced into isogenic lines of hiPSCs using CRISPR/CAS9 genome editing tools. hiPSCs were then differentiated into cortical neurons to understand how the variant and gene may be contributing to disease. We observed a significant (~25%) decrease in sodium current in the V1282F neurons compared to control neurons,suggesting the mutation is causing a loss-of-channel function. These results were supported by recordings in recombinant cells overexpressing either the mutant or wildtype NaV1.2, with the mutant channel having significantly (~75%) lower current amplitude than wildtype. We hypothesize that this phenotype maycontribute to disease either through the direct loss of neuronal activity or through subsequent abnormal neurodevelopment.Publication link2022 A calibrated functional patch-clamp assay to enhance clinical variant interpretation in KCNH2-related long QT syndromeSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication inThe American Journal of Human Genetics (2022) Authors: Jiang C., Richardson E., Farr J., Hill A.P., Ullah R., Kroncke B.M., Harrison S.M., Thomson K.L., Ingles J., Vandenberg J.I., CA Ng.Modern sequencing technologies have revolutionized our detection of gene variants. However, in most genes, includingKCNH2, the majority of missense variants are currently classified as variants of uncertain significance (VUSs). The aim of this study was to investigate the utility of an automated patch-clamp assay for aiding clinical variant classification inKCNH2. The assay was designed according to recommendations proposed by the Clinical Genome Sequence Variant Interpretation Working Group. Thirty-one variants (17 pathogenic/likely pathogenic, 14 benign/likely benign) were classified internally as variant controls. They were heterozygously expressed in Flp-In HEK293 cells for assessing the effects of variants on current density and channel gating in order to determine the sensitivity and specificity of the assay. All 17 pathogenic variant controls had reduced current density, and 13 of 14 benign variant controls had normal current density, which enabled determination of normal and abnormal ranges for applying evidence of moderate or supporting strength for VUS reclassification. Inclusion of functional assay evidence enabled us to reclassify 6 out of 44KCNH2VUSs as likely pathogenic. The high-throughput patch-clamp assay can provide moderate-strength evidence for clinical interpretation of clinicalKCNH2variants and demonstrates the value of developing automated patch-clamp assays for functional characterization of ion channel gene variants.Publication link2021 The insecticide deltamethrin enhances sodium channel slow inactivation of human NaV1.9, NaV1.8 and NaV1.7SyncroPatch 384i (a predecessor model of the SyncroPatch 384) Publication in Toxicology and Applied Pharmacology (2021) Authors: Bothe S.N., Lampert A.The insecticide deltamethrin of the pyrethroid class mainly targets voltage-gated sodium channels (NaVs). Deltamethrin prolongs the opening of NaVs by slowing down fast inactivation and deactivation. Pyrethroids are supposedly safe for humans, however, they have also been linked to the gulf-war syndrome, a neuropathic pain condition that can develop following exposure to certain chemicals. Inherited neuropathic pain conditions have been linked to mutations in the NaV subtypes NaV1.7, NaV1.8, and NaV1.9. Here, we examined the effect of deltamethrin on the human isoforms NaV1.7, NaV1.8, and NaV1.9_C4 (chimera containing the C-terminus of rat NaV1.4) heterologously expressed in HEK293T and ND7/23 cells using whole-cell patch-clamp electrophysiology. For all three NaV subtypes, we observed increased persistent and tail currents that are typical for NaV channels modified by deltamethrin. The most surprising finding was an enhanced slow inactivation induced by deltamethrin in all three NaV subtypes. An enhanced slow inactivation is contrary to the prolonged opening caused by pyrethroids and has not been described for deltamethrin or any other pyrethroid before. Furthermore, we found that the fraction of deltamethrin-modified channels increased use-dependently. However, for NaV1.8, the use-dependent potentiation occurred only when the holding potential was increased to 90mV, a potential at which the tail currents decay more slowly. This indicates that use-dependent modification is due to an accumulation of tail currents. In summary, our findings support a novel mechanism whereby deltamethrin enhances slow inactivation of voltage-gated sodium channels, which may, depending on the cellular resting membrane potential, reduce neuronal excitability and counteract the well-described pyrethroid effects on channel activation.Publication link2021 The M1 and pre-M1 segments contribute differently to ion selectivity in ASICs and ENaCsSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication in Journal of General Physiology(2021) Authors: Sheikh Z.P. , Wulf M., Friis S., Althaus M., Lynagh T., Pless S.A.The ability to discriminate between different ionic species, termed ion selectivity, is a key feature of ion channels and forms the basis for their physiological function. Members of the degenerin/epithelial sodium channel (DEG/ENaC) superfamily of trimeric ion channels are typically sodium selective, but to a surprisingly variable degree. While acid-sensing ion channels (ASICs) are weakly sodium selective (sodium:potassium ratio 10:1), ENaCs show a remarkably high preference for sodium over potassium (>500:1). This discrepancy may be expected to originate from differences in the pore-lining second transmembrane segment (M2). However, these show a relatively high degree of sequence conservation between ASICs and ENaCs, and previous functional and structural studies could not unequivocally establish that differences in M2 alone can account for the disparate degrees of ion selectivity. By contrast, surprisingly little is known about the contributions of the first transmembrane segment (M1) and the preceding pre-M1 region. In this study, we used conventional and noncanonical amino acidbased mutagenesis in combination with a variety of electrophysiological approaches to show that the pre-M1 and M1 regions of mASIC1a channels are major determinants of ion selectivity. Mutational investigations of the corresponding regions in hENaC show that these regions contribute less to ion selectivity, despite affecting ion conductance. In conclusion, our work suggests that the remarkably different degrees of sodium selectivity in ASICs and ENaCs are achieved through different mechanisms. These results further highlight how M1 and pre-M1 are likely to differentially affect pore structure in these related channels.Publication link2021 Neurogranin, Encoded by the Schizophrenia Risk Gene NRGN, Bidirectionally Modulates Synaptic Plasticity via Calmodulin-Dependent Regulation of the Neuronal PhosphoproteomeSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication in Biological Psychiatry(2021) Authors: Hwang H., Szucs M.J., Ding L.J., Allen A., Ren X., Haensgen H., Gao F., Rhim H., Andrade A., Pan J.Q., Carr S.A., Ahmad R., Xu W.Neurogranin (Ng), encoded by the schizophrenia risk gene NRGN, is a calmodulin-binding protein enriched in the postsynaptic compartments, and its expression is reduced in the postmortem brains of patients with schizophrenia. Experience-dependent translation of Ng is critical for encoding contextual memory, and Ng regulates developmental plasticity in the primary visual cortex during the critical period. However, the overall impact of Ng on the neuronal signaling that regulates synaptic plasticity is unknown.Publication link2021 Small molecule modulation of the Drosophila Slo channel elucidated by cryo-EMSyncroPatch 384 Publication in Nature Communications (2021) Authors: Raisch T., Brockmann A., Ebbinghaus-Kintscher U., Freigang J., Gutbrod O., Kubicek J., Maertens B., Hofnagel O., Raunser S.Slowpoke (Slo) potassium channels display extraordinarily high conductance, are synergistically activated by a positive transmembrane potential and high intracellular Ca2+ concentrations and are important targets for insecticides and antiparasitic drugs. However, it is unknown how these compounds modulate ion translocation and whether there are insect-specific binding pockets. Here, we report structures of Drosophila Slo in the Ca2+-bound and Ca2+-free form and in complex with the fungal neurotoxin verruculogen and the anthelmintic drug emodepside. Whereas the architecture and gating mechanism of Slo channels are conserved, potential insect-specific binding pockets exist. Verruculogen inhibits K+ transport by blocking the Ca2+-induced activation signal and precludes K+ from entering the selectivity filter. Emodepside decreases the conductance by suboptimal K+ coordination and uncouples ion gating from Ca2+ and voltage sensing. Our results expand the mechanistic understanding of Slo regulation and lay the foundation for the rational design of regulators of Slo and other voltage-gated ion channels.Publication link2021 Mechanism of hERG inhibition by gating-modifier toxin, APETx1, deduced by functional characterizationSyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publication inBMC Molecular and Cell Biology(2021) Authors: Matsumura K., Shimomura T., Kubo Y., Oka T., Kobayashi N., Imai S., Yanase N., Akimoto M., Fukuda M., Yokogawa M., Ikeda K., Kurita J., Nishimura Y., Shimada I., Osawa M.Human ether--go-go-related gene potassium channel 1 (hERG) is a voltage-gated potassium channel, the voltage-sensing domain (VSD) of which is targeted by a gating-modifier toxin, APETx1. APETx1 is a 42-residue peptide toxin of sea anemone Anthopleura elegantissima and inhibits hERG by stabilizing the resting state. A previous study that conducted cysteine-scanning analysis of hERG identified two residues in the S3-S4 region of the VSD that play important roles in hERG inhibition by APETx1. However, mutational analysis of APETx1 could not be conducted as only natural resources have been available until now. Therefore, it remains unclear where and how APETx1 interacts with the VSD in the resting state.Publication link2021 Neuroactive Type-A -Aminobutyric Acid Receptor Allosteric Modulator Steroids from the Hypobranchial Gland of Marine Mollusk, Conus geographusSyncroPatch 384i (a predecessor model of the SyncroPatch 384) Publication in Journal of Medicinal Chemistry (2021) Authors: Niu C., Leavitt L. S., Lin Z., Paguigan N. D., Sun L., Zhang J., Torres J. P., Raghuraman S., Chase K., Cadeddu R., Karthikeyan M., Bortolato M., Reilly C. A., Hughes R. W., Light A. R., Olivera B. M., Schmidt E. W.In a program to identify pain treatments with low addiction potential, we isolated five steroids, conosteroids AE (15), from the hypobranchial gland of the mollusk Conus geographus. Compounds 15 were active in a mouse dorsal root ganglion (DRG) assay that suggested that they might be analgesic. A synthetic analogue 6 was used for a detailed pharmacological study. Compound 6 significantly increased the pain threshold in mice in the hot-plate test at 2 and 50 mg/kg. Compound 6 at 500 nM antagonizes type-A -aminobutyric acid receptors (GABAARs). In a patch-clamp experiment, out of the six subunit combinations tested, 6 exhibited subtype selectivity, most strongly antagonizing 112 and 432 receptors (IC50 1.5 and 1.0 M, respectively). Although the structures of 16 differ from those of known neuroactive steroids, they are cell-type-selective modulators of GABAARs, expanding the known chemical space of neuroactive steroids.Publication link2021 Induced Pluripotent Stem Cell-Derived Cardiomyocytes with SCN5A R1623Q Mutation Associated with Severe Long QT Syndrome in Fetuses and Neonates Recapitulates Pathophysiological PhenotypesSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication in Biology(2021) Authors: Hayama E., Furutani Y., Kawaguchi N., Seki A., Nagashima Y., Okita K., Takeuchi D., Matsuoka R., Inai K., Hagiwara N., Nakanishi T.The SCN5A R1623Q mutation is one of the most common genetic variants associated with severe congenital long QT syndrome 3 (LQT3) in fetal and neonatal patients. To investigate the properties of the R1623Q mutation, we established an induced pluripotent stem cell (iPSC) cardiomyocyte (CM) model from a patient with LQTS harboring a heterozygous R1623Q mutation. The properties and pharmacological responses of iPSC-CMs were characterized using a multi-electrode array system. The biophysical characteristic analysis revealed that R1623Q increased open probability and persistent currents of sodium channel, indicating a gain-of-function mutation. In the pharmacological study, mexiletine shortened FPDcF in R1623Q-iPSC-CMs, which exhibited prolonged field potential duration corrected by Fridericias formula (FPDcF, analogous to QTcF). Meanwhile, E4031, a specific inhibitor of human ether-a-go-go-related gene (hERG) channel, significantly increased the frequency of arrhythmia-like early after depolarization (EAD) events. These characteristics partly reflect the patient phenotypes. To further analyze the effect of neonatal isoform, which is predominantly expressed in the fetal period, on the R1623Q mutant properties, we transfected adult form and neonatal isoform SCN5A of control and R1623Q mutant SCN5A genes to 293T cells. Whole-cell automated patch-clamp recordings revealed that R1623Q increased persistent Na+ currents, indicating a gain-of-function mutation. Our findings demonstrate the utility of LQT3-associated R1623Q mutation-harboring iPSC-CMs for assessing pharmacological responses to therapeutic drugs and improving treatment efficacy. Furthermore, developmental switching of neonatal/adult NaV1.5 isoforms may be involved in the pathological mechanisms underlying severe long QT syndrome in fetuses and neonates.Publication link2021 Machine Learning Strategies When Transitioning between Biological AssaysSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication in Journal of Chemical Infromation and Modeling(2021) Authors: McShane S. A., Ahlberg E., Noeske T., Spjuth O.Machine learning is widely used in drug development to predict activity in biological assays based on chemical structure. However, the process of transitioning from one experimental setup to another for the same biological endpoint has not been extensively studied. In a retrospective study, we here explore different modeling strategies of how to combine data from the old and new assays when training conformal prediction models using data from hERG and NaV assays. We suggest to continuously monitor the validity and efficiency of models as more data is accumulated from the new assay and select a modeling strategy based on these metrics. In order to maximize the utility of data from the old assay, we propose a strategy that augments the proper training set of an inductive conformal predictor by adding data from the old assay but only having data from the new assay in the calibration set, which results in valid (well-calibrated) models with improved efficiency compared to other strategies. We study the results for varying sizes of new and old assays, allowing for discussion of different practical scenarios. We also conclude that our proposed assay transition strategy is more beneficial, and the value of data from the new assay is higher, for the harder case of regression compared to classification problems.Publication link2021 High-throughput characterization of photocrosslinker-bearing ion channel variants to map residues critical for function and pharmacologySyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publicationin PLoS Biology (2021) Authors: Braun N., Friis S., Ihling C., Sinz A., Andersen J., Pless S.A.Incorporation of noncanonical amino acids (ncAAs) can endow proteins with novel functionalities, such as crosslinking or fluorescence. In ion channels, the function of these variants can be studied with great precision using standard electrophysiology, but this approach is typically labor intensive and low throughput. Here, we establish a high-throughput protocol to conduct functional and pharmacological investigations of ncAA-containing human acid-sensing ion channel 1a (hASIC1a) variants in transiently transfected mammalian cells. We introduce 3 different photocrosslinking ncAAs into 103 positions and assess the function of the resulting 309 variants with automated patch clamp (APC). We demonstrate that the approach is efficient and versatile, as it is amenable to assessing even complex pharmacological modulation by peptides. The data show that the acidic pocket is a major determinant for current decay, and live-cell crosslinking provides insight into the hASIC1apsalmotoxin 1 (PcTx1) interaction. Further, we provide evidence that the protocol can be applied to other ion channels, such as P2X2 and GluA2 receptors. We therefore anticipate the approach to enable future APC-based studies of ncAA-containing ion channels in mammalian cells.Publication link2021 High-Throughput Screening of TRPV1 Ligands in the Light of the Bioluminescence Resonance Energy Transfer TechniqueSyncroPatch 384 Publication in Molecular Pharmacology (2021) Authors: Chappe Y., Michel P., Joushomme A., Barbeau S., Pierredon S., Baron L., Garenne A., Poulletier De Gannes F., Hurtier A., Mayer S., Lagroye I., Quignard J-F., Ducret T., Compan V., Franchet C., Percherancier Y.Ion channels are attractive drug targets for many therapeutic applications. However, high-throughput screening (HTS) of drug candidates is difficult and remains very expensive. We thus assessed the suitability of the bioluminescence resonance energy transfer (BRET) technique as a new HTS method for ion-channel studies by taking advantage of our recently characterized intra- and intermolecular BRET probes targeting the transient receptor potential vanilloid type 1 (TRPV1) ion channel. These BRET probes monitor conformational changes during TRPV1 gating and subsequent coupling with calmodulin, two molecular events that are intractable using reference techniques such as automated calcium assay (ACA) and automated patch-clamp (APC). We screened the small-sized Prestwick chemical library, encompassing 1200 compounds with high structural diversity, using either intra- and intermolecular BRET probes or ACA. Secondary screening of the detected hits was done using APC. Multiparametric analysis of our results shed light on the capability of calmodulin inhibitors included in the Prestwick library to inhibit TRPV1 activation by capsaicin. BRET was the lead technique for this identification process. Finally, we present data exemplifying the use of intramolecular BRET probes to study other transient receptor potential (TRP) channels and non-TRPs ion channels. Knowing the ease of use of BRET biosensors and the low cost of the BRET technique, these assays may advantageously be included for extending ion-channel drug screening.Publication link2021 From High-Throughput Screening to Target Validation: Benzo[d]isothiazoles as Potent and Selective Agonists of Human Transient Receptor Potential Cation Channel Subfamily M Member 5 Possessing In Vivo Gastrointestinal Prokinetic Activity in RodentsSyncroPatch 384 Publicationin Journal of Medicinal Chemistry (2021) Authors: Barilli A., Aldegheri L., Bianchi F., Brault L., Brodbeck D., Castelletti L., Feriani A., Lingard I., Myers R., Nola S., Piccoli L., Pompilio D.,Raveglia L.F., Salvagno C., Tassini S., Virginio C., Sabat M.Transient receptor potential cation channel subfamily M member 5 (TRPM5) is a nonselective monovalent cation channel activated by intracellular Ca2+increase. Within the gastrointestinal system, TRPM5 is expressed in the stoma, small intestine, and colon. In the search for a selective agonist of TRPM5 possessing in vivo gastrointestinal prokinetic activity, a high-throughput screening was performed and compound 1 was identified as a promising hit. Hit validation and hit to lead activities led to the discovery of a series of benzo[d]isothiazole derivatives. Among these, compounds 61 and 64 showed nanomolar activity and excellent selectivity (>100-fold) versus related cation channels. The in vivo drug metabolism and pharmacokinetic profile of compound 64 was found to be ideal for a compound acting locally at the intestinal level, with minimal absorption into systemic circulation. Compound 64 was tested in vivo in a mouse motility assay at 100 mg/kg, and demonstrated increased prokinetic activity.Publication link2021 Heterozygous KCNH2 variant phenotyping using Flp-In HEK293 and high-throughput automated patch clamp electrophysiologySyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication in Biol. Methods Protoc.(2021) Authors: Ng C-A., Farr J., Young P., Windley M.J., Perry M.D., Hill A.P., Vandenberg J.I.KCNH2 is one of the 59 medically actionable genes recommended by the American College of Medical Genetics for reporting of incidental findings from clinical genomic sequencing. However, half of the reported KCNH2 variants in the ClinVar database are classified as variants of uncertain significance. In the absence of strong clinical phenotypes, there is a need for functional phenotyping to help decipher the significance of variants identified incidentally. Here, we report detailed methods for assessing the molecular phenotype of any KCNH2 missense variant. The key components of the assay include quick and cost-effective generation of a bicistronic vector to co-express WT and any KCNH2 variant allele, generation of stable Flp-In HEK293 cell lines and high-throughput automated patch-clamp electrophysiology analysis of channel function. Stable cell lines take 3-4 weeks to produce and can be generated in bulk, which will then allow up to 30 variants to be phenotyped per week after 48 hours of channel expression. This high throughput functional genomics assay will enable a much more rapid assessment of the extent of loss of function of any KCNH2 variant.Publication link2021 Fluorescent- and tagged-protoxin II peptides: potent markers of the NaV1.7 channel pain targetSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publicationin British Journal of Pharmacological (2021) Authors: Montnach J., De Waard S., Nicolas S., Burel S., Osorio N., Zoukimian C., Mantegazza M., Boukaiba R., Broud R., Partiseti M., Delmas P., Marionneau C., De Waard M.Protoxin II (ProTx II) is a high affinity gating modifier that is thought to selectively block the NaV1.7 voltage-dependent Na+channel, a major therapeutic target for the control of pain. We aimed at producing ProTx II analogues entitled with novel functionalities for cell distribution studies and biochemical characterization of its NaVchannel targets.Publication link2021 Fragment-Based Design of a Potent MAT2a Inhibitor and in Vivo Evaluation in an MTAP Null Xenograft ModelSyncroPatch 384 Publication in Journal of Medicinal Chemistry (2021) Authors: De Fusco C., Schimpl M., Brjesson U., Cheung T., Collie I., Evans L., Narasimhan P., Stubbs C., Vazquez-Chantada M., Wagner D. J., Grondine M., Sanders M. G., Tentarelli S., Underwood E., Argyrou A., Smith J. M., Lynch J. T., Chiarparin E., Robb G., Bagal S. K., Scott J. S.MAT2a is a methionine adenosyltransferase that synthesizes the essential metabolite S-adenosylmethionine (SAM) from methionine and ATP. Tumors bearing the co-deletion of p16 and MTAP genes have been shown to be sensitive to MAT2a inhibition, making it an attractive target for treatment of MTAP-deleted cancers. A fragment-based lead generation campaign identified weak but efficient hits binding in a known allosteric site. By use of structure-guided design and systematic SAR exploration, the hits were elaborated through a merging and growing strategy into an arylquinazolinone series of potent MAT2a inhibitors. The selected in vivo tool compound 28 reduced SAM-dependent methylation events in cells and inhibited proliferation of MTAP-null cells in vitro. In vivo studies showed that 28 was able to induce antitumor response in an MTAP knockout HCT116 xenograft model.Publication link2021 Disease-linked super-trafficking of a potassium channelSyncroPatch 768 PE(a predecessor model of the SyncroPatch 384 instrument) Publication in Journal of Biological Chemistry (2021) Authors: Huang H.,Chamness L.M., Vanoye C.G., Kuenze G., Meiler J., George A.L., Schlebach J.P., Sanders C.R.Gain-of-function (GOF) mutations in the KCNQ1 voltage-gated potassium channel can induce cardiac arrhythmia. Here it was tested whether any of the known human GOF disease mutations in KCNQ1 act by increasing the amount of KCNQ1 that reaches the cell surfacesuper-trafficking. Seven out of the 15 GOF mutants tested were seen to surface-traffic more efficiently than the wild type (WT) channel. Among these we found that levels of R231C KCNQ1 in the plasma membrane were 5-fold higher than the WT channel. This was shown to arise from the combined effects of enhanced efficiency of translocon-mediated membrane integration of the S4 voltage-sensor helix and from enhanced post-translational folding/trafficking that is related to energetic linkage of C231 with the V129 and F166 side chains. Whole-cell electrophysiology recordings confirmed that R231C KCNQ1 in complex with KCNE1 exhibits constitutive conductance, but also revealed that the single channel activity of this mutant is only 20% that of WT. The GOF phenotype associated with R231C therefore reflects the effects of super-trafficking and constitutive channel activation, which together offset reduced channel activity. These investigations show that membrane protein super-trafficking can contribute to human disease.Publication link2021 Dyshomeostatic modulation of Ca2+-activated K+ channels in a human neuronal model of KCNQ2 encephalopathySyncroPatch 768 PE(a predecessor model of the SyncroPatch 384 instrument) Publication in Nature (2021) Authors: Simkin D., Marshall K.A., Vanoye C.G., Desai R.R., Bustos B.I., Piyevsky B.N., Ortega J.A., Forrest M., Robertson G.L., Penzes P., Laux L.C., Lubbe S.J., Millichap J.J., George Jr A.L., Kiskinis E.Mutations in KCNQ2, which encodes a pore-forming K+channel subunit responsible for neuronal M-current, cause neonatal epileptic encephalopathy, a complex disorder presenting with severe early-onset seizures and impaired neurodevelopment. The condition is exceptionally difficult to treat, partially because the effects of KCNQ2 mutations on the development and function of human neurons are unknown. Here, we used induced pluripotent stem cells (iPSCs) and gene editing to establish a disease model and measured the functional properties of differentiated excitatory neurons. We find that patient iPSC-derived neurons exhibit faster action potential repolarization, larger post-burst afterhyperpolarization and a functional enhancement of Ca2+-activated K+channels. These properties, which can be recapitulated by chronic inhibition of M-current in control neurons, facilitate a burst-suppression firing pattern that is reminiscent of the interictal electroencephalography pattern in patients. Our findings suggest that dyshomeostatic mechanisms compound KCNQ2 loss-of-function leading to alterations in the neurodevelopmental trajectory of patient iPSC-derived neurons.Publication link2021 Computer modeling of whole-cell voltage-clamp analyses to delineate guidelines for good practice of manual and automated patch-clampSyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publication in Scientific Reports (2021) Authors: Montnach J., Lorenzini M., Lesage A., Simon I., Nicolas S., Moreau E., Marionneau C., Bar I., De Waard M., Loussouar G.The patch-clamp technique and more recently the high throughput patch-clamp technique have contributed to major advances in the characterization of ion channels. However, the whole-cell voltage-clamp technique presents certain limits that need to be considered for robust data generation. One major CaVeat is that increasing current amplitude profoundly impacts the accuracy of the biophysical analyses of macroscopic ion currents under study. Using mathematical kinetic models of a cardiac voltage-gated sodium channel and a cardiac voltage-gated potassium channel, we demonstrated how large current amplitude and series resistance artefacts induce an undetected alteration in the actual membrane potential and affect the characterization of voltage-dependent activation and inactivation processes. We also computed how doseresponse curves are hindered by high current amplitudes. This is of high interest since stable cell lines frequently demonstrating high current amplitudes are used for safety pharmacology using the high throughput patch-clamp technique. It is therefore critical to set experimental limits for current amplitude recordings to prevent inaccuracy in the characterization of channel properties or drug activity, such limits being different from one channel type to another. Based on the predictions generated by the kinetic models, we draw simple guidelines for good practice of whole-cell voltage-clamp recordings.Publication link2021 Discovery of the First Orally Available, Selective KNa1.1 Inhibitor: In Vitro and In Vivo Activity of an Oxadiazole SeriesSyncroPatch 384 Publication in ACS Medicinal Chemistry Letters (2021) Authors: Griffin A. M., Kahlig K. M., Hatch R. J., Hughes Z. A., Chapman M. L., Antonio B., Marron B. E., Wittmann M., Martinez-Botella G.The gene KCNT1 encodes the sodium-activated potassium channel KNa1.1 (Slack, Slo2.2). Variants in the KCNT1 gene induce a gain-of-function (GoF) phenotype in ionic currents and cause a spectrum of intractable neurological disorders in infants and children, including epilepsy of infancy with migrating focal seizures (EIMFS) and autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). Effective treatment options for KCNT1-related disease are absent, and novel therapies are urgently required. We describe the development of a novel class of oxadiazole KNa1.1 inhibitors, leading to the discovery of compound 31 that reduced seizures and interictal spikes in a mouse model of KCNT1 GoF.Publication link2021 Chemical modulation of KV7 potassium channelsSyncroPatch 384 Publication in RSC Medicinal Chemistry (2021) Authors: Borgini M., Mondal P., Liu R., Wipf P.The rising interest in KV7 modulators originates from their ability to evoke fundamental electrophysiological perturbations in a tissue-specific manner. A large number of therapeutic applications are, in part, based on the clinical experience with two broad-spectrum KV7 agonists, flupirtine and retigabine. Since precise molecular structures of human KV7 channel subtypes in closed and open states have only very recently started to emerge, computational studies have traditionally been used to analyze binding modes and direct the development of more potent and selective KV7 modulators with improved safety profiles. Herein, the synthetic and medicinal chemistry of small molecule modulators and the representative biological properties are summarized. Furthermore, new therapeutic applications supported by in vitro and in vivo assay data are suggested.Publication link2022 Pathophysiological metabolic changes associated with disease modify the proarrhythmic risk profile of drugs with potential to prolong repolarisationSyncroPatch 384PE(a predecessor model of the SyncroPatch 384 instrument) Publication in Br. J. Pharmacol.(2022) Authors: TeBay C., McArthur J., Mangala M., Kerr N., Heitmann S., Perry M., Windley M., Vandenberg J., Hill A.Hydroxychloroquine, chloroquine and azithromycin are three drugs that were proposed to treat coronavirus disease 2019 (COVID-19). While concern already existed around their proarrhythmic potential, there are little data regarding how altered physiological states encountered in patients such as febrile state, electrolyte imbalances or acidosis might change their risk profiles. Potency of human ether--go-go related gene (hERG) block was measured using high-throughput electrophysiology in the presence of variable environmental factors. These potencies informed simulations to predict population risk profiles. Effects on cardiac repolarisation were verified in human induced pluripotent stem cell-derived cardiomyocytes from multiple individuals. Chloroquine and hydroxychloroquine blocked hERG with IC50 of 1.470.07 and 3.780.17M, respectively, indicating proarrhythmic risk at concentrations effective against severe acute respiratory syndrome-coronovirus-2 (SARS-CoV-2) in vitro. Hypokalaemia and hypermagnesaemia increased potency of chloroquine and hydroxychloroquine, indicating increased proarrhythmic risk. Acidosis significantly reduced potency of all drugs, whereas increased temperature decreased potency of chloroquine and hydroxychloroquine against hERG but increased potency for azithromycin. In silico simulations demonstrated that proarrhythmic risk was increased by female sex, hypokalaemia and heart failure and identified specific genetic backgrounds associated with emergence of arrhythmia.Our study demonstrates how proarrhythmic risk can be exacerbated by metabolic changes and pre-existing disease. More broadly, the study acts as a blueprint for how high-throughput in vitro screening, combined with in silico simulations, can help guide both preclinical screening and clinical management of patients in relation to drugs with potential to prolong repolarisation.Publication link2021 Cation and anion channelrhodopsins: Sequence motifs and taxonomic distributionSyncroPatch 384i (a predecessor model of the SyncroPatch 384) Publication in ASM Journals(2021) Authors: Govorunova E.G., Sineshchekov O.A., Li H., Wang Y., Brown L.S., Palmateer A., Melkonian M., Cheng S., Carpenter E., Patterson J., Wong G. K-S., Spudich J.L.Cation and anion channelrhodopsins (CCRs and ACRs, respectively) primarily from two algal species, Chlamydomonas reinhardtii and Guillardia theta, have become widely used as optogenetic tools to control cell membrane potential with light. We mined algal and other protist polynucleotide sequencing projects and metagenomic samples to identify 75 channelrhodopsin homologs from three channelrhodopsin families, including one revealed in dinoflagellates in this study. We carried out electrophysiological analysis of 33 natural channelrhodopsin variants from different phylogenetic lineages and 10 metagenomic homologs in search of sequence determinants of ion selectivity, photocurrent desensitization, and spectral tuning in channelrhodopsins. Our results show that association of a reduced number of glutamates near the conductance path with anion selectivity depends on a wider protein context, because prasinophyte homologs with the identical glutamate pattern as in cryptophyte ACRs are cation-selective. Desensitization is also broadly context-dependent, as in one branch of stramenopile ACRs and their metagenomic homologs its extent roughly correlates with phylogenetic relationship of their sequences. Regarding spectral tuning, two prasinophyte CCRs exhibit red-shifted spectra to 585 nm, although their retinal-

KCNH2 traffickingSyncroPatch 384PE (a predecessor model of the SyncroPatch 384i instrument) Pre-Publication in bioRxiv Biology (2020) Authors: Kozek K.A., Glazer A.M., Ng C.-A., Blackwell D., Egly C.L., Vanags L.R., Blair M., Mitchell D., Matreyek K.A., Fowler D.M., Knollmann B.C., Vandenberg J., Roden D.M., Kroncke B.M.Background: KCHN2 encodes the KV11.1 potassium channel responsible for IKr, a major repolarization current during the cardiomyocyte action potential. Variants in KCNH2 that decrease IKrcan cause Type 2 Long QT syndrome, usually due to mistrafficking to the cell surface. Accurately discriminating between variants with normal and abnormal trafficking would help clinicians identify and treat individuals at risk of a major cardiac event. The volume of reported non-synonymous KCNH2 variants preclude the use of conventional electrophysiologic methods for functional study. Objective: To report a high-throughput, multiplexed screening method for KCNH2 genetic variants capable of measuring the cell surface abundance of hundreds of missense variants in KCNH2. Methods: We develop a method to quantitate KCNH2 variant trafficking on a pilot region of 11 residues in the S5 helix, and generate trafficking scores for 220/231 missense variants in this region. Results: For 5/5 variants, high-throughput trafficking scores validated when tested in single variant flow cytometry and confocal microscopy experiments. We additionally compare our results with planar patch electrophysiology and find that loss-of-trafficking variants do not produce IKr, but that some variants which traffic normally may still be functionally compromised. Conclusions: Here, we describe a new method for detecting trafficking-deficient variants in KCNH2 in a multiplexed assay. This new method accurately generates trafficking data for variants in KCNH2 and can be readily extended to all residues in KV11.1 and to other cell surface proteins. Clinical Implications: Hundreds of KCNH2 variants have been observed to date, and thousands more will be found as clinical and population sequencing efforts become increasingly widespread. The major mechanism of KV11.1 loss of function is misfolding and failure to traffic to the cell surface. Deep mutational scanning of KCNH2 trafficking is a scalable, high-throughput method that can help identify new loss of function variants and decipher the large number of KCNH2 variants being found in the population.Publication link2020 Conservation and divergence in NaChBac and NaV1.7 pharmacology reveals novel drug interaction mechanismsSyncroPatch 768PE (a predecessor model of the SyncroPatch 768i instrument) Publication in Nature Scientific Reports (2020) Authors: Zhu W., Li T., Silva J. R., Chen J.Voltage-gated Na+(NaV) channels regulate homeostasis in bacteria and control membrane electrical excitability in mammals. Compared to their mammalian counterparts, bacterial NaVchannels possess a simpler, fourfold symmetric structure and have facilitated studies of the structural basis of channel gating. However, the pharmacology of bacterial NaVremains largely unexplored. Here we systematically screened 39 NaVmodulators on a bacterial channel (NaChBac) and characterized a selection of compounds on NaChBac and a mammalian channel (human NaV1.7). We found that while many compounds interact with both channels, they exhibit distinct functional effects. For example, the local anesthetics ambroxol and lidocaine block both NaV1.7 and NaChBac but affect activation and inactivation of the two channels to different extents. The voltage-sensing domain targeting toxin BDS-I increases NaV1.7 but decreases NaChBac peak currents. The pore binding toxins aconitine and veratridine block peak currents of NaV1.7 and shift activation (aconitine) and inactivation (veratridine) respectively. In NaChBac, they block the peak current by binding to the pore residue F224. Nonetheless, aconitine has no effect on activation or inactivation, while veratridine only modulates activation of NaChBac. The conservation and divergence in the pharmacology of bacterial and mammalian NaVchannels provide insights into the molecular basis of channel gating and will facilitate organism-specific drug discovery.Publication link2020 Discovery of AZD9833, a Potent and Orally Bioavailable Selective Estrogen Receptor Degrader and AntagonistSyncroPatch 768PE (a predecessor model of the SyncroPatch 768i instrument) Publication in Medicinal Chemistry (2020) Authors: Scott J.S., Moss T.A., Balazs A., Barlaam B., Breed J., Carbajo R.J., Chiarparin E., Davey P.R.J., Delpuech O., Fawell S., Fisher D.I., Gagrica S., Gangl E.T., Grebe T., Greenwood R.D., Hande S., Hatoum-Mokdad H., Herlihy K., Hughes S., Hunt T.A., Huynh H., Janbon S.L.M., Johnson T., Kavanagh S., Klinowska T., Lawson M., Lister A.S., Marden S., McGinnity D.F., Morrow C.J., Nissink W.M., O'Donovan D.H., Peng B., Polanski R., Stead D.S., Stokes S., Thakur K., Throner S.R., Tucker M.J., Varnes J., Wang H., Wilson D.M., Wu D., Wu Y., Yang B., Yang W.Herein we report the optimization of a series of tricyclic indazoles as selective estrogen receptor degraders (SERD) and antagonists for the treatment of ER+breast cancer. Structure based design together with systematic investigation of each region of the molecular architecture led to the identification ofN-[1-(3-fluoropropyl)azetidin-3-yl]-6-[(6S,8R)-8-methyl-7-(2,2,2-trifluoroethyl)-6,7,8,9-tetrahydro-3H-pyrazolo[4,3-f]isoquinolin-6-yl]pyridin-3-amine (28). This compound was demonstrated to be a highly potent SERD that showed a pharmacological profile comparable to fulvestrant in its ability to degrade ER in both MCF-7 and CAMA-1 cell lines. A stringent control of lipophilicity ensured that28had favorable physicochemical and preclinical pharmacokinetic properties for oral administration. This, combined with demonstration of potent in vivo activity in mouse xenograft models, resulted in progression of this compound, also known as AZD9833, into clinical trials.Publication link2020 Allosteric mechanism for KCNE1 modulation of KCNQ1 potassium channel activationSyncropatch 768 PE Publication in eLife (2020) Authors: Kuenze G., Vanoye C.G., Desai R.R., Adusumili S., Brewer K.R., Woods H., McDonald E.F., Sanders C.R., George Jr. A.L.The function of the voltage-gated KCNQ1 potassium channel is regulated by co-assembly with KCNE auxiliary subunits. KCNQ1-KCNE1 channels generate the slow delayed rectifier current, IKs, which contributes to the repolarization phase of the cardiac action potential. A three amino acid motif (F57-T58-L59, FTL) in KCNE1 is essential for slow activation of KCNQ1-KCNE1 channels. However, how this motif interacts with KCNQ1 to control its function is unknown. Combining computational modeling with electrophysiological studies, we developed structural models of the KCNQ1-KCNE1 complex that suggest how KCNE1 controls KCNQ1 activation. The FTL motif binds at a cleft between the voltage-sensing and pore domains and appears to affect the channel gate by an allosteric mechanism. Comparison with the KCNQ1-KCNE3 channel structure suggests a common transmembrane-binding mode for different KCNEs and illuminates how specific differences in the interaction of their triplet motifs determine the profound differences in KCNQ1 functional modulation by KCNE1 versus KCNE3.Publication link2020 Application of High-Throughput Automated Patch-Clamp Electrophysiology to Study Voltage-Gated Ion Channel Function in Primary Cortical CulturesSyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in SLAS Discovery (2020) Authors: Toh M.F., Brooks J.M., Strassmaier T., Haedo R.J., Puryear C.B., Roth B.L., Ouk K., Pin S.S.Conventionally, manual patch-clamp electrophysiological approaches are the gold standard for studying ion channel function in neurons. However, these approaches are labor-intensive, yielding low-throughput results, and are therefore not amenable for compound profiling efforts during the early stages of drug discovery. The SyncroPatch 384PE has been successfully implemented for pharmacological experiments in heterologous overexpression systems that may not reproduce the function of voltage-gated ion channels in a native, heterogeneous environment. Here, we describe a protocol allowing the characterization of endogenous voltage-gated potassium (KV) and sodium (NaV) channel function in developing primary rat cortical cultures, allowing investigations at a significantly improved throughput compared with manual approaches. Key neuronal marker expression and microelectrode array recordings of electrophysiological activity over time correlated well with neuronal maturation. Gene expression data revealed high molecular diversity in KVand NaVsubunit composition throughout development. Voltage-clamp experiments elicited three major current components composed of inward and outward conductances. Further pharmacological experiments confirmed the endogenous expression of functional KVand NaVchannels in primary cortical neurons. The major advantages of this approach compared with conventional manual patch-clamp systems include unprecedented improvements in experimental ease and throughput for ion channel research in primary neurons. These efforts demonstrated feasibility for primary neuronal ion channel investigation with the SyncroPatch, providing the foundation for future studies characterizing biophysical changes in endogenous ion channels in primary systems associated with disease or development.Publication link2020 A general procedure to select calibration drugs for lab-specific validation and calibration of proarrhythmia risk prediction models: An illustrative example using the CiPA modelSyncroPatch 768PE (a predecessor model of the SyncroPatch 768i instrument) Publication in theJournal of Pharmacological and Toxicological Methods (2020) Authors: Han X., Samieegohar M., Ridder B.J., Wu W.W., Randolph A., Tran P., Sheng J., Stoelzle-Feiz S., Brinkwirth N., Rotordam M.G., Becker N., Friis S., Rapedius M., Goteze T.A., Strassmaier T., Okeyo G., Kramer J., Kuryshev Y., Li Z.In response to the ongoing shift of the regulatory cardiac safety paradigm, a recent White Paper proposed general principles for developing and implementing proarrhythmia risk prediction models. These principles included development strategies to validate models, and implementation strategies to ensure a model developed by one lab can be used by other labs in a consistent manner in the presence of lab-to-lab experimental variability. While the development strategies were illustrated through the validation of the model under the Comprehensive In vitro Proarrhythmia Assay (CiPA), the implementation strategies have not been adopted yet.Publication link2020 Accounting for variability in ion current recordings using a mathematical model of artefacts in voltage-clamp experimentsSyncroPatch 384PE (a predecessor model of the SyncroPatch 384i) Publication in Philosophical Transactions of the Royal Society A (2020) Authors: Lei, C.L., Clerx, M., Whittaker, D.G., Gavaghan, D.J., de Boer, T.P., Mirams, G.R.Mathematical models of ion channels, which constitute indispensable components of action potential models, are commonly constructed by fitting to whole-cell patch-clamp data. In a previous study, we fitted cell-specific models to hERG1a (KV11.1) recordings simultaneously measured using an automated high-throughput system, and studied cell-cell variability by inspecting the resulting model parameters. However, the origin of the observed variability was not identified. Here, we study the source of variability by constructing a model that describes not just ion current dynamics, but the entire voltage-clamp experiment. The experimental artefact components of the model include: series resistance, membrane and pipette capacitance, voltage offsets, imperfect compensations made by the amplifier for these phenomena, and leak current. In this model, variability in the observations can be explained by either cell properties, measurement artefacts, or both. Remarkably, by assuming that variability arises exclusively from measurement artefacts, it is possible to explain a larger amount of the observed variability than when assuming cell-specific ion current kinetics. This assumption also leads to a smaller number of model parameters. This result suggests that most of the observed variability in patch-clamp data measured under the same conditions is caused by experimental artefacts, and hence can be compensated for in post-processing by using our model for the patch-clamp experiment. This study has implications for the question of the extent to which cell-cell variability in ion channel kinetics exists, and opens up routes for better correction of artefacts in patch-clamp data.Publication link2019 Structure- and Ligand-Based Discovery of Chromane Arylsulfonamide NaV1.7 Inhibitors for the Treatment of Chronic PainSyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in Journal of Medicinal Chemistry (2019) Authors: McKerrall S.J., Nguyen T., Lai K.W., Bergeron P., Deng L., DiPasquale A., Chang J.H., Chen J., Chernov-Rogan T., Hackos D.H., Maher J., Ortwine D.F., Pang J., Payandeh J., Proctor W.R., Shields S.D., Vogt J., Ji P., Liu W., Ballini E., Schumann L., Tarozzo G., Bankar G., Chowdhury S., Hasan A., Johnson J.P. Jr., Khakh K., Lin S., Cohen C.J., Dehnhardt C.M., Safina B.S., Sutherlin D.P.Using structure- and ligand-based design principles, a novel series of piperidyl chromane arylsulfonamide NaV1.7 inhibitors was discovered. Early optimization focused on improvement of potency through refinement of the low energy ligand conformation and mitigation of high in vivo clearance. An in vitro hepatotoxicity hazard was identified and resolved through optimization of lipophilicity and lipophilic ligand efficiency to arrive at GNE-616 (24), a highly potent, metabolically stable, subtype selective inhibitor of NaV1.7. Compound 24 showed a robust PK/PD response in a NaV1.7-dependent mouse model, and site-directed mutagenesis was used to identify residues critical for the isoform selectivity profile of 24.Publication link2019 Synthesis by native chemical ligation and characterization of the scorpion toxin AmmTx3SyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in Bioorganic & Medicinal Chemistry (2019) Authors: Zoukimian C., Meudal H., De Waard S., Ait Quares K., Nicolas S., Canepari M., Broud R., Landon C., De Waard M., Boturyn D.The scorpion toxin AmmTx3 is a specific blocker of KV4 channels. It was shown to have interesting potential for neurological disorders. In this study, we report the first chemical synthesis of AmmTx3 by using the native chemical ligation strategy and validate its biological activity. We determined its 3D structure by nuclear magnetic resonance spectroscopy, and pointed out that AmmTx3 possesses the well-known CS structural motif, which is found in a large number of scorpion toxins. Overall, this study establishes an easy synthetic access to biologically active AmmTx3 toxin.Publication link2019 Spectrum of KV2.1 Dysfunction in KCNB1Associated Neurodevelopmental DisordersSyncroPatch 768PE (a predecessor model of the SyncroPatch 384/768i) Publication Annals of Neurology (2019) Authors: Kang, S.K., Vanoye, C.G., Misra, S.N., Echevarria, D.M., Calhoun, J.D., OConnor, J.B., Fabre, K.L., McKnight, D., Demmer, L., Goldenberg, P., Grote, L.E., Thiffault, I., Saunders, C., Strauss, K.A., Torkamani, A., van der Smagt, J., van Gassen, K., Carson, R.P., Diaz, J., Leon, E., Jacher, J.E., Hannibal, M.C., Litwin, J., Friedman, N.R., Schreiber, A., Lynch, B., Poduri, A., Marsh, E.D., Goldberg, E.M., Millichap, J.J., George Jr., A.L., Kearney, J.A.Objective Pathogenic variants inKCNB1, encoding the voltagegated potassium channel KV2.1, are associated with developmental and epileptic encephalopathy (DEE). Previous functional studies on a limited number ofKCNB1variants indicated a range of molecular mechanisms by which variants affect channel function, including loss of voltage sensitivity, loss of ion selectivity, and reduced cellsurface expression.Methods We evaluated a series of 17KCNB1variants associated with DEE or other neurodevelopmental disorders (NDDs) to rapidly ascertain channel dysfunction using highthroughput functional assays. Specifically, we investigated the biophysical properties and cellsurface expression of variant KV2.1 channels expressed in heterologous cells using highthroughput automated electrophysiology and immunocytochemistryflow cytometry.Results Pathogenic variants exhibited diverse functional defects, including altered current density and shifts in the voltage dependence of activation and/or inactivation, as homotetramers or when coexpressed with wildtype KV2.1. Quantification of protein expression also identified variants with reduced total KV2.1 functional defects caused byKCNB1variants associated with DEE and other NDDs. This will aid in establishingKCNB1variant pathogenicity and the mechanism of dysfunction, which will enable targeted strategies for therapeutic intervention based on molecular phenotype.Publication link2019 Structural Basis of NaV1.7 Inhibition by a Gating-Modifier Spider ToxinSyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in Cell (2019) Authors: Xu H.,Li T.,Rohou A.,Arthur C.P.,Tzakoniati F.,Wong E.,Estevez A.,Kugel C.,Franke Y.,Chen J.,Ciferri C.,Hackos D.H.,Koth C.M.,Payandeh J.Voltage-gated sodium (NaV) channels are targets of disease mutations, toxins, and therapeutic drugs. Despite recent advances, the structural basis of voltage sensing, electromechanical coupling, and toxin modulation remains ill-defined. Protoxin-II (ProTx2) from the Peruvian green velvet tarantula is an inhibitor cystine-knot peptide and selective antagonist of the human NaV1.7 channel. Here, we visualize ProTx2 in complex with voltage-sensor domain II (VSD2) from NaV1.7 using X-ray crystallography and cryoelectron microscopy. Membrane partitioning orients ProTx2 for unfettered access to VSD2, where ProTx2 interrogates distinct features of the NaV1.7 receptor site. ProTx2 positions two basic residues into the extracellular vestibule to antagonize S4 gating-charge movement through an electrostatic mechanism. ProTx2 has trapped activated and deactivated states of VSD2, revealing a remarkable 10 translation of the S4 helix, providing a structural framework for activation gating in voltage-gated ion channels. Finally, our results deliver key templates to design selective NaV channel antagonists.Publication link2019 Rapid characterisation of hERG channel kinetics I: using an automated high-throughput systemSyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in Biophysical Journal (2019) Authors: Lei C.L., Clerx M., Beattie K.A., Gavaghan D.J., Polonchuk L., Mirams G.R.,Wang K.Predicting how pharmaceuticals may affect heart rhythm is a crucial step in drug-development, and requires a deep understanding of a compounds action on ion channels. In vitro hERG-channel current recordings are an important step in evaluating the pro-arrhythmic potential of small molecules, and are now routinely performed using automated high-throughput patch clamp platforms. These machines can execute traditional voltage clamp protocols aimed at specific gating processes, but the array of protocols needed to fully characterise a current is typically too long to be applied in a single cell. Shorter high-information protocols have recently been introduced which have this capability, but they are not typically compatible with high-throughput platforms. We present a new high-information 15 s protocol to characterise hERG (KV11.1) kinetics, suitable for both manual and high-throughput systems. We demonstrate its use on the Nanion SyncroPatch 384PE, a 384 well automated patch clamp platform, by applying it to CHO cells stably expressing hERG1a. From these recordings we construct 124 cell-specific variants/parameterisations of a hERG model at 25 C. A further 8 independent protocols are run in each cell, and are used to validate the model predictions. We then combine the experimental recordings using a hierarchical Bayesian model, which we use to quantify the uncertainty in the model parameters, and their variability from cell to cell, which we use to suggest reasons for the variability. This study demonstrates a robust method to measure and quantify uncertainty, and shows that it is possible and practical to use high-throughput systems to capture full hERG channel kinetics quantitatively and rapidly. Statement of SignificanceWe present a method for high-throughput characterisation of hERG potassium channel kinetics, via fitting a mathematical model to results of over one hundred single cell patch clamp measurements collected simultaneously on an automated voltage clamp platform. The automated patch clamp data are used to parameterise a mathematical ion channel model fully, opening a new era of automated and rapid development of mathematical models from quick and cheap experiments. The method also allows ample data for independent validation of the models and enables us to study experimental variability and propose its origins. In future the method can be applied to characterise changes to hERG currents in different conditions, for instance at different temperatures (see Part II of the study) or under mutations or the action of pharmaceuticals; and should be easily adapted to study many other currents.Publication link2019 Rapid characterisation of hERG channel kinetics II: temperature dependenceSyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in Biophysical Journal (2019) Authors: Lei C.L., Clerx M., Beattie K.A., Melgari D., Hancox J.C., Gavaghan D.J., Polonchuk L., Wang K., Mirams G.R.Ion channel behaviour can depend strongly on temperature, with faster kinetics at physiological temperatures leading to considerable changes in currents relative to room temperature. These temperature-dependent changes in voltage-dependent ion channel kinetics (rates of opening, closing and inactivating) are commonly represented with Q10coefficients or an Eyring relationship. In this paper we assess the validity of these representations by characterising channel kinetics at multiple temperatures. We focus on the hERG channel, which is important in drug safety assessment and commonly screened at room temperature, so that results require extrapolation to physiological temperature. In Part I of this study we established a reliable method for high-throughput characterisation of hERG1a (KV11.1) kinetics, using a 15 second information-rich optimised protocol. In this Part II, we use this protocol to study the temperature dependence of hERG kinetics using CHO cells over-expressing hERG1a on the Nanion SyncroPatch 384PE, a 384-well automated patch clamp platform, with temperature control. We characterise the temperature dependence of hERG gating by fitting the parameters of a mathematical model of hERG kinetics to data obtained at five distinct temperatures between 25 and 37 C, and validate the models using different protocols. Our models reveal that activation is far more temperature sensitive than inactivation, and we observe that the temperature dependency of the kinetic parameters is not represented well by Q10coefficients: it broadly follows a generalised, but not the standardly-used, Eyring relationship. We also demonstrate that experimental estimations of Q10coefficients are protocol-dependent. Our results show that a direct fit using our 15 second protocol best represents hERG kinetics at any given temperature, and suggests that predictions from the Generalised Eyring theory may be preferentially used if no experimentally-derived data are available. Statement of SignificanceIon channel currents are highly sensitive to temperature changes. Yet because many experiments are performed more easily at room temperature, it is common to extrapolate findings to physiological temperatures through the use of Q10coefficients or Eyring rate theory. By applying short, information-rich protocols that we developed in Part I of this study we identify how kinetic parameters change over temperature. We find that the commonly-used Q10and Eyring formulations are incapable of describing the parameters temperature dependence, a more Generalised Eyring relationship works well, but remeasuring kinetics and refitting a model is optimal. The findings have implications for the accuracy of the many applications of Q10coefficients in electrophysiology, and suggest that care is needed to avoid misleading extrapolations in their many scientific and industrial pharmaceutical applications.Publication link2019 In vitro and in vivo characterization of a synthetic scorpion toxin AmmTx3, a potent inhibitor of cardiac voltage-gated potassium channel KV4.2SyncroPatch 384PE (a predecessor model of the SyncroPatch 384i) Publication in Archives of Cardiovascular Diseases Supplements (2019) Authors: Nicolas S., Zoukimian C.,Meuda H., De Waard S., Ait Ouares K., Canepari M., Beroud R., Landon C., De Waard M., Boturyn D.Background: Voltage-gated potassium channel KV4.2 (encoded by KCND2 gene) contributes to the cardiac transient outward potassium current (Ito1). This current is the main contributor to the repolarisation phase 1 of the cardiac action potential. The toxin AmmTx3, identified from the venom of the scorpion Androctonus mauretanicus, is a blocker of KV4.x channels, and have interesting therapeutic potential for neurological disorders due to its effect in cerebellar granule neurons. Its effects on cardiac KV4.2 channels remains unclear. Conclusion :AmmTx3 toxin can be chemically synthesized and used as a KV4.2 channel inhibitor to contributed to the better understanding of the exact role of Ito1 in cardiac electrophysiology. Those first results seem to be a promising evidence that AmmTx3 could a potential inhibitor of Ito current in early repolarisation syndrome.Publication link2019 Pharmacological activation of IKr in models of long QT Type 2 risks overcorrection of repolarizationSyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in Cardiovascular Research (2019) Authors: Perry M.D., Ng C-A., Mangala M.M., Ng T.Y.M., Hines A.D., Liang W., Xu M.J.O., Hill A.P., Vandenberg J.I.AimsCurrent treatment for congenital long QT syndrome Type 2 (cLQTS2), an electrical disorder that increases the risk of life-threatening cardiac arrhythmias, is aimed at reducing the incidence of arrhythmia triggers (beta-blockers) or terminating the arrhythmia after onset (implantable cardioverter-defibrillator). An alternative strategy is to target the underlying disease mechanism, which is reduced rapid delayed rectifier current (IKr) passed by KV11.1 channels. Small molecule activators of KV11.1 have been identified but the extent to which these can restore normal cardiac signalling in cLQTS2 backgrounds remains unclear. Here, we examined the ability of ICA-105574, an activator of KV11.1 that impairs transition to the inactivated state, to restore function to heterozygous KV11.1 channels containing either inactivation enhanced (T618S, N633S) or expression deficient (A422T) mutations. Methods and results ICA-105574 effectively restored KV11.1 current from heterozygous inactivation enhanced or expression defective mutant channels in heterologous expression systems. In a human-induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) model of cLQTS2 containing the expression defective KV11.1 mutant A422T, cardiac repolarization, estimated from the duration of calcium transients in isolated cells and the rate corrected field potential duration (FPDc) in culture monolayers of cells, was significantly prolonged. The KV11.1 activator ICA-105574 was able to reverse the prolonged repolarization in a concentration-dependent manner. However, at higher doses, ICA-105574 produced a shortening of the FPDc compared to controls. In vitro and in silico analysis suggests that this overcorrection occurs as a result of a temporal redistribution of the peak IKrto much earlier in the plateau phase of the action potential, which results in early repolarization. Conclusions KV11.1 activators, which target the primary disease mechanism, provide a possible treatment option for cLQTS2, with the CaVeat that there may be a risk of overcorrection that could itself be pro-arrhythmic.Publication link2019 High Throughput Characterization of KCNB1 Variants Associated with Developmental and Epileptic EncephalopathySyncroPatch 768PE (a predecessor model of the SyncroPatch 384/768i) Pre-Publication in bioRxiv (2019) Authors: Kang, S.K., Vanoye, C.G., Misra, S.N., Echevarria, D.M., Calhoun, J.D., OConnor, J.B., Fabre, K.L., McKnight, D., Demmer, L., Goldenberg, P., Grote, L.E., Thiffault, I., Saunders, C., Strauss, K.A., Torkamani, A., van der Smagt, J., van Gassen, K., Carson, R.P., Diaz, J., Leon, E., Jacher, J.E., Hannibal, M.C., Litwin, J., Friedman, N.R., Schreiber, A., Lynch, B., Poduri, A., Marsh, E.D., Goldberg, E.M., Millichap, J.J., George Jr., A.L., Kearney, J.A.Pathogenic variants inKCNB1, encoding the voltage-gated potassium channel KV2.1, are associated with developmental and epileptic encephalopathies (DEE). Previous functional studies on a limited number ofKCNB1variants indicated a range of molecular mechanisms by which variants affect channel function, including loss of voltage sensitivity, loss of ion selectivity, and reduced cell-surface expression. We evaluated a series of 17KCNB1variants associated with DEE or neurodevelopmental disorder (NDD) to rapidly ascertain channel dysfunction using high-throughput functional assays. Specifically, we investigated the biophysical properties and cell-surface expression of variant KV2.1 channels expressed in heterologous cells using high-throughput automated electrophysiology and immunocytochemistry-flow cytometry. Pathogenic variants exhibited diverse functional defects, including altered current density and shifts in the voltage-dependence of activation and/or inactivation, as homotetramers or when co-expressed with wild-type KV2.1. Quantification of protein expression also identified variants with reduced total KV2.1 expression or deficient cell-surface expression. Our study establishes a platform for rapid screening of functional defects ofKCNB1variants associated with DEE and other NDDs, which will aid in establishingKCNB1variant pathogenicity and may enable discovery of targeted strategies for therapeutic intervention based on molecular phenotype.Publication link2019 High-throughput phenotyping of heteromeric human ether--go-go-related gene potassium channel variants can discriminate pathogenic from rare benign variantsSyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in Heart Rhythm (2019) Authors: Ng C-A., Perry M.D., Liang W., Smith N.J., Foo B., Shrier A., Lukacs G.L., Hill A.P., Vandenberg J.I.Background KCNH2 encodes the human ether--go-go-related gene (hERG) potassium channel, which passes the rapid delayed rectifier potassium current, IKr. Loss-of-function variants in KCNH2 cause long QT syndrome type 2 (LQTS2) which is associated with a markedly increased risk of cardiac arrhythmias. The majority of rare KCNH2 variants however are likely to be benign. Objective To develop a high-throughput assay for discriminating between pathogenic and benign KCNH2 variants. Methods Nonsynonymous homozygous KCNH2 variants stably expressed in Flp-In human embryonic kidney 293 (HEK293) cell lines were phenotyped using an automated patch-clamp platform (SyncroPatch 384PE) and a cell surface ELISA assay. Functional phenotyping of heterozygous KCNH2 variants stably expressed in Flp-In HEK293 using a bicistronic vector was performed using SyncroPatch 384PE. Results Inhomozygous KCNH2 variant cell lines, discrepancies between current density and cell surface expression levels measured by ELISA can be explained by changes in gating properties of the variant channels. Amongst 30 heterozygous KCNH2 variant cell lines studied, the assay correctly predicted the ClinVar ascribed classification for 17/17 pathogenic/likely pathogenic/benign variants. Of 13 pore-domain variants studied, 11 had a dominant-negative expression defect whilst the remaining two had enhanced inactivation gating resulting in a dominant-negative phenotype. Conclusions High-throughput electrophysiological phenotyping of heterozygous KCNH2 variants can accurately distinguish between dominant-negative, haploinsufficient loss-of-function, and benign variants. This assay will help with future classification of KCNH2 variantsPublication link2019 Electrophysiological evaluation of pentamidine and 17-AAG in human stem cell-derived cardiomyocytes for safety assessmentSyncroPatch 384PE (a predecessor model of SyncroPatch 384i) article in European Journal of Pharmacology (2019) Authors: Asahi Y., Nomura F., Abe Y., Doi M., Sakakura T., Takasuna K., Yasuda K.Human ether-a-go-go-related gene (hERG) trafficking inhibition is known to be one of the mechanisms of indirect hERG inhibition, resulting in QT prolongation and lethal arrhythmia. Pentamidine, an antiprotozoal drug, causes QT prolongation/Torsades de Pointes (TdP) via hERG trafficking inhibition, but 17-AAG, a geldanamycin derivative heat shock protein 90 (Hsp90) inhibitor, has not shown torsadogenic potential clinically, despite Hsp90 inhibitors generally being hypothesized to cause TdP by hERG trafficking inhibition. In the present study, we investigated the underlying mechanisms of both drugs actions on hERG channels using hERG-overexpressing CHO cells (hERG-CHOs) and human embryonic stem cell-derived cardiomyocytes (hES-CMs). The effects on hERG tail current and protein levels were evaluated using population patch clamp and Western blotting in hERG-CHOs. The effects on field potential duration (FPD) were recorded by a multi-electrode array (MEA) in hES-CMs. Neither drug affected hERG tail current acutely. Chronic treatment with each drug inhibited hERG tail current and decreased the mature form of hERG protein in hERG-CHOs, whereas the immature form of hERG protein was increased by pentamidine but decreased by 17-AAG. In MEA assays using hES-CMs, pentamidine time-dependently prolonged FPD, but 17-AAG shortened it. The FPD prolongation in hES-CMs upon chronic pentamidine exposure is relevant to its clinically reported arrhythmic risk. CaV1.2 or NaV1.5 current were not reduced by chronic application of either drug at a relevant concentration to hERG trafficking inhibition in human embryonic kidney (HEK293) cells. Therefore, the reason why chronic 17-AAG shortened the FPD despite the hERG trafficking inhibition occur is still unknown.Publication link2019 Functional consequences of a KCNT1 variant associated with status dystonicus and earlyonset infantile encephalopathySyncroPatch 384PE (a predeccessor model of the SyncroPatch 384i) Publication in Annals of Clinical and Translational Neurology (2019) Authors: Gertler T.S., Thompson C.H., Vanoye C.G., Millichap J.J., George Jr A.L.Objective: We identified a novel de novo KCNT1 variant in a patient with earlyinfantile epileptic encephalopathy (EIEE) and status dystonicus, a lifethreatening movement disorder. We determined the functional consequences of this variant on the encoded KNa1.1 channel to investigate the molecular mechanisms responsible for this disorder. Methods: A retrospective case review of the proband is presented. We performed manual and automated electrophysiologic analyses of the KCNT1L437F variant expressed heterologously in Chinese hamster ovary (CHO) cells in the presence of channel activators/blockers. Results: The KCNT1L437F variant, identified in a patient with refractory EIEE and status dystonicus, confers a gainoffunction channel phenotype characterized by instantaneous, voltagedependent activation. Channel openers do not further increase L437F channel function, suggesting maximal activation, whereas channel blockers similarly block wildtype and variant channels. We further demonstrated that KCNT1 current can be measured on a highthroughput automated electrophysiology platform with potential value for future screening of novel and repurposed pharmacotherapies. Interpretation: A novel pathogenic variant in KCNT1 associated with earlyonset, medicationrefractory epilepsy and dystonia causes gainoffunction with rapid activation kinetics. Our findings extend the genotypephenotype relationships of KCNT1 variants to include severe dystonia.Publication link2019 Design, synthesis and characterization of novel N-heterocyclic-1-benzyl-1H-benzo[d]imidazole-2-amines as selective TRPC5 inhibitors leading to the identification of the selective compound, AC1903SyncroPatch 384PE (a predecessor model of SyncroPatch 384i) article in Bioorganic and Medicinal Chemistry Letters (2019) Authors: Sharma, S.H., Pablo J.L., Montesinos, M.S., Greka, A., Hopkins, C.R.The transient receptor potential cation channel 5 (TRPC5) has been previously shown to affect podocyte survival in the kidney. As such, inhibitors of TRPC5 are interesting candidates for the treatment of chronic kidney disease (CKD). Herein, we report the synthesis and biological characterization of a series of N-heterocyclic-1-benzyl-1H-benzo[d]imidazole-2-amines as selective TRPC5 inhibitors. Work reported here evaluates the benzimidazole scaffold and substituents resulting in the discovery of AC1903, a TRPC5 inhibitor that is active in multiple animal models of CKD.Publication link2019 Development of Photocrosslinking Probes Based on Huwentoxin-IV to Map the Site of Interaction on NaV1.7SyncroPatch 768PE (a predecessor model of the SyncroPatch 384/768i) Publication in Cell Chemical Biology (2019) Authors: Tzakoniati F., Xu H., Li T., Garcia N., Kugel C., Payandeh J., Koth C.M., Tate E.W.Highlights:Development of six potent diazirine-containing photoprobes based on Huwentoxin-IV. Photoprobes specifically photolabel purified bacterial-NaV1.7 VSD2 chimeric channels. Proteomic mass spectrometry identifies binding site on S1-S2 loop and S3 helix. Proposed model of HwTx-IV binding reveals importance of K27 and R29. Summary:Voltage-gated sodium (NaV) channels respond to changes in the membrane potential of excitable cells through the concerted action of four voltage-sensor domains (VSDs). Subtype NaV1.7 plays an important role in the propagation of signals in pain-sensing neurons and is a target for the clinical development of novel analgesics. Certain inhibitory cystine knot (ICK) peptides produced by venomous animals potently modulate NaV1.7; however, the molecular mechanisms underlying their selective binding and activity remain elusive. This study reports on the design of a library of photoprobes based on the potent spider toxin Huwentoxin-IV and the determination of the toxin binding interface on VSD2 of NaV1.7 through a photocrosslinking and tandem mass spectrometry approach. Our Huwentoxin-IV probes selectively crosslink to extracellular loop S1-S2 and helix S3 of VSD2 in a chimeric channel system. Our results provide a strategy that will enable mapping of sites of interaction of other ICK peptides on NaV channels.Publication link2019 Chemical Synthesis, Proper Folding, NaV Channel Selectivity Profile and Analgesic Properties of the Spider Peptide Phlotoxin 1SyncroPatch 384PE (a predecessor model of the SyncroPatch 384i) Publication in Toxins (2019) Authors: Nicolas S., Zoukimian C., Bosmans F., Montnach J., Diochot S., Cuypers E., De Waard S., Broud R., Mebs D., Craik D., Boturyn D., Lazdunski M., Tytgat J., De Waard M.Phlotoxin-1 (PhlTx1) is a peptide previously identified in tarantula venom (Phlogius species) that belongs to the inhibitory cysteine-knot (ICK) toxin family. Like many ICK-based spider toxins, the synthesis of PhlTx1 appears particularly challenging, mostly for obtaining appropriate folding and concomitant suitable disulfide bridge formation. Herein, we describe a procedure for the chemical synthesis and the directed sequential disulfide bridge formation of PhlTx1 that allows for a straightforward production of this challenging peptide. We also performed extensive functional testing of PhlTx1 on 31 ion channel types and identified the voltage-gated sodium (NaV) channel NaV1.7 as the main target of this toxin. Moreover, we compared PhlTx1 activity to 10 other spider toxin activities on an automated patch-clamp system with Chinese Hamster Ovary (CHO) cells expressing human NaV1.7. Performing these analyses in reproducible conditions allowed for classification according to the potency of the best natural NaV1.7 peptide blockers. Finally, subsequent in vivo testing revealed that intrathecal injection of PhlTx1 reduces the response of mice to formalin in both the acute pain and inflammation phase without signs of neurotoxicity. PhlTx1 is thus an interesting toxin to investigate NaV1.7 involvement in cellular excitability and pain.Publication link2019 Compounds commonly used in equine medicine inhibits the voltage-gated potassium channel KV11.1SyncroPatch 384PE (a predecessor model of SyncroPatch 384i) Publication in Research in Veterinary Science (2019) Authors: Calloe K., Rognant, S., Friis S., Shaughnessy C., Klaerke D.A., Trachsel D.Background The voltage-gated K+-channel KV11.1 has a central role in cardiac repolarization. Blockage of KV11.1 has been linked to severe cardiovascular side effects, such as acquired long QT syndrome (aLQTS), torsade de pointes arrhythmia and sudden cardiac death (SCD). KV11.1 is susceptible to unspecific drug interactions due to the presence of two aromatic amino acids residing in the inner vestibule of the pore. These aromatic residues are also present in the equine orthologue of KV11.1. This suggests that equine KV11.1 may also be prone to high-affinity block by a range of different chemical entities, which potentially could cause severe cardiac side effects and SCD in horses. Aim To screen a series of commonly used drugs in equine medicine for interaction with KV11.1. Methods High-throughput screening of selected compounds on human KV11.1 expressed in a mammalian cell line was performed using an automated patch clamp system, the SyncroPatch 384PE (Nanion Technologies, Munich, Germany).Results were validated on equine KV11.1 expressed in CHO-K1 cells by manual patch clamp. Results Acepromazine maleat (IC50=0.5M) trimethoprim (IC50=100M), diphenhydramine hydrochloride (IC50=2M) and cypro

membrane (SSM)based electrophysiology, a powerful but unexploited tool for functional studies of electrogenic transporter proteins. We show that NCX_Mj is highly selective for Na+, whereas Ca2+can be replaced by Mg2+and Sr2+and that NCX_Mj can be inhibited by divalent ions, particularly Cd2+. By directly comparing the apparent affinities of Na+and Ca2+for NCX_Mj with those for human NCX1, we show excellent agreement, indicating a strong functional similarity between NCX_Mj and its eukaryotic isoforms. We also provide detailed instructions to facilitate the adaption of this method to other electrogenic transporter proteins. Our findings demonstrate that NCX_Mj can serve as a model for the NCX family and highlight several possible applications for SSM-based electrophysiology.Webinar2019 SURFE2R as a service closing the gap between technology and applicationPresenter: Dr. Ralf Schwandner, CEO of Assay.Works GmbH, GermanyAssay.Works offers assay and screening services using the SURFE2R 96SE.Solid-supported membrane (SSM)-based electrophysiology allows functional characterization of transporters which are otherwise difficult to investigate due to their slow transport conductivity or intracellular localization. The "SURFE2R 96SE" platform makes this technology suitable for compound characterization at higher throughputs.For this case study, we prepared membrane vesicles from a PepT1-expressing cell line and screened an ion-channel/transporter-focused library of 237 bioactive compounds for substrate and inhibitory activity. With the SURFE2R 96SE, the screen was completed in less than 8 hours with a success rate of 98%, demonstrating utility of the technology for compound screening.On the downside, application of SSM-based electrophysiology is limited to targets with an electrogenic net transport, assay setup is rather complex, and cost of ownership is significant. This may be an obstacle for implementing SURFE2R technology in-house. In response, Assay.Works entered into a partnership with Nanion, providing SURFE2R assays as a service. Backed by Nanions extensive expertise, we utilize our assay development and screening capabilities, including cell and membrane production, compound libraries, and sample management, to help clients realizing proof-of-concept, compound characterization, or profiling studies.Poster PDF2020 A novel approach to detect electrogenic transporter activity in intact cells applied to investigate iPSC derived cardiomyocytes and neuronsSURFE2R N1 and SURFE2R 96SE poster, 64th Annual Meeting of the Biophysical SocietyPoster PDF2018 Label-free analysis of Na+/Ca2+ exchanger (NCX) isolated from iPSC-derived cardiomyocytesSURFE2R N1 and SURFE2R 96SE poster, Europhysiology Meeting 2018Application Note PDFPepT1 Electrophysiological recordings of PepT1 (SLC15A1) activity on Nanions SURFE2RSURFE2R N1 application note: The human peptide transporter PepT1 is an uptake transporter responsible for initial absorption and renal reabsorption of dietary oligopeptides. It is primarily located in the plasma membranes of enterocytes of the small intestine as well as the renal proximal tubular cells. PepT1 functions as a co-transporter, coupling the uphill peptide transport into the cells to the electrochemical proton gradient. Due to the movement of protons, PepT1 is an electrogenic transporter. PepT1 shows a very high capacity but a low affinity and substrate specificity. Its ability to transport a large range of compounds has enabled the rational design of drugs and pro-drugs (e.g. penicillins, ACE inhibitors) which have good oral bio-availability using delivery via PepT1. Designing pro-drugs with higher affinity for PepT1 is a successful strategy to increase the bio-availability of poorly absorbed drugs. Here we present electric real-time PepT1 activity measurements on the SURFE2R instruments using purified plasma membranes of CHO cells overexpressing PepT1. Peptide transport was activated on the SURFE2R N1 using a sensor with attached PepT1- containing membrane fragments which was inserted into the device. This was perfused with a buffer containing the dipeptide glycyl-glycine as the substrate. The data presented here show activation of PepT1 by glyclyglycine and inhibition by Lys[Z(No2)]-Val on the SURFE2R N1 and scale-up of the assay on the SURFE2R N96.Poster PDF2017 An emerging technique for the characterization of transport proteins: SSM-based electrophysiologySURFE2R N1 and SURFE2R SE96 poster, 19th IUPAB / 11th EBSA congress 2017Application Note PDFEAAT3 Electrophysiological recordings of EAAT3 (SLC1A1) activity on Nanions SURFE2R N1SURFE2R N1 application note:The excitatory amino acid transporter 3 (EAAT3; also known as EAAC1) is a sodium-dependent neuronal uptake transporter encoded by the slc1a1 gene. It plays amajor role in the reuptake of glutamate from the synaptic cleft, thereby maintaining a low extra- cellular concentration of glutamate and regulating the excitatory neurotransmission. EAAT3 is also involved in the uptake of aspartate and cysteine into the cells. The transporter is highly expressed in mature neurons, where it is distributed in somata and dendrites. EAAT3 functions as a cotransporter, coupling the uphill substrate transport into the cells to the electrochemical gradients of sodium and potassium. The stoichiometry of transport is 1 glutamate with 3 Na+and 1 H+moving into the cell to 1 K+moving out of the cell. Therefore EAAT3 is an electrogenic transporter, generating a net charge flow.Dysfunction of glutamate transporters leads to increased extracellular glutamate levels, thereby causing neuro- toxicity and neurodegeneration. Regulatory mechanisms facilitating EAAT3 function are, therefore, interesting as targets for the treatment of neurodegenerative diseases.Here we present EAAT3 activity measurements on the SURFE2R N1 instrument using purified plasma membrane of CHO cells expressing EAAT3.Application Note PDFGAT1 Electrophysiological recordings of hGAT1 (SLC6A1) activity on Nanions SURFE2R N1SURFE2R N1 application note:GABA is the major inhibitory neurotransmitter in the brain and is important in controlling excitability. After release, GABA is removed from the extracellular space by GABA transporters (GATs), thus terminating inhibitory synaptic transmission. The GABA transporters belong to the family of neurotransmitter:sodium symporters referred to as the solute carrier 6 (SLC6) family in humans. GATs co-transport GABA, Na+and Cl-with the proposed stiochiometry 1 GABA: 2 Na+: 1 Cl-, resulting in a net influx of 1 positive charge per cycle. So far, 4 GATs have been identified, GAT1, GAT2, GAT3 and BGT1.GAT1 is expressed throughout the brain in both GABAergic and non-GABAergic neurons, and is expressed in particularly high levels in the olfactory bulb, basal ganglia, cerebellum and retina. The physiological function of GAT1 is primarily to terminate synaptic transmission but also to ensure the fidelity of synaptic transmission by preventing the spread of neurotransmitter to neighbouring synapses. GABA transporters also play an important role in neurotransmitter reutilization. In certain circumstances, e.g. when the sodium gradient increases during ischemia or following seizures, GATs can act in reverse which may have a protective effect during seizures, by inhibiting electrical excitability. There is some evidence that GATs may play a role in neurodegenerative diseases such as Parkinsons and Alzheimers and may provide a novel target for treating these conditions.Here we present human GAT1 activity measurements on the SURFE2R N1 instrument using purified plasma membranes from HEK cells. GABA affinity and effect of inhibitors were investigated.Application Note PDFCIC Electrophysiological recordings of the H+/ Cl- exchanger Ec-ClC on the SURFE2R N1SURFE2R N1 application note: Samples kindly provided by Dr. Merritt Maduke, Stanford University, USAClCs are a family of chloride ion channels and transporters with important physiological roles including regulation of the membrane potential, transepithelial salt transport and ion homeostasis1,2. To date, 9 members of the ClC family have been identified in mammals1,2, the first 4 (ClC-1, ClC-2, ClC-Ka, and ClC-Kb) are located on the plasmamembrane where they act as chloride ion channelswhereas the remaining 5 are located in intracellular organelles (ClC-3-7) and are chloride-proton exchangers1-3. These transporters are important for endosome, lysosome and synaptic vesicle acidification1,2, and mutations in, e.g. ClC-5 underlie the rare chronic kidney disorder, Dents Disease, and mutations in ClC-7 underlie osteopetrosis2,4, a rare inherited bone hardening disorder. Given their ubiquitous expression and importance in physiologicalprocesses, they are important potential drug targets.ClC from Escherichia coli (Ec-ClC or ClC-ec1) is closely ClC from Escherichia coli (Ec-ClC or ClC-ec1) is closely related to its mammalian counterparts and is a Cl-/H+exchanger3,5. It transports 2 Cl- into the cell, coupled to the efflux of 1 H+3,5. Therefore Ec-ClC is an electrogenic transporter, generating a net charge flow. In E. coli, the Ec-ClC mediates acid resistance of enteric bacteria by promoting H+extrusion1,5,6. Here we present Ec-ClC activity measurements on the SURFE2R N1 instrument using proteoliposomes reconstituted with purified Ec-ClC at different lipid-to-protein ratios.Application Note PDFCNT1 Electrophysiological recordings of CNT1 (SLC28A1) activity on Nanions SURFE2R N1SURFE2R N1 application note:The concentrative nucleoside transporter 1 (CNT1) is a sodium-dependent uptake transporter encoded by the SLC28A1 gene. CNT1 functions as a co-transporter, coupling the uphill nucleoside transport into the cells to the electrochemical gradient of sodium. The stoichiometry of transport is proposed to be 1:1, but a stoichiometry of 2 Na+: 1 nucleoside has also been suggested. CNT1 is an electrogenic transporter, generating a net charge flow. It plays a major role in the uptake of pyrimidines, including uridine and cytidine, from the extracellular milieu into the cytoplasm in nucleoside salvage pathways which is the first step of nucleoside biosynthesis. The transporter is expressed in epithelial tissues including liver, kidney and small intestine where it is localized to the apical membrane. CNTs are important targets for many antiviral and anticancer agents, and CNT1 has been proposed to play a role in tumor biology via a mechanism beyond nucleoside transport. In fact, tumors expressing high levels of CNT1 can indicate a higher risk of relapse for breast cancer patients, suggesting that nucleoside salvage may interfere with chemosensitivity. On the other hand, high expression of the CNT1 protein could promote drug- induced cytotoxicity if patients were treated with suitable hCNT substrates. In any case, hCNT1 is an important mediator in the transport of anticancer and antiviral nucleoside drugs by mechanisms that require further study.Here we present CNT1 activity measurements on the SURFE2R N1 instrument using purified plasma membrane of CHO cells expressing CNT1.Publication link2022 Structure, mechanism and lipid-mediated remodeling of the mammalian Na+/H+ exchanger NHA2SURFE2RN1 Publication in Nature Structural & Molecular Biology (2022) Authors: Matsuoka R., Fudim R., Jung S., Zhang C., Bazzone A., Chatzikyriakidou Y., Robinson C. V., Nomura N., Iwata S., Landreh M., Orellana L., Beckstein O., Drew D.The Na+/H+ exchanger SLC9B2, also known as NHA2, correlates with the long-sought-after Na+/Li+ exchanger linked to the pathogenesis of diabetes mellitus and essential hypertension in humans. Despite the functional importance of NHA2, structural information and the molecular basis for its ion-exchange mechanism have been lacking. Here we report the cryo-EM structures of bison NHA2 in detergent and in nanodiscs, at 3.0 and 3.5 resolution, respectively. The bison NHA2 structure, together with solid-state membrane-based electrophysiology, establishes the molecular basis for electroneutral ion exchange. NHA2 consists of 14 transmembrane (TM) segments, rather than the 13 TMs previously observed in mammalian Na+/H+ exchangers (NHEs) and related bacterial antiporters. The additional N-terminal helix in NHA2 forms a unique homodimer interface with a large intracellular gap between the protomers, which closes in the presence of phosphoinositol lipids. We propose that the additional N-terminal helix has evolved as a lipid-mediated remodeling switch for the regulation of NHA2 activity.Publication link2022 Structures and mechanism of the plant PIN-FORMED auxin transporterSURFE2RN1 Publication in Nature (2022) Authors: Ung K.L., Winkler M., Schulz L., Kolb M., Janacek D.P., Dedic E., Stokes D.L., Hammes U.Z., Pedersen B.P.Auxins are hormones that have central roles and control nearly all aspects of growth and development in plants. The proteins in the PIN-FORMED (PIN) family (also known as the auxin efflux carrier family) are key participants in this process and control auxin export from the cytosol to the extracellular space. Owing to a lack of structural and biochemical data, the molecular mechanism of PIN-mediated auxin transport is not understood. Here we present biophysical analysis together with three structures of Arabidopsis thaliana PIN8: two outward-facing conformations with and without auxin, and one inward-facing conformation bound to the herbicide naphthylphthalamic acid. The structure forms a homodimer, with each monomer divided into a transport and scaffold domain with a clearly defined auxin binding site. Next to the binding site, a prolineproline crossover is a pivot point for structural changes associated with transport, which we show to be independent of proton and ion gradients and probably driven by the negative charge of the auxin. The structures and biochemical data reveal an elevator-type transport mechanism reminiscent of bile acid/sodium symporters, bicarbonate/sodium symporters and sodium/proton antiporters. Our results provide a comprehensive molecular model for auxin recognition and transport by PINs, link and expand on a well-known conceptual framework for transport, and explain a central mechanism of polar auxin transport, a core feature of plant physiology, growth and development.Publication link2022 SSM-based electrophysiology, a label-free real-time method reveals sugar binding & transport events in SGLT1SURFE2RN1 Publication in Biosensors and Bioelectronics(2022) Authors: Bazzone A., Krner A., Meincke M., Bhatt M., Srujan S., Barthmes M., Kubick S., Fertig N.Here, we present a solid-supported membrane (SSM)-based electrophysiological approach to study sugar binding and Na+/glucose cotransport by SGLT1 in membrane vesicles. SSM-based electrophysiology delivers a cumulative real-time current readout from numerous SGLT1 proteins simultaneously using a gold-coated sensor chip.In contrast to conventional techniques, which mainly operate with voltage steps, currents are triggered by sugar or sodium addition. Sugar concentration jumps in the presence of sodium lead to transport currents between 5 and 10 nA. Remarkably, in the absence of sodium (i.e. no transport), we observed fast pre-steady-state (PSS) currents with time constants between 3 and 10 ms. These PSS currents mainly originate from sugar binding. Sodium binding does not induce PSS currents. Due to high time resolution, PSS currents were distinguished from transport and eventually correlated with conformational transitions within the sugar translocation pathway.In addition, we analyzed the impact of driving forces on transport and binding currents, showing that membrane voltage and sodium concentration gradients lead to an increased transport rate without affecting sugar binding kinetics. We also compared Na+/sugar efflux with physiologically relevant influx and found similar transport rates, but lower affinity in efflux mode.SSM-based electrophysiology is a powerful technique, which overcomes bottlenecks for transport measurements observed in other techniques such as the requirement of labels or the lack of real-time data. Rapid solution exchange enables the observation of substrate-induced electrogenic events like conformational transitions, opening novel perspectives for in-depth functional studies of SGLT1 and other transporters.Publication link2022 Structure and lipid-mediated remodelling mechanism of the Na+/H+ exchanger NHA2SURFE2R N1 Publicationin Nature Structural & Molecular Biology (2022) Authors: Matsuoka R., Fudim R., Jung S., Zhang C., Bazzone A., Chatzikyriakidou Y., Nomura N., Iwata S., Orellana L., Beckstein O., Drew D.Na+/H+exchangers catalyse an ion-exchange activity that is carried out in most, if not all cells. SLC9B2, also known as NHA2, correlates with the long-sought after sodium/lithium (Na+/Li+) exchanger linked to the pathogenesis of diabetes mellitus and essential hypertension in humans. Despite its functional importance, structural information and the molecular basis of its ion-exchange mechanism have been lacking. Here, we report the cryo EM structures ofbisonNHA2 in detergent and in nanodiscs at 3.0 and 3.5 resolution, respectively. NHA2 shares closest structural similarity to the bacterial electrogenic Na+/H+antiporter NapA, rather than other mammalian SLC9A members. Nevertheless, SSM-based electrophysiology results with NHA2 show the catalysis of electroneutral rather than electrogenic ion exchange, and the ion-binding site is quite distinctive, with a tryptophan-arginine- glutamate triad separated from the well-established ion-binding aspartates. These triad residues fine-tune ion binding specificity, as demonstrated by a salt-bridge swap mutant that converts NHA2 into a Li+-specific transporter. Strikingly, an additional N-terminal helix in NHA2 establishes a unique homodimer with a large 25 intracellular gap between protomers. In the presence of phosphatidylinositol lipids, the N-terminal helix rearranges and closes this gap. We confirm that dimerization of NHA2 is required for activityin vivo, and propose that the N- terminal helix has evolved as a lipid-mediated remodelling switch for regulation of transport activity.Publication link2022 Mechanistic basis of choline import involved in teichoic acids and lipopolysaccharide modificationSURFE2RN1 Publication in Science Advances (2022) Authors: Brland N., Rueff A.S., Cebrero G.,Hutter C.A.J., Seeger M.A., Veening J.W., Perez C.Phosphocholine molecules decorating bacterial cell wall teichoic acids and outer-membrane lipopolysaccharide have fundamental roles in adhesion to host cells, immune evasion, and persistence. Bacteria carrying the operon that performs phosphocholine decoration synthesize phosphocholine after uptake of the choline precursor by LicB, a conserved transporter among divergent species. Streptococcus pneumoniae is a prominent pathogen where phosphocholine decoration plays a fundamental role in virulence. Here, we present cryoelectron microscopy and crystal structures of S. pneumoniae LicB, revealing distinct conformational states and describing architectural and mechanistic elements essential to choline import. Together with in vitro and in vivo functional characterization, we found that LicB displays proton-coupled import activity and promiscuous selectivity involved in adaptation to choline deprivation conditions, and describe LicB inhibition by synthetic nanobodies (sybodies). Our results provide previously unknown insights into the molecular mechanism of a key transporter involved in bacterial pathogenesis and establish a basis for inhibition of the phosphocholine modification pathway across bacterial phyla.Publication link2022 Mechanistic basis of choline import involved in teichoic acids and lipopolysaccharide modificationSURFE2RN1 Publication in Sci Adv. (2022) Authors: Brland N., Rueff A-S., Cebrero G., Hutter C. A. J., Seeger M. A., Veening J-W., Perez C.Phosphocholine molecules decorating bacterial cell wall teichoic acids and outer-membrane lipopolysaccharide have significant roles in adhesion to host cells, immune evasion, and persistence. Bacteria carrying the operon that performs phosphocholine decoration, synthesize phosphocholine after uptake of the choline precursor by LicB, a conserved transporter among divergent species. Streptococcus pneumoniae is a prominent pathogen where phosphocholine decoration plays a fundamental role in virulence. Here we present cryo-electron microscopy and crystal structures of S. pneumoniae LicB, revealing distinct conformational states and describing architectural and mechanistic elements essential to choline import. Together with in vitro and in vivo functional characterization, we found that LicB displays proton-coupled import activity and promiscuous selectivity involved in adaptation to choline deprivation conditions, and describe LicB inhibition by synthetic nanobodies (sybodies) and hemicholinium-3. Our results provide novel insights into the molecular mechanism of a key transporter involved in bacterial pathogenesis and establish a basis for inhibition of the phosphocholine modification pathway across bacterial phyla.Publication link2022 Electrogenic reaction step and phospholipid translocation pathway of the mammalian P4-ATPase ATP8A2SURFE2RN1 Publication in FEBS Letters (2022) Authors: Tadini-Buoninsegni F., Mikkelsen S.A., Mogensen L.S., Holm R., Molday R.S., Andersen J.P.ATP8A2 is a mammalian P4-ATPase (flippase) that translocates the negatively charged lipid substrate phosphatidylserine from the exoplasmic leaflet to the cytoplasmic leaflet of cellular membranes. Using an electrophysiological method based on solid supported membranes, we investigated the electrogenicity of specific reaction steps of ATP8A2 and explored a potential phospholipid translocation pathway involving residues with positively charged side chains. Changes to the current signals caused by mutations show that the main electrogenic event occurs in connection with release of the bound phosphatidylserine to the cytoplasmic leaflet and support the hypothesis that the phospholipid interacts with specific lysine and arginine residues near the cytoplasmic border of the lipid bilayer during the translocation and/or reorientation required for insertion into the cytoplasmic leaflet.Publication link2022 Expression, purification and characterization of human proton-coupled oligopeptide transporter 1 hPEPT1SURFE2RN1 Publication in Protein Expression and Purification (2022) Authors: Rafiq M., Ernst H.A., Aduri N.G., Prabhala B.K., Tufail S., Rahman M., Bloch M.B., Mirza N., Taylor N., Boesen T., Gajhede M., Mirzaa O.The human peptide transporter hPEPT1 (SLC15A1) is responsible for uptake of dietary di- and tripeptides and a number of drugs from the small intestine by utilizing the proton electrochemical gradient, and hence an important target for peptide-like drug design and drug delivery. hPEPT1 belongs to the ubiquitous major facilitator superfamily that all contain a 12TM core structure, with global conformational changes occurring during the transport cycle. Several bacterial homologues of these transporters have been characterized, providing valuable insight into the transport mechanism of this family. Here we report the overexpression and purification of recombinant hPEPT1 in a detergent-solubilized state. Thermostability profiling of hPEPT1 at different pH values revealed that hPEPT1 is more stable at pH 6 as compared to pH 7 and 8. Micro-scale thermophoresis (MST) confirmed that the purified hPEPT1 was able to bind di- and tripeptides respectively. To assess the in-solution oligomeric state of hPEPT1, negative stain electron microscopy was performed, demonstrating a predominantly monomeric state.Publication link2022 Coexistence of Ammonium Transporter and Channel Mechanisms in Amt-Mep-Rh Twin-His Variants Impairs the Filamentation Signaling Capacity of Fungal Mep2 TransceptorsSURFE2RN1 Publication in mBio (2022) Authors: Williamson G., Brito A. S., Bizior A., Tamburrino G., Mirandela G. D., Harris T., Hoskisson P. A., Zachariae U., Marini A. M., Boeckstaens M., Javelle A.Ammonium translocation through biological membranes, by the ubiquitous Amt-Mep-Rh family of transporters, plays a key role in all domains of life. Two highly conserved histidine residues protrude into the lumen of the pore of these transporters, forming the familys characteristic Twin-His motif. It has been hypothesized that the motif is essential to confer the selectivity of the transport mechanism. Here, using a combination of in vitro electrophysiology on Escherichia coli AmtB, in silico molecular dynamics simulations, and in vivo yeast functional complementation assays, we demonstrate that variations in the Twin-His motif trigger a mechanistic switch between a specific transporter, depending on ammonium deprotonation, to an unspecific ion channel activity. We therefore propose that there is no selective filter that governs the specificity in Amt-Mep transporters but the inherent mechanism of translocation, dependent on the fragmentation of the substrate, ensures the high specificity of the translocation. We further show that both mechanisms coexist in fungal Mep2 Twin-His variants, disrupting the transceptor function and so inhibiting the filamentation process. These data strongly support a transport mechanism-mediated signalling process in the long-standing debate on the sensory function of Mep2-like transporters.Publication link2021 A Solid Supported Membrane Electrophysiology Assay for Efficient Characterization of Ion-Coupled TransportSURFE2RN1 Publication in Journal of Biological Chemistry(2021) Authors: Thomas N.E., Feng W., Henzler-Wildman K.A.Transport stoichiometry determination can provide great insight into the mechanism and function of ion-coupled transporters. Traditional reversal potential assays are a reliable, general method for determining the transport stoichiometry of ion-coupled transporters, but the time and material costs of this technique hinder investigations of transporter behavior under multiple experimental conditions. Solid supported membrane electrophysiology (SSME) allows multiple recordings of liposomal or membrane samples adsorbed onto a sensor, and is sensitive enough to detect transport currents from moderate-flux transporters that are inaccessible to traditional electrophysiology techniques. Here, we use SSME to develop a new method for measuring transport stoichiometry with greatly improved throughput. Using this technique, we were able to verify the recent report of a fixed 2:1 stoichiometry for the proton:guanidinium antiporter Gdx, reproduce the 1H+:2Cl- antiport stoichiometry of CLC-ec1, and confirm loose proton:nitrate coupling for CLC-ec1. Furthermore, we were able to demonstrate quantitative exchange of internal contents of liposomes adsorbed onto SSME sensors to allow multiple experimental conditions to be tested on a single sample. Our SSME method provides a fast, easy, general method for measuring transport stoichiometry, which will facilitate future mechanistic and functional studies of ion-coupled transporters.Publication link2020 The structural basis of promiscuity in small multidrug resistance transportersSURFE2R N1 Publication in Nature Communications (2020) Authors: Kermani A.A., Macdonald C.B., Burata O.E., Koff B.B., Koide A., Denbaum E., Koide S., Stockbridge R.B.By providing broad resistance to environmental biocides, transporters from the small multidrug resistance (SMR) family drive the spread of multidrug resistance cassettes among bacterial populations. A fundamental understanding of substrate selectivity by SMR transporters is needed to identify the types of selective pressures that contribute to this process. Using solid-supported membrane electrophysiology, we find that promiscuous transport of hydrophobic substituted cations is a general feature of SMR transporters. To understand the molecular basis for promiscuity, we solved X-ray crystal structures of a SMR transporter Gdx-Clo in complex with substrates to a maximum resolution of 2.3. These structures confirm the familys extremely rare dual topology architecture and reveal a cleft between two helices that provides accommodation in the membrane for the hydrophobic substituents of transported drug-like cations.Publication link2020 Unlocking the Reversal Potential of Solid Supported Membrane Electrophysiology to Determine Transport StoichiometrySURFE2R N1 pre-Publication in bioRxiv (2020) Authors: Henzler-Wildman K.A., Thomas N.E.Transport stoichiometry provides insight into the mechanism and function of ion-coupled transporters, but measuring transport stoichiometry is time-consuming and technically difficult. With the increasing evidence that many ion-coupled transporters employ multiple transport stoichiometries under different conditions, improved methods to determine transport stoichiometry are required to accurately characterize transporter activity. Reversal potential was previously shown to be a reliable, general method for determining the transport stoichiometry of ion-coupled transporters (Fitzgerald & Mindell, 2017). Here, we develop a new technique for measuring transport stoichiometry with greatly improved throughput using solid supported membrane electrophysiology (SSME). Using this technique, we are able to verify the recent report of a fixed 2:1 stoichiometry for the proton:guanidinium antiporter Gdx. Our SSME method requires only small amounts of transporter and provides a fast, easy, general method for measuring transport stoichiometry, which will facilitate future mechanistic and functional studies of ion-coupled transporters.Publication link2020 Electrophysiology Measurements of Metal Transport by MntH2 from Enterococcus faecalisSURFE2R N1 Publication in Membranes (2020) Authors: Gantner M., Laftsoglou T., Rong H., Postis V.L.G., Jueken L.J.C.Transition metals are essential trace elements and their high-affinity uptake is required for many organisms. Metal transporters are often characterised using metal-sensitive fluorescent dyes, limiting the metals and experimental conditions that can be studied. Here, we have tested whether metal transport byEnterococcus faecalisMntH2 can be measured with an electrophysiology method that is based on the solid-supported membrane technology.E. faecalisMntH2 belongs to the Natural Resistance-Associated Macrophage Protein (Nramp) family of proton-coupled transporters, which transport divalent transition metals and do not transport the earth metals. Electrophysiology confirms transport of Mn(II), Co(II), Zn(II) and Cd(II) by MntH2. However, no uptake responses for Cu(II), Fe(II) and Ni(II) were observed, while the presence of these metals abolishes the uptake signals for Mn(II). Fluorescence assays confirm that Ni(II) is transported. The data are discussed with respect to properties and structures of Nramp-type family members and the ability of electrophysiology to measure charge transport and not directly substrate transport.Publication link2020 Energy Coupling in Cation-Pumping Pyrophosphatase Back to MitchellSURFE2R N1 Publication in Frontiers in Plant Science (2020) Authors: Baykov A.A.Those of a certain age may remember (and their younger colleagues can read) accounts of the vivid debate in the 1970s surrounding the coupling mechanism involved in oxidative and photo phosphorylation. By that time, Mitchell's chemiosmotic hypothesis had already gained credence, and the debated issue was how a transmembrane H+potential difference drives ATP synthesis by Ftype ATP synthases. The major mechanisms that were considered assumed that the membrane (Fo) and peripheral (F1) parts were functionally connected in different ways. Peter Mitchell proposed a direct coupling mechanism in which protons are translocated through Fo into the catalytic site of F1, where they participate directly in ADP phosphorylation and form water as the second product (Mitchell, 1974). Paul Boyer, the proponent of the main competing mechanism, advocated an indirect coupling mechanism (successively termed alternating site, binding change, or rotational) that implied that protons transfer their energy to the catalytic site indirectly, via distant conformational strain (Boyer, 1997). The debate was resolved in favor of Boyer's mechanism when it became clear that the alternative mechanism is inconsistent with H+/ATP stoichiometry and, finally, when the three-dimensional structure of the F-ATPase was determined (Abrahams et al., 1994).Publication link2019 Phosphatidylserine flipping by the P4-ATPase ATP8A2 is electrogenicSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in PNAS (2019) Authors: Tadini-Buoninsegni F., Mikkelsen S.A., Mogensen L.S., Molday R.S., and Andersen J.P.Significance: Phospholipid flippases constitute the largest subfamily of P-type ATPases and have in eukaryotic organisms evolved as a central transport system for selective translocation of phospholipids across biological membranes to generate membrane lipid asymmetry, a property essential for numerous cellular processes. The importance of flippases is highlighted by severe neurological disorders and liver diseases caused by flippase dysfunction in humans. The electrogenicity of phospholipid transport by flippases has not previously been explored. We demonstrated that phosphatidylserine translocation by the flippase ATP8A2 generates electrical current, resulting from specific steps in the flippase reaction cycle moving the charged lipid head group between the membrane bilayer leaflets, and that no charged substrate is being countertransported. These findings unravel key features of phospholipid flippases. Abstract: Phospholipid flippases (P4-ATPases) utilize ATP to translocate specific phospholipids from the exoplasmic leaflet to the cytoplasmic leaflet of biological membranes, thus generating and maintaining transmembrane lipid asymmetry essential for a variety of cellular processes. P4-ATPases belong to the P-type ATPase protein family, which also encompasses the ion transporting P2-ATPases: Ca2+-ATPase, Na+/K+-ATPase, and H+,K+-ATPase. In comparison with the P2-ATPases, understanding of P4-ATPases is still very limited. The electrogenicity of P4-ATPases has not been explored, and it is not known whether lipid transfer between membrane bilayer leaflets can lead to displacement of charge across the membrane. A related question is whether P4-ATPases countertransport ions or other substrates in the opposite direction, similar to the P2-ATPases. Using an electrophysiological method based on solid supported membranes, we observed the generation of a transient electrical current by the mammalian P4-ATPase ATP8A2 in the presence of ATP and the negatively charged lipid substrate phosphatidylserine, whereas only a diminutive current was generated with the lipid substrate phosphatidylethanolamine, which carries no or little charge under the conditions of the measurement. The current transient seen with phosphatidylserine was abolished by the mutation E198Q, which blocks dephosphorylation. Likewise, mutation I364M, which causes the neurological disorder cerebellar ataxia, mental retardation, and disequilibrium (CAMRQ) syndrome, strongly interfered with the electrogenic lipid translocation. It is concluded that the electrogenicity is associated with a step in the ATPase reaction cycle directly involved in translocation of the lipid. These measurements also showed that no charged substrate is being countertransported, thereby distinguishing the P4-ATPase from P2-ATPases.Publication link2020 A two-lane mechanism for selective biological ammonium transportSURFE2R N1 Publication in eLife (2020) Authors: Williamson G., Tamburrino G., Bizior A.,Boeckstaens M.,Mirandela G.D., Bage M.G., Pisliakov A., Ives C.M., Terras E., Hoskisson P.A., Marini A.M., Zachariae U., Javelle A.The transport of charged molecules across biological membranes faces the dual problem of accommodating charges in a highly hydrophobic environment while maintaining selective substrate translocation. This has been the subject of a particular controversy for the exchange of ammonium across cellular membranes, an essential process in all domains of life. Ammonium transport is mediated by the ubiquitous Amt/Mep/Rh transporters that includes the human Rhesus factors. Here, using a combination of electrophysiology, yeast functional complementation and extended molecular dynamics simulations, we reveal a unique two-lane pathway for electrogenic NH4+transport in two archetypal members of the family, the transporters AmtB fromEscherichia coliand Rh50 from Nitrosomonas europaea. The pathway underpins a mechanism by which charged H+and neutral NH3are carried separately across the membrane after NH4+deprotonation. This mechanism defines a new principle of achieving transport selectivity against competing ions in a biological transport process.Publication link2018 The lipid environment determines the activity of the E. coli ammonium transporter, AmtBSURFE2R N1 Publication in Faseb J (2018) Authors: Mirandela G.D., Tamburrino G., Hoskisson P.A., Zachariae U., Javelle A.The movement of ammonium across biological membranes is a fundamental process in all living organisms and is mediated by the ubiquitous Amt/Mep/Rh family of transporters. Recent structural analysis and coupled mass spectrometry studies have shown that the Escherichia coli ammonium transporter, AmtB, specifically binds phosphatidylglycerol (PG). Upon PG binding, several residues of AmtB undergo a small conformational change, which stabilizes the protein against unfolding. However, no studies have so far been conducted to explore if PG binding to AmtB has functional consequences. Here, we used an in vitro experimental assay with purified components together with molecular dynamics simulations to characterise the relation between PG binding and AmtB activity. Firstly, our results indicate that the function of Amt in archaebacteria and eubacteria may differ. Secondly, we show that PG is an essential cofactor for AmtB activity and that in the absence of PG AmtB cannot complete the full translocation cycle. Furthermore, our simulations reveal previously undiscovered PG binding sites on the intracellular side of the lipid bilayer between the AmtB subunits. The possible molecular mechanisms explaining the functional role of PG are discussed.Publication link2019 Mutation of two key aspartate residues alters stoichiometry of the NhaB Na+/H+ exchanger from Klebsiella pneumoniaeSURFE2R N1 Publication in Nature Scientific Reports (2019) Authors: Patio-Ruiz M., Fendler K., & Clinescu O.Bacterial NhaB Na+/H+exchangers belonging to the Ion Transporter superfamily are poorly characterized in contrast to Na+/H+exchangers of the Cation Proton Antiporter superfamily which have NhaA from Escherichia coli as a prominent member. For a more detailed understanding of the intricacies of the exchangers transport mechanism, mutational studies are essential. Therefore, we mutated two protonatable residues present in the putative transmembrane region of NhaB from Klebsiella pneumoniae (KpNhaB), which could serve as substrate binding sites, Asp146 and Asp404, to either glutamate or alanine and analyzed transport function and stability of the mutants using electrophysiological and fluorimetric techniques. While mutation of either Asp residue to Glu only had slight to moderate effects on the transport activity of the exchanger, the mutations D404A and D146A, in particular, had more profound effects on the transport function. Furthermore, a double mutant, D146A/D404A, exhibited a remarkable behavior at alkaline pH, where recorded electrical currents changed polarity, showing steady-state transport with a stoichiometry of H+:Na+1, as opposed to the H+:Na+>1 stoichiometry of the WT. Thus, we showed that Asp146 and Asp404 are part of the substrate binding site(s) of KpNhaB and engineered a Na+/H+exchanger with a variable stoichiometry.Publication link2017 Insights into the mechanism of membrane pyrophosphatases by combining experiment and computer simulationSURFE2R N1 Publication in Structural Dynamics (2017) Authors: Shah N.R., Wilkinson C., Harborne S.P.D., Turku A., Li K.-M., Sun Y.-J., Harris S., Goldman A.Membrane-integral pyrophosphatases (mPPases) couple the hydrolysis of pyrophosphate (PPi) to the pumping of Na+, H+, or both these ions across a membrane. Recently solved structures of the Na+-pumpingThermotoga maritimamPPase (TmPPase) and H+-pumpingVigna radiatamPPase revealed the basis of ion selectivity between these enzymes and provided evidence for the mechanisms of substrate hydrolysis and ion-pumping. Our atomistic molecular dynamics (MD) simulations of TmPPase demonstrate that loop 56 is mobile in the absence of the substrate or substrate-analogue bound to the active site, explaining the lack of electron density for this loop in resting state structures. Furthermore, creating anapomodel of TmPPase by removing ligands from the TmPPase:IDP:Na structure in MD simulations resulted in increased dynamics in loop 56, which results in this loop moving to uncover the active site, suggesting that interactions between loop 56 and the imidodiphosphate and its associated Mg2+are important for holding a loop-closed conformation. We also provide further evidence for the transport-before-hydrolysis mechanism by showing that the non-hydrolyzable substrate analogue, methylene diphosphonate, induces low levels of proton pumping by VrPPase.Publication link2017 Mechanisms of charge transfer in human copper ATPases ATP7A and ATP7BSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in IUBMB Life (2017) Authors: Tadini-Buoninsegni F., Smeazzetto S.ATP7A and ATP7B are Cu+-transporting ATPases of subclass IB and play a fundamental role in intracellular copper homeostasis. ATP7A/B transfer Cu+ions across the membrane from delivery to acceptor proteins without establishing a free Cu+gradient. Transfer of copper across the membrane is coupled to ATP hydrolysis. Current measurements on solid supported membranes (SSM) were performed to investigate the mechanism of copper-related charge transfer across ATP7A and ATP7B. SSM measurements demonstrated that electrogenic copper displacement occurs within ATP7A/B following addition of ATP and formation of the phosphorylated intermediate. Comparison of the time constants for cation displacement in ATP7A/B and sarcoplasmic reticulum Ca2+-ATPase is consistent with the slower phosphoenzyme formation in copper ATPases. Moreover, ATP-dependent copper transfer in ATP7A/B is not affected by varying the pH, suggesting that net proton counter-transport may not occur in copper ATPases. Platinum anticancer drugs activate ATP7A/B and are subjected to ATP-dependent vectorial displacement with a mechanism analogous to that of copper.Publication link2017 Discovery of Compounds that Positively Modulate the High Affinity Choline TransporterSURFE2R N1 Publication in Frontiers in Molecular Neuroscience (2017) Authors: Choudhary P., Armstrong E.J., Jorgensen C.C., Piotrowski M., Barthmes M., Torella R., Johnston S.E., Maruyama Y., Janiszewski J.S., Storer R.I., Skerratt S.E., Benn C.L.Cholinergic hypofunction is associated with decreased attention and cognitive deficits in the central nervous system in addition to compromised motor function. Consequently, stimulation of cholinergic neurotransmission is a rational therapeutic approach for the potential treatment of a variety of neurological conditions. High affinity choline uptake (HACU) into acetylcholine (ACh)-synthesizing neurons is critically mediated by the sodium- and

that doubles as a defense system during the enteric life of the bacterium. Structural and functional assays are described, which shed light on the transport mechanism of this important molecular machine.Publication link2016 Charge translocation by mitochondrial NADH:ubiquinone oxidoreductase (complex I) from Yarrowia lipolytica measured on solid-supported membranesSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in Biochemical and Biophysical Research Communications (2016) Authors: Siebels I., Drse S.The charge translocation by purified reconstituted mitochondrial complex I from the obligate aerobic yeast Yarrowia lipolytica was investigated after adsorption of proteoliposomes to solid-supported membranes. In presence of n-decylubiquinone (DBQ), pulses of NADH provided by rapid solution exchange induced charge transfer reflecting steady-state pumping activity of the reconstituted enzyme. The signal amplitude increased with time, indicating 'deactiveactive' transition of the Yarrowia complex I. Furthermore, an increase of the membrane-conductivity after addition of 5-(N-ethyl-N-isopropyl)amiloride (EIPA) was detected which questiones the use of EIPA as an inhibitor of the Na+/H+-antiporter-like subunits of complex I. This investigation shows that electrical measurements on solid-supported membranes are a suitable method to analyze transport events and 'active/deactive' transition of mitochondrial complex I.Publication link2015 A universal mechanism for transport and regulation of CPA sodium proton exchangersSURFE2R-technology (custom-built system) Publication in Biological Chemistry (2015) Authors: Clinescu O., Fendler K.Recent studies performed on a series of Na+/H+exchangers have led us to postulate a general mechanism for Na+/H+exchange in the monovalent cation/proton antiporter superfamily. This simple mechanism employs a single binding site for which both substrates compete. The developed kinetic model is self-regulatory, ensuring down-regulation of transport activity at extreme pH, and elegantly explains the pH-dependent activity of Na+/H+exchangers. The mechanism was experimentally verified and shown to describe both electrogenic and electroneutral exchangers. Using a small number of parameters, exchanger activity can be modeled under different conditions, providing insights into the physiological role of Na+/H+exchangers.Publication link2015 Hofmeister effect of anions on calcium translocation by sarcoplasmic reticulum Ca2+-ATPaseSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in Nature Scientific Reports (2015) Authors: Tadini-Buoninsegni F., Moncelli M.R., Peruzzi N., Ninham B.W., Dei L., Lo Nostroa P.The occurrence of Hofmeister (specific ion) effects in various membrane-related physiological processes is well documented. For example the effect of anions on the transport activity of the ion pump Na+, K+-ATPase has been investigated. Here we report on specific anion effects on the ATP-dependent Ca2+translocation by the sarcoplasmic reticulum Ca2+-ATPase (SERCA). Current measurements following ATP concentration jumps on SERCA-containing vesicles adsorbed on solid supported membranes were carried out in the presence of different potassium salts. We found that monovalent anions strongly interfere with ATP-induced Ca2+translocation by SERCA, according to their increasing chaotropicity in the Hofmeister series. On the contrary, a significant increase in Ca2+translocation was observed in the presence of sulphate. We suggest that the anions can affect the conformational transition between the phosphorylated intermediates E1P and E2P of the SERCA cycle. In particular, the stabilization of the E1P conformation by chaotropic anions seems to be related to their adsorption at the enzyme/water and/or at the membrane/water interface, while the more kosmotropic species affect SERCA conformation and functionality by modifying the hydration layers of the enzyme.Publication link2014 Substrate-bound outward-open state of the betaine transporter BetP provides insights into Na+ couplingSURFE2R-technology (custom-built system) Publication in Nature Communications (2014) Authors: Perez C., Faust B., Mehdipour A.R., Francesconi K.A., Forrest L.R., Ziegler C.The Na+-coupled betaine symporter BetP shares a highly conserved fold with other sequence unrelated secondary transporters, for example, with neurotransmitter symporters. Recently, we obtained atomic structures of BetP in distinct conformational states, which elucidated parts of its alternating-access mechanism. Here, we report a structure of BetP in a new outward-open state in complex with an anomalous scattering substrate, adding a fundamental piece to an unprecedented set of structural snapshots for a secondary transporter. In combination with molecular dynamics simulations these structural data highlight important features of the sequential formation of the substrate and sodium-binding sites, in which coordinating water molecules play a crucial role. We observe a strictly interdependent binding of betaine and sodium ions during the coupling process. All three sites undergo progressive reshaping and dehydration during the alternating-access cycle, with the most optimal coordination of all substrates found in the closed state.Publication link2015 A sulfurbased transport pathway in Cu+ATPasesSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in EMBO Reports (2015) Authors: Mattle D., Zhang L., Sitsel O., Pedersen L.T., Moncelli M.R., Tadini-Buoninsegni F., Gourdon P., Rees D.C., Nissen P., Meloni G.Cells regulate copper levels tightly to balance the biogenesis and integrity of copper centers in vital enzymes against toxic levels of copper. PIBtype Cu+ATPases play a central role in copper homeostasis by catalyzing the selective translocation of Cu+across cellular membranes. Crystal structures of a copperfree Cu+ATPase are available, but the mechanism of Cu+recognition, binding, and translocation remains elusive. Through Xray absorption spectroscopy, ATPase activity assays, and charge transfer measurements on solidsupported membranes using wildtype and mutant forms of the Legionella pneumophila Cu+ATPase (LpCopA), we identify a sulfurlined metal transport pathway. Structural analysis indicates that Cu+is bound at a highaffinity transmembranebinding site in a trigonalplanar coordination with the Cys residues of the conserved CPC motif of transmembrane segment 4 (C382 and C384) and the conserved Met residue of transmembrane segment 6 (M717 of the MXXXS motif). These residues are also essential for transport. Additionally, the studies indicate essential roles of other conserved intramembranous polar residues in facilitating copper binding to the highaffinity site and subsequent release through the exit pathway.Publication link2014 NhaA Na+/H+ antiporter mutants that hardly react to the membrane potentialSURFE2R-technology (custom-built system) Publication in PLoS ONE (2014) Authors: Alkoby D., Rimon A., Budak M., Patino-Ruiz M., Clinescu O., Fendler K., Padan E..pH and Na+homeostasis in all cells requires Na+/H+antiporters. The crystal structure, obtained at pH 4, of NhaA, the main antiporter of Escherichia coli, has provided general insights into an antiporter mechanism and its unique pH regulation. Here, we describe a general method to select various NhaA mutants from a library of randomly mutagenized NhaA. The selected mutants, A167P and F267C are described in detail. Both mutants are expressed in Escherichia coli EP432 cells at 70-95% of the wild type but grow on selective medium only at neutral pH, A167P on Li+(0.1 M) and F267C on Na+(0.6 M). Surprising for an electrogenic secondary transporter, and opposed to wild type NhaA, the rates of A167P and F267C are almost indifferent to membrane potential. Detailed kinetic analysis reveals that in both mutants the rate limiting step of the cation exchange cycle is changed from an electrogenic to an electroneutral reaction.Publication link2014 Species differences in bacterial NhaA Na+/H+ exchangersSURFE2R-technology (custom-built system) Publication in FEBS Letters (2014) Authors: Clinescu O., Danner E., Bhm M., Hunte C., Fendler K..Bacteria have adapted their NhaA Na+/H+exchangers responsible for salt homeostasis to their different habitats. We present an electrophysiological and kinetic analysis of NhaA from Helicobacter pylori and compare it to the previously investigated exchangers from Escherichia coli and Salmonella typhimurium. Properties of all three transporters are described by a simple model using a single binding site for H+and Na+. We show that H. pylori NhaA only has a small acidic shift of its pH-dependent activity profile compared to the other transporters and discuss why a more drastic change in its pH activity profile is not physiologically required.Publication link2014 Keeping it simple, transport mechanism and pH regulation in Na+/H+ exchangersSURFE2R-technology (custom-built system) Publication in Journal of Biological Chemistry (2014) Authors: Clinescu O., Paulino C., Khlbrandt W., Fendler K.Na+/H+exchangers are essential for regulation of intracellular proton and sodium concentrations in all living organisms. We examined and experimentally verified a kinetic model for Na+/H+exchangers, where a single binding site is alternatively occupied by Na+or one or two H+ions. The proposed transport mechanism inherently down-regulates Na+/H+exchangers at extreme pH, preventing excessive cytoplasmic acidification or alkalinization. As an experimental test system we present the first electrophysiological investigation of an electroneutral Na+/H+exchanger, NhaP1 from Methanocaldococcus jannaschii (MjNhaP1), a close homologue of the medically important eukaryotic NHE Na+/H+exchangers. The kinetic model describes the experimentally observed substrate dependences of MjNhaP1, and the transport mechanism explains alkaline down-regulation of MjNhaP1. Because this model also accounts for acidic down-regulation of the electrogenic NhaA Na+/H+exchanger from Escherichia coli (EcNhaA, shown in a previous publication) we conclude that it applies generally to all Na+/H+exchangers, electrogenic as well as electroneutral, and elegantly explains their pH regulation. Furthermore, the electrophysiological analysis allows insight into the electrostatic structure of the translocation complex in electroneutral and electrogenic Na+/H+exchangers.Publication link2014 Molecular characterization of the Na+/H+-antiporter NhaA from Salmonella TyphimuriumSURFE2R-technology (custom-built system) Publication in PLoS ONE (2014) Authors: Lentes C.J., Mir S.H., Boehm M., Ganea C., Fendler K., Hunte C.Na+/H+antiporters are integral membrane proteins that are present in almost every cell and in every kingdom of life. They are essential for the regulation of intracellular pH-value, Na+-concentration and cell volume. These secondary active transporters exchange sodium ions against protons via an alternating access mechanism, which is not understood in full detail. Na+/H+antiporters show distinct species-specific transport characteristics and regulatory properties that correlate with respective physiological functions. Here we present the characterization of the Na+/H+antiporter NhaA from Salmonella enterica serovar Thyphimurium LT2, the causing agent of food-born human gastroenteritis and typhoid like infections. The recombinant antiporter was functional in vivo and in vitro. Expression of its gene complemented the Na+-sensitive phenotype of an E. coli strain that lacks the main Na+/H+antiporters. Purified to homogeneity, the antiporter was a dimer in solution as accurately determined by size-exclusion chromatography combined with multi-angle laser-light scattering and refractive index monitoring. The purified antiporter was fully capable of electrogenic Na+(Li+)/H+-antiport when reconstituted in proteoliposomes and assayed by solid-supported membrane-based electrophysiological measurements. Transport activity was inhibited by 2-aminoperimidine. The recorded negative currents were in agreement with a 1Na+(Li+)/2H+stoichiometry. Transport activity was low at pH 7 and up-regulation above this pH value was accompanied by a nearly 10-fold decrease of KmNa (16 mM at pH 8.5) supporting a competitive substrate binding mechanism. K+does not affect Na+affinity or transport of substrate cations, indicating that selectivity of the antiport arises from the substrate binding step. In contrast to homologous E. coli NhaA, transport activity remains high at pH values above 8.5. The antiporter from S. Typhimurium is a promising candidate for combined structural and functional studies to contribute to the elucidation of the mechanism of pH-dependent Na+/H+antiporters and to provide insights in the molecular basis of species-specific growth and survival strategies.Publication link2014 Anticancer Ruthenium(III) Complex KP1019 Interferes with ATP-Dependent Ca2+ Translocation by Sarco-Endoplasmic Reticulum Ca2+-ATPase (SERCA)SURFE2R N1 Publication in ChemMedChem (2014) Authors: Sadafi F.Z., Massai L., Bartolommei G., Moncelli M.R., Messori L., Tadini-Buoninsegni F.Sarco-endoplasmic reticulum Ca2+-ATPase (SERCA), a P-type ATPase that sustains Ca2+transport and plays a major role in intracellular Ca2+homeostasis, represents a therapeutic target for cancer therapy. Here, we investigated whether ruthenium-based anticancer drugs, namely KP1019 (indazolium [trans-tetrachlorobis(1H-indazole)ruthenate(III)]), NAMI-A (imidazolium [trans-tetrachloro(1H-imidazole)(S-dimethylsulfoxide)ruthenate(III)]) and RAPTA-C ([Ru(6-p-cymene)dichloro(1,3,5-triaza-7-phosphaadamantane)]), and cisplatin (cis-diammineplatinum(II) dichloride) might act as inhibitors of SERCA. Charge displacement by SERCA adsorbed on a solid-supported membrane was measured after ATP or Ca2+concentration jumps. Our results show that KP1019, in contrast tocancer the other metal compounds, is able to interfere with ATP-dependent translocation of Ca2+ions. An IC50value of 1Mwas determined for inhibition of calcium translocation by KP1019. Conversely, it appears that KP1019 does not significantly affect Ca2+binding to the ATPase from the cytoplasmic side. Inhibition of SERCA at pharmacologically relevant concentrations may represent a crucial aspect in the overall pharmacological and toxicological profile of KP1019.Publication link2014 Direct observation of electrogenic NH4(+) transport in ammonium transport (Amt) proteinsSURFE2R-technology (custom-built system) Publication in PNAS (2014) Authors: Wacker T., Garcia-Celma J.J., Lewe P., Andrade S.L.Significance: We have detected and analyzed electrogenic transport of ammonium and methylammonium by members of the ammonium transport (Amt) family of membrane proteins using solid-supported membrane electrophysiology. Amt transport is pH-dependent and occurs at a rate of 30300 ions per s per trimer, well in the range of other transport proteins. The study establishes, to our knowledge, the first in vitro assay system for Amt transport in a fully controlled setup and settles debate about whether Amt proteins function as passive ammonia channels or active ammonium transporters.Abstract:Significance: We have detected and analyzed electrogenic transport of ammonium and methylammonium by members of the ammonium transport (Amt) family of membrane proteins using solid-supported membrane electrophysiology. Amt transport is pH-dependent and occurs at a rate of 30300 ions per s per trimer, well in the range of other transport proteins. The study establishes, to our knowledge, the first in vitro assay system for Amt transport in a fully controlled setup and settles debate about whether Amt proteins function as passive ammonia channels or active ammonium transporters. Abstract: Ammonium transport (Amt) proteins form a ubiquitous family of integral membrane proteins that specifically shuttle ammonium across membranes. In prokaryotes, archaea, and plants, Amts are used as environmental NH4+sCaVengers for uptake and assimilation of nitrogen. In the eukaryotic homologs, the Rhesus proteins, NH4+/NH3transport is used instead in acidbase and pH homeostasis in kidney or NH4+/NH3(and eventually CO2) detoxification in erythrocytes. Crystal structures and variant proteins are available, but the inherent challenges associated with the unambiguous identification of substrate and monitoring of transport events severely inhibit further progress in the field. Here we report a reliable in vitro assay that allows us to quantify the electrogenic capacity of Amt proteins. Using solid-supported membrane (SSM)-based electrophysiology, we have investigated the three Amt orthologs from the euryarchaeon Archaeoglobus fulgidus. Af-Amt1 and Af-Amt3 are electrogenic and transport the ammonium and methylammonium cation with high specificity. Transport is pH-dependent, with a steep decline at pH values of 5.0. Despite significant sequence homologies, functional differences between the three proteins became apparent. SSM electrophysiology provides a long-sought-after functional assay for the ubiquitous ammonium transporters.Publication link2013 Functional characterization of a ClC transporter by solid-supported membrane electrophysiologySURFE2R-technology (custom-built system) Publication in Journal of General Physiology (2013) Authors: Garcia-Celma J., Szydelko A., Dutzler R.EcClC, a prokaryotic member of the ClC family of chloride channels and transporters, works as coupled H+/Cl-exchanger. With a known structure and the possibility of investigating its behavior with different biochemical and biophysical techniques, the protein has become an important model system for the family. Although many aspects of its function have been previously characterized, it was difficult to measure transport on the same sample under different environmental conditions. To overcome this experimental limitation, we have studied EcClC by solid-supported membrane electrophysiology. The large transport-related transient currents and a simple way of relating transport rates to the measured signal have allowed a thorough investigation of ion selectivity, inhibition, and the dependence of transport on changes in ion concentration and pH. Our results confirm that the protein transports larger anions with about similar rates, whereas the smaller fluoride is not a substrate. We also show that 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS), a known inhibitor of other anion transport protein, irreversibly inhibits EcClC from the intracellular side. The chloride dependence shows an apparent saturation at millimolar concentrations that resembles a similar behavior in eukaryotic ClC channels. Our experiments have also allowed us to quantify the pH dependence of transport. EcClC shows a strong activation at low pH with an apparent pKa of 4.6. The pronounced pH dependence is lost by the mutation of a conserved glutamate facing the extracellular solution that was previously shown to be an acceptor for transported protons, whereas it is largely retained by the mutation of an equivalent residue at the intracellular side. Our results have provided a quantitative basis for the transport behavior of EcClC, and they will serve as a reference for future investigations of novel electrogenic transporters with still-uncharacterized properties.Publication link2013 Photocycle and vectorial proton transfer in a rhodopsin from the eukaryote Oxyrrhis marinaSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in Biochemistry (2013) Authors: Janke C., Scholz F., Becker-Baldus J., Glaubitz C., Wood P.G., Bamberg E., Wachtveitl J., Bamann C.Retinylidene photoreceptors are ubiquitously present in marine protists as first documented by the identification of green proteorhodopsin (GPR). We present a detailed investigation of a rhodopsin from the protist Oxyrrhis marina (OR1) with respect to its spectroscopic properties and to its vectorial proton transport. Despite its homology to GPR, OR1's features differ markedly in its pH dependence. Protonation of the proton acceptor starts at pH below 4 and is sensitive to the ionic conditions. The mutation of a conserved histidine H62 did not influence the pK(a) value in a similar manner as in other proteorhodopsins where the charged histidine interacts with the proton acceptor forming the so-called His-Asp cluster. Mutational and pH-induced effects were further reflected in the temporal behavior upon light excitation ranging from femtoseconds to seconds. The primary photodynamics exhibits a high sensitivity to the environment of the proton acceptor D100 that are correlated to the different initial states. The mutation of the H62 does not affect photoisomerization at neutral pH. This is in agreement with NMR data indicating the absence of the His-Asp cluster. The subsequent steps in the photocycle revealed protonation reactions at the Schiff base coupled to proton pumping even at low pH. The main electrogenic steps are associated with the reprotonation of the Schiff base and internal proton donor. Hence, OR1 shows a different theme of the His-Asp organization where the low pK(a) of the proton acceptor is not dominated by this interaction, but by other electrostatic factors.Publication link2013 Electrophysiological characterization of uncoupled mutants of LacYSURFE2R-technology (custom-built system) Publication in Biochemistry (2013) Authors: Gaiko O., Bazzone A., Fendler K., Kaback H.R.In this study of the lactose permease of Escherichia coli (LacY), five functionally irreplaceable residues involved specifically in H+translocation (Arg302 and Glu325) or in the coupling between protonation and sugar binding (Tyr236, Glu269, and His322) were mutated individually or together with mutant Glu325 Ala. The wild type and each mutant were purified and reconstituted into proteoliposomes, which were then examined using solid-supported-membrane-based electrophysiology. Mutants Glu325 Ala or Arg302 Ala, in which H+symport is abolished, exhibit a weakly electrogenic rapid reaction triggered by sugar binding. The reaction is essentially absent in mutant Tyr236 Phe, Glu269 Ala, and His322 Ala, and each of these mutations blocks the electrogenic reaction observed in the Glu325 Ala mutant. The findings are consistent with the interpretation that the electrogenic reaction induced by sugar binding is due to rearrangement of charged residues in LacY and that this reaction is blocked by mutation of each member of the Tyr236/Glu269/His322 triad. In addition, further support is provided for the conclusion that deprotonation is rate limiting for downhill lactose/H+symport.Publication link2013 Enhanced adsorption of Ca-ATPase containing vesicles on a negatively charged solid supported membrane for the investigation of membrane transportersSURFE2R N1 Publication in Langmuir (2013) Authors: Sacconi A., Moncelli M.R., Mergheri G., Tadini-Buoninsegni F.A convenient model system for a biological membrane is a solid-supported membrane (SSM), which consists of a gold-supported alkanethiol|phospholipid bilayer. In combination with a concentration jump method, SSMs have been used for the investigation of several membrane transporters. Vesicles incorporating sarcoplasmic reticulum Ca-ATPase (SERCA) were adsorbed on a negatively charged SSM (octadecanethiol|phosphatidylserine bilayer). The current signal generated by the adsorbed vesicles following an ATP concentration jump was compared to that produced by SERCA-containing vesicles adsorbed on a conventional SSM (octadecanethiol|phosphatidylcholine bilayer). A significantly higher current amplitude was recorded on the serine-based SSM. The adsorption of SERCA-incorporating vesicles on the SSM was then characterized by surface plasmon resonance (SPR). The SPR measurements clearly indicate that in the presence of Ca2+and Mg2+, the amount of adsorbed vesicles on the serine-based SSM is about twice that obtained using the conventional SSM, thereby demonstrating that the higher current amplitude recorded on the negatively charged SSM is correlated with a greater quantity of adsorbed vesicles. The enhanced adsorption of membrane vesicles on the PS-based SSM may be useful to study membrane preparations with a low concentration of transport protein generating small current signals, as in the case of various recombinantly expressed proteins.Publication link2012 The nitrite transport protein NirC from Salmonella typhimurium is a nitrite/proton antiporterSURFE2R-technology (custom-built system) Publication in Biochimica et Biophysica Acta (BBA) - Biomembranes (2012) Authors: Rycovska A., Hatahet L., Fendler K., Michel H.In anaerobically grown bacteria, transport of nitrite is catalyzed by an integral membrane protein of the form atenitrite transporter family, NirC, which in Salmonella typhimurium plays a critical role in intracellular virulence. We present a functional characterization of the S. typhimurium nitrite transporter StmNirC in native membrane vesicles as well as purified and reconstituted into proteoliposomes. Using an electrophysiological technique based on solid supported membranes, we show nitrite induced translocation of negative charges into proteoliposomes reconstituted with purified StmNirC. These data demonstrate the electrogenicity of StmNirC and its substrate specificity for nitrite. Monitoring changes in pH on everted membrane vesicles containing overexpressed StmNirC using acridine orange as a pH indicator we demonstrate that StmNirC acts as a secondary active transporter. It promotes low affinity transport of nitrite coupled to H+antiport with a pH independent profile in the pH range from 6 to 8. In addition to nitrite also nitrate is transported by StmNirC, but with reduced flux and complete absence of proton antiport activity. Taken together, these data suggest a bispecific anion selectivity of StmNirC with an ion specific transport mode. This may play a role in regulating nitrite transport under physiological conditions.Publication link2013 Differential Effects of Mutations on the Transport Properties of the Na+/H+ Antiporter NhaA from Escherichia coliSURFE2R-technology (custom-built system) Publication in Journal of Biological Chemistry (2013) Authors: Mager T., Braner M., Kubsch B., Hatahet L., Alkoby D., Rimon A., Padan E., Fendler K.Na+/H+antiporters show a marked pH dependence, which is important for their physiological function in eukaryotic and prokaryotic cells. In NhaA, the Escherichia coli Na+/H+antiporter, specific single site mutations modulating the pH profile of the transporter have been described in the past. To clarify the mechanism by which these mutations influence the pH dependence of NhaA, the substrate dependence of the kinetics of selected NhaA variants was electrophysiologically investigated and analyzed with a kinetic model. It is shown that the mutations affect NhaA activity in quite different ways by changing the properties of the binding site or the dynamics of the transporter. In the first case, pK and/or KDNa are altered, and in the second case, the rate constants of the conformational transition between the inside and the outside open conformation are modified. It is shown that residues as far apart as 1520 from the binding site can have a significant impact on the dynamics of the conformational transitions or on the binding properties of NhaA. The implications of these results for the pH regulation mechanism of NhaA are discussed.Publication link2012 Distinctive features of catalytic and transport mechanisms in mammalian sarco-endoplasmic reticulum Ca2+ ATPase (SERCA) and Cu+ (ATP7A/B) ATPasesSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in Journal of Biological Chemistry (2012) Authors: Lewis D., Pilankatta R., Inesi G., Bartolommei G., Moncelli M.R., Tadini-Buoninsegni F.Ca2+(sarco-endoplasmic reticulum Ca2+ATPase (SERCA)) and Cu+(ATP7A/B) ATPases utilize ATP through formation of a phosphoenzyme intermediate (E-P) whereby phosphorylation potential affects affinity and orientation of bound cation. SERCA E-P formation is rate-limited by enzyme activation by Ca2+, demonstrated by the addition of ATP and Ca2+to SERCA deprived of Ca2+(E2) as compared with ATP to Ca2+-activated enzyme (E12Ca2+). Activation by Ca2+is slower at low pH (2H+E2 to E12Ca2+) and little sensitive to temperature-dependent activation energy. On the other hand, subsequent (forward or reverse) phosphoenzyme processing is sensitive to activation energy, which relieves conformational constraints limiting Ca2+ translocation. A H+-gated pathway, demonstrated by experiments on pH variations, charge transfer, and Glu-309 mutation allows luminal Ca2+release by H+/Ca2+exchange. As compared with SERCA, initial utilization of ATP by ATP7A/B is much slower and highly sensitive to temperature-dependent activation energy, suggesting conformational constraints of the headpiece domains. Contrary to SERCA, ATP7B phosphoenzyme cleavage shows much lower temperature dependence than EP formation. ATP-dependent charge transfer in ATP7A and -B is observed, with no variation of net charge upon pH changes and no evidence of Cu+/H+exchange. As opposed to SERCA after Ca2+chelation, ATP7A/B does not undergo reverse phosphorylation with Pi after copper chelation unless a large N-metal binding extension segment is deleted. This is attributed to the inactivating interaction of the copper-deprived N-metal binding extension with the headpiece domains. We conclude that in addition to common (P-type) phosphoenzyme intermediate formation, SERCA and ATP7A/B possess distinctive features of catalytic and transport mechanisms.Publication link2012 Investigation of the sodium-binding sites in the sodium-coupled betaine transporter BetPSURFE2R-technology (custom-built system) Publication in PNAS (2012) Authors: Khafizov K., Perez C., Koshy C., Quick M., Fendler K., Ziegler C., Forrest L.R.Sodium-coupled substrate transport plays a central role in many biological processes. However, despite knowledge of the structures of several sodium-coupled transporters, the location of the sodium-binding site(s) often remains unclear. Several of these structures have the five transmembrane-helix inverted-topology repeat, LeuT-like (FIRL) fold, whose pseudosymmetry has been proposed to facilitate the alternating-access mechanism required for transport. Here, we provide biophysical, biochemical, and computational evidence for the location of the two cation-binding sites in the sodium-coupled betaine symporter BetP. A recent X-ray structure of BetP in a sodium-bound closed state revealed that one of these sites, equivalent to the Na2 site in related transporters, is located between transmembrane helices 1 and 8 of the FIRL-fold; here, we confirm the location of this site by other means. Based on the pseudosymmetry of this fold, we hypothesized that the second site is located between the equivalent helices 6 and 3. Molecular dynamics simulations of the closed-state structure suggest this second sodium site involves two threonine sidechains and a backbone carbonyl from helix 3, a phenylalanine from helix 6, and a water molecule. Mutating the residues proposed to form the two binding sites increased the apparent Kmand Kdfor sodium, as measured by betaine uptake, tryptophan fluorescence, and22Na+binding, and also diminished the transient currents measured in proteoliposomes using solid supported membrane-based electrophysiology. Taken together, these results provide strong evidence for the identity of the residues forming the sodium-binding sites in BetP.Publication link2011 Transport Mechanism and pH Regulation of the Na+/H+ Antiporter NhaA from Escherichia coliSURFE2R-technology (custom-built system) Publication in Journal of Biological Chemistry (2011) Authors: Mager T., Rimon A., Padan E., Fendler K.Using an electrophysiological assay the activity of NhaA was tested in a wide pH range from pH 5.0 to 9.5. Forward and reverse transport directions were investigated at zero membrane potential using preparations with inside-out and right side-out-oriented transporters with Na+or H+gradients as the driving force. Under symmetrical pH conditions with a Na+gradient for activation, both the wt and the pH-shifted G338S variant exhibit highly symmetrical transport activity with bell-shaped pH dependences, but the optimal pH was shifted 1.8 pH units to the acidic range in the variant. In both strains the pH dependence was associated with a systematic increase of the Kmfor Na+at acidic pH. Under symmetrical Na+concentration with a pH gradient for NhaA activation, an unexpected novel characteristic of the antiporter was revealed; rather than being down-regulated, it remained active even at pH as low as 5. These data allowed a transport mechanism to advance based on competing Na+and H+binding to a common transport site and a kinetic model to develop quantitatively explaining the experimental results. In support of these results, both alkaline pH and Na+induced the conformational change of NhaA associated with NhaA cation translocation as demonstrated here by trypsin digestion. Furthermore, Na+translocation was found to be associated with the displacement of a negative charge. In conclusion, the electrophysiological assay allows the revelation of the mechanism of NhaA antiport and sheds new light on the concept of NhaA pH regulation.Publication link2012 Assaying the proton transport and regulation of UCP1 using solid supported membranesSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in European Biophysics Journal (2012) Authors: Blesneac I., Ravaud S., Machillot P., Zoonens M., Masscheylen S., Miroux B., Vivaudou M., Pebay-Peyroula E.The uncoupling protein 1 (UCP1) is a mitochondrial protein that carries protons across the inner mitochondrial membrane. It has an important role in non-shivering thermogenesis, and recent evidence suggests its role in human adult metabolism. Using rapid solution exchange on solid supported membranes, we succeeded in measuring electrical currents generated by the transport activity of UCP1. The protein was purified from mouse brown adipose tissue, reconstituted in liposomes and absorbed on solid supported membranes. A fast pH jump activated the ion transport, and electrical signals could be recorded. The currents were characterized by a fast rise and a slow decay, were stable over time, inhibited by purine nucleotides and activated by fatty acids. This new assay permits direct observation of UCP1 activity in controlled cell-free conditions, and opens up new possibilities for UCP1 functional characterization and drug screening because of its robustness and its potential for automation.Publication link2011 G117C MelB, a mutant melibiose permease with a changed conformational equilibriumSURFE2R-technology (custom-built system) Publication in Biochimica et Biophysica Acta (BBA) - Biomembranes (2011) Authors: Ganea C., Meyer-Lipp K., Lemonnier R., Krah A., Leblanc G., Fendler K.Replacement of the glycine at position 117 by a cysteine in the melibiose permease creates an interesting phenotype: while the mutant transporter shows still transport activity comparable to the wild type its pre steady-state kinetic properties are drastically altered. The transient charge displacements after substrate concentration jumps are strongly reduced and the fluorescence changes disappear. Together with its maintained transport activity this indicates that substrate translocation in G117C melibiose permease is not impaired but that the initial conformation of the mutant transporter differs from that of the wild type permease. A kinetic model for the G117C melibiose permease based on a rapid dynamic equilibrium of the substrate free transporter is proposed. Implications of the kinetic model for the transport mechanism of the wild type permease are discussed.Publication link2011 Robust Electrophysiological Assays using Solid Supported Membranes: the Organic Cation Transporter OCT2SURFE2R ONE (a predecessor model of the SURFE2R N1) Publication in Australian Journal of Chemistry (2011) Authors: Gaiko O., Janausch I., Geibel S., Vollert H., Arndt P., Gonski S., Fendler K.An electrophysiological assay platform based on solid supported membranes (SSM) for the organic cation transporter (OCT) is presented. Stable Chinese hamster ovary (CHO) cell lines overexpressing the human (hOCT2) and rat transporters (rOCT2) were generated and validated. Membrane preparations from the cell lines were investigated using SSM-based electrophysiology. Baculovirus transfected insect cells (HighFive and Mimic Sf9) were also tested with the same assay but yielded less than optimal results. The assays were validated by the determination of substrate affinities and inhibition by standard inhibitors. The study demonstrates the suitability of the SSM-based electrophysiological OCT assay for rapid and automatic screening of drug candidates.Publication link2011 Characterisation of the purified human sodium/iodide symporter reveals that the protein is mainly present in a dimeric form and permits the detailed study of a native C-terminal fragmentSURFE2R-technology (custom-built system) Publication in Biochimica et Biophysica Acta (BBA) - Biomembranes (2011) Authors: Huc-Brandt S., Marcellin D., Graslin F., Averseng O., Bellanger L., Hivin P., Quemeneur E., Basquin C., NaVarro V., Pourcher T., Darrouzet E.The sodium/iodide symporter is an intrinsic membrane protein that actively transports iodide into thyroid follicular cells. It is a key element in thyroid hormone biosynthesis and in the radiotherapy of thyroid tumours and their metastases. Sodium/iodide symporter is a very hydrophobic protein that belongs to the family of sodium/solute symporters. As for many other membrane proteins, particularly mammalian ones, little is known about its biochemistry and structure. It is predicted to contain 13 transmembrane helices, with an N-terminus oriented extracellularly. The C-terminal, cytosolic domain contains approximately one hundred amino acid residues and bears most of the transporter's putative regulatory sites (phosphorylation, sumoylation, di-acide, di-leucine or PDZ-binding motifs). In this study, we report the establishment of eukaryotic cell lines stably expressing various human sodium/iodide symporter recombinant proteins, and the development of a purification protocol which allowed us to purify milligram quantities of the human transporter. The quaternary structure of membrane transporters is considered to be essential for their function and regulation. Here, the oligomeric state of human sodium/iodide symporter was analysed for the first time using purified protein, by size exclusion chromatography and light scattering spectroscopy, revealing that the protein exists mainly as a dimer which is stabilised by a disulfide bridge. In addition, the existence of a sodium/iodide symporter C-terminal fragment interacting with the protein was also highlighted. We have shown that this fragment exists in various species and cell types, and demonstrated that it contains the amino-acids [512-643] from the human sodium/iodide symporter protein and, therefore, the last predicted transmembrane helix. Expression of either the [1-512] truncated domain or the [512-643] domain alone, as well as co-expression of the two fragments, was performed, and revealed that co-expression of [1-512] with [512-643] allowed the reconstitution of a functional protein. These findings constitute an important step towards an understanding of some of the post-translational mechanisms that finely tune iodide accumulation through human sodium/iodide symporter regulation.Publication link2011 Development of an assay for Complex I/Complex III of the respiratory chain using solid supported membranes and its application in mitochondrial toxicity screening in drug discoverySURFE2R ONE (a predecessor model of SURFE2R N1) Publication in ASSAY and Drug Development Technologies (2011) Authors: Preissl S., Bick I., Obrdlik P., Diekert K., Gul S., Gribbon P.Membrane-bound transporter proteins are involved in cell signal transduction and metabolism as well as influencing key pharmacological properties such as drug bioavailability. The functional activity of transporters that belong to the group of electrically active membrane proteins can be directly monitored using the solid-supported membrane-based SURFE(2)R technology (SURFace Electrogenic Event Reader; Scientific Devices Heidelberg GmbH, Heidelberg, Germany). The method makes use of membrane fragments or vesicles containing transport proteins adsorbed onto solid-supported membrane-covered electrodes and allows the direct measurement of their activity. This technology has been used to develop a robust screening compatible assay for Complex I/Complex III, key components of the respiratory chain in 96-well microtiter plates. The assay was screened against 1,000 compounds from the ComGenex Lead-like small molecule library to ascertain whether mitochondrial liabilities might be an underlying, although undesirable feature of typical commercial screening libraries. Some 105 hits (compounds exhibiting >50% inhibition of Complex I/Complex III activity at 10M) were identified and their activities were subsequently confirmed in duplicate, yielding a confirmation rate of 68%. Analysis of the confirmed hits also provided evidence of structure-activity relationships and two compounds from one structural class were further evaluated in dose-response experiments. This study provides evidence that profiling of compounds for potential mitochondrial liabilities, even at an early stage of drug discovery, may be a necessary additional quality filter that should be considered during the compound screening and profiling cascade.Publication link2010 Solid-supported membrane technology for the investigation of the influenza A virus M2 channel activitySURFE2R ONE (a predecessor model of SURFE2R N1) Publication in Pflgers Archiv - European Journal of Physiology (2010) Authors: Balannik V., Obrdlik P., Inayat S., Steensen C., Wang J., Rausch J.M., DeGrado W.F., Kelety B., Pinto L.H.Influenza A virus encodes an integral membrane protein, A/M2, that forms a pH-gated proton channel that is essential for viral replication. The A/M2 channel is a target for the anti-influenza drug amantadine, although the effectiveness of this drug has been diminished by the appearance of naturally occurring point mutations in the channel pore. Thus, there is a great need to discover novel anti-influenza therapeutics, and, since the A/M2 channel is a proven target, approaches are needed to screen for new classes of inhibitors for the A/M2 channel. Prior in-depth studies of the activity and drug sensitivity of A/M2 channels have employed labor-intensive electrophysiology techniques. In this study, we tested the validity of electrophysiological measurements with solid-supported membranes (SSM) as a less labor-intensive alternative technique for the investigation of A/M2 ion channel properties and for drug screening. By comparing the SSM-based measurements of the activity and drug sensitivity of A/M2 wild-type and mutant channels with measurements made with conventional electrophysiology methods, we show that SSM-based electrophysiology is an efficient and reliable tool for functional studies of the A/M2 channel protein and for screening compounds for inhibitory activity against the channel.Publication link2010 The G215R Mutation in the Cl/H+-Antiporter ClC-7 Found in ADO II Osteopetrosis Does Not Abolish Function but Causes a Severe Trafficking DefectSURFE2R ONE (a predecessor model of SURFE2R N1) Publication in PLoS ONE (2010) Authors: Schulz P., Werner J., Stauber T., Henriksen K., Fendler K.BACKGROUND:ClC-7 is a ubiquitous transporter which is broadly expressed in mammalian tissues. It is implied in the pathogenesis of lysosomal storage disease and osteopetrosis. Because of its endosomal/lysosomal localization it is still poorly characterized.METHODOLOGY/PRINCIPAL FINDINGS:An electrophysiological characterization of rat ClC-7 using solid-supported membrane-based electrophysiology is presented. The measured currents show the characteristics of ClC-7 and confirm its function as a Cl-/H+-antiporter. We have used rat ClC-7 in CHO cells as a model system to investigate the functionality and cellular localization of the wt transporter and its variant G213R ClC-7 which is the analogue of human G215R ClC-7 responsible for autosomal dominant osteopetrosis type II. Our study shows

Escherichia coli and GltPPhfrom Pyrococcus horikoshii were overexpressed in E. coli and purified to homogeneity with a yield of 1-2 mg/L of culture. Single-particle analysis and electron microscopy indicate that GltP(Ph) is a trimer in detergent solution. Electron microscopy of negatively stained GltPPhtwo-dimensional crystals shows that the transporter is a trimer also in the membrane. Gel filtration of GltPEcindicates a reversible equilibrium of two oligomeric states in detergent solution that we identified as a trimer and hexamer by blue-native gel electrophoresis and cross-linking. The purified transporters were fully active upon reconstitution into liposomes, as demonstrated by the uptake of radioactively labeled L-aspartate or L-glutamate. L-aspartate/L-glutamate transport of GltPEcinvolves the cotransport of protons and depends only on pH, whereas GltP(Ph) catalyzes L-glutamate transport with a cotransport of H+or Na+. L-glutamate induces a fast transient current in GltP(Ph) proteoliposomes coupled to a solid supported membrane (SSM). We show that the electric signal depends on the concentration of Na+or H+outside the proteoliposomes and that GltP(Ph) does not require K+inside the proteoliposomes. In addition, the electrical currents are inhibited by TBOA and HIP-B. The half-saturation concentration for activation of GltPPhglutamate transport (K0.5glut) is 194 M.Publication link2004 Time-resolved charge translocation by sarcoplasmic reticulum Ca-ATPase measured on a solid supported membraneSURFE2R-technology (custom-built system) Publication in Biophysical Journal (2004) Authors: Tadini Buoninsegni F., Bartolommei G., Moncelli M.R., Inesi G., Guidelli R.Sarcoplasmic reticulum vesicles were adsorbed on an octadecanethiol/phosphatidylcholine mixed bilayer anchored to a gold electrode, and the Ca-ATPase contained in the vesicles was activated by ATP concentration jumps both in the absence and in the presence of K(+) ions and at different pH values. Ca2+concentration jumps in the absence of ATP were also carried out. The resulting capacitive current transients were analyzed together with the charge under the transients. The relaxation time constants of the current transients were interpreted on the basis of an equivalent circuit. The current transient after ATP concentration jumps and the charge after Ca2+concentration jumps in the absence of ATP exhibit almost the same dependence upon the Ca2+concentration, with a half-saturating value of approximately 1.5 M. The pH dependence of the charge after Ca2+translocation demonstrates the occurrence of one H+ per one Ca2+countertransport at pH 7 by direct charge-transfer measurements. The presence of K+decreases the magnitude of the current transients without altering their shape; this decrease is explained by K+binding to the cytoplasmic side of the pump in the E1conformation and being released to the same side during the E1-E2transition.Publication link2005 Kinetics of charge translocation in the passive downhill uptake mode of the Na+/H+ antiporter NhaA of Escherichia coliSURFE2R 500 (a predecessor model of SURFE2R N1) Publication in Biochimica et Biophysica Acta (BBA) - Bioenergetics (2005) Authors: Zuber D., Krause R., Venturi M., Padan E., Bamberg E., Fendler K.The Na+/H+antiporter NhaA is the main Na+extrusion system in E. coli. Using direct current measurements combined with a solid supported membrane (SSM), we obtained electrical data of the function of NhaA purified and reconstituted in liposomes. These measurements demonstrate NhaA's electrogenicity, its specificity for Li+and Na+and its pronounced pH dependence in the range pH 6.5-8.5. The mutant G338S, in contrast, presents a pH independent profile, as reported previously. A complete right-side-out orientation of the NhaA antiporter within the proteoliposomal membrane was determined using a NhaA-specific antibody based ELISA assay. This allowed for the first time the investigation of NhaA in the passive downhill uptake mode corresponding to the transport of Na+from the periplasmic to the cytoplasmic side of the membrane. In this mode, the transporter has kinetic properties differing significantly from those of the previously investigated efflux mode. The apparent Kmvalues were 11 mM for Na+and 7.3 mM for Li+at basic pH and 180 mM for Na+and 50 mM for Li+at neutral pH. The data demonstrate that in the passive downhill uptake mode pH regulation of the carrier affects both apparent Kmas well as turnover (Vmax).Publication link2004 Calcium transport by sarcoplasmic reticulum Ca-ATPase can be investigated on a solid-supported membraneSURFE2R-technology (custom-built system) Publication in Bioelectrochemistry (2004) Authors: Bartolommei G., Buoninsegni F.T., Moncelli M.R.Sarcoplasmic reticulum (SR) native vesicles incorporating Ca-ATPase are adsorbed on a solid-supported lipid membrane (SSM). Upon adsorption, the ion pumps are chemically activated by concentration jumps of ATP and the capacitive current transients generated by SR Ca-ATPase are measured under potentiostatic conditions. The Michaelis-Menten constant, KM, for ATP is evaluated by varying the concentration of ATP in the activating solution. This preliminary result shows that ion transport by SR Ca-ATPase can be suitably investigated by a technique based on concentration jumps on an SSM.Publication link2004 Charge translocation during cosubstrate binding in the Na+/proline transporter of E.coliSURFE2R-technology (custom-built system) Publication in Journal of Molecular Biology (2004) Authors: Zhou A., Wozniak A., Meyer-Lipp K., Nietschke M., Jung H., Fendler K.Charge translocation associated with the activity of the Na+/proline cotransporter PutP of Escherichia coli was analyzed for the first time. Using a rapid solution exchange technique combined with a solid-supported membrane (SSM), it was demonstrated that Na+and/or proline individually or together induce a displacement of charge. This was assigned to an electrogenic Na+and/or proline binding process at the cytoplasmic face of the enzyme with a rate constant of k>50 s1which preceeds the rate-limiting step. Based on the kinetic analysis of our electrical signals, the following characteristics are proposed for substrate binding in PutP. (1) Substrate binding is electrogenic not only for Na+, but also for the uncharged cosubstrate proline. The charge displacement associated with the binding of both substrates is of comparable size and independent of the presence of the respective cosubstrate. (2) Both substrates can bind individually to the transporter. Under physiological conditions, an ordered binding mechanism prevails, while at sufficiently high concentrations, each substrate can bind in the absence of the other. (3) Both substrate binding sites interact cooperatively with each other by increasing the affinity and/or the speed of binding of the respective cosubstrate. (4) Proline binding proceeds in a two-step process: low affinity (1 mM) electroneutral substrate binding followed by a nearly irreversible electrogenic conformational transition.Publication link2002 Photocurrents Generated by Bacteriorhodopsin Adsorbed on Thiol/Lipid Bilayers Supported by MercurySURFE2R-technology (custom-built system) Publication in Langmuir (2002) Authors: Dolfi A., Tadini-Buoninsegni F., Moncelli M.R., Guidelli R.The kinetics of light-driven proton transport by bacteriorhodopsin (bR) were investigated over a broad pH range upon adsorbing purple membrane (PM) fragments on a mercury-supported mixed alkanethiol/phospholipid bilayer. The light-on and light-off capacitive photocurrents were measured under short-circuit conditions in the absence of photoartifacts. Using dioleoylphosphatidylcholine as the lipid monolayer, a bell-shaped curve of the peak current versus pH, with a maximum in the proximity of 6, was obtained. The analysis of the biphasic decay kinetics of the light-on and light-off currents allows an estimate of the pKavalues for the steps releasing protons to, and taking up protons from, the bathing solution. In particular, the pKavalues obtained from the light-off current (pK1= 3.5, pK2= 5.3, pK3= 7.5, and pK4= 9.0) suggest a mechanism similar to that proposed by Balashov et al. for dark adaptation, albeit in the opposite direction (Balashov, S. P.; Imasheva, E. S.; Govindjee, R.; Sheves, M.; Ebrey, T. G. Biophys. J. 1996, 70, 473). The time dependence of the light-on and light-off currents in the proximity of pH 6 is interpreted on the basis of both a simple equivalent circuit and a kinetic model making use of spectroscopic data available in the literature. When using dioleoylphosphatidylserine (DOPS) as the lipid monolayer, an inversion in the sign of both light-on and light-off currents, as well as a change in their shape and magnitude, was observed by increasing the pH above 9 and then, at all pH values from 9 to 1, by subsequently decreasing the pH on the same mercury-supported mixed alkanethiol/DOPS bilayer. The normal situation was restored only by adding sodium azide. This inversion in current and the notable hysteresis observed under these conditions are critically discussed.Publication link2003 Charge displacements during ATP-hydrolysis and synthesis of the Na+-transporting FoF1-ATPase of Ilyobacter tartaricusSURFE2R-technology (custom-built system) Publication in Biophysical Journal (2003) Authors: Burzik C., Kaim G., Dimroth P., Bamberg E., Fendler K.Transient electrical currents generated by the Na+-transporting FoF1-ATPase of Ilyobacter tartaricus were observed in the hydrolytic and synthetic mode of the enzyme. Two techniques were applied: a photochemical ATP concentration jump on a planar lipid membrane and a rapid solution exchange on a solid supported membrane. We have identified an electrogenic reaction in the reaction cycle of the FoF1-ATPase that is related to the translocation of the cation through the membrane bound Fosubcomplex of the ATPase. In addition, we have determined rate constants for the process: For ATP hydrolysis this reaction has a rate constant of 1530s1 if H+is transported and 3060s1if Na+ is transported. For ATP synthesis the rate constant is 5070s1.Publication link1999 Kinetics of electrogenic transport by the ADP/ATP carrierSURFE2R-technology (custom-built system) Publication in Biophysical Journal (1999) Authors: Gropp T., Brustovetsky N., Klingenberg M., Mller V., Fendler K., Bamberg E.The electrogenic transport of ATP and ADP by the mitochondrial ADP/ATP carrier (AAC) was investigated by recording transient currents with two different techniques for performing concentration jump experiments: 1) the fast fluid injection method: AAC-containing proteoliposomes were adsorbed to a solid supported membrane (SSM), and the carrier was activated via ATP or ADP concentration jumps. 2) BLM (black lipid membrane) technique: proteoliposomes were adsorbed to a planar lipid bilayer, while the carrier was activated via the photolysis of caged ATP or caged ADP with a UV laser pulse. Two transport modes of the AAC were investigated, ATPex-0inand ADPex-0in. Liposomes not loaded with nucleotides allowed half-cycles of the ADP/ATP exchange to be studied. Under these conditions the AAC transports ADP and ATP electrogenically. Mg2+inhibits the nucleotide transport, and the specific inhibitors carboxyatractylate (CAT) and bongkrekate (BKA) prevent the binding of the substrate. The evaluation of the transient currents yielded rate constants of 160s1for ATP and 400s1for ADP translocation. The function of the carrier is approximately symmetrical, i.e., the kinetic properties are similar in the inside-out and right-side-out orientations. The assumption from previous investigations, that the deprotonated nucleotides are exclusively transported by the AAC, is supported by further experimental evidence. In addition, caged ATP and caged ADP bind to the carrier with similar affinities as the free nucleotides. An inhibitory effect of anions (200300mM) was observed, which can be explained as a competitive effect at the binding site. The results are summarized in a transport model.Publication link2001 Evidence for intraprotein charge transfer during the transport activity of the melibiose permease from Escherichia coliSURFE2R-technology (custom-built system) Publication in Biochemistry (2001) Authors: Ganea C., Pourcher T., Leblanc G., Fendler K.Electrogenic activity associated with the activity of the melibiose permease (MelB) of Escherichia coli was investigated by using proteoliposomes containing purified MelB adsorbed onto a solid-supported membrane. Transient currents were selectively recorded by applying concentration jumps of Na+ions (or Li+) and/or of different sugar substrates of MelB (melibiose, thio-methyl galactoside, raffinose) using a fast-flow solution exchange system. Characteristically, the transient current response was fast, including a single decay exponential component (15 ms) on applying a Na+(or Li+) concentration jump in the absence of sugar. On imposing a Na+(or Li+) jump on proteoliposomes preincubated with the sugar, a sugar jump in a preparation preincubated with the cation, or a simultaneous jump of the cation and sugar substrates, the electrical transients were biphasic and comprised both the fast and an additional slow (350 ms) decay components. Finally, selective inactivation of the cosubstrate translocation step by acylation of MelB cysteins with N-ethyl maleimide suppressed the slow response components and had no effect on the fast transient one. We suggest that the fast transient response reflects charge transfer within MelB during cosubstrate binding while the slow component is associated with charge transfer across the proteoliposome membrane. From the time course of the transient currents, we estimate a rate constant for Na+binding in the absence and presence of melibiose of k > 50 s-1and one for melibiose binding in the absence of Na+of k 10 s-1.Publication link1999 Charge Translocation by the Na/K-ATPase Investigated on Solid Supported Membranes: Rapid Solution Exchange with a New TechniqueSURFE2R-technology (custom-built system) Publication in Biophysical Journal (1999) Authors: Pintschovius J., Fendler K.Adsorption of Na+/K+-ATPase containing membrane fragments from pig kidney to lipid membranes allows the detection of electrogenic events during the Na+/K+-ATPase reaction cycle with high sensitivity and time resolution. High stability preparations can be obtained using solid supported membranes (SSM) as carrier electrodes for the membrane fragments. The SSMs are prepared using an alkanethiol monolayer covalently linked to a gold surface on a glass substrate. The hydrophobic surface is covered with a lipid monolayer (SAM, self-assembled monolayer) to obtain a double layer system having electrical properties similar to those of unsupported bilayer membranes (BLM). As we have previously shown (, Biophys. J. 64:384-391), the Na+/K+-ATPase on a SSM can be activated by photolytic release of ATP from caged ATP. In this publication we show the first results of a new technique which allows rapid solution exchange at the membrane surface making use of the high mechanical stability of SSM preparations. Especially for substrates, which are not available as a caged substance-such as Na+and K+-this technique is shown to be capable of yielding new results. The Na+/K+-ATPase was activated by rapid concentration jumps of ATP and Na+(in the presence of ATP). A time resolution of up to 10 ms was obtained in these experiments. The aim of this paper is to present the new technique together with the first results obtained from the investigation of the Na+/K+-ATPase. A comparison with data taken from the literature shows considerable agreement with our experiments.Publication link1999 Charge Translocation by the Na+/K+-ATPase Investigated on Solid Supported Membranes: Cytoplasmic Cation Binding and ReleaseSURFE2R-technology (custom-built system) Publication in Biophysical Journal (1999) Authors: Pintschovius J., Fendler K., Bamberg E.In the preceding publication (Pintschovius and Fendler, 1999. Biophys. J. 76:000000) a new technique was described that was able to produce concentration jumps of arbitrary ion species at the surface of a solid supported membrane (SSM). This technique can be used to investigate the kinetics of ion translocating proteins adsorbed to the SSM. Charge translocation of the Na+/K+-ATPase in the presence of ATP was investigated. Here we describe experiments carried out with membrane fragments containing Na+/K+-ATPase from pig kidney and in the absence of ATP. Electrical currents are measured after rapid addition of Na+. We demonstrate that these currents can be explained only by a cation binding process on the cytoplasmic side, most probably to the cytoplasmic cation binding site of the Na+/K+-ATPase. An electrogenic reaction of the protein was observed only with Na+, but not with other monovalent cations (K+, Li+, Rb+, Cs+). Using Na+activation of the enzyme after preincubation with K+we also investigated the K+-dependent half-cycle of the Na+/K+-ATPase. A rate constant for K+translocation in the absence of ATP of 0.20.3 s1 was determined. In addition, these experiments show that K+deocclusion, and cytoplasmic K+release are electroneutral.Publication link1993 Charge transport by ion translocating membrane proteins on solid supported membranesSURFE2R-technology (custom-built system) Publication in Biophysical Journal (1993) Authors: Seifert K., Fendler K., Bamberg E.A new method for the investigation of ion translocating membrane proteins is presented. Protein containing membrane fragments or vesicles are adsorbed to a solid supported membrane. The solid supported membrane consists of a lipid monolayer on a gold evaporated or gold sputtered glass substrate which is coated with a long chained mercaptan (CH3(CH2)mSH, m = 15, 17). Specific conductance and specific capacitance of the solid supported membrane are comparable to those of a black lipid membrane. However, the solid supported membrane has the advantage of a much higher mechanical stability. The electrical activity of bacteriorhodopsin, Na,K-ATPase, H,K-ATPase, and Ca-ATPase on the solid supported membrane is measured and compared to signals obtained on a conventionally prepared black lipid membrane. It is shown that both methods yield similar results. The solid supported membrane therefore represents an alternative method for the investigation of electrical properties of ion translocating transmembrane proteins.Presentation PDF2020 Unlocking the (Reversal) Potential of SSM Electrophysiology: Transporter Stoichiometry with the SURFE2R N1Presenter: Nathan Thomas 5th Year PhD student at University of Wisconsin-Madison, USA (Dr. Henzler-Wildman's lab) Source:The 64th Annual Meeting of the Biophysical Society, San Diego, CA (USA), Feb 15-19. 2020Webinar19.05.2020 | Webinar: Insights and New Approaches in Transporter Characterization SURFE2R N1Speakers: Dr. Maria Barthmes (Product Manager, Nanion Technologies)Nathan Thomas (5th Year PhD, University of Wisconsin-Madison [Dr. Henzler-Wildman's lab])Prof. Dr. Camilo Perez (Head of Research Group - BIOZENTRUM, University of Basel, Switzerland)Maria will introduce SSM-based Electrophysiology going over basic features and principles of the method and have a look at experimental workflows.Nathan Thomas"Unlocking the (Reversal) Potential of SSM Electrophysiology: Transporter Stoichiometry with the SURFE2R N1Camillo Perez:"Characterization of a choline uniporter by SSM-based electrophysiology" disclaimer: due to a pending manuscript submission, this presentation will be made available in full in the coming weeks. Please stay tuned.Webinar28.06.2022 | Webinar: Structures and mechanism of the plant PIN-FORMED auxin transporterSpeakers:Associate Professor Bjrn Panyella Pedersen(Aarhus University, Denmark, Dept of Molecular Biology and Genetics)Associate Professor Ulrich Hammes(Technical University of Munich, Germany, Plant Systems Biology)Auxins are central hormones in all plants and controls virtually all aspects of growth and development. The PIN-FORMED (PIN) protein family is a key player in this process. Here we present biophysical analysis and structures of Arabidopsis thaliana PIN8 at 2.9-3.4 resolution; two outward facing conformations with and without auxin bound, and one inward facing conformation with the known inhibitor and herbicide naphthylphthalamic acid (NPA) bound. The structure forms a homo-dimer with each monomer divided into a transport and scaffold domain with a clearly defined auxin binding site. Next to the binding site, a proline-proline crossover is a pivot point for structural changes associated with transport, which we show by biophysical analysis to be independent of proton and ion gradients and likely driven by the negative charge of the auxin. Our results provide the first comprehensive molecular model for auxin recognition and transport by PINs.Webinar16.06.2020 | Webinar: Functional studies on membrane transporters using the SURFE2R N1Speakers: Dr. Maria Barthmes (Product Manager, Nanion Technologies)Dr. Andre Bazzone (Application Scientist, Nanion Technologies)SSM (solid supported membrane)-based electrophysiology employed by the SURFE2R instruments is a capacitive sensor-based method to detect membrane currents generated by low turnover proteins such as transporters and membrane pumps. By resolving protein activity in real time and label free it introduces the advantages of electrophysiology to the field of membrane transporters. During this webinar you will learn all about SSM-based electrophysiology and its applications.First, we discuss the basics and principles behind this technology in depth. Then we focus on practical topics, like preparation of samples and experimental workflows, and finally introduce some datasets to highlight the potential and possibilities for membrane transporter studies.aria will introduce SSM-based Electrophysiology going over basic features and principles of the method and have a look at experimental workflows.Webinar18.03.2021 | Webinar: Understanding Drug Efflux Small Multidrug Resistance Family Stoichiometry and SpecificitySpeakers:Randy Stockbridge(Assistant Professor, Department of Molecular, Cellular, and Developmental Biology - University of Michigan)Katherine Henzler-Wildman(Professor Biochemistry, Department of Biochemistry - University of Wisconsin-Madison)Title: Determining transport stoichiometry using SSMERandy Stockbridge(Assistant Professor, Department of Molecular, Cellular, and Developmental Biology - University of Michigan)Abstract: Transporters from the small multidrug resistance (SMR) family provide broad resistance to environmental biocides, driving the spread of multidrug resistance cassettes among bacterial populations. Understanding substrate specificity is essential to understand this process. Using solid-supported membrane electrophysiology, we measure the transport of different substrates by SMR family members, and show that promiscuous transport of hydrophobic substituted cations is a general feature of all SMR transporters, including those whose primary physiological role is in bacterial nitrogen metabolism.Title: Basis of promiscuity in small multidrug resistance transportersKatherine Henzler-Wildman(Professor Biochemistry, Department of Biochemistry - University of Wisconsin-Madison)Abstract: Transport stoichiometry can provide great insight into the mechanism and function of ion-coupled transporters. Traditional reversal potential assays are a reliable, general method for determining the transport stoichiometry of ion-coupled transporters, but the time and material costs of this technique hinder investigations of transporter behavior under multiple experimental conditions. Our prior work on EmrE has demonstrated that it is not a tightly coupled transporter and that the net transport stoichiometry is likely to vary with pH and substrate identity. This has motivated us to develop an SSME-based assay for assessing transport stoichiometry that is rapid and easily adaptable to different substrates and pH conditions. Here we present results for Gdx and CLC-Ec1, two well-characterized transporters that demonstrate the success of our approach. Our SSME-based method reproduces the fixed 2H+:1 guanidinium+ antiport stoichiometry of Gdx, the 1H+:2Cl- antiport stoichiometry of CLC-ec1, and loose proton:nitrate coupling for CLC-ec1. This method requires only small amounts of transporter and provides a fast, easy method to characterize transport stoichiometry under varied conditions, which will facilitate future mechanistic and functional studies of ion-coupled transporters.Webinar06.05.2021 | Webinar: Secondary transport inhibition and regulation by SSM-electrophysiologyProfessor Dr. Christine Ziegler(University of Regensburg, Faculty of Biology and Pre-Clinics, Institute of Biophysics and physical Biochemistry, Structural Biology-Biophysics II) Professor Dr. Camilo Perez(University of Basel, Biozentrum, Center for Molecular Life Sciences)Title: The K+ switch in BetP: from coupling to regulationProfessor Dr. Christine Ziegler(University of Regensburg, Faculty of Biology and Pre-Clinics, Institute of Biophysics and physical Biochemistry, Structural Biology-Biophysics II)Abstract: The bacterial betaine transporter BetP is a prime example for an efficient osmotic stress sensor and regulated secondary transporter, respectively. BetPs full activation depends on the presence of 300mM internal K+, however, K+ is not transported. Several K+ binding sites were identified at the osmo-sensor, but also close to a Na+ site. We introduced a point mutation based in one of the two sodium binding sites in order to switch BetP from Na+ to K+ coupling. Using cryoEM/X-ray crystallography combined with SSM/Stopped-Flow Trp-fluorescence we discovered an intriguing competition between Na+ and K+ binding in BetP, which hints towards a change in the functional role of K+ in LeuT-fold transporter during evolution. BetP shares its overall fold with SLC6 neurotransmitter transporters, which also show differences in their ability to facilitate K+-coupled antiport. Therefore, our structure-function study provides new insights into an evolutionary switching of K+ between coupling to regulatory ion.Title: Selection of transporter-targeted inhibitory nanobodies by SSM-based electrophysiologyProfessor Dr. Camilo Perez(University of Basel, Biozentrum, Center for Molecular Life Sciences)Abstract: Single domain antibodies (nanobodies) have been extensively used in machanistic and structural studies of protein and pose an enormous potential as tools for developing clinical therapies, many of which depend on inhibition of membrane proteins such as transporters.However, most of the methods used to determine inhibition of transport activity are difficult to perform in high-throughput routines and depend on labeled substrates availability. This complicates the screening of large nanobody libraries. Solid-supported membrane (SSM) electrophysiology to select inhibitory and non-inhibitory nanobodies targeting an electrogenic secondary transporter and to calculate nanobodies inhibitory constants. This technique may be especially useful for selecting inhibitory nanobodies targeting transporters for which labeled substrates are not available.Webinar13.10.2020 | Webinar: Employing SSM electrophysiology to capture electrogenic fluxes in membrane proteinsSpeakers:Dr. Matthias Quick (Columbia University; NY, USA)This is an on-demand webinar from Nanji[on and Friends 2020.My lab has been focusing on the study of ion-dependent transporters with special emphasis on Na+ or H+-coupled symporters. Whereas flux studies with radiolabeled solutes using the target protein reconstituted in proteoliposomes provided a wealth of information, the determination of the thermodynamically-coupled solute transport-associated flux of H+ or Na+ has been challenging. This can be attributed in part to the low transport turnover numbers of these transporters and difficulties associated with their functional expression in suitable model systems that allow for their characterization with traditional electrophysiological methods (e.g., two-electrode voltage clamp or patch-clamp methods).By using the SURFE2R N1 SSM platform, our team was able to quickly collect data of solute transport-associated flux of co-transported ions across the membrane of proteoliposomes containing different target proteins. With this technology it is possible to collect data for a full kinetic characterization of a target protein such as its dependence on substrate and ion concentrations, pH, and potential essential additives, as well as its substrate recognition profile. The SURFE2R system also enables the use of a wide range of substrates that are readily commercially available, avoiding the use of radiolabeled compounds.Webinar01.07.2021 | Webinar: SSM-electrophysiology as a Tool for Uncovering Transporter FunctionSpeakers:Olga Boudker, Professor of Physiology and Biophysics(Weill Cornell Medical College)Lars Jeuken, Professor of Molecular Biophysics(Faculty of Biological Sciences, University of Leeds)Olga Boudker, Professor of Physiology and Biophysics(Weill Cornell Medical College)Abstract:Human excitatory amino acid transporter 3 (hEAAT3) mediates glutamate uptake in neurons, intestine, and kidney. We have determined cryo-EM structures of hEAAT3 in several functional states where the transporter is empty, bound to coupled sodium ions only, or fully loaded with three sodium ions, a proton, and the substrate aspartate. The structures suggest that hEAAT3 operates by an elevator mechanism involving three functionally independent subunits. When the substrate-binding site is near the cytoplasm, it has a remarkably low affinity for the substrate, perhaps facilitating its release and allowing the rapid transport turnover. The mechanism of the coupled uptake of the sodium ions and the substrate is conserved across evolutionarily distant families and is augmented by coupling to protons in EAATs. The structures further suggest a mechanism by which a conserved glutamate residue mediates proton symport.Title: SSM-based electrophysiological characterization of a metal transporterLars Jeuken, Professor of Molecular Biophysics,(Faculty of Biological Sciences, University of Leeds)Abstract:Transition metals are essential trace elements and their high-affinity uptake is required for many organisms. Metal transporters are often characterized using metal-sensitive fluorescent dyes encapsulated in proteoliposomes, limiting the metals and experimental conditions that can be studied. Here, we have tested whether metal transport by Enterococcus faecalis MntH2 can be measured with the solid-supported membrane (SSM) technology. SSM uptake assays confirm transport of Mn(II), Co(II), Zn(II), and Cd(II) by MntH2. However, no uptake responses for Cu(II), Fe(II) nor Ni(II) were observed, while the presence of these metals abolishes the uptake signals for Mn(II). Although E. faecalis MntH2 is hypothesized to be a proton-metal symporter, no proton symport could be detected with either SSM or fluorescence assays. These data are discussed with respect to fluorescence uptake assays with Mn(II) and Ni(II), where transport was measured on the time scale of minutes, in sharp contrast to the sub-second timescale of the SSM technology.Webinar02.12.2021 | Webinar: Structure and Function of Na+ and H+ dependent transportersSpeakers: Professor David Drew(Stockholm University, Professor in Biochemistry)Associate Professor Dr. Matthias Quick(Columbia University Irving Medical Center, Departments of Psychiatry and Physiology and Cellular Biophysics, Associate Professor of Neurobiology)Title: Structure and mechanism of the Na+/H+ exchanger NHA2Professor David Drew(Stockholm University, Professor in Biochemistry)Abstract: SLC9B2, also known as NHA2, correlates with the long-sought after sodium/lithium (Na+/Li+) exchanger linked to the pathogenesis of diabetes mellitus and essential hypertension in humans. Despite its functional importance, structural information and the molecular basis of its ion-exchange mechanism have been lacking. Here, we I briefly present the cryo EM structures of bison NHA2 in detergent and in nanodiscs at 3.0 and 3.5 resolution, respectively. I will then show how SSM-based electrophysiology has enabled us to conclude that NHA2 catalyses the electroneutral rather than electrogenic exchange of ions. The ion-binding site is quite distinctive, with a tryptophan-arginine-glutamate triad separated from the well-established ion-binding aspartates. These triad residues fine-tune ion binding specificity, as demonstrated by a salt-bridge swap mutant that converts NHA2 into a Li+-specific transporter.Title: The molecular mechanism of the Na+/H+ antiporter NhaA from binding to fluxAssociate Professor Dr. Matthias Quick(Columbia University Irving Medical Center, Departments of Psychiatry and Physiology and Cellular Biophysics, Associate Professor of Neurobiology)Abstract: The Na+/H+ antiporter NhaA represents the archetype of Na+/H+ exchangers, evolutionarily conserved proteins in all kingdoms of life that are essential in cellular ion homeostasis. While structural information has provided excellent starting points in developing mechanistic models of NhaA-mediated transport, the correlation between the correlation of Na+ and H+ binding and flux remains still enigmatic. Since structural information about the composition of the Na+ and H+ sites in NhaA is missing, functional assays are required to gain insight into the molecular events that regulate NhaA activity. By using the SURFE2R N1 SSM platform, our team was able to collect data of NhaA-mediated ion flux across the membrane of NhaA-containing proteoliposomes. Direct Na+ binding studies in conjunction with flux studies reveal that, whereas Na+ transport is impaired at low pH, NhaA can bind Na+ in pH-independent fashion, providing new insight into the interplay of the two cations during transport.Product Sheet PDFSURFE2R N1 Product SheetProduct video2020 SURFE2R N1 Product VideoLabel free, real time measurements - electrophysiological approach. Record the action of billion transporters simultaneously.Label free, real time measurements - electrophysiological approach. Record the action of billion transporters simultaneously.Poster PDF2018 Transported by light: optogenetic control of NCX1SURFE2R N1 poster, Biophysics Annual Meeting 2018Poster PDF2022 Functional investigation of GABA and Glutamate re-uptake transporters EAAT3 and GAT1 using SSM-based electrophysiologyPublication link2021 An Overview of Cell-Based Assay Platforms for the Solute Carrier Family of TransportersSURFE2R N1 and SURFE2R 96SE review Publication in Frontiers in Pharmacology (2021) Authors: Dvorak V., Wiedmer T., Ingles-Prieto A., Altermatt P., Batoulis H., Brenz F., Bender E., Digles D., Drrenberger F., Heitman L.H., IJzerman A.P., Kell D.B., Kickinger S., Krz D., Leippe P., Licher T., Manolova V., Rizzetto R., Sassone F., Scarabottolo L., Schlessinger A., Schneider V., Sijben H.J., Steck A-L., Sundstrm H., Tremolada S., Wilhelm M., Wright Muelas M., Zindel D., Steppan C-M., Superti-Furga G.The solute carrier (SLC) superfamily represents the biggest family of transporters with important roles in health and disease. Despite being attractive and druggable targets, the majority of SLCs remains understudied. One major hurdle in research on SLCs is the lack of tools, such as cell-based assays to investigate their biological role and for drug discovery. Another challenge is the disperse and anecdotal information on assay strategies that are suitable for SLCs. This review provides a comprehensive overview of state-of-the-art cellular assay technologies for SLC research and discusses relevant SLC characteristics enabling the choice of an optimal assay technology. The Innovative Medicines Initiative consortium RESOLUTE intends to accelerate research on SLCs by providing the scientific community with high-quality reagents, assay technologies and data sets, and to ultimately unlock SLCs for drug discovery.Poster PDF2015 Functional Characterization of Prokaryotic NCX by Solid Supported Membrane TechnologySURFE2R N1 poster, Gordon Research Conference 2015Publication link2020 Protein Adsorption on Solid Supported Membranes: Monitoring the Transport Activity of P-Type ATPasesSURFE2R 96SE andSURFE2R N1 Review in Molecules (2020) Authors: Tadini-Buoninsegni F.P-type ATPases are a large family of membrane transporters that are found in all forms of life. These enzymes couple ATP hydrolysis to the transport of various ions or phospholipids across cellular membranes, thereby generating and maintaining crucial electrochemical potential gradients. P-type ATPases have been studied by a variety of methods that have provided a wealth of information about the structure, function, and regulation of this class of enzymes. Among the many techniques used to investigate P-type ATPases, the electrical method based on solid supported membranes (SSM) was employed to investigate the transport mechanism of various ion pumps. In particular, the SSM method allows the direct measurement of charge movements generated by the ATPase following adsorption of the membrane-bound enzyme on the SSM surface and chemical activation by a substrate concentration jump. This kind of measurement was useful to identify electrogenic partial reactions and localize ion translocation in the reaction cycle of the membrane transporter. In the present review, we discuss how the SSM method has contributed to investigate some key features of the transport mechanism of P-type ATPases, with a special focus on sarcoplasmic reticulum Ca2+-ATPase, mammalian Cu+-ATPases (ATP7A and ATP7B), and phospholipid flippase ATP8A2.Publication link2021 A Solid Supported Membrane-Based Technology for Electrophysical Screening of B0AT1-Modulating CompoundsSURFE2R 96SE Publicationin SLAS DISCOVERY: Advancing the Science of Drug Discovery.(2021) Authors: Gerbeth-Kreul C., Pommereau A., Ruf S., Kane Jr J.L., Kuntzweiler T., Hessler G., Engel C.K., Shum P., Wei L., Czech J., Licher T.Classical high-throughput screening (HTS) technologies for the analysis of ionic currents across biological membranescan be performed using fluorescence-based, radioactive, and mass spectrometry (MS)-based uptake assays. These assaysprovide rapid results for pharmacological HTS, but the underlying, indirect analytical character of these assays can be linkedto high false-positive hit rates. Thus, orthogonal and secondary assays using more biological target-based technologies areindispensable for further compound validation and optimization. Direct assay technologies for transporter proteins areelectrophysiology-based, but are also complex, time-consuming, and not well applicable for automated profiling purposes.In contrast to conventional patch clamp systems, solid supported membrane (SSM)-based electrophysiology is a sensitive,membrane-based method for transporter analysis, and current technical developments target the demand for automated,accelerated, and sensitive assays for transporter-directed compound screening. In this study, the suitability of the SSM based technique for pharmacological compound identification and optimization was evaluated performing cell-free SSMbased measurements with the electrogenic amino acid transporter B0AT1 (SLC6A19). Electrophysiological characterizationof leucine-induced currents demonstrated that the observed signals were specific to B0AT1. Moreover, B0AT1-dependentresponses were successfully inhibited using an established in-house tool compound. Evaluation of current stability anddata reproducibility verified the robustness and reliability of the applied assay. Active compounds from primary screensof large compound libraries were validated, and false-positive hits were identified. These results clearly demonstrate thesuitability of the SSM-based technique as a direct electrophysiological method for rapid and automated identification ofsmall molecules that can inhibit B0AT1 activityPublication link2020 Label-Free Bioelectrochemical Methods for Evaluation of Anticancer Drug Effects at a Molecular LevelSURFE2R 96SE and SURFE2R N1 Review article in Frontiers in Sensors (2020) Authors: Tadini-Buoninsegni F.,Palchetti I.Cancer is a multifactorial family of diseases that is still a leading cause of death worldwide. More than 100 different types of cancer affecting over 60 human organs are known. Chemotherapy plays a central role for treating cancer. The development of new anticancer drugs or new uses for existing drugs is an exciting and increasing research area. This is particularly important since drug resistance and side effects can limit the efficacy of the chemotherapy. Thus, there is a need for multiplexed, cost-effective, rapid, and novel screening methods that can help to elucidate the mechanism of the action of anticancer drugs and the identification of novel drug candidates. This review focuses on different label-free bioelectrochemical approaches, in particular, impedance-based methods, the solid supported membranes technique, and the DNA-based electrochemical sensor, that can be used to evaluate the effects of anticancer drugs on nucleic acids, membrane transporters, and living cells. Some relevant examples of anticancer drug interactions are presented which demonstrate the usefulness of such methods for the characterization of the mechanism of action of anticancer drugs that are targeted against various biomolecules.Publication link2017 SSM-Based Electrophysiology for Transporter ResearchSURFE2R N1 and SURFE2R 96SE book chapter in Methody in Enzymology Authors: Bazzone A., Barthmes M., Fendler K.Functional characterization of transport proteins using conventional electrophysiology can be challenging, especially for low turnover transporters or transporters from bacteria and intracellular compartments. Solid-supported membrane (SSM)-based electrophysiology is a sensitive and cell-free assay technique for the characterization of electrogenic membrane proteins. Purified proteins reconstituted into proteoliposomes or membrane vesicles from cell culture or native tissues are adsorbed to the sensor holding an SSM. A substrate or a ligand is applied via rapid solution exchange. The electrogenic transporter activity charges the sensor, which is recorded as a transient current. The high stability of the SSM allows cumulative measurements on the same sensor using different experimental conditions. This allows the determination of kinetic properties including EC50, IC50, Km, KD, and rate constants of electrogenic reactions. About 100 different transporters have been measured so far using this technique, among them symporters, exchangers, uniporters, ATP-, redox-, and light-driven ion pumps, as well as receptors and ion channels. Different instruments apply this technique: the laboratory setups use a closed flow-through arrangement, while the commercially available SURFE2R N1 resembles a pipetting robot. For drug screening purposes high-throughput systems, such as the SURFE2R 96SE enable the simultaneous measurement of up to 96 sensors.Publication link2014 Industrializing Electrophysiology: HT Automated Patch Clamp on SyncroPatch 96 Using Instant Frozen CellsSURFE2R N96 (predecessor model of SURFE2R 96SE) book chapter in Patch-Clamp Methods and Protocols Authors: Polonchuk L.Patch-clamping is a powerful technique for investigating the ion channel function and regulation. However, its low throughput hampered profiling of large compound series in early drug development. Fortunately, automation has revolutionized the area of experimental electrophysiology over the past decade. Whereas the first automated patch-clamp instruments using the planar patch-clamp technology demonstrated rather a moderate throughput, few second-generation automated platforms recently launched by various companies have significantly increased ability to form a high number of high-resistance seals. Among them is SyncroPatch 96 (Nanion Technologies GmbH, Munich, Germany), a fully automated giga-seal patch-clamp system with the highest throughput on the market. By recording from up to 96 cells simultaneously, the SyncroPatch 96 allows to substantially increase throughput without compromising

components we crystallized LL-37 in the presence of detergents and obtained the structure of a narrow tetrameric channel with a strongly charged core. The formation of a tetramer was further studied by cross-linking in the presence of detergents and lipids. Using planar lipid membranes a small but defined conductivity of this channel could be demonstrated. Molecular dynamic simulations underline the stability of this channel in membranes and demonstrate pathways for the passage of water molecules. Time lapse studies ofE. colicells treated with LL-37 show membrane discontinuities in the outer membrane followed by cell wall damage and cell death. Collectively, our results open a venue to the understanding of a novel AMP killing mechanism and allows the rational design of LL-37 derivatives with enhanced bactericidal activity.Publication link2021 Norfluoxetine inhibits TREK-2 K2P channels by multiple mechanisms including state-independent effects on the selectivity filter gatePort-a-Patch andVesicle Prep Pro Publication in Journal of General Physiology (2021) Authors: Proks P., Schewe M., Conrad L.J., Rao S., Rathje S.,Rdstrm K.E.J., Carpenter E.P.,Baukrowitz T., Tucker S.J.The TREK subfamily of two-pore domain K+(K2P) channels are inhibited by fluoxetine and its metabolite, norfluoxetine (NFx). Although not the principal targets of this antidepressant, TREK channel inhibition by NFx has provided important insights into the conformational changes associated with channel gating and highlighted the role of the selectivity filter in this process. However, despite the availability of TREK-2 crystal structures with NFx bound, the precise mechanisms underlying NFx inhibition remain elusive. NFx has previously been proposed to be a state-dependent inhibitor, but its binding site suggests many possible ways in which this positively charged drug might inhibit channel activity. Here we show that NFx exerts multiple effects on single-channel behavior that influence both the open and closed states of the channel and that the channel can become highly activated by 2-APB while remaining in the down conformation. We also show that the inhibitory effects of NFx are unrelated to its positive charge but can be influenced by agonists which alter filter stability, such as ML335, as well as by an intrinsic voltage-dependent gating process within the filter. NFx therefore not only inhibits channel activity by altering the equilibrium between up and down conformations but also can directly influence filter gating. These results provide further insight into the complex allosteric mechanisms that modulate filter gating in TREK K2P channels and highlight the different ways in which filter gating can be regulated to permit polymodal regulation.Publication link2020 Ionophore constructed from non-covalent assembly of a G-quadruplex and liponucleoside transports K+-ion across biological membranesPort-a-Patch andVesicle Prep Pro Publication in Nature Communications (2020) Authors: Debnath M., Chakraborty S., Kumar Y.P., Chaudhuri R., Jana B., Dash J.The selective transport of ions across cell membranes, controlled by membrane proteins, is critical for a living organism. DNA-based systems have emerged as promising artificial ion transporters. However, the development of stable and selective artificial ion transporters remains a formidable task. We herein delineate the construction of an artificial ionophore using a telomeric DNA G-quadruplex (h-TELO) and a lipophilic guanosine (MG). MG stabilizes h-TELO by non-covalent interactions and, along with the lipophilic side chain, promotes the insertion of h-TELO within the hydrophobic lipid membrane. Fluorescence assays, electrophysiology measurements and molecular dynamics simulations reveal that MG/h-TELO preferentially transports K+-ions in a stimuli-responsive manner. The preferential K+-ion transport is presumably due to conformational changes of the ionophore in response to different ions. Moreover, the ionophore transports K+-ions across CHO and K-562 cell membranes. This study may serve as a design principle to generate selective DNA-based artificial transporters for therapeutic applications.Publication link2020 Purification and initial characterization of Plasmodium falciparum K+ channels, PfKch1 and PfKch2 produced in Saccharomyces cerevisiaeVesicle Prep Pro andPort-a-Patch Publication in Microbial Cell Factories (2020) Authors: Molbaek K., Tejada M., Ricke C.H., Scharff-Poulsen P., ElleKVist P., Helix-Nielsen C., Kumar N., Klaerke D.A., Pedersen P.A.Resistance towards known antimalarial drugs poses a significant problem, urging for novel drugs that target vital proteins in the malaria parasitePlasmodium falciparum. However, recombinant production of malaria proteins is notoriously difficult. To address this, we have investigated two putative K+channels, PfKch1 and PfKch2, identified in the P.falciparum genome. We show that PfKch1 and PfKch2 and a C-terminally truncated version of PfKch1 (PfKch111094)could indeed be functionally expressed in vivo, since a K+-uptake deficient Saccharomyces cerevisiae strain was complemented by the P. falciparum cDNAs. PfKch111094-GFP and GFP-PfKch2 fusion proteins were overexpressedin yeast, purified and reconstituted in lipid bilayers to determine their electrophysiological activity. Single channel conductance amounted to 16 1 pS for PfKch111094-GFP and 28 2 pS for GFP-PfKch2. We predicted regulator of K+-conductance (RCK) domains in the C-terminals of both channels, and we accordingly measured channel activity in the presence of Ca2+.Publication link2020 Hepatitis C Virus p7 Induces Membrane Permeabilization by Interacting with PhosphatidylserinePort-a-Patch and Vesicle Prep Pro Publication in International Journal of Molecular Sciences (2020) Authors: Lee H-R., Lee G.Y., You D-G., Kim H.K., Yoo Y.D.Hepatitis C virus (HCV) p7 is known to be a nonselective cation channel for HCV maturation. Because the interaction of HCV proteins with host lipids in the endoplasmic reticulum membrane is crucial for the budding process, the identification of p7lipid interactions could be important for understanding the HCV life cycle. Here, we report that p7 interacts with phosphatidylserine (PS) to induce membrane permeabilization. The interaction of p7 with PS was not inhibited by Gd3+ ions, which have been known to interact with negatively charged lipids, but channel activity and p7-induced mitochondrial depolarization were inhibited by Gd3+ ions. From the present results, we suggest that the p7PS interaction plays an essential role in regulating its ion channel function and could be a potential molecular target for anti-HCV therapy.Publication link2020 Human TRPA1 is an inherently mechanosensitive bilayer-gated ion channelPort-a-Patch and Vesicle Prep Pro Publication in Cell Calcium (2020) Authors: Moparthi L., Zygmunt P.M.The role of mammalian Transient Receptor Potential Ankyrin 1 (TRPA1) as a mechanosensor is controversial. Here, we report that purified human TRPA1 (hTRPA1) with and without its N-terminal ankyrin repeat domain responded with pressure-dependent single-channel current activity when reconstituted into artificial lipid bilayers. The hTRPA1 activity was abolished by the thiol reducing agent TCEP. Thus, depending on its redox state, hTRPA1 is an inherent mechanosensitive ion channel gated by force-from-lipids.Publication link2020 Calcium activates purified human TRPA1 with and without its N-terminal ankyrin repeat domain in the absence of calmodulinPort-a-Patch and Vesicle Prep Pro Publication in Cell Calcium (2020) Authors: Moparthi L., Moparthi S.B., Wenger J., Zygmunt P.M.Extracellular influx of calcium or release of calcium from intracellular stores have been shown to activate mammalian TRPA1 as well as to sensitize and desensitize TRPA1 electrophilic activation. Calcium binding sites on both intracellular N- and C-termini have been proposed. Here, we demonstrate based on Frster resonance energy transfer (FRET) and bilayer patch-clamp studies, a direct calmodulin-independent action of calcium on the purified human TRPA1 (hTRPA1), causing structural changes and activation without immediate subsequent desensitization of hTRPA1 with and without its N-terminal ankyrin repeat domain (N-ARD). Thus, calcium alone activates hTRPA1 by a direct interaction with binding sites outside the N-ARD.Publication link2020 Conformational equilibrium shift underlies altered K+ channel gating as revealed by NMRPort-a-Patch and Vesicle Prep Pro Publication in Nature Communications (2020) Authors: Iwahasi Y., Toyama Y., Imai S., Itoh H., Osawa M., Inoue M., Shimada I.The potassium ion (K+) channel plays a fundamental role in controlling K+permeation across the cell membrane and regulating cellular excitabilities. Mutations in the transmembrane pore reportedly affect the gating transitions of K+channels, and are associated with the onset of neural disorders. However, due to the lack of structural and dynamic insights into the functions of K+channels, the structural mechanism by which these mutations cause K+channel dysfunctions remains elusive. Here, we used nuclear magnetic resonance spectroscopy to investigate the structural mechanism underlying the decreased K+-permeation caused by disease-related mutations, using the prokaryotic K+channel KcsA. We demonstrated that the conformational equilibrium in the transmembrane region is shifted toward the non-conductive state with the closed intracellular K+-gate in the disease-related mutant. We also demonstrated that this equilibrium shift is attributable to the additional steric contacts in the open-conductive structure, which are evoked by the increased side-chain bulkiness of the residues lining the transmembrane helix. Our results suggest that the alteration in the conformational equilibrium of the intracellular K+-gate is one of the fundamental mechanisms underlying the dysfunctions of K+channels caused by disease-related mutations.Publication link2019 Temporin L and aurein 2.5 have identical conformations but subtly distinct membrane and antibacterial activitiesPort-a-Patch andVesicle Prep Pro Publication in Nature Scientific Reports (2019) Authors: Manzo G., Ferguson P.M., Hind C.H., Clifford M., Gustilo V.B., Ali H., Bansal S.S., Bui T.T., Drake A.F., Atkinson R.A., Sutton J.M., Lorenz C.D., Phoenix D.A., Mason A.J.Frogs such as Rana temporaria and Litoria aurea secrete numerous closely related antimicrobial peptides (AMPs) as an effective chemical dermal defence. Damage or penetration of the bacterial plasma membrane is considered essential for AMP activity and such properties are commonly ascribed to their ability to form secondary amphipathic, -helix conformations in membrane mimicking milieu. Nevertheless, despite the high similarity in physical properties and preference for adopting such conformations, the spectrum of activity and potency of AMPs often varies considerably. Hence distinguishing apparently similar AMPs according to their behaviour in, and effects on, model membranes will inform understanding of primary-sequence-specific antimicrobial mechanisms. Here we use a combination of molecular dynamics simulations, circular dichroism and patch-clamp to investigate the basis for differing anti-bacterial activities in representative AMPs from each species; temporin L and aurein 2.5. Despite adopting near identical, -helix conformations in the steady-state in a variety of membrane models, these two AMPs can be distinguished both in vitro and in silico based on their dynamic interactions with model membranes, notably their differing conformational flexibility at the N-terminus, ability to form higher order aggregates and the characteristics of induced ion conductance. Taken together, these differences provide an explanation of the greater potency and broader antibacterial spectrum of activity of temporin L over aurein 2.5. Consequently, while the secondary amphipathic, -helix conformation is a key determinant of the ability of a cationic AMP to penetrate and disrupt the bacterial plasma membrane, the exact mechanism, potency and spectrum of activity is determined by precise structural and dynamic contributions from specific residues in each AMP sequence.Publication link2020 A pleurocidin analogue with greater conformational flexibility, enhanced antimicrobial potency and in vivo therapeutic efficacyVesicle Prep Pro andPort-a-PatchPublication inCommunications Biology(2020) Authors: Manzo G., Hind C.K., Ferguson P.M., Amison R.T., Hodgson-Casson A.C., Ciazynska K.A., Weller B.J., Clarke M., Lam C., Man R.C.H., O'Shaughnessy B.G., Clifford M., Bui T.T., Drake A.F., Atkinson R.A., Lam J.K.W., Pitchford S.C., Page C.P., Phoenix D.A., Lorenz C.D., Sutton J.M., Mason A.J.Antimicrobial peptides (AMPs) are a potential alternative to classical antibiotics that are yet to achieve a therapeutic breakthrough for treatment of systemic infections. The antibacterial potency of pleurocidin, an AMP from Winter Flounder, is linked to its ability to cross bacterial plasma membranes and seek intracellular targets while also causing membrane damage. Here we describe modification strategies that generate pleurocidin analogues with substantially improved, broad spectrum, antibacterial properties, which are effective in murine models of bacterial lung infection. Increasing peptidelipid intermolecular hydrogen bonding capabilities enhances conformational flexibility, associated with membrane translocation, but also membrane damage and potency, most notably against Gram-positive bacteria. This negates their ability to metabolically adapt to the AMP threat. An analogue comprising D-amino acids was well tolerated at an intravenous dose of 15mg/kg and similarly effective as vancomycin in reducing EMRSA-15 lung CFU. This highlights the therapeutic potential of systemically delivered, bactericidal AMPs.Publication link2019 Minor sequence modifications in temporin B cause drastic changes in antibacterial potency and selectivity by fundamentally altering membrane activityPort-a-Patch and Vesicle Prep Pro Publication in Nature Scientific Reports (2019) Authors: Manzo G., Ferguson P.M., Gustilo V.B., Ali H., Bui T.T., Drake A.F., Atkinson R.A., Batoni G., Lorenz C.D., Phoenix D.A., Mason A.J.Antimicrobial peptides (AMPs) are a potential source of new molecules to counter the increase in antimicrobial resistant infections but a better understanding of their properties is required for effective translation as therapeutics. Details of the mechanism of their interaction with the bacterial plasma membrane are desired since damage or penetration of this structure is considered essential for AMP activity. Relatively modest modifications to AMP primary sequence can induce substantial changes in potency and/or spectrum of activity but, hitherto, have not been predicted to substantially alter the mechanism of interaction with the bacterial plasma membrane. Here we use a combination of molecular dynamics simulations, circular dichroism, liquid- and solid-state NMR and patch clamp to investigate the extent to which temporin B and its analogues can be distinguished both in vitro and in silico on the basis of their interactions with model membranes. Enhancing the hydrophobicity of the N-terminus and cationicity of the C-terminus in temporin B improves its membrane activity and potency against both Gram-negative and Gram-positive bacteria. In contrast, enhancing the cationicity of the N-terminus abrogates its ability to trigger channel conductance and renders it ineffective against Staphylococcus aureus while nevertheless enhancing its potency against Escherichia coli. Our findings suggest even closely related AMPs may target the same bacterium with fundamentally differing mechanisms of action.Publication link2019 Molecular understanding of calcium permeation through the open Orai channelPort-a Patch and Vesicle Prep Pro Publication in PLOS Computational Biology (2019) Authors: Liu X., Wu G., Yu Y., Chen X., Ji R., Lu J., Li X., Zhang X., Yang X., Shen Y.The Orai channel is characterized by voltage independence, low conductance, and high Ca2+selectivity and plays an important role in Ca2+influx through the plasma membrane (PM). How the channel is activated and promotes Ca2+permeation is not well understood. Here, we report the crystal structure and cryo-electron microscopy (cryo-EM) reconstruction of a Drosophila melanogaster Orai (dOrai) mutant (P288L) channel that is constitutively active according to electrophysiology. The open state of the Orai channel showed a hexameric assembly in which 6 transmembrane 1 (TM1) helices in the center form the ion-conducting pore, and 6 TM4 helices in the periphery form extended long helices. Orai channel activation requires conformational transduction from TM4 to TM1 and eventually causes the basic section of TM1 to twist outward. The wider pore on the cytosolic side aggregates anions to increase the potential gradient across the membrane and thus facilitate Ca2+permeation. The open-state structure of the Orai channel offers insights into channel assembly, channel activation and Ca2+permeation.Publication link2018 Single channel recording of a mitochondrial calcium uniporterPort-a-Patch andVesicle Prep Pro Publication in BBRC (2018) Authors: Wu G., Li S., Zong G., Liu, X., Fei S., Shen L., Guan X., Yanga X., Shen Y.Mitochondrial calcium uniporter (MCU) is the pore-forming subunit of the entire uniporter complex and plays an important role in mitochondrial calcium uptake. However, the single channel recording of MCU remains controversial. Here, we expressed and purified different MCU proteins and then reconstituted them into planar lipid bilayers for single channel recording. We showed that MCU alone from Pyronema omphalodes (pMCU) is active with prominent single channel Ca2+currents. In sharp contrast, MCU alone from Homo sapiens (hMCU) is inactive. The essential MCU regulator (EMRE) activates hMCU, and therefore, the complex (hMCU-hEMRE) shows prominent single channel Ca2+currents. These single channel currents are sensitive to the specific MCU inhibitor Ruthenium Red. Our results clearly demonstrate that active MCU can conduct large amounts of calcium into the mitochondria.Publication link2019 Golgi anti-apoptotic proteins are evolutionarily conserved ion channels that regulate cell death in plantsPort-a-Patch andVesicle Prep Pro Pre-Publication in BioRxiv (2019) Authors: Sierla M., Prole D.L., Saraiva N., Carrara G., Dinischiotu N., Vaattovaara A., Wrzaczek M., Taylor C.W., Smith F.L., Feys B.Programmed cell death regulates developmental and stress responses in eukaryotes. Golgi anti-apoptotic proteins (GAAPs) are evolutionarily conserved cell death regulators. Human and viral GAAPs inhibit apoptosis and modulate intracellular Ca2+fluxes, and viral GAAPs form cation selective channels. Although most mammalian cell death regulators are not conserved at the sequence level in plants, the GAAP gene family shows expansion, with five paralogues (AtGAAP1-5) in the Arabidopsis genome. We pursued molecular and physiological characterization of AtGAAPs making use of the advanced knowledge of their human and viral counterparts. Structural modeling of AtGAAPs predicted the presence of a channel-like pore, and electrophysiological recordings from purified AtGAAP3 reconstituted into lipid bilayers confirmed that plant GAAPs can function as ion channels. AtGAAP1 and AtGAAP4 localized exclusively to the Golgi within the plant cell, while AtGAAP2, AtGAAP3 and AtGAAP5 also showed tonoplastic localization. Gene expression analysis revealed differential spatial expression and abundance of transcript for AtGAAP paralogues in Arabidopsis tissues. We demonstrate that AtGAAP1-5 inhibit Bax-induced cell death in yeast. However, overexpression of AtGAAP1 induces cell death in Nicotiana benthamiana leaves and lesion mimic phenotype in Arabidopsis. We propose that AtGAAPs function as Golgi-localized ion channels that regulate cell death by affecting ionic homeostasis within the cell.Publication link2018 Reconstitution and Electrophysiological Characterization of Ion Channels in Lipid BilayersPort-a-Patch and Vesicle Prep Pro Publication in Current Protocols in Pharmacology (2018) Authors: Klaerke D.A., de los Angeles Tejada M., Grsfjeld Christensen V., Lassen M., Amstrup Pedersen P., Calloe K.Detergentsolubilized purified ion channels can be reconstituted into lipid bilayers for electrophysiological analysis. Traditionally, ion channels were inserted into vesicles and subsequently fused with planar black lipid membranes formed from lipids dissolved in a hydrophobic solvent such as decane. Provided in this article is a stepbystep guide to reconstitute purified ion channel proteins into giant unilamellar vesicles (GUVs). This procedure results in the formation of proteoliposomes that can be used for planar bilayer formation and electrophysiological characterization of singlechannel currents. By using preformed GUVs it is possible to omit the membrane solvent. Compared to traditional preparations, the lipid bilayers formed from GUVs provide an environment that more closely resembles the native cell membrane. Also described is an alternate protocol that entails the production of planar lipid bilayers from GUVs onto which proteins in detergent are added.Publication link2018 Romo1 is a mitochondrial nonselective cation channel with viroporin-like characteristicsPort-a-Patch andVesicle Prep Pro Publication in Journal of Cell Biology (2018) Authors: Lee G.Y., You D.G., Lee H.R., Hwang S.W., Lee C.J., Do Yoo Y.Reactive oxygen species (ROS) modulator 1 (Romo1) is a nuclear-encoded mitochondrial inner membrane protein known to regulate mitochondrial ROS production and to act as an essential redox sensor in mitochondrial dynamics. Although its physiological roles have been studied for a decade, the biophysical mechanisms that explain these activities of Romo1 are unclear. In this study, we report that Romo1 is a unique mitochondrial ion channel that differs from currently identified eukaryotic ion channels. Romo1 is a highly conserved protein with structural features of class II viroporins, which are virus-encoded nonselective cation channels. Indeed, Romo1 forms a nonselective cation channel with its amphipathic helical transmembrane domain necessary for pore-forming activity. Notably, channel activity was specifically inhibited by Fe2+ ions, an essential transition metal ion in ROS metabolism. Using structural bioinformatics, we designed an experimental data-guided structural model of Romo1 with a rational hexameric structure. We propose that Romo1 establishes a new category of viroporin-like nonselective cation channel in eukaryotes.Publication link2018 Parallel evolution of frog antimicrobial peptides produces identical conformations but subtly distinct membrane and antibacterial activitiesPort-a-Patch andVesicle Prep Pro pre-Publication in bioRxiv (2018) Authors: Manzo G., Ferguson P.M., Hind C., Clifford M., Gustilo V.B., Ali H., Bansal S.S., Bui T.T., Drake A.F., Atkinson R.A., Sutton M.J., Lorenz C.D., Phoenix D.A., Mason A.J.Frogs such as Rana temporaria and Litoria aurea secrete numerous closely related antimicrobial peptides (AMPs) as an effective chemical dermal defence. Despite the high similarity in physical properties and preference for adopting secondary amphipathic, -helix conformations in membrane mimicking milieu, their spectrum of activity and potency often varies considerably. Damage or penetration of the bacterial plasma membrane is considered essential for AMP activity and hence distinguishing apparently similar AMPs according to their behaviour in, and effects on, model membranes will inform understanding of species specific effective antimicrobial mechanisms. Here we use a combination of molecular dynamics simulations, circular dichroism and patch-clamp to investigate the basis for differing anti-bacterial activities in representative AMPs from each species; temporin L and aurein 2.5. Despite adopting near identical, -helix conformations in the steady-state in a variety of membrane models, these two AMPs can be distinguished both in vitro and in silico based on their dynamic interactions with model membranes; the greater conformational flexibility and the higher amplitude channel conductance induced offers a rationale for the greater potency and broader spectrum of activity of temporin L over aurein 2.5. Specific contributions from individual residues are identified that define the mechanisms of action of each AMP. Our findings suggest AMPs in frogs are examples of parallel evolution whose utility is based on apparently similar but subtly distinct mechanisms of action.Publication link2018 Quantifying Permeation of Small Charged Molecules across Channels: Electrophysiology in Small VolumesPort-a-Patch and Vesicle Prep Pro Publication in ACS Omega (2018) Authors: Wang J., Benier L., Winterhalter M.A major bottleneck in the development of small molecule antibiotics is to achieve good permeability across the outer membrane in Gram-negative bacteria. Optimization with respect to permeability surprisingly lacks appropriate methods. Recently we proposed to use the diffusion potential for charged molecules created by their difference in electrophoretic mobility while crossing the outer membrane channel under a concentration gradient. The latter provides semi-quantitative values but the current available setups require large volumes and thus exclude several classes of molecules. Here we propose a simple approach capturing proteoliposomes at aperture of glass surface (planar aperture or conical glass capillary) decreasing the necessary volume below 50 L. We measured the transport of two charged molecules sulbactam and ceftazidime across the two major porins in E.coli. Both molecules permeate through these porins were observed with sulbactam owes higher permeability.Publication link2018 An aromatic cluster in Lysinibacillus sphaericus BinB involved in toxicity and proper in-membrane foldingPort-a-Patch andVesicle Prep Pro Publication in Archives of Biochemistry and Biophysics (2018) Authors: Chooduang A., Surya W., Torres J., Boonserm P.The binary toxin from Lysinibacillus sphaericus has been successfully used for controlling mosquito-transmitted diseases. Based on structural alignments with other toxins, an aromatic cluster in the C-terminal domain of BinB (termed here BC) has been proposed to be important for toxicity. We tested this experimentally using BinB mutants bearing single mutations in this aromatic cluster. Consistent with the hypothesis, two of these mutations, F311A and F315A, were not toxic to Culex quinquefasciatus larvae and were unable to permeabilize liposomes or elicit ion channel activity, in contrast to wild-type BinB. Despite these effects, none of these mutations altered significantly the interaction between the activated forms of the two subunits in solution. These results indicate that these aromatic residues on the C-terminal domain of BinB are critical for toxin insertion in membranes. The latter can be by direct contact of these residues with the membrane surface, or by facilitating the formation a membrane-inserting oligomer.Publication link2018 Molecular mechanism of the Orai channel activationPort-a-Patch andVesicle Prep Pro Pre-publicatin in bioRxiv (2018) Authors: Liu, X.,Wu, G.,Yu, Y.,Chen, X.,Ji, R., Lu, J., Li, X., Zhang, X.,Yang, X., Shen, Y.The Orai channel is characterized by voltage independence, low conductance and high Ca2+selectivity and plays an important role in Ca2+influx through the plasma membrane. How the channel is activated and promotes Ca2+permeation are not well understood. Here, we report the crystal structure and cryo-electron microscopy reconstruction of a Drosophila melanogaster Orai mutant (P288L) channel that is constitutively active according to electrophysiology. The open state of the Orai channel showed a hexameric assembly in which six TM1 helices in the center form the ion-conducting pore, and six TM4 helices in the periphery form extended long helices. Orai channel activation requires conformational transduction from TM4 to TM1 and eventually causes the basic section of TM1 to twist outward. The wider pore on the cytosolic side aggregates anions to increase the potential gradient across the membrane and thus facilitate Ca2+permeation. The open-state structure of the Orai channel offers insights into channel assembly, channel activation and Ca2+permeation.Publication link2017 Polydim-I antimicrobial activity against MDR bacteria and its model membrane interactionPort-a-Patch andVesicle Prep Pro Publication in PLoS ONE (2017) Authors: Rangel M., Fernandes dos Santos Castro F., Mota-Lima L.D.,Clissa P.B., Martins D.B., Perez dos Santos Cabrera M.,Mortari M.R.The rapid spread of multi-drug resistant pathogens represents a serious threat to public health, considering factors such as high mortality rates, treatment restrictions and high prevalence of multi-drug resistant bacteria in the hospital environment. Antimicrobial peptides (AMPs) may exhibit powerful antimicrobial activity against different and diverse microorganisms, also presenting the advantage of absence or low toxicity towards animal cells. In this study, the evaluation of the antimicrobial activity against multi-drug resistant bacteria of a recently described AMP from wasp, Polydim-I, was performed. Polydim-I presented activity against standard strains (non-carriers of multi-resistant genes) that are susceptible to commercial antimicrobials, and also against multi-drug resistant strains at concentrations bellow 1g/ml (0.41 M). This is a rather low concentration among those reported for AMPs. At this concentration we found out that Polydim-I inhibits almost 100% of the tested pathogens growth, while with the ATCC strains the minimum inhibitory concentration (MIC100) is 400 times higher. Also, in relation toin vitroactivity of conventional drugs against multi-drug resistant bacteria strains, Polydim-I is almost 10 times more efficient and with broader spectrum. Cationic AMPs are known as multi-target compounds and specially for targeting the phospholipid matrix of bacterial membranes. Exploring the interactions of Polydim-I with lipid bilayers, we have confirmed that this interaction is involved in the mechanism of action. Circular dichroism experiments showed that Polydim-I undergoes a conformational transition from random coil to a mostly helical conformation in the presence of membrane mimetic environments. Zeta potential measurements confirmed the binding and partial charge neutralization of anionic asolectin vesicles, and also suggested a possible aggregation of peptide molecules. FTIR experiments confirmed that some peptide aggregation occurs, which is minimized in the presence of strongly anionic micelles of sodium dodecyl sulfate. Also, Polydim-I induced channel-like structures formation to asolectin lipid bilayers, as demonstrated in the electrophysiology experiments. We suggest that cationic Polydim-I targets the membrane lipids due to electrostatic attraction, partially accumulates, neutralizing the opposite charges and induces pore formation. Similar mechanism of action has already been suggested for other peptides from wasp venoms, especially mastoparans.Publication link2017 The Rotavirus NSP4 Viroporin Domain is a Calcium-conducting Ion ChannelPort-a-Patch and Vesicle Prep Pro Publication in Nature Scientific Reports Authors: Pham T., Perry J.L., Dosey T.L., Delcour L.H.,Hyser J.M.Viroporins are small virus-encoded ion channel proteins. Most viroporins are monovalent selective cation channels, with few showing the ability to conduct divalent cations, like calcium (Ca2+). Nevertheless, some viroporins are known to disrupt host cell Ca2+homeostasis, which is critical for virus replication and pathogenesis. Rotavirus nonstructural protein 4 (NSP4) is an endoplasmic reticulum transmembrane glycoprotein that has a viroporin domain (VPD), and NSP4 viroporin activity elevates cytosolic Ca2+in mammalian cells. The goal of this study was to demonstrate that the NSP4 VPD forms an ion channel and determine whether the channel can conduct Ca2+. Using planar lipid bilayer and liposome patch clamp electrophysiology, we show that a synthetic peptide of the NSP4 VPD has ion channel activity. The NSP4 VPD was selective for cations over anions and channel activity was observed to have both well-defined square top openings as well as fast current fluctuations, similar to other viroporins. Importantly, the NSP4 VPD showed similar conductance of divalent cations (Ca2+and Ba2+) as monovalent cations (K+), but a viroporin defective mutant lacked Ca2+conductivity. These data demonstrate that the NSP4 VPD is a Ca2+-conducting viroporin and establish the mechanism by which NSP4 disturbs host cell Ca2+homeostasis.Publication link2017 Evaluation of the antimicrobial activity of the mastoparan Polybia-MPII isolated from venom of the social wasp Pseudopolybia vespiceps testacea (Vespidae, Hymenoptera)Port-a-Patch andVesicle Prep Pro Publication in International Journal of Antimicrobial Agents (2017) Authors: Silva J.C., Neto L.M., Neves R.C., Gonalves J.C., Trentini M.M., Mucury-Filho R., Smidt K.S., Fensterseifer I.C., Silva O.N., Lima L.D., Clissa P.B., Vilela N., Guilhelmelli F., Silva L.P., Rangel M., Kipnis A., Silva-Pereira I., Franco O.L., Junqueira-Kipnis A.P., Bocca A.L., Mortari M.R.Mastoparans, a class of peptides found in wasp venom, have significant effects following a sting as well as useful applications in clinical practice. Among these is their potential use in the control of micro-organisms that cause infectious diseases with a significant impact on society. Thus, the present study describes the isolation and identification of a mastoparan peptide from the venom of the social waspPseudopolybia vespicepsand evaluated its antimicrobial profile against bacteria (Staphylococcus aureusandMycobacterium abscessussubsp.massiliense), fungi (Candida albicansandCryptococcus neoformans) and in vivoS. aureusinfection. The membrane pore-forming ability was also assessed. The mastoparan reduced in vitro and ex vivo mycobacterial growth by 80% at 12.5M in infected peritoneal macrophages but did not affect the shape of bacterial cells at the dose tested (6.25M). The peptide also showed potent action againstS. aureusin vitro (EC50and EC90values of 1.83M and 2.90M, respectively) and reduced the in vivo bacterial load after 6 days of topical treatment (5mg/kg). Antifungal activity was significant, with EC50and EC90values of 12.9M and 15.3M, respectively, forC. albicans, and 11M and 22.70M, respectively, forC. neoformans. Peptides are currently attracting interest for their potential in the design of antimicrobial drugs, particularly due to the difficulty of micro-organisms in developing resistance to them. In this respect, Polybia-MPII proved to be highly effective, with a lower haemolysis rate compared with peptides of the same family.Publication link2017 Organelle membrane derived patches: reshaping classical methods for new targetsPort-a-Patch and Vesicle Prep Pro Publication in Nature Scientific Reports(2017) Authors: Shapovalov G., Ritaine A., Bidaux G., Slomianny, C., Borowiec A-S., Gordienko D., Bultynck G., Skryma R., Prevarskaya N.Intracellular ion channels are involved in multiple signaling processes, including such crucial ones as regulation of cellular motility and fate. With 95% of the cellular membrane belonging to intracellular organelles, it is hard to overestimate the importance of intracellular ion channels. Multiple studies have been performed on these channels over the years, however, a unified approach allowing not only to characterize their activity but also to study their regulation by partner proteins, analogous to the patch clamp golden standard, is lacking. Here, we present a universal approach that combines the extraction of intracellular membrane fractions with the preparation of patchable substrates that allows to characterize these channels in endogenous protein environment and to study their regulation by partner proteins. We validate this method by characterizing activity of multiple intracellular ion channels localized to different organelles and by providing detailed electrophysiological characterization of the regulation of IP3R activity by endogenous Bcl-2. Thus, after synthesis and reshaping of the well-established approaches, organelle membrane derived patch clamp provides the means to assess ion channels from arbitrary cellular membranes at the single channel level.Publication link2017 Asymmetric mechanosensitivity in a eukaryotic ion channelPort-a-Patch and Vesicle Prep Pro Publication in PNAS (2017) Authors: Clausen M.V., Jarerattanachata V., Carpenterc E.P., Mark S. , Sansomb P., Tucker S.J.Living organisms perceive and respond to a diverse range of mechanical stimuli. A variety of mechanosensitive ion channels have evolved to facilitate these responses, but the molecular mechanisms underlying their exquisite sensitivity to different forces within the membrane remains unclear. TREK-2 is a mammalian two-pore domain (K2P) K+channel important for mechanosensation, and recent studies have shown how increased membrane tension favors a more expanded conformation of the channel within the membrane. These channels respond to a complex range of mechanical stimuli, however, and it is uncertain how differences in tension between the inner and outer leaflets of the membrane contribute to this process. To examine this, we have combined computational approaches with functional studies of oppositely oriented single channels within the same lipid bilayer. Our results reveal how the asymmetric structure of TREK-2 allows it to distinguish a broad profile of forces within the membrane, and illustrate the mechanisms that eukaryotic mechanosensitive ion channels may use to detect and fine-tune their responses to different mechanical stimuli. Significance: One important way in which living organisms are able to detect and respond to their environment is via the conversion of mechanical forces into electrical signals. However, the molecular mechanisms that enable mammalian mechanosensitive ion channels to detect a wide profile of forces within the membrane remain unclear. By studying the functional activity of individual TREK-2 K2Pchannels inserted in different directions into a lipid bilayer, we are now able to describe how the asymmetric structure of this channel enables it to sense such a broad profile of forces. These results help us understand how eukaryotic ion channels respond to a rich variety of sensory stimuli.Publication link2017 Bilayer-Mediated Structural Transitions Control Mechanosensitivity of the TREK-2 K2P ChannelPort-a-Patch andVesicle Prep Pro Publication in Structure (2017) Authors: Arya P, Jarerattanachat V., Clausen M.V., Schewe M., McClenaghan C., Argent L., Conrad L.J., Dong Y.Y., Pike A.C.W., Carpenter E.P., Baukrowitz T., Sansom M.S.P., Tucker S.J.The mechanosensitive two-pore domain (K2P) K+channels (TREK-1, TREK-2, and TRAAK) are important for mechanical and thermal nociception. However, the mechanisms underlying their gating by membrane stretch remain controversial. Here we use molecular dynamics simulations to examine theirbehavior in a lipid bilayer. We show that TREK-2 moves from the down to up conformation in direct response to membrane stretch, and examine the role of the transmembrane pressure profile in this process. Furthermore, we show how state-dependent interactions with lipids affect the movement of TREK-2, and how stretch influences both the inner pore and selectivity filter. Finally, we present functional studies that demonstrate why direct pore block by lipid tails does not represent the principal mechanism of mechanogating. Overall, this study provides a dynamic structural insight into K2P channel mechanosensitivity and illustrates how the structure of a eukaryotic mechanosensitive ion channel responds to changes in forces within the bilayer.Publication link2016 Structural and functional characterization of a calcium-activated cation channel from Tsukamurella paurometabolaPort-a-Patch and Vesicle Prep Pro Publication in Nature Communications (2016) Authors: Dhakshnamoorthy B., Rohaim A., Rui H., Blachowicz L., Roux B.The selectivity filter is an essential functional element of K+channels that is highly conserved both in terms of its primary sequence and its three-dimensional structure. Here, we investigate the properties of an ion channel from the Gram-positive bacteriumTsukamurella paurometabolawith a selectivity filter formed by an uncommon proline-rich sequence. Electrophysiological recordings show that it is a non-selective cation channel and that its activity depends on Ca2+concentration. In the crystal structure, the selectivity filter adopts a novel conformation with Ca2+ions bound within the filter near the pore helix where they are coordinated by backbone oxygen atoms, a recurrent motif found in multiple proteins. The binding of Ca2+ion in the selectivity filter controls the widening of the pore as shown in crystal structures and in molecular dynamics simulations. The structural, functional and computational data provide a characterization of this calcium-gated cationic channel.Publication link2016 The N-terminal Ankyrin Repeat Domain Is Not Required for Electrophile and Heat Activation of the Purified Mosquito TRPA1 ReceptorPort-a-Patch and Vesicle Prep Pro Publication in Nature (2016) Authors: Survery S., Moparthi L., Kjellbom P., Hgesttt E.D., Zygmunt P.M., Johanson U.Temperature sensors are crucial for animals to optimize living conditions. The temperature response of the ion channel transient receptor potential A1 (TRPA1) is intriguing, some orthologs have been reported to be activated by cold and others by heat, but the molecular mechanisms responsible for its activation remain elusive. Single-channel electrophysiological recordings of heterologously expressed and purified Anopheles gambiae TRPA1 (AgTRPA1), with and without the N-terminal ankyrin repeat domain, demonstrate that both proteins are functional as they responded to the electrophilic compounds allyl isothiocyanate (AITC) and cinnamaldehyde as well as heat. The proteins similar intrinsic fluorescence properties and corresponding quenching when activated by AITC or heat, suggest lipid bilayer-independent conformational changes outside the N-terminal domain. The results show that AgTRPA1 is an inherent temperature- and chemoreceptor, and analogous to what has been reported for the human TRPA1 ortholog the N-terminal domain may tune the response but is not required for the activation by these stimuli.Publication link2016 Photosensitization in Porphyrias and Photodynamic Therapy Involves TRPA1 and TRPV1Port-a-Patch and Vesicle Prep Pro Publication in The Journal of Neuroscience (2016) Authors: Babes A., Sauer S.K., Moparthi L., Kichko T.I., Neacsu C., Namer B., Filipovic M., Zygmunt P.M., Reeh P.W., Fischer M.J.Photosensitization, an exaggerated sensitivity to harmless light, occurs genetically in rare diseases, such as porphyrias, and in photodynamic therapy where short-term toxicity is intended. A common feature is the experience of pain from bright light. In human subjects, skin exposure to 405 nm light induced moderate pain, which was intensified by pretreatment with aminolevulinic acid. In heterologous expression systems and cultured sensory neurons, exposure to blue light activated TRPA1 and, to a lesser extent, TRPV1 channels in the absence of additional photosensitization. Pretreatment with aminolevulinic acid or with protoporphyrin IX dramatically increased the light sensitivity of both TRPA1 and TRPV1 via generation of reactive oxygen species. Artificial lipid bilayers equip

reconstituted into artificial liposomes. Using bacterial MS channels as a model, we have shown by reconstituting these channels into liposomes that there is a close relationship between the physico-chemical properties of the lipid bilayer and structural dynamics bringing about the function of these channels.Publication link2009 Amyloid-beta-Induced Ion Flux in Artificial Lipid Bilayers and Neuronal Cells: Resolving a ControversyPort-a-Patch and Vesicle Prep Pro Publication in Neurotoxicity Research (2009) Authors: Capone R., Quiroz F.G., Prangkio P., Saluja I., Sauer A.M., Bautista M.R., Turner R.S., Yang J., Mayer M.Understanding the pathogenicity of amyloid-beta (A) peptides constitutes a major goal in research on Alzheimers disease (AD). One hypothesis entails that A peptides induce uncontrolled, neurotoxic ion flux through cellular membranes. The exact biophysical mechanism of this ion flux is, however, a subject of an ongoing controversy which has attenuated progress toward understanding the importance of A-induced ion flux in AD. The work presented here addresses two prevalent controversies regarding the nature of transmembrane ion flux induced by peptides. First, the results clarify that can induce stepwise ion flux across planar lipid bilayers as opposed to a gradual increase in transmembrane current; they show that the previously reported gradual thinning of membranes with concomitant increase in transmembrane current arises from residues of the solvent hexafluoroisopropanol, which is commonly used for the preparation of amyloid samples. Second, the results provide additional evidence suggesting that A peptides can induce ion channel-like ion flux in cellular membranes that is independent from the postulated ability of to modulate intrinsic cellular ion channels or transporter proteins.Publication link2009 The M34A mutant of Connexin26 reveals active conductance states in pore-suspending membranesPort-a-Patch and Vesicle Prep Pro Publication in Journal of Structural Biology (2009) Authors: Gamann O., Kreir M., Ambrosi C., Pranskevich J., Oshima A, Rling C., Sosinsky G., Fertig N., Steinem C.Connexin26 (Cx26) is a member of the connexin family, the building blocks for gap junction intercellular channels. These dodecameric assemblies are involved in gap junction-mediated cellcell communication allowing the passage of ions and small molecules between two neighboring cells. Mutations in Cx26 lead to the disruption of gap junction-mediated intercellular communication with consequences such as hearing loss and skin disorders. We show here that a mutant of Cx26, M34A, forms an active hemichannel in lipid bilayer experiments. A comparison with the Cx26 wild-type is presented. Two different techniques using micro/nano-structured substrates for the formation of pore-suspending lipid membranes are used. We reconstituted the Cx26 wild-type and Cx26M34A into artificial lipid bilayers and observed single channel activity for each technique, with conductance levels of around 35, 70 and 165 pS for the wild-type. The conductance levels of Cx26M34A were found at around 45 and 70 pS.Publication link2006 High-resolution electrophysiology on a chip: Transient dynamics of alamethicin channel formationPort-a-Patch and Vesicle Prep Pro Publication in Biochimica et Biophysica Acta - Biomembranes (2006) Authors: Sondermann M., George M., Fertig N., Behrends J.C.Microstructured planar substrates have been shown to be suitable for patch clamp recording from both whole cells and isolated patches of membrane, as well as for measurements from planar lipid bilayers. Here, we further explore this technology with respect to high-resolution, low noise single-channel recording. Using solvent-free lipid bilayers from giant unilamellar vesicles obtained by electro-swelling, we recorded channels formed by the peptaibol alamethicin, a well-studied model system for voltage-dependent channels, focusing on the transient dynamics of single-channel formation upon application of a voltage step. With our setup, we were able to distinctly resolve dwell times well below 100 mus and to perform a thorough statistical analysis of alamethicin gating. Our results show good agreement with models that do not rely on the existence of non-conducting preaggregate states. Microstructured apertures in glass substrates appear promising with respect to future experiments on cellular ion channels reconstituted in suspended lipid membranes.Publication link2008 Rapid screening of membrane protein activity: electrophysiological analysis of OmpF reconstituted in proteoliposomesPort-a-Patch and Vesicle Prep Pro Publication in Lab-on-a-chip (2008) Authors: Kreir M., Farre C., Beckler M., George M., Fertig N.Solvent-free planar lipid bilayers were formed in an automatic manner by bursting of giant unilamellar vesicles (GUVs) after gentle suction application through micron-sized apertures in a borosilicate glass substrate. Incubation of GUVs with the puried ion channel protein of interest yielded proteoliposomes. These proteoliposomes allow for immediate recording of channel activity after GUV sealing. This approach reduces the time-consuming, laborious and sometimes difcult protein reconstitution processes normally performed after bilayer formation. Bilayer recordings are attractive for investigations of membrane proteins not accessible to patch clamp analysis, like e.g. proteins from organelles. In the presented work, we show the example of the outer membrane protein OmpF from Escherichiacoli. We reconstituted OmpF in proteoliposomes and observed the characteristic trimeric conductance levels and the typical gating induced by pH and transmembrane voltage. Moreover, OmpF is the main entrance for beta-lactam antibiotics and we investigated translocation processes of antibiotics and modulation of OmpF by spermine. We suggest that the rapid formation of porin containing lipid bilayers is of potential for the efcient electrophysiological characterization of the OmpF protein, for studying membrane permeation processes and for the rapid screening of antibiotics.Application Note PDFCiPA- Identification of cardiac liability in drug discovery using the Port-a-PatchPort-a-Patch application note: Cells were kindly provided by NEXEL and Charles River, USA.In 2013 the Cardiac Safety Research Consortium (CSRC), the Health and Environmental Sciences Institute (HESI), and the US Food and Drug Administration (FDA) proposed a paradigm to improve assessment of the proarrhythmic risk of therapeutic compounds. This paradigm, the Comprehensive In-vitro Proarrhythmia Assay (CiPA), was introduced to provide a more complete assessment of proarrhythmic risk by evaluating and implementing currently available high throughput methods. An important part of this is the electrophysiological evaluation of hERG, and also other cardiac channels including NaV1.5 and CaV1.2. The Q&A draft from August 2020 describes how nonclinical assays such as patch clamp can be used as a part of an integrated risk assessment prior to first-in-human studies, and in later stages of clinical development.Following up on hERG and NaV1.5 best practices and calibration standards which have been published recently on automated patch clamp devices, we show here cardiac ion channel recordings from human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) or overexpressing cell lines generated with the worlds smallest patch clamp setups: Port-a-Patch and Port-a-Patch mini. Recordings at RT or physiological temperature of hERG recorded from HEK cells, and peak or late INacurrent recorded from iPSC-CMs or CHO cells are shown. INa-Late was activated by ATX-II and blocked by ranolazine, INa-Peak was blocked by tetracaine in a concentration-dependent manner, and hERG was blocked by increasing concentrations of dofetilide.Webinar07.09.2021 | Webinar: Automated patch clamp assay development for the study of red blood cells (RBCs) in health and diseasePresenters: Nicoletta Murciano (PhD Candidate - Nanion Technologies), Dr. Lars Kaestner (Professor - Saarland University)Calcium (Ca2+) is a universal signalling molecule and is critically important in regulating many physiological functions and survival of RBCs. Amongst others, intracellular Ca2+ controls cell volume and deformability. This process plays a substantial role in RBCs since their volume needs to adapt when passing blood vessel constrictions during the flow. Excessive Ca2+ uptake also leads to accelerated cell clearance causing anaemia.Therefore, studying Ca2+ regulation is crucial to understand RBC diseases. Piezo1, KCa3.1 (Gardos channel) and NMDA receptors are three channels present in the RBC membrane and critical for Ca2+ regulation.We developed functional assays to measure these channels in healthy and diseased RBCs populations using electrophysiological tools, contributing to the characterization of RBC diseases.User meeting video14.10.2020 | Webinar: Measurement of Transient receptor potential cation (TRP) channels using the Patchliner and Port-a-PatchDr. Andrs Horvth (Nanion Technologies; Germany) This is an on-demand webinar from Nan]i[on and Friends 2020.CHO cells expressing transient receptor potential cation channel V (TRPV) members 1, 3 and 4 and subfamily M member 8 were studied using our automated patch clamp systems, the Patchliner Octo (PL) and Port-a-Patch Perfusion (PaPP). During the recordings, heat, cold and/or ligand activation was performed. A classical ramp pulse-protocol (100 mV to 100 mV) was applied.Heat activation of TRPV1, 3, 4 channels was performed repeatedly by the heated pipetted (37-45 C) of the PL. Interestingly ruthenium red (RR, 50 and 200 M) was not able to prevent heat activation. Experiments involving TRPV4 were also performed on the PaPP. The cannel could be activated by heat and only partially blocked by RR. Ligand activation could be also performed on the PL (10 M Capsaicin TRPV1, 200 M 2-APB TRPV3, 100 nM GSK1016790 TRPV4) and TRPV4 on the PaPP. In all cases the effect could be inhibited using blockers. TRPM8 channel could be repetitively activated using solution at 10C on the PaPP at 10 C. Capsazepine (10 M) was used to block the activated current.Both the PL and PaPP are powerful tools to study TRP channel physiology (both using heat activation and ligand activation) and could be used to find compounds which block the temperature and ligand response separately.Webinar20.11.2018 | Webinar: The RELEVANCE of ion channel interplay Voltage-activated channels in non-excitable cellsRELEVANCE is an EU funded innovative training network and investigates in five scientific work packages different aspects, were the characterisation of red blood cells has an societal importance, such as in transfusion medicine, anaemias, diagnostics or in sports medicine. This will unavoidably result in the investigation of ion channels. The webinar is a joined presentation by a principle investigator of RELEVANCE, Prof. Lars Kaestner (Saarland University) and an early stage researcher, Maria Giustina Rotordam (Nanion Technologies, Munich).Piezo1, KCa3.1 (Gardos channel) and CaV2.1 are three channels present in the red blood cell membrane. We will highlight the role of these channels in Hereditary Xerocytosis as well as in the Gardos Channelopathy using electrophysiological tools. Since red blood cells are everything but under suspicion to be excitable cells, we will take these cells as an example to show that KCa3.1, CaV2.1 and Piezo1 present an intimate interplay providing evidence that voltage-activated channels can well play a substantial role in non-excitable cells.Publication link2018 Functional Analysis of Membrane Proteins Produced by Cell-Free TranslationPort-a-Patch andOrbit mini article in Protein Engineering (2018) Authors: Dondapati S.K., Wstenhagen D.A., Kubick S.Cell-free production is a valuable and alternative method for the synthesis of membrane proteins. This system offers openness allowing the researchers to modify the reaction conditions without any boundaries. Additionally, the cell-free reactions are scalable from 20 L up to several mL, faster and suitable for the high-throughput protein production. Here, we present two cell-free systems derived from Escherichia coli (E. coli) and Spodoptera frugiperda (Sf21) lysates. In the case of the E. coli cell-free system, nanodiscs are used for the solubilization and purification of membrane proteins. In the case of the Sf21 system, endogenous microsomes with an active translocon complex are present within the lysates which facilitate the incorporation of the bacterial potassium channel KcsA within the microsomal membranes. Following cell-free synthesis, these microsomes are directly used for the functional analysis of membrane proteins.Publication link2022 Prediction and verification of potential lead analgesic and antiarrhythmic components in Corydalis yanhusuo W. T. Wang based on voltage-gated sodium channel proteinsPort-a-Patch and Buffer Solution PublicationinInternational Journal of Biological Macromolecules (2022) Authors: Sun J., Liu X., Zhao S., Zhang S., Yang L., Zhang J., Zhao M., Xu Y.Corydalis yanhusuoW. T. Wang, a traditional Chinese herbal medicine, has been used as an analgesic for thousands of years and it also promotes blood circulation. In this study, 33Corydalis yanhusuoalkaloidactive components were acquired from Traditional Chinese Medicine Database and Analysis Platform (TCMSP). A total of 543 pain-related targets, 1774 arrhythmia targets, and 642 potential targets of these active components were obtained using Swiss Target Prediction, GeneCards, Open Target Platform, and Therapeutic Target Database. Fifty intersecting targets were visualized through a Venn diagram, KEGG and GO pathway enrichment analysis. The analysis proposed that sodium ion channels are likely potential targets ofCorydalis yanhusuoactive components as analgesia and anti-arrhythmia agents.Molecular dockingshowed that the 33 components could bind toNaV1.7andNaV1.5(two subtypes of ion channel proteins) with different binding energies. In apatch clampstudy, dihydrosanguinarine and dihydrochelerythrine, two monomers with the strongest binding effects, could inhibit the peak currents and promote both activation and inactivation phases of NaV1.5. Meanwhile, dihydrosanguinarine and dihydrochelerythrine could also inhibit peak currents and promote the activation phase of NaV1.7. Therefore, the findings from this study provide valuable information for future uses of traditional Chinese medicines to treat pain and cardiovascular disease.Publication link2021 Active components of Bupleurum chinense and Angelica biserrata showed analgesic effects in formalin induced pain by acting on NaV1.7Port-a-Patch and Buffer Solution Publication in Journal of Ethnopharmacology(2021) Authors: Xu Y., Yu Y., Wang Q., Li W., Zhang S., Liao X., Liu Y., Su Y., Zhao M., Zhang J.Ethnopharmacology relevance -Pain is an unpleasant sensory and emotional experience, often accompanied by the occurrence of a variety of diseases. More than 800 kinds of traditional Chinese medicines (TCM) has now been reported for pain relief and several monomers have been developed into novel analgesic drugs. Bupleurum chinense and Angelica biserrata were representatives of the TCM that are currently available for the treatment of pain.Product videoPort-a-Patch mini Product Video-2019Do you want to patch a cell in 60 seconds?The Port-a-Patch mini is built on the success of the Port-a-Patch. We miniaturized this patch clamp system even further by integrating the amplifier into the system. Supporting giga-seal recordings from one cell at a time, the Port-a-Patch mini offers fast and easy access to high quality patch clamp data with only minimal training. Not only a powerful research tool but also ideal for educational purposes and quick tests of cells and ion channels.Available at an attractive price, the Port-a-Patch mini is a powerful research tool for studying ion channels and also the ideal technology platform for teaching basic electrophysiological concepts in academic institutions.Product Sheet PDFPort-a-Patch mini Product SheetWebinar14.10.2020 | Webinar: TRP channels in human corneal cells and their role in ophthalmologyProf. Dr. Stefan Mergler (Charit -Universittsmedizin Berlin; Germany) This is an on-demand webinar from Nan]i[on and Friends 2020.Product Sheet PDFPort-a-Patch Product SheetWebinar07.10.2015 | Webinar: Patch Clamp Made Easy, Fast Track to ExcellenceThis webinar shows applications that go way beyond possibilities of conventional patch-clamping, where the Port-a-Patch facilitates completely novel scientific directions.The Port-a-Patch platform, used in the scientific work presented in this webinar, is a highly versatile patch clamp platform for high quality recordings form cells, organelles and artificial membranes.The Port-a-Patch is a semi-automated patch clamp device supporting stable giga-seals and excellent voltage-control of the membrane.The Port-a-Patch replaces a classical patch clamp rig, is easy to learn, still with the same quality and accuracy as known from conventional gold standard patch clamp. However, the planar geometry, compact size and versatile add-ons, allows an unprecedented experimental freedom of this platform.This webinar shows applications that go way beyond possibilities of conventional patch-clamping, where the Port-a-Patch facilitates completely novel scientific directions. The high scientific impact of the Port-a-Patch is illustrated by its vast publications list, including high rank journals such as Science, Nature, PNAS.Flyer PDFPort-a-Patch Product Flyer Internal PerfusionFlyer PDFPort-a-Patch Product Flyer Suction Control ProApplication Note PDFTRPV4 Activation and inhibition of TRPV4 using different stimuli on the Port-a-PatchPort-a-Patch application note: TRPV4 is a member of the transient receptor potential TRPV4 is a member of the transient receptor potential channel (TRP) family. Transient receptor potential vanilloidtype 4 (TRPV4) shares approximately 40% identity with TRPV1 and TRPV2 and is a Ca2+-permeable non-selectivecation channel1-3expressed in a wide range of tissues including neurons of the central and peripheral nervous systems, and in non-neuronal tissue including human T cells, corneal and retinal epithelial cells, endothelial cells of the eye, liver, heart, kidney, synoviocytes, epitheliallining of trachea and lung airways, stellate cells of thepancreas, and many more.Flyer PDFPort-a-Patch Accessories CatalogApplication Note PDFTRPM8 Cold activation of TRPM8 using the External Perfusion System and the Port-a-PatchPort-a-Patch application note: TRPM8 is a member of the transient receptor potentialchannel (TRP) family. TRPM8 is known to be a thermosensitivechannel, activated by cold temperatures(below ~25C) and ligands such as menthol, Eucalyptoland icilin1-4. It belongs to the melastatin subfamily ofTRP channels5and shows an outward rectification with arelatively high permeability for calcium ions andlittle selectivity between monovalent cations. Menthol,a secondary alcohol produced by the peppermintherb, Mentha piperita, is widely used in the food andpharmaceutical industries as a cooling/soothingcompound and odorant.Here we present data of hTRPM8 collected on the Port-a-Patch using cooled solution via the External Perfusion System. Cold activated hTRPM8 was blocked by capsazepine with an IC50in good agreement with the literature.Here we present data of hTRPM8 collected on the Port-a-Patch using cooled solution via the External Perfusion System. Cold activated hTRPM8 was blocked by capsazepine with an IC50in good agreement with the literature.Application Note PDFTRPM8 / TRPV1 TRPV1 and TRPM8 recorded on Nanions Port-a-PatchPort-a-Patch application note:TRPV1 and TRPM8 are members of the transient receptor potential channel (TRP) family. This family was designated TRP because of a spontaneously occurring Drosophila mutant lacking TRP that responded to a continuous lightwith a transient receptor potential. TRPV1 is mainly expressed in sensory nerves. Its presence is essential for transduction of nociception as well as inflammatory and hypothermic effects of vanniloid compounds.It also contributes to acute thermal nociception and thermal hyperalgesia following tissue injury. TRPV1 forms a relatively Ca2+-selective ion channel with outwardly rectifying properties. It is activated by vanilloids such as capsaicin, by protons, increased temperatures, lipoxygenase products, as well as anandamide (Huang et al., 2002). Menthol, a secondary alcohol produced by the peppermint herb, Mentha piperita, is widely used in the food and pharmaceutical industries as a cooling/soothing compound and odorant. It induces Ca2+influx in a subset ofsensory neurons from dorsal root and trigeminal ganglia, due to activation of TRPM8, a Ca2+-permeable, cold-activated member of the TRP superfamily of cation channels (McKemy et al., 2002; Peier et al., 2002). Here we present data of TRPV1 and TRPM8 collected on the Port-a-Patch. Channel activation with capsaicin as well as menthol are shown.Application Note PDFTRPA1 TRPA1 activation by allyl isothiocyanate recorded on the Port-a-PatchPort-a-Patch application note:Transient receptor potential (TRP) channels are animportant class of receptors found widely distributedthroughout the mammalian central and peripheralnervous systems. They have been shown to beactivated by many stimuli including temperature,mechano-stimulation, divalent cations and pH, amongstothers. TRP channels are receiving much attention aspotential targets for the treatment of, for example, pain,respiratory diseases such as asthma, cancer and immunedisorders (for review see ref. 1).The TRPA1 receptor was first cloned from culturedhuman lung fibroblasts but has subsequently been foundto be expressed in sensory neurones and is often foundco-localised with TRPV1. TRPA1 isactivated by a number of chemical stimuli includingallyl isothiocyanate (mustard oil), cinnamaldehyde (theactive ingredient of cinnamon), chlorobenzylidenemalononitrile (CS tear gas), hydrogen peroxide andhyperchlorite (chlorine gas). It is thought that TRPA1,together with TRPV1, may contribute to chemicalhypersensitivity, chronic cough, and airway inflammationin asthma.Here we present data recorded on the Port-aPatchwith external perfusion showing recordings ofhuman TRPA1 (hTRPA1) activated by increasingconcentrations of allyl isothiocyanate (AITC). hTRPA1could be repetitively stimulated using low concentrationsof AITC but was desensitized by high concentrations ofAITC (>10 M).Application Note PDFTRPM7 TRPM7 activation by internal sequestering of Mg2+ ions recorded on the Port-a-PatchPort-a-Patch application note:Transient receptor potential (TRP) channels are animportant class of receptors found widely distributedthroughout the mammalian central and peripheralnervous systems. They have been shown to beactivated by many stimuli including temperature,mechano-stimulation, divalent cations and pH, amongstothers. TRP channels are receiving much attention aspotential targets for the treatment of, for example, pain,respiratory diseases such as asthma, cancer and immunedisorders.The TRPM7 receptor is thought to play a role inmagnesium homeostasis. A role for TRPM7 in intracellularpH sensing, the pathological response to bloodvessel wall injuryand cell adhesion has also beensuggested. In electrophysiological studies TRPM7 canbe recorded using a voltage ramp protocol. It displaysa characteristic large outward current with little inwardcurrent and can be blocked by the presence of internalMg2+ions.Here we present data recorded on the Port-a-Patch with internal perfusion showing recordings of mouseTRPM7 (mTRPM7) and block of this channel by the internalperfusion of Mg2+ions.Application Note PDFMitochondria Recordings from mitochondria and mitoplastsPort-a-Patch application note: Mitochondria are often referred to as the power house of the cell since they are responsible for making most of the cells energy supply in the form of adenosine triphosphate (ATP). In addition to providing the cell with energy, mitochondria are thought to have roles in cell signalling, cellular differentiation and apoptosis. They have also been implicated in the pathophysiology of neurodegenerative disorders such as Parkinsons Disease , and may also play a role in diabetes and in the ageing process. Mitochondria are usually rod shaped and range in size from approximately 1 - 10 m. They have an outer membrane and a highly folded inner membrane. The outer membrane is highly permeable and contains one of the most well studied mitochondrial proteins, the voltage-dependent anion channel (VDAC). The inner membrane contains many ion channels, including the Ca2+uniporter, a KATP channel, the Ca2+-activated K+channel (KCa) and the inner membrane anion channel (IMAC). To study the mitochondrial inner membrane using the patch clamp technique, mitoplasts were formed. This is a process whereby the mitochondria are swelled, thus rupturing the outer membrane and exposing the inner membrane.Application Note PDFNaV1.7 Patch clamp recordings of hNaV1.7 on Nanions Port-a-PatchPort-a-Patch application note: Cells were kindly provided byAnaxonThe NaV1.7 gene (SCN9A) encodes a voltage-gated sodium (NaV) channel, primarily expressed in the peripheral nervous system and has been isolated from rat dorsal root ganglion (DRG) neurons, human medullary thyroid cancer cells (hNE-Na) and PC12 cells.Different NaVchannels play a key role in modulation of action potentials in the central and peripheral nervous systems. In particular, the fast upstroke of the action potential is mediated by NaVchannels. NaVchannels are in part characterized by their TTX-sensitivity (TTX-resistant [TTXr], TTX-sensitive [TTXs]). NaV1.7 is a TTXs channel and is sensitive to TTX in the nanomolar range. The role of hNaV1.7 has yet to be fully elucidated but is proposed to play an important role in nociception and pain sensing. NaV1.7 has beenimplicated to play a role in disease pain states, in particular inflammatory pain and hypersensitivity to heat following burn injury. Common to many of the voltagegated ion channels, a number of compounds display a higher affinity for the inactivated state of the channel. For this reason, it is important to be able to reliably record both activation and inactivation kinetics of the channel.In this Application Note we present data using the Port-a-Patch characterizing CHO cells stably expressing hNaV1.7. The hNaV1.7 activation andinactivation properties and TTX sensitivity are consistent with those reported in the literature.Application Note PDFKV1.3 Automated internal perfusion of Jurkat cells expressing KV1.3 channelsPort-a-Patch application note: KV1.3 is a voltage-gated potassium channel which plays arole in human T cell activation and proliferation, cell-mediatedcytotoxicity and volume regulation. It is therefore an important target for the therapeutic control of T cellresponses. The KV1.3 channel is endogenously expressedin Jurkat cells, an immortalised T lymphocyte cell line. Thechannel is activated by membrane depolarisation at voltagestypically more positive than -40 mV with rapid activationkinetics and slower inactivation.In common with many other voltage-gated potassiumchannels, KV1.3 can be blocked by replacing K+with Cs+ions in the pipette solution. Using Nanions Internal PerfusionSystem, we were able to record stable KV1.3 currentsfrom Jurkat cells and reliably block this potassium currentby perfusing the internal side of the chip with a Cs+-containingsolution. The KV1.3 mediated current could also becompletely recovered by perfusing the inside of the chipwith K+-containing solution.KV1.3 mediated currents can also be blocked by internalperfusion with tetraethylammonium (TEA). We presentdata here showing block of KV1.3 by increasing concentrationsof TEA using the Internal Perfusion System. The IC50that we obtained for TEA applied internally was in goodagreement with the literature.Application Note PDFMitochondria Mitoplasts from HEK cells on Nanions Port-a-PatchPort-a-Patch application note: Mitochondria play an important role in metabolism by providing the cell with ATP due to oxidative phosphorylation. But they are also supposed to be involved in apoptosis and cytoprotection. The mitochondrial membranes contain a large number of ion channels, transporters and pores. However the physiological role of mitochondrial channels is largely unknown. The main channel of the outer membrane is VDAC, a well described anion conductance. In the inner membrane most prominent are the permeability transition pore (PTP) and the inner membrane anion channel IMAC. Different potassium channels are described as well (mitoBK, mitoKATP and Kv1.3) and a number of calcium conducting channels and receptors (for example MCU). To study the channels of the inner mitochondrial membrane the outer membrane can be stripped of by osmotic swelling. This method was used for the examples of electrophysiological measurement with the Port-a-Patch shown below.Application Note PDFhERG Pharmacology of hERG recorded on Nanions Port-a-PatchPort-a-Patch application note: Cells were kindly provided by Millipore.The hERG gene (KCNH2) encodes a potassium ionchannel responsible for the repolarizing IKr current in thecardiac action potential (Sanguinetti et al., 1995).Abnormalities in this channel may lead to either Long QTsyndrome (LQT2) (with loss-of-function mutations) or ShortQT syndrome (with gain-of-function mutations), bothpotentially fatal cardiac arrhythmia, due to repolarizationdisturbances of the cardiac action potential.Given the importance of this channel in maintainingcardiac function, it has become an important target incompound safety screening.A large range of therapeutic agents with diverse chemicalstructures have been reported to induce long QTsyndrome. These include antihistamines (e.g. Terfenadine),gastrointestinal prokinetic agents (e.g. Cisapride) andothers.Here we present data collected on the Port-a-Patch.Astemizole, Terfenadine, Cisapride and Flunarizinedose-response curves on hERG expressed in CHO cellsare shown. The mean current amplitude in these cells was1076 79 pA (n= 88) at -40 mV.Application Note PDFhERG Temperature controlled hERG recordings on the Port-a-PatchPort-a-Patch application note: Cells were kindly provided by Millipore.The hERG gene encodes a potassium channelresponsible for the repolarization of the IKr current incardiac cells. This channel is important in the repolarizationof the cardiac action potential. Abnormalitiesin this channel can cause long orshort QT syndrome, leading to potentially fatalcardiac arrhythmia. Given the importance of thischannel in maintaining cardiac function, anddisturbances of channel activity by certain compoundssuch as anti-arrhythmias and anti-psychotics, it hasbecome an important target in compound safetyscreening. It is a desirable option to study this channelat physiological temperature since compounds candisplay different actions or potencies at physiologicaltemperature.Here, we present data collected on the Port-a-Patchusing the External Perfusion System coupled with NanionsTemperature Control. Cells were captured and sealedat room temperature and control recordings made beforeraising the temperature to 35C so that parameterssuch as current peak amplitude could be compared atthe two different temperatures. Furthermore, the hERGactive compound, quinidine, was used at physiologicaltemperature and a full dose response curve wasachieved. This demonstrates the stability of therecordings on the Port-a-Patch at this temperature. Theconcentration response curve generated an IC50similarto that obtained at room temperature, and similar to thatpublished in the literature.Application Note PDFhERG Characterization of hERG (CHO) on Nanions Port-a-PatchPort-a-Patch application note: Cells were kindly provided by Millipore.The hERG gene (KCNH2) encodes a potassium ion channelresponsible for the repolarizing IKrcurrent in the cardiacaction potential (Sanguinetti et al., 1995).Abnormalities in this channel may lead to either Long QTSyndrome (LQT2) (with loss-of-function mutations) or ShortQT syndrome (with gain-of-function mutations), both potentiallyfatal cardiac arrhythmia, due to repolarizationdisturbances of the cardiac action potential.Given the importance of this channel in maintaining cardiacfunction, it has become an important target in compoundsafety screening.A large range of therapeutic agents with diverse chemicalstructures have been reported to induce long QT syndrome.These include antihistamines (e.g. Terfenadine),gastrointestinal prokinetic agents (e.g. Cisapride) andothers.In this report we present data that were collected on thePort-a-Patch. Cells (CHO permanently expressing hERG,supplied by Millipore) were tested. Current amplitudes, IVsand cisapride as well as quinidine dose response curveswere analyzed.Application Note PDFhERG Characterization of hERG (HEK293) on Nanions Port-a-PatchPort-a-Patch application note: Cells were kindly provided by Millipore.The hERG gene (KCNH2) encodes a potassium ion channelresponsible for the repolarizing IKrcurrent in the cardiacaction potential (Sanguinetti et al., 1995).Abnormalities in this channel may lead to either Long QTSyndrome (LQT2) (with loss-of-function mutations) or ShortQT syndrome (with gain-of-function mutations), both potentiallyfatal cardiac arrhythmia, due to repolarizationdisturbances of the cardiac action potential.Given the importance of this channel in maintaining cardiacfunction, it has become an important target in compoundsafety screening.A large range of therapeutic agents with diverse chemicalstructures have been reported to induce long QT syndrome.These include antihistamines (e.g. terfenadine),gastrointestinal prokinetic agents (e.g. cisapride) andothers.In this report we present data that were collected on thePort-a-Patch. Cells (HEK293 stably expressing hERG, suppliedby Millipore) were tested. Current amplitudes, IVsand cisapride aswell as quinidine dose-response curves were analyzed.Application Note PDFCaV3.2 Characterization of CaV3.2 on Nanions Port-a-PatchPort-a-Patch application note: Cells were kindly provided by Millipore.The gene CACNA1H encodes the 1H subunit of the voltage-gatedcalcium channel CaV3.2. It belongs to the lowvoltage-activated T-type calcium channels. CaV3.2 displaysthe typical characteristics of the T-type channels:activation at low depolarization of the membrane andtransient kinetics.T-type Ca2+channels are involved in diverse, mainly rhythmicprocesses like e.g. pacemaking and generation ofthalamocortical rhythms in sleep or epilepsy. CaV3.2 isexpressed in a wide variety of cells. Amongst others ithas been found in kidney, smooth muscle, brain, adrenaland cardiac cells. It seems to be involved in contractionof smooth muscle and the secretion of the adrenal hormonesaldosterone and cortisol. Pharmacological blockof T-type channels may lead to new drugs for the treatmentof hypertension and epilepsy.The biophysical and pharmacological properties of thecells are presented in this Application Note.Application Note PDFGABAA a5b3g2 Activation and inhibition of GABAA a5b3g2 receptors on the Port-a-PatchPort-a-Patch application note: Cells were kindly provided by Merck Millipore.Gamma aminobutyric acid type A (GABAA) receptors are the most important inhibitory neurotransmitter receptors in the mammalian central nervous system (CNS). They are opened by GABA allowing the passage of chloride ions across the membrane. GABAAchannels are modulated by a variety of different drugs including benzodiazepines, barbiturates, neuroactive steroids, anesthetics, and convulsants. The receptors are heteropentameric and depending on the subunit combination, they exhibit different electrophysiological and pharmacological properties. Six a-, three b-, three g-, one d-, one e-, one p-, one - and three r-subunits have been cloned, including splice variants of some of these subunits. Functional GABAAreceptors typically assemble with two a, two b, and one g subunit, with alternating a and b subunits connected by a g subunit. GABAAreceptors play a critical role in regulating excitability of the brain, anxiety, vigilance, as well as learning and memory. The a5 subunit is highly expressed in the hippocampus and olfactory bulb and expressed in low levels in other brain regions including the cortex, subiculum, hypothalamus, sympathetic preganglionic neurons, and amygdala. GABAAreceptors containing the a5 subunit cluster at both extrasynaptic sites as well as synaptic sites thus contributing to tonic currents and synaptic GABA-ergic neurotransmission. a5 -containing receptors exhibit unique physiology and pharmacology and they are potential pharmacological targets for the treatment of neurodevelopmental disorders, depression, schizophrenia, and mild cognitive impairment. The Port-a-Patch with External Perfusion System was used to record a5b3g2 receptors expressed in HEK293 cells.Application Note PDFCardiomyocytes Action Potentials in Mouse ES Cell-Derived Cor.At Cardiomyocytes on Nanions Port-a-PatchPort-a-Patch application note: Cells were kindly provided by Axiogenesis.To provide scientists in basic or applied cardiology andtoxicology with a standardized and pure cardiac myocytemodel with functional expression of all essential cardiacion channels, Axiogenesis has developed Cor.Atcardiomyocytes. These mouse embryonic stem cell derivedcardiomyocytes are ready to use and 99.9 % purewithout contamination by other cell types. Using the Porta-Patch,Cor.Atcardiomyocytes have experimentallybeen shown to functionally express at least three essentialcardiac currents INa, ICa, and IK, and to exhibit typicalcardiac action potentials.Application Note PDFCaV2.2 Characterization of CaV2.2 on Nanions Port-a-PatchPort-a-Patch application note: Cells were kindly provided by Millipore.The voltage gated N-type calcium channel (CaV2.2) isencoded by the gene CACNA1B. CaV2.2 is a high voltage activated calcium channel.CaV2.2 is found mainly in the brain, where it mediates neurotransmitterrelease at the synapse. The strong depolarizationof neuronal action potentials causes the openingof the channel. Calcium can then enter the cell and initiatesthe fusion of the neurotransmitter vesicles with themembrane.CaV2.2 is inhibited by w-conotoxin, a neurotoxin of the fishhunting snail, with high specificity. CaV2.2 has been implicatedin the transmission of pain. Pharmacological blockof CaV2.2 by compounds based on w-conotoxin has beenshown to be effective against strong chronical pain.The biophysical and pharmacological properties of thecells are presented in this Application Note.Publication link2022 Structural basis of the negative allosteric modulation of 5-BDBD at human P2X4 receptorsPort-a-Patch Publicationin Molecular Pharmacology (2022) Authors: Bidula S., Nadzirin I.B., Cominetti M.MD., Hickey H., Cullum S. A., Searcey M., Schmid R., Fountain S.The P2X4 receptor is a ligand-gated ion channel activated by extracellular ATP. P2X4 activity is associated with neuropathic pain, vasodilation, and pulmonary secretion and is therefore of therapeutic interest. The structure-activity relationship of P2X4 antagonists is poorly understood. Here we elucidate the structure-activity of 5-(3-bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one (5-BDBD) at human P2X4 by combining pharmacology, electrophysiology, molecular modeling, and medicinal chemistry. 5-BDBD antagonized P2X4 in a noncompetitive manner but lacked effect at human P2X2. Molecular modeling and site-directed mutagenesis suggested an allosteric binding site for 5-BDBD located between two subunits in the body region of P2X4, with M109, F178, Y300, and I312 on one subunit and R301 on the neighboring subunit as key residues involved in antagonist binding. The bromine group of 5-BDBD was redundant for the antagonist activity of 5-BDBD, although an interaction between the carbonyl group of 5-BDBD and R301 in P2X4 was associated with 5-BDBD activity. 5-BDBD could inhibit the closed channel but poorly inhibited the channel in the open/desensitizing state. We hypothesize that this is due to constriction of the allosteric site after transition from closed to open channel state. We propose that M109, F178, Y300, R301, and I312 are key residues for 5-BDBD binding; provide a structural explanation of how they contribute to 5-BDBD antagonism; and highlight that the limited action of 5-BDBD on open versus closed channels is due to a conformational change in the allosteric site.Application Note PDFBacterial Spheroplasts Preparation and recordings on the Port-a-Patch of native E. Coli spheroplastsPort-a-Patch application note: Bacterial membranes are not easy to patch clamp.Since bacterial ion channels are of increasing interest,we started to optimize the protocols for patch clampingbacterial spheroplasts with the Port-a-Patch.Bacterial spheroaplasts can be prepared up to a size of5 m. They consist of the inner bacterial membrane. Thistechnique was first used for patch clamp experimentsby Boris Matrinac in 1987 and let to the discovery ofmechanosensitive channels in E. coli. Here we describethe preparation of Spheroplasts out of E. coli and showion channel currents recorded with the Port-a-Patch.Publication link2022 A chemically mediated artificial neuronPort-a-Patch Publication in Nature Electronics (2022) Authors: Wang T., Wang M., Wang J., Yang L., Ren X., Song G., Chen S., Yuan Y., Liu R., Pan L., Li Z., Leow W.R., Luo Y., Ji S., Cui Z., He K., Zhang F., Lv F., Tian Y., Cai K., Yang B., Niu J., Zou H., Liu S., Xu G., Fan X., Hu B., Loh X.J., Wang L. Chen X.Brainmachine interfaces typically rely on electrophysiological signals to interpret and transmit neurological information. In biological systems, however, neurotransmitters are chemical-based interneuron messengers. This mismatch can potentially lead to incorrect interpretation of the transmitted neuron information. Here we report a chemically mediated artificial neuron that can receive and release the neurotransmitter dopamine. The artificial neuron detects dopamine using a carbon-based electrochemical sensor and then processes the sensory signals using a memristor with synaptic plasticity, before stimulating dopamine release through a heat-responsive hydrogel. The system responds to dopamine exocytosis from rat pheochromocytoma cells and also releases dopamine to activate pheochromocytoma cells, forming a chemical communication loop similar to interneurons. To illustrate the potential of this approach, we show that the artificial neuron can trigger the controllable movement of a mouse leg and robotic hand.Publication link2022 Mutagenicity and safety pharmacology of a standardized antidiabetic polyherbal formulationPort-a-Patch Publicationin Nature Scientific Reports (2022) Authors: Majid F.A.A., Fadhlina A., Ismail H.F., Zainol S.N., Mamillapalli A.K., Venkatesan V., Eswarappa R., Pillai R.Synacinn is a standardized polyherbal extract formulated for the treatment of diabetes mellitus and its complications. This study aims to assess the mutagenicity potential of Synacinn by

after removal of unbound photoactive drug from the solution. Mutation of the key residue of the local anesthetic binding site (F1579A) did not fully prevent ligand binding and inhibition, however, it eliminated most of the modulation caused by ligand binding. Our results indicate that riluzole binds with highest affinity to the local anesthetic binding site, which transmits inhibition by the unique non-blocking modulation mechanism. Our results also suggest the existence of one or more additional binding sites, with lower affinity, and different inhibition mechanism.Publication link2020 New natural agonists of the transient receptor potential Ankyrin 1 (TRPA1) channelPort-a-Patch Publication in Nature Scientific Reports (2020) Authors: Legrand C., Merlini J.M., de SenarclensBezenon C., Michlig S.The transient receptor potential (TRP) channels family are cationic channels involved in various physiological processes as pain, inflammation, metabolism, swallowing function, gut motility, thermoregulation or adipogenesis. In the oral CaVity, TRP channels are involved in chemesthesis, the sensory chemical transduction of spicy ingredients. Among them, TRPA1 is activated by natural molecules producing pungent, tingling or irritating sensations during their consumption. TRPA1 can be activated by different chemicals found in plants or spices such as the electrophiles isothiocyanates, thiosulfinates or unsaturated aldehydes. TRPA1 has been as well associated to various physiological mechanisms like gut motility, inflammation or pain. Cinnamaldehyde, its well known potent agonist from cinnamon, is reported to impact metabolism and exert anti-obesity and anti-hyperglycemic effects. Recently, a structurally similar molecule to cinnamaldehyde, cuminaldehyde was shown to possess anti-obesity and anti-hyperglycemic effect as well. We hypothesized that both cinnamaldehyde and cuminaldehyde might exert this metabolic effects through TRPA1 activation and evaluated the impact of cuminaldehyde on TRPA1. The results presented here show that cuminaldehyde activates TRPA1 as well. Additionally, a new natural agonist of TRPA1, tiglic aldehyde, was identified and p-anisaldehyde confirmed.Publication link2020 Homocysteine-induced electrical remodeling via the mediation of IP 3 R1/NaV1.5 signaling pathwayPort-a-Patch Publication in American Journal of Translational Research (2020) Authors: Han L., Wu A., Li Q., Xia Z., Wu Y., Hong K., Xia Z., Li J.Inositol-1,4,5-triphosphate-receptor 1 (IP3R1), a Ca2+ channel in the sarcoplasmic reticulum membrane, is an effective regulator of Ca2+ release involved in the pathology of most cardiovascular diseases. Our study aim to investigate the underlying mechanism by which IP3R1 signaling mediates the process of homocysteine (Hcy)-induced Ca2+ accumulation via interaction with sodium current (NaV1.5) in atrium. We utilized whole-cell patch-clamp analysis and flow cytometry to detect the abnormal electrical activity in mouse atrial myocytes (MACs) obtained from C57B6 mice fed with high-Hcy diet. The results represented not only an increase in protein levels of NaV1.5 and IP3R1, but also an enhanced intracellular levels of Ca2+, and prolonged action potential duration (APD). However, the inhibition of IP3R1 or NaV1.5 gene could both attenuate Ca2+ accumulation in MACs triggered by Hcy, as well as abnormal electrical activity. In addition, Hcy increased the interaction between IP3R1 and NaV1.5. These data suggest that Hcy induced Ca2+ accumulation is mediated by the IP3R1/NaV1.5 signaling pathway, accompanied with the influx of Na+ and Ca2+, which act as triggers for electrical remodeling.Publication link2020 Interferon- and high glucose-induced opening of Cx43 hemichannels causes endothelial cell dysfunction and damagePort-a-Patch Publication in Biochimica et Biophysica Acta (BBA) - Molecular Cell Research (2020) Authors: Sez J.C., Contreras-Duarte S., Labra V.C., Santibaez C.A., Mellado L.A., Inostroza C.A., Alvear T.F., Retamal M.A., Velarde V., Orellana J.A..Both IFN- or high glucose have been linked to systemic inflammatory imbalance with serious repercussions not only for endothelial function but also for the formation of the atherosclerotic plaque. Although the uncontrolled opening of connexin hemichannels underpins the progression of various diseases, whether they are implicated in endothelial cell dysfunction and damage evoked by IFN- plus high glucose remains to be fully elucidated. In this study, by using live cell imaging and biochemical approaches, we demonstrate that IFN- plus high glucose augment endothelial connexin43 hemichannel activity, resulting in the increase of ATP release, ATP-mediated Ca2+dynamics and production of nitric oxide and superoxide anion, as well as impaired insulin-mediated uptake and intercellular diffusion of glucose and cell survival. Based on our results, we propose that connexin 43 hemichannel inhibition could serve as a new approach for tackling the activation of detrimental signaling resulting in endothelial cell dysfunction and death caused by inflammatory mediators during atherosclerosis secondary to diabetes mellitus.Publication link2020 Chronic hepatitis C virus infection impairs insulin secretion by regulation of p38 MAPK-dependent exocytosis in pancreatic -cellsPort-a-Patch Publication in Clinical Science (2020) Authors: Chen J., Wang F., Zhou Y., Jiang J., Ksimu S., Zhang X., Li J.Z., Niu J., Wang Q.Chronic hepatitis C virus (HCV) infection has a close association with type 2 diabetes mellitus. Although the mechanisms of insulin resistance in chronic hepatitis C (CHC) patients have been extensively studied, little attention has been given to the role of -cell function in HCV-associated diabetes. Here, we analysed -cell function in CHC patients and HCV-infected mouse model and found in addition to insulin resistance, impaired pancreatic -cell function occurred in CHC patients and HCV-infected C/OTg mice, not only in diabetic individuals but also in individuals with impaired fasting glucose levels. Both first-phase and second-phase insulin secretion were impaired, at least partially due to the reduction of exocytosis of secretory insulin-containing granules following HCV infection. Up-regulated p38 in HCV-infected -cells resulted in inactivation of protein kinase D (PKD), which was responsible for impaired insulin secretory capacity of -cells. Thus, impaired insulin secretion due to HCV infection in -cells contributes to HCV-associated type 2 diabetes. These findings provided a new inspiration for the important prognostic and therapeutic implications in the management of CHC patients with impaired fasting glucose.Publication link2020 Fenamates Inhibit Human Sodium Channel NaV1.2 and Protect Glutamate-Induced Injury in SH-SY5Y CellsPort-a-Patch Publication in Cellular and Molecular Neurobiology (2020) Authors: Sun J.F., Zhao M.Y., Xu Y.J., Su Y., Kong X.H., Wang Z.Y.Voltage-gated sodium channels are crucial mediators of neuronal damage in ischemic and excitotoxicity disease models. Fenamates have been reported to have anti-inflammatory properties following a decrease in prostaglandin synthesis. Several researches showed that fenamates appear to be ion channel modulators and potential neuroprotectants. In this study, the neuroprotective effects of tolfenamic acid, flufenamic acid, and mefenamic acid were tested by glutamate-induced injury in SH-SY5Y cells. Following this, fenamates' effects were examined on both the expression level and the function of hNaV1.1 and hNaV1.2, which were closely associated with neuroprotection, using Western blot and patch clamp. Finally, the effect of fenamates on the expression of apoptosis-related proteins in SH-SY5Y cells was examined. The results showed that both flufenamic acid and mefenamic acid exhibited neuroprotective effects against glutamate-induced injury in SH-SY5Y cells. They inhibited peak currents of both hNaV1.1 and hNaV1.2. However, fenamates exhibited decreased inhibitory effects on hNaV1.1 when compared to hNaV1.2. Correspondingly, the inhibitory effect of fenamates was found to be consistent with the level of neuroprotective effects in vitro. Fenamates inhibited glutamate-induced apoptosis through the modulation of the Bcl-2/Bax-dependent cell death pathways. Taken together, NaV1.2 might play a part in fenamates' neuroprotection mechanism. NaV1.2 and NMDAR might take part in the neuroprotection mechanism of the fenamates. The fenamates inhibited glutamate-induced apoptosis through modulation of the Bcl-2/Bax-dependent cell death pathways.Publication link2020 Ascorbate-induced oxidative stress mediates TRP channel activation and cytotoxicity in human etoposide-sensitive and -resistant retinoblastoma cellsPort a PatchPublication in Laboratory Investigation (2020) Authors: Oronowicz J., Reinhard J., Reinach P.S., Ludwiczak S., Luo H., Salem M.H.O.B., Kraemer M.M., Biebermann H., Kakkassery V., Mergler S.There are indications that pharmacological doses of ascorbate (Asc) used as an adjuvant improve the chemotherapeutic management of cancer. This favorable outcome stems from its cytotoxic effects due to prooxidative mechanisms. Since regulation of intracellular Ca2+ levels contributes to the maintenance of cell viability, we hypothesized that one of the effects of Asc includes disrupting regulation of intracellular Ca2+ homeostasis. Accordingly, we determined if Asc induced intracellular Ca2+ influx through activation of pertussis sensitive Gi/o-coupled GPCR which in turn activated transient receptor potential (TRP) channels in both etoposide-resistant and -sensitive retinoblastoma (WERI-Rb1) tumor cells. Ca2+ imaging, whole-cell patch-clamping, and quantitative real-time PCR (qRT-PCR) were performed in parallel with measurements of RB cell survival using Trypan Blue cell dye exclusion. TRPM7 gene expression levels were similar in both cell lines whereas TRPV1, TRPM2, TRPA1, TRPC5, TRPV4, and TRPM8 gene expression levels were downregulated in the etoposide-resistant WERI-Rb1 cells. In the presence of extracellular Ca2+, 1mM Asc induced larger intracellular Ca2+ transients in the etoposide-resistant WERI-Rb1 than in their etoposide-sensitive counterpart. With either 100M CPZ, 500M La3+, 10mM NAC, or 100M 2-APB, these Ca2+ transients were markedly diminished. These inhibitors also had corresponding inhibitory effects on Asc-induced rises in whole-cell currents. Pertussis toxin (PTX) preincubation blocked rises in Ca2+ influx. Microscopic analyses showed that after 4 days of exposure to 1mM Asc cell viability fell by nearly 100% in both RB cell lines. Taken together, one of the effects underlying oxidative mediated Asc-induced WERI-Rb1 cytotoxicity stems from its promotion of Gi/o coupled GPCR mediated increases in intracellular Ca2+ influx through TRP channels. Therefore, designing drugs targeting TRP channel modulation may be a viable approach to increase the efficacy of chemotherapeutic treatment of RB. Furthermore, Asc may be indicated as a possible supportive agent in anti-cancer therapies.Publication link2020 Bioluminescence Methodology for Ion Channel StudiesPort-a-Patch Chapter in Patch Clamp Electrophysiology (2020) Authors: Wadsworth P.A., Singh A.K., Nguyen N., Stephan C., Laezza F.As key players in cell function, ion channels are important targets for drug discovery and therapeutic development against a wide range of health conditions. Thus, developing assays to reconstitute ion channel macromolecular complexes in physiological conditions and screen for chemical modifiers of proteinprotein interactions within these complexes is timely in drug discovery campaigns. For most ion channels, expressing their pore-forming subunit in heterologous mammalian cells has now become a routine procedure. However, reconstituting protein-channel complexes in physiological environments is still challenging, limiting our ability to identify tools and probes based on allosteric mechanisms, which could lead to more targeted and precise modulation of the channel function. Here, we describe the assay development steps to stably reconstitute the interaction between voltage-gated Na+ (NaV) channel NaV1.6 and its accessory protein, fibroblast growth factor 14 (FGF14) using the split-luciferase complementation assay (LCA), followed by assay miniaturization and optimization in 384-well plates for in-cell high-throughput screening (HTS) against protein-channel interactions. This optimized LCA can subsequently be used for rapid estimation of hit potency and efficacy via dose-dependency studies, enabling ranking of hits prior to more labor-intensive validation studies. Lastly, we introduce the methodology for rapid functional hit validation studies using semi-automated planar patch-clamp electrophysiology. Our robust, in-cell HTS platform can be adapted to any suitable ion channel complex to explore regulatory pathways of cellular signaling using kinase inhibitors, as well as to screen small molecules for probe development and drug repurposing toward new targets/areas of medicine. Overall, the flexibility of this assay allows users to broadly explore therapeutic options for channelopathy-associated diseases at a fast pace, enabling rapid hypothesis generation in early phase drug discovery campaigns and narrowing down targets prior to more labor-intensive in vivo studies.Publication link2020 Addressing hERG activity while maintaining favorable potency, selectivity and pharmacokinetic properties of PPAR modulatorsPort-a Patch Publication in Bioorganic & Medicinal Chemistry Letters (2020) Authors: Lagu B., Senaiar R.S., Kluge A.F., Mallesh B., Ramakrishna M., Bhat R., Patane M.A.One of the most commonly used strategies to reduce hERG (human ether-a-go-go) activity in the drug candidates is introduction of a carboxylic acid group. During the optimization of PPAR modulators, some of the compounds containing a carboxylic acid were found to inhibit the hERG channel in a patch clamp assay. By modifying the basicity of the imidazole core, potent and selective PPAR modulators that do not inhibit hERG channel were identified. Some of the modulators have excellent pharmacokinetic profiles in mice.Publication link2020 Arterial Medial Calcification through Enhanced small Extracellular Vesicle Release in Smooth Muscle-Specific Asah1 Gene Knockout MicePort-a-Patch Publication in Nature Scientific Reports (2020) Authors: Bhat O.M., Li G., Yuan X., Huang D., Gulbins E., Kukreja R.C., Li P-L.Arterial medial calcification (AMC) involves an increased small extracellular vesicle (sEV) secretion and apatite calcium precipitation in the arterial wall. The mechanisms mediating AMC remain poorly understood. In the present study, smooth muscle-specific acid ceramidase (Ac) gene knockout mice (Asah1fl/fl/SMCre) were used to demonstrate the role of lysosomal ceramide signaling pathway in AMC. Asah1fl/fl/SMCremice were found to have more severe AMC in both aorta and coronary arteries compared to their littermates (Asah1fl/fl/SMwtand WT/WT mice) after receiving a high dose vitamin D. These mice also had pronounced upregulation of osteopontin and RUNX2 (osteogenic markers), CD63, AnX2 (sEV markers) and ALP expression (mineralization marker) in the arterial media. In cultured coronary arterial smooth muscle cells (CASMCs) from Asah1fl/fl/SMCremice, high dose of Pi led to a significantly increased calcium deposition, phenotypic change and sEV secretion compared to WT CASMCs, which was associated with reduced lysosome-multivesicular body (MVB) interaction. Also, GW4869, sEV release inhibitor decreased sEV secretion and calcification in these cells. Lysosomal transient receptor potential mucolipin 1 (TRPML1) channels regulating lysosome interaction with MVBs were found remarkably inhibited in Asah1fl/fl/SMCreCASMCs as shown by GCaMP3 Ca2+imaging and Port-a-Patch patch clamping of lysosomes. Lysosomal Ac in SMCs controls sEV release by regulating lysosomal TRPML1 channel activity and lysosome-MVB interaction, which importantly contributes to phenotypic transition and AMC.Publication link2019 Postpartum hormones oxytocin and prolactin cause pro-arrhythmic prolongation of cardiac repolarization in long QT syndrome type 2Port-a Patch Publication in EP Europace (2019) Authors: Bodi I., Sorge J., Castiglione A., Glatz S.M., Wuelfers E.M., Franke G., Perez-Feliz S., Koren G., Zehender M., Bugger H., Seemann G., Brunner M., Bode C., Odening K.E.AimsWomen with long QT syndrome 2 (LQT2) have a particularly high postpartal risk for lethal arrhythmias. We aimed at investigating whether oxytocin and prolactin contribute to this risk by affecting repolarization.Methods and resultsIn female transgenic LQT2rabbits (HERG-G628S, loss of IKr), hormone effects on QT/action potential duration (APD) were assessed (0.2200ng/L). Hormone effects (200ng/L) on ion currents and cellular APD were determined in transfected cells and LQT2cardiomyocytes. Hormone effects on ion channels were assessed with qPCR and western blot. Experimental data were incorporated intoin silicomodels to determine the pro-arrhythmic potential. Oxytocin prolonged QTc and steepened QT/RR-slopein vivoand prolonged ex vivo APD75in LQT2hearts. Prolactin prolonged APD75at high concentrations. As underlying mechanisms, we identified an oxytocin- and prolactin-induced acute reduction of IKs-tail and IKs-steady (25.5%, oxytocin; 13.3%, prolactin, P0.05) in CHO-cells and LQT2-cardiomyocytes. IKrcurrents were not altered. This oxytocin-/prolactin-induced IKsreduction caused APD90prolongation (+11.9%/+13%, P0.05) in the context of reduced/absent IKrin LQT2cardiomyocytes. Hormones had no effect on IK1and ICa,Lin cardiomyocytes. Protein and mRNA levels of CACNA1C/CaV1.2 and RyR2 were enhanced by oxytocin and prolactin. Incorporating these hormone effects into computational models resulted in reduced repolarization reserve and increased propensity to pro-arrhythmic permanent depolarization, lack of capture and early afterdepolarizations formation.ConclusionsPostpartum hormones oxytocin and prolactin prolong QT/APD in LQT2by reducing IKsand by increasing CaV1.2 and RyR2 expression/transcription, thereby contributing to the increased postpartal arrhythmic risk in LQT2.Publication link2019 Screening by changes in stereotypical behavior during cell motilityPort-a Patch Publication in Scientific Reports (2019) Authors: Tweedy L., Witzel P., Heinrich D., Insall R.H., Endres R.G.Stereotyped behaviors are series of postures that show very little variability between repeats. They have been used to classify the dynamics of individuals, groups and species without reference to the lower-level mechanisms that drive them. Stereotypes are easily identified in animals due to strong constraints on the number, shape, and relative positions of anatomical features, such as limbs, that may be used as landmarks for posture identification. In contrast, the identification of stereotypes in single cells poses a significant challenge as the cell lacks these landmark features, and finding constraints on cell shape is a non-trivial task. Here, we use the maximum caliber variational method to build a minimal model of cell behavior during migration. Without reference to biochemical details, we are able to make behavioral predictions over timescales of minutes using only changes in cell shape over timescales of seconds. We use drug treatment and genetics to demonstrate that maximum caliber descriptors can discriminate between healthy and aberrant migration, thereby showing potential applications for maximum caliber methods in automated disease screening, for example in the identification of behaviors associated with cancer metastasis.Publication link2019 NMDA Receptor-Mediated Signaling Pathways Enhance Radiation Resistance, Survival and Migration in Glioblastoma Cells-A Potential Target for Adjuvant RadiotherapyPort-a Patch Publication in Cancers (2019) Authors: Mller-Lngle A., Lutz H., Hehlgans S., Rdel F., Rau K., Laube B.Glioblastoma is one of the most aggressive malignant brain tumors, with a survival time less than 15 months and characterized by a high radioresistance and the property of infiltrating the brain. Recent data indicate that the malignancy of glioblastomas depends on glutamatergic signaling via ionotropic glutamate receptors. In this study we revealed functional expression of Ca2+-permeable NMDARs in three glioblastoma cell lines. Therefore, we investigated the impact of this receptor on cell survival, migration and DNA double-strand break (DSB) repair in the presence of both, glutamate and NMDAR antagonists, and after clinically relevant doses of ionizing radiation. Our results indicate that treatment with NMDAR antagonists slowed the growth and migration of glutamate-releasing LN229 cells, suggesting that activation of NMDARs facilitate tumor expansion. Furthermore, we found that DSB-repair upon radiation was more effective in the presence of glutamate. In contrast, antagonizing the NMDAR or the Ca2+-dependent transcription factor CREB impaired DSB-repair similarly and resulted in a radiosensitizing effect in LN229 and U-87MG cells, indicating a common link between NMDAR signaling and CREB activity in glioblastoma. Since the FDA-approved NMDAR antagonists memantine and ifenprodil showed differential radiosensitizing effects, these compounds may constitute novel optimizations for therapeutic interventions in glioblastoma..Publication link2019 NPPB prevents postoperative peritoneal adhesion formation by blocking volume-activated Cl currentPort-a Patch Publication in Naunyn-Schmiedeberg's Archives of Pharmacology (2019) Authors: Zhong, J., Qin Z., Yu H., Liu X., Li C., Shi J., Mao J., Xu B.5-Nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) is a non-specific chloride channel blocker. Peritoneal adhesion is an inevitable complication of abdominal surgery and remains an important clinical problem, leading to chronic pain, intestinal obstruction, and female infertility. The aim of this study is to observe the effects of NPPB on peritoneal adhesions and uncover the underlying mechanism. The formation of postoperative peritoneal adhesions was induced by mechanical injury to the peritoneum of rats. MTT assay and wound-healing assay were used to evaluate proliferation and migration of primary cultured adhesion fibroblasts (AFB) respectively. Whole-cell chloride currents were measured using a fully automated patch-clamp workstation. Cell volume changes were monitored by light microscopy and video imaging. Our results demonstrated that NPPB could significantly prevent the formation of peritoneal adhesion in rats and inhibit the proliferation of AFB in a concentration-dependent manner. NPPB also reduced the migration of AFB cells with an IC50 of 53.09 M. A 47% hypotonic solution successfully activated the ICl,vol in AFB cells. The current could be blocked by extracellular treatment with NPPB. Moreover, 100 M NPPB almost completely eliminated the capacity of regulatory volume decrease (RVD) in these cells. These data indicate that NPPB could prevent the formation of postoperative peritoneal adhesions. The possible mechanism may be through the inhibition of the proliferation and migration of AFB cells by modulating ICl,vol and cell volume. These results suggest a potential clinical use of NPPB for preventing the formation of peritoneal adhesions.Publication link2019 Mitragynine, an euphoric compound inhibits hERG1a/1b channel current and upregulates the complexation of hERG1a-Hsp90 in HEK293-hERG1a/1b cellsPort-a Patch Publication in Nature Scientific Reports (2019) Authors: Tay Y.L., Amanah A., Adenan M.I., Wahab H.A., Tan M.L.Mitragyna speciosaKorth (M. speciosa) has been widely used as a recreational product, however, there are growing concerns on the abuse potentials and toxicity of the plant. Several poisoning and fatal cases involving kratom and mitragynine have been reported but the underlying causes remain unclear. The human ether-a-go-go-related gene 1 (hERG1) encodes the pore-forming subunit underlying cardiac rapidly delayed rectifier potassium current (IKr). Pharmacological blockade of the IKr can cause acquired long QT syndrome, leading to lethal cardiac arrhythmias. This study aims to elucidate the mechanisms of mitragynine-induced inhibition on hERG1a/1b current. Electrophysiology experiments were carried out using Port-a-Patch system. Quantitative RT-PCR, Western blot analysis, immunofluorescence and co-immunoprecipitation methods were used to determine the effects of mitragynine on hERG1a/1b expression and hERG1-cytosolic chaperones interaction. Mitragynine was found to inhibit the IKr current with an IC50 value of 332.70nM. It causes a significant reduction of the fully-glycosylated (fg) hERG1a protein expression but upregulates both core-glycosylated (cg) expression and hERG1a-Hsp90 complexes, suggesting possible impaired hERG1a trafficking. In conclusion, mitragynine inhibits hERG1a/1b current through direct channel blockade at lower concentration, but at higher concentration, it upregulates the complexation of hERG1a-Hsp90 which may be inhibitory towards channel trafficking.Publication link2019 Modulation of the potassium channel KcsA by anionic phospholipids: Role of arginines at the non-annular lipid binding sitesPort-a Patch Publication in BBA - Biomembranes (2019) Authors: Poveda J.A., Giudici A.M., Renart M.L., Millet O., Morales A., Gonzlez-Ros J.M., Oakes V., Furini S., Domene CThe role of arginines R64 and R89 at non-annular lipid binding sites of KcsA, on the modulation of channel activity by anionic lipids has been investigated. In wild-type (WT) KcsA reconstituted into asolectin lipid membranes, addition of phosphatidic acid (PA) drastically reduces inactivation in macroscopic current recordings. Consistent to this, PA increases current amplitude, mean open time and open probability at the single channel level. Moreover, kinetic analysis reveals that addition of PA causes longer open channel lifetimes and decreased closing rate constants. Effects akin to those of PA on WT-KcsA are observed when R64 and/or R89 are mutated to alanine, regardless of the added anionic lipids. We interpret these results as a consequence of interactions between the arginines and the anionic PA bound to the non-annular sites. NMR data shows indeed that at least R64 is involved in binding PA. Moreover, molecular dynamics (MD) simulations predict that R64, R89 and surrounding residues such as T61, mediate persistent binding of PA to the non-annular sites. Channel inactivation depends on interactions within the inactivation triad (E71-D80-W67) behind the selectivity filter. Therefore, it is expected that such interactions are affected when PA binds the arginines at the non-annular sites. In support of this, MD simulations reveal that PA binding prevents interaction between R89 and D80, which seems critical to the effectiveness of the inactivation triad. This mechanism depends on the stability of the bound lipid, favoring anionic headgroups such as that of PA, which thrive on the positive charge of the arginines.Publication link2019 Inhibition of Polyamine Biosynthesis Reverses Ca2+ Channel Remodeling in Colon Cancer CellsPort-a-Patch Publication in Cancers (2019) Authors: Gutirrez L.G., Hernndez-Morales M., Nez L., Villalobos C.Store-operated Ca2+entry (SOCE) is the most important Ca2+entry pathway in non-excitable cells. Colorectal cancer (CRC) shows decreased Ca2+store content and enhanced SOCE that correlate with cancer hallmarks and are associated to remodeling of store-operated channels (SOCs). Normal colonic cells display small, Ca2+-selective currents driven by Orai1 channels. In contrast, CRC cells display larger, non-selective currents driven by Orai1 and transient receptor potential canonical type 1 channels (TRPC1). Difluoromethylornithine (DFMO), a suicide inhibitor of ornithine decarboxylase (ODC), the limiting step in polyamine biosynthesis, strongly prevents CRC, particularly when combined with sulindac. We asked whether DFMO may reverse SOC remodeling in CRC. We found that CRC cells overexpress ODC and treatment with DFMO decreases cancer hallmarks including enhanced cell proliferation and apoptosis resistance. Consistently, DFMO enhances Ca2+store content and decreases SOCE in CRC cells. Moreover, DFMO abolish selectively the TRPC1-dependent component of SOCs characteristic of CRC cells and this effect is reversed by the polyamine putrescine. Combination of DFMO and sulindac inhibit both SOC components and abolish SOCE in CRC cells. Finally, DFMO treatment inhibits expression of TRPC1 and stromal interaction protein 1 (STIM1) in CRC cells. These results suggest that polyamines contribute to Ca2+channel remodeling in CRC, and DFMO may prevent CRC by reversing channel remodelingPublication link2019 KCa1.1 and KV1.3 channels regulate the interactions between fibroblast-like synoviocytes and T lymphocytes during rheumatoid arthritisPort-a-Patch Publication in Arthritis Research & Therapy (2019) Authors: Tanner M.R.,Pennington M.W.,Chauhan S.S.,Laragione T.,Gulko P.S.,Beeton C.Background: Fibroblast-like synoviocytes (FLS) and CCR7-effector memory T (TEM) cells are two of the major cell types implicated in the progression of rheumatoid arthritis (RA). In particular, FLS become highly invasive, whereas TEMcells proliferate and secrete proinflammatory cytokines, during RA. FLS and T cells may also interact and influence each other's phenotypes. Inhibition of the pathogenic phenotypes of both FLS and TEMcells can be accomplished by selectively blocking the predominant potassium channels that they upregulate during RA: KCa1.1 (BK, Slo1, MaxiK, KCNMA1) upregulated by FLS and KV1.3 (KCNA3) upregulated by activated TEMcells. In this study, we investigated the roles of KCa1.1 and KV1.3 in regulating the interactions between FLS and TEMcells and determined if combination therapies of KCa1.1- and KV1.3-selective blockers are more efficacious than monotherapies in ameliorating disease in rat models of RA.Methods: We used in vitro functional assays to assess the effects of selective KCa1.1 and KV1.3 channel inhibitors on the interactions of FLS isolated from rats with collagen-induced arthritis (CIA) with syngeneic TEMcells. We also used flow cytometric analyses to determine the effects of KCa1.1 blockers on the expression of proteins used for antigen presentation on CIA-FLS. Finally, we used the CIA and pristane-induced arthritis models to determine the efficacy of combinatorial therapies of KCa1.1 and KV1.3 blockers in reducing disease severity compared with monotherapies. Results: We show that the interactions of FLS from rats with CIA and of rat TEM cells are regulated by KCa1.1 and KV1.3. Inhibiting KCa1.1 on FLS reduces the ability of FLS to stimulate TEM cell proliferation and migration, and inhibiting KV1.3 on TEM cells reduces TEM cells' ability to enhance FLS expression of KCa1.1 and major histocompatibility complex class II protein, as well as stimulates their invasion. Furthermore, we show that combination therapies of selective KCa1.1 and KV1.3 blockers are more efficacious than monotherapies at reducing signs of disease in two rat models of RA.Conclusions: Our results demonstrate the importance of KCa1.1 and KV1.3 in regulating FLS and TEMcells during RA, as well as the value of combined therapies targeting both of these cell types to treat RA.Publication link2019 Fenamates inhibit human sodium channel NaV1.7 and NaV1.8Port-a-Patch Publication in Neuroscience Letters (2019) Authors: Sun, J-F., Xu, Y-J., Kong, X-H., Su, Y., Wang, Z-Y.Fenamates are N-substituted anthranilic acid derivatives, clinically used as nonsteroidal anti-inflammatory drugs (NSAIDs) in fever, pain and inflammation treatments. Previous studies have shown that they are also modulators of diverse ion channels, exhibiting either activation or inhibitory effects. However, the effects of fenamates on sodium channel subtypes are still unknown. In this study, fenamates, including mefenamic acid, flufenamic acid and tolfenamic acid, were examined by whole-cell patch clamp techniques on the sodium channels hNaV1.7 and hNaV1.8, which are closely associated with pain. The results showed that the mefenamic acid, flufenamic acid, and tolfenamic acid inhibited the peak currents of hNaV1.7 and hNaV1.8 in CHO cells stably expressing hNaV1.7 and hNaV1.8. However, much lighter inhibition effects of hNaV1.8 were registered in the experimental system. Furthermore, the mefenamic acid, flufenamic acid and tolfenamic acid significantly affected the inactivation processes of hNaV1.7 and hNaV1.8 with I-V curves left-shifted to hyperpolarized direction. These data indicate that the inhibition effects of NaV1.7 and NaV1.8 by mefenamic acid, flufenamic acid and tolfenamic acid might contribute to their analgesic activity in addition to their inhibition of cyclooxygenase. These findings provide a basis for further studies in the discovery of other potential targets for NSAIDs.Publication link2019 High-resolution experimental and computational electrophysiology reveals weak -lactam binding events in the porin PorBPort-a-PatchPublication in Nature Scientific Reports (2019) Authors: Bartsch A., Llabrs S., Pein F., Kattner C., Schn M., Diehn M., Tanabe M., Munk A., Zachariae U., Steinem C.The permeation of most antibiotics through the outer membrane of Gram-negative bacteria occurs through porin channels. To design drugs with increased activity against Gram-negative bacteria in the face of the antibiotic resistance crisis, the strict constraints on the physicochemical properties of the permeants imposed by these channels must be better understood. Here we show that a combination of high-resolution electrophysiology, new noise-filtering analysis protocols and atomistic biomolecular simulations reveals weak binding events between the beta-lactam antibiotic ampicillin and the porin PorB from the pathogenic bacterium Neisseria meningitidis. In particular, an asymmetry often seen in the electrophysiological characteristics of ligand-bound channels is utilised to characterise the binding site and molecular interactions in detail, based on the principles of electro-osmotic flow through the channel. Our results provide a rationale for the determinants that govern the binding and permeation of zwitterionic antibiotics in anion-selective porin channels.Publication link2019 Breaching the Barrier: Quantifying Antibiotic Permeability across Gram-Negative Bacterial MembranesPort-a Patch Publication in Journal of Molecular Biology (2019) Authors: Cama J., MaeHenney A., Winterhalter M.The double membrane cell envelope of Gram negative bacteria is a sophisticated barrier that facilitates the uptake of nutrients and protects the organism from toxic compounds. An antibiotic molecule must find its way through the negatively charged lipopolysaccharide layer on the outer surface, pass through either a porin or the hydrophobic layer of the outer membrane, then traverse the hydrophilic peptidoglycan layer only to find another hydrophobic lipid bilayer before it finally enters the cytoplasm, where it typically finds its target. This complex uptake pathway with very different physico-chemical properties is one reason that Gram-negatives are intrinsically protected against multiple classes of antibiotic-like molecules, and is likely the main reason that in vitro target based screening programmes have failed to deliver novel antibiotics for these organisms. Due to the lack of general methods available for quantifying the flux of drugs into the cell, little is known about permeation rates, transport pathways and accumulation at the target sites for particular molecules. Here we summarise the current tools available for measuring antibiotic uptake across the different compartments of Gram-negative bacteria.Publication link2019 Control of Lysosomal TRPML1 Channel Activity and Exosome Release by Acid Ceramidase in Mouse PodocytesPort-a Patch Publication in American Journal of Physiology Cell Physiology (2019) Authors: Li G., Huang D., Hong J., Bhat O.M., Yuan X., Li P.L.The transient receptor potential mucolipin 1 (TRPML1) channel has been reported to mediate lysosomal Ca2+release that is involved in Ca2+-dependent lysosome trafficking and autophagic flux. However, this regulatory mechanism of lysosomal TRPML1 channel activity in podocytes remains poorly understood. In the present study, we tested whether TRPML1 channel in podocytes mediates lysosome trafficking which is essential for multivesicular body (MVB) degradation by lysosomes. We first demonstrated the abundant expression of TRPML1 channel in podocytes. By GCaMP3 Ca2+imaging, we characterized the lysosomal specificity of TRPML1 channel-mediated Ca2+release in podocytes. Given the important role of acid ceramidase (AC) in lysosome function and podocyte injury, we tested whether AC regulates this TRPML1 channel-mediated Ca2+release and consequent lysosome-dependent MVB degradation in podocytes. Pharmacologically, it was found that TRPML1 channel activity was remarkably attenuated by AC inhibitor, carmofur. Sphingosine, as an AC product, was demonstrated to induce TRPML1-mediated Ca2+release, which was inhibited by a TRPML1 blocker, verapamil. Using a Port-a-Patch planar patch-clamp system, we found that AC-associated sphingolipids, sphingomyelin, ceramide, and sphingosine had different effects on TRPML1 channel activity in podocytes. Functionally, the inhibition of AC or blockade of TRPML1 channels was found to suppress the interaction of lysosomes and MVBs, leading to increased exosome release from podocytes. These results suggest that AC is critical for TRPML1 channel-mediated Ca2+ release, which controls lysosome-MVB interaction and exosome release in podocytes.Publication link2019 Abolishing cAMP sensitivity in HCN2 pacemaker channels induces generalized seizuresPort-a Patch and Internal Perfusion Publication in JCI Insight (2019) Authors: Hammelmann, V., Stieglitz, M.S., Hlle, H., Le Meur, K., Kass, J., Brmmer, M., Gruner, C., Rtzer, R.D., Fenske, S., Hartmann, J., Zott, B., Lthi, A., Spahn, S., Moser, M., Isbrandt, D., Ludwig, A., Konnerth, A., Wahl-Schott, C., Biel M.Hyperpolarization-activated cyclic nucleotidegated (HCN) channels are dually gated channels that are operated by voltage and by neurotransmitters via the cAMP system. cAMP-dependent HCN regulation has been proposed to play a key role in regulating circuit behavior in the thalamus. By analyzing a knockin mouse model (HCN2EA), in which binding of cAMP to HCN2 was abolished by 2 amino acid exchanges (R591E, T592A), we found that cAMP gating of HCN2 is essential for regulating the transition between the burst and tonic modes of firing in thalamic dorsal-lateral geniculate (dLGN) and ventrobasal (VB) nuclei. HCN2EA mice display impaired visual learning, generalized seizures of thalamic origin, and altered NREM sleep properties. VB-specific deletion of HCN2, but not of HCN4, also induced these generalized seizures of the absence type, corroborating a key role of HCN2 in this particular nucleus for controlling consciousness. Together, our data define distinct pathological phenotypes resulting from the loss of cAMP-mediated gating of a neuronal HCN channel.Publication link2019 Action of MK7264 (Gefapixant) at human P2X3 and P2X2/3 receptors and in vivo efficacy in models of sensitisationPort-a-Patch Publication in British Journal of Pharmacology (2019) Authors: Richards D., Gever J.R., Ford A.P., Fountain S.J.Background & Purpose:The P2X3 receptor is an ATPgated ion channel expressed by sensory afferent neurons, and is as a target to treat chronic sensitisation conditions. The firstinclass, selective P2X3 and P2X2/3 receptor antagonist, the diaminopyrimidine MK7264 (Gefapixant), has progressed to Phase III trials for refractory or unexplained chronic cough. We have used patchclamp to elucidate the pharmacology and kinetics of MK7264 and rat models of hypersensitivity and hyperalgesia to test efficacy in these conditions.Experimental Approach:Wholecell patchclamp of 1321N1 cells expressing human P2X3 and P2X2/3 receptors was used to determine mode of MK7264 action, potency and kinetics. The analgesic efficacy was assessed using paw withdrawal threshold and limb weight distribution in rat models of inflammatory, osteoarthritic and neuropathic sensitisation.Key Results:MK7264 is a reversible allosteric antagonist at human P2X3 and P2X2/3 receptors with IC50 values of 153 and 220nM, respectively. Experiments with the slowly desensitising P2X2/3 heteromer revealed concentration and statedependency to washon, with faster rates and greater inhibition when applied before agonist compared to during agonist application. Washon rate (value) for MK7264 at maximal concentrations was 19s and 146s when applied before and during agonist application, respectively. In vivo, MK7264 (30 mg/kg) displayed efficacy comparable to naproxen (20 mg/kg) in inflammatory and osteoarthritic sensitisation models, and gabapentin (100 mg/kg) in neuropathic sensitisation models, increasing paw withdrawal threshold and decreasing weight bearing discomfort.Conclusions and Implications:MK7264 is a reversible and selective P2X3 and P2X2/3 antagonist, exerting allosteric antagonism via preferential activity at closed channels. Efficacy in rat models supports clinical investigation of chronic sensitisation conditions.Publication link2018 TRPM8 Activation via 3-Iodothyronamine Blunts VEGF-Induced Transactivation of TRPV1 in Human Uveal Melanoma CellsPort-a-Patch Publication in Frontiers in Pharmacology (2018) Authors: Walcher, L., Budde, C., Bhm, A., Re

Gehrmann E.J., Rodarte K.E., Atik M.A., Norton R.S., Pennington R.W., Beeton C.Effector memory T lymphocytes (TEMcells) that lack expression of CCR7 are major drivers of inflammation in a number of autoimmune diseases, including multiple sclerosis and rheumatoid arthritis. The KV1.3 potassium channel is a key regulator of CCR7TEMcell activation. Blocking KV1.3 inhibits TEMcell activation and attenuates inflammation in autoimmunity, and as such, KV1.3 has emerged as a promising target for the treatment of TEMcell-mediated autoimmune diseases. The scorpion venom-derived peptide HsTX1 and its analog HsTX1[R14A] are potent KV1.3 blockers and HsTX1[R14A] is selective for KV1.3 over closely-related KV1 channels. PEGylation of HsTX1[R14A] to create a KV1.3 blocker with a long circulating half-life reduced its affinity but not its selectivity for KV1.3, dramatically reduced its adsorption to inert surfaces, and enhanced its circulating half-life in rats. PEG-HsTX1[R14A] is equipotent to HsTX1[R14A] in preferential inhibition of human and rat CCR7TEMcell proliferation, leaving CCR7+nave and central memory T cells able to proliferate. It reduced inflammation in an active delayed-type hypersensitivity model and in the pristane-induced arthritis (PIA) model of rheumatoid arthritis (RA). Importantly, a single subcutaneous dose of PEG-HsTX1[R14A] reduced inflammation in PIA for a longer period of time than the non-PEGylated HsTX1[R14A]. Together, these data indicate that HsTX1[R14A] and PEG-HsTX1[R14A] are effective in a model of RA and are therefore potential therapeutics for TEMcell-mediated autoimmune diseases. PEG-HsTX1[R14A] has the additional advantages of reduced non-specific adsorption to inert surfaces and enhanced circulating half-life.Publication link2017 Cytotoxic effect of zinc oxide nanoparticles on murine photoreceptor cells via potassium channel block and Na+/K+ATPase inhibitionPort-a-PatchPublication inCell Proliferation (2017) Authors: Chen C. Bu W., Ding H., Li Q., Wang D., Bi H., Guo D.Objective: Zinc oxide (ZnO) nanoparticles can exhibit toxicity towards organisms and oxidative stress is often hypothesized to be one of the most important factors. Nevertheless, the detailed mechanism of toxicityinduced by ZnO nanoparticles has not been completely addressed. The present study aimed to investigate the toxic effects of ZnO nanoparticles on the expression and activity of Na+/K+ATPase and on potassium channel block. Materials and methods: In the present study, we explored the cytotoxic effect of ZnO nanoparticles on murine photoreceptor cells using lactate dehydrogenase (LDH) release assay, reactive oxygen species (ROS) determination, mitochondrial membrane potential (m) measurement, delayed rectifier potassium current recordings and Na+/K+ATPase expression and activity monitoring. Results: The results indicated that ZnO nanoparticles could increase the LDH release in medium, aggravate the ROS level within cells, collapse the m, block the delayed rectifier potassium current, and attenuate the expressions of Na+/K+ATPase at both mRNA and protein levels and its activity, and thus exert cytotoxic effects on murine photoreceptor cells, finally damaging target cells. Conclusion: Our findings will facilitate the understanding of the mechanism involved in ZnO nanoparticleinduced cytotoxicity in murine photoreceptor cells via potassium channel block and Na+/K+ATPase inhibition.Publication link2017 Mitochondria sustain store-operated currents in colon cancer cells but not in normal colonic cells: reversal by non-steroidal anti-inflammatory drugsPort-a-Patch Publication in Oncotarget (2017) Authors: Hernndez-Morales M., Sobradillo D., Valero R.A., Muoz E., Ubierna D., Moyer M.P., Nez L., Villalobos C.Tumor cells undergo a critical remodeling of intracellular Ca2+homeostasis that contribute to important cancer hallmarks. Store-operated Ca2+entry (SOCE), a Ca2+entry pathway modulated by mitochondria, is dramatically enhanced in colon cancer cells. In addition, most cancer cells display the Warburg effect, a metabolic switch from mitochondrial metabolism to glycolysis that provides survival advantages. Accordingly, we investigated mitochondria control of store-operated currents (SOCs) in two cell lines previously selected for representing human normal colonic cells and colon cancer cells. We found that, in normal cells, mitochondria are important for SOCs activity but they are unable to prevent current inactivation. In contrast, in colon cancer cells, mitochondria are dispensable for SOCs activation but are able to prevent the slow, Ca2+-dependent inactivation of SOCs. This effect is associated to increased ability of tumor cell mitochondria to take up Ca2+due to increased mitochondrial potential () linked to the Warburg effect. Consistently with this view, selected non-steroidal anti-inflammatory drugs (NSAIDs) depolarize mitochondria, inhibit mitochondrial Ca2+uptake and promote SOC inactivation, leading to inhibition of both SOCE and cancer cell proliferation. Thus, mitochondria sustain store-operated currents in colon cancer cells but not in normal colonic cells and this effect is counteracted by selected NSAIDs providing a mechanism for cancer chemoprevention.Publication link2017 A mutant of the Buthus martensii Karsch antitumor-analgesic peptide exhibits reduced inhibition to hNaV1.4 and hNaV1.5 channels while retaining analgesic activityPort-a-PatchPublication in Journal of Biological Chemistry (2017) Authors: Xu Y., Meng X., Hou X., Sun J., Kong X., Sun Y., Liu Z., Ma Y., Niu Y., Song Y., Cui Y., Zhao M., Zhang J.Scorpion toxins can kill other animals by inducing paralysis and arrhythmia, which limits the potential applications of these agents in the clinical management of diseases. Antitumor-analgesic peptide (AGAP), purified from Buthus martensii Karsch, has been proved to possess analgesic and antitumor activities. Trp38, a conserved aromatic residue of AGAP, might play an important role in mediating AGAP activities according to the sequence and homology-modeling analyses. Therefore, an AGAP mutant, W38G, was generated, and effects of both AGAP and the mutant W38G were examined by whole-cell patch clamp techniques on the sodium channels hNaV1.4 and hNaV1.5, which were closely associated with the biotoxicity of skeletal and cardiac muscles, respectively. The data showed that both W38G and AGAP inhibited the peak currents of hNaV1.4 and hNaV1.5; however, W38G induced a much weaker inhibition of both channels than AGAP. Accordingly, W38G exhibited much less toxic effect on both skeletal and cardiac muscles than AGAP in vivo. The analgesic activity of W38G and AGAP were verified in vivo as well, and W38G retained analgesic activity similar to AGAP. Inhibition to both NaV1.7 and NaV1.8 was involved in the analgesic mechanism of AGAP and W38G. These findings indicated that Trp38 was a key amino acid involved in the biotoxicity of AGAP, and the AGAP mutant W38G might be a safer alternative for clinical application because it retains the analgesic efficacy with less toxicity to skeletal and cardiac muscles.Publication link2017 Buserelin alleviates chloride transport defect in human cystic fibrosis nasal epithelial cellsPort-a-PatchPublication in PLoS ONE (2017) Authors: Calvez M.L., Benz N., Huguet F., Saint-Pierre A., Rouill E., Coraux C., Frec C., Kerbiriou M., Trouv P.Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride (Cl-) channel regulated by protein kinases, phosphatases, divalent cations and by protein-protein interactions. Among protein-protein interactions, we previously showed that Annexin A5 (AnxA5) binds to CFTR and is involved in the channel localization within membranes and in its Cl- channel function. The deletion of phenylalanine at position 508 (F508del) is the most common mutation in CF which leads to an altered protein (F508del-CFTR) folding with a nascent protein retained within the ER and is quickly degraded. We previously showed that AnxA5 binds to F508del-CFTR and that its increased expression due to a Gonadoliberin (GnRH) augments Cl- efflux in cells expressing F508del-CFTR. The aim of the present work was to use the GnRH analog buserelin which is already used in medicine. Human nasal epithelial cells from controls and CF patients (F508del/F508del) were treated with buserelin and we show here that the treatment alleviates Cl- channel defects in CF cells. Using proteomics we highlighted some proteins explaining this result. Finally, we propose that buserelin is a potential new pharmaceutical compound that can be used in CF and that bronchus can be targeted since we show here that they express GnRH-R.Publication link2016 Upregulation of Transient Receptor Potential Vanilloid Type-1 Channel Activity and Ca2+ Influx Dysfunction in Human Pterygial CellsPort-a-Patch Publication in Investigative Opthalmology & Visual Science (2016) Authors: Garreis F., Schrder A., Reinach P.S., Zoll S., Khajavi N., Dhandapani P., Lucius A., Pleyer U., Paulsen F., Mergler S.Purpose:The heat-sensitive transient receptor potential vanilloid type-1 (TRPV1) channel (i.e., capsaicin [CAP] receptor) is upregulated in numerous cancers. This study determined if this response occurs in fresh and cultured hyperplastic human pterygial epithelial tissues.Methods:Reverse transcriptase PCR and quantitative real-time PCR, along with immunohistochemistry and Western blotting, characterized TRPV1 expression patterns in pterygial and healthy conjunctival tissue, primary and immortalized pterygial cells (hPtEC), and primary and immortalized conjunctival epithelial cells (HCjEC). Imaging of Ca2+and planar whole-cell patch-clamping evaluated TRP channel activity. An MTS assay measured cell metabolic activity and a cell growth assay monitored proliferation.Results:Capsaicin (20 M) and elevating bath temperature above 43C activated Ca2+transients more in hPtEC than HCjEC. Capsaicin induced corresponding changes in inward currents that were inhibited by 20 M capsazepine (CPZ). Vascular endothelial growth factor (VEGF) also increased Ca2+-influx and induced corresponding inward currents more in hPtEC than in HCjEC, whereas CPZ (20 M), BCTC (20 M), or La3+(500 M) reduced these responses, respectively. Whereas epidermal growth factor (EGF) increased proliferation more in hPtEC than in HCjEC, VEGF had no effect on this response. Capsazepine suppressed hPtEC proliferation induced by EGF and VEGF, whereas it was cytotoxic to HCjEC.Conclusions:Mitogenic responses to EGF and VEGF are mediated through TRPV1 transactivation. Only in hPtEC do the increases in proliferation induced by EGF exceed those in HCjEC. Therefore, TRPV1 is a potential drug target whose clinical relevance in treating pterygium warrants further assessment.Publication link2016 Voltage Dependence of Conformational Dynamics and Subconducting States of VDAC-1Port-a-Patch Publication in Biophysical Journal (2016) Authors: Briones R., Weichbrodt C., Paltrinieri L., Mey I., Villinger S., Giller K., Lange A., Zweckstetter M., Griesinger C., Becker S., Steinem C., de Groot B.L.The voltage-dependent anion channel 1 (VDAC-1) is an important protein of the outer mitochondrial membrane that transports energy metabolites and is involved in apoptosis. The available structures of VDAC proteins show a wide -stranded barrel pore, with its N-terminal -helix (N-) bound to its interior. Electrophysiology experiments revealed that voltage, its polarity, and membrane composition modulate VDAC currents. Experiments with VDAC-1 mutants identified amino acids that regulate the gating process. However, the mechanisms for how these factors regulate VDAC-1, and which changes they trigger in the channel, are still unknown. In this study, molecular dynamics simulations and single-channel experiments of VDAC-1 show agreement for the current-voltage relationships of an open channel and they also show several subconducting transient states that are more cation selective in the simulations. We observed voltage-dependent asymmetric distortions of the VDAC-1 barrel and the displacement of particular charged amino acids. We constructed conformational models of the protein voltage response and the pore changes that consistently explain the protein conformations observed at opposite voltage polarities, either in phosphatidylethanolamine or phosphatidylcholine membranes. The submicrosecond VDAC-1 voltage response shows intrinsic structural changes that explain the role of key gating amino acids and support some of the current gating hypotheses. These voltage-dependent protein changes include asymmetric barrel distortion, its interaction with the membrane, and significant displacement of N- amino acids.Publication link2016 Distinct regions that control ion selectivity and calcium-dependent activation in the bestrophin ion channelPort-a-Patch Publication in Proceedings of the National Academy of Sciences of the United States of America (2016) Authors: Vaisey G., Miller A.N., Long S.B.Abstract:Cytoplasmic calcium (Ca2+) activates the bestrophin anion channel, allowing chloride ions to flow down their electrochemical gradient. Mutations in bestrophin 1 (BEST1) cause macular degenerative disorders. Previously, we determined an X-ray structure of chicken BEST1 that revealed the architecture of the channel. Here, we present electrophysiological studies of purified wild-type and mutant BEST1 channels and an X-ray structure of a Ca2+-independent mutant. From these experiments, we identify regions of BEST1 responsible for Ca2+activation and ion selectivity. A Ca2+clasp within the channels intracellular region acts as a sensor of cytoplasmic Ca2+. Alanine substitutions within a hydrophobic neck of the pore, which widen it, cause the channel to be constitutively active, irrespective of Ca2+. We conclude that the primary function of the neck is as a gate that controls chloride permeation in a Ca2+-dependent manner. In contrast to what others have proposed, we find that the neck is not a major contributor to the channels ion selectivity. We find that mutation of a cytosolic aperture of the pore does not perturb the Ca2+dependence of the channel or its preference for anions over cations, but its mutation dramatically alters relative permeabilities among anions. The data suggest that the aperture functions as a size-selective filter that permits the passage of small entities such as partially dehydrated chloride ions while excluding larger molecules such as amino acids. Thus, unlike ion channels that have a single selectivity filter, in bestrophin, distinct regions of the pore govern anion-vs.-cation selectivity and the relative permeabilities among anions.Significance:BEST1 is a Ca2+-activated chloride channel found in a variety of cell types that allows chloride to traverse the plasma membrane. Mutations in BEST1 can cause macular degeneration. The mechanisms for anion selectivity and Ca2+-dependent activation of BEST1 are unknown. Here, we show that a hydrophobic neck region of the channels pore does not play a major role in ion selectivity but acts as an effective gate, responding to Ca2+binding at a cytosolic sensor. Mutation of a cytosolic aperture dramatically affects relative permeabilities among anions. These insights help rationalize how disease-causing mutations in BEST1 affect channel behavior and contribute to a broader understanding of ion channel gating and selectivity mechanisms.Publication link2016 Reversible control of current across lipid membranes by local heatingPort-a-Patch Publication in Scientific Reports (2016) Authors: Urban P., Kirchner S.R., Mhlbauer C., Lohmller T., Feldmann J.Lipid membranes are almost impermeable for charged molecules and ions that can pass the membrane barrier only with the help of specialized transport proteins. Here, we report how temperature manipulation at the nanoscale can be employed to reversibly control the electrical resistance and the amount of current that flows through a bilayer membrane with pA resolution. For this experiment, heating is achieved by irradiating gold nanoparticles that are attached to the bilayer membrane with laser light at their plasmon resonance frequency. We found that controlling the temperature on the nanoscale renders it possible to reproducibly regulate the current across a phospholipid membrane and the membrane of living cells in absence of any ion channels.Publication link2015 The Functional Property Changes of Muscular NaV1.4 and Cardiac NaV1.5 Induced by Scorpion Toxin BmK AGP-SYPU1 Mutants Y42F and Y5FPort-a-Patch Publication in Biochemistry (2015) Authors: Meng x., Xu Y., Zhao M., Wang F., Ma Y., Jin Y., Liu Y., Song Y., Zhang J.Scorpion toxins are invaluable therapeutic leads and pharmacological tools which influence the voltage-gated sodium channels. However, the details were still unclear about the structurefunction relationship of scorpion toxins on VGSC subtypes. In the previous study, we reported one -type scorpion toxin Bmk AGP-SYPU1 and its two mutants (Y5F and Y42F) which had been demonstrated to ease pain in mice acetic acid writhing test. However, the function of Bmk AGP-SYPU1 on VGSCs is still unknown. In this study, we examined the effects of BmK AGP-SYPU1 and its two mutants (Y5F and Y42F) on hNaV1.4 and hNaV1.5 heterologously expressed CHO cell lines by using Na+-specialized fluorescent dye and whole-cell patch clamp. The data showed that BmK AGP-SYPU1 displayed as an activator of hNaV1.4 and hNaV1.5, which might indeed contribute to its biotoxicity to muscular and cardiac system and exhibited the functional properties of both the -type and -type scorpion toxin. Notably, Y5F mutant exhibited lower activatory effects on hNaV1.4 and hNaV1.5 compared with BmK AGP-SYPU1. Y42F was an enhanced activator and confirmed that the conserved Tyr42 was the key amino acid involved in bioactivity or biotoxicity. These data provided a deep insight into the structurefunction relationship of BmK AGP-SYPU1, which may be the guidance for engineering -toxin with high selectivity on VGSC subtypes.Publication link2016 Defined Medium Conditions for the Induction and Expansion of Human Pluripotent Stem Cell-Derived Retinal Pigment EpitheliumPort-a-Patch Publication in Stem Cell Reviews and Reports (2016) Authors: Lidgerwood G.E., Lim S.Y., Crombie D.E., Ali R., Gill K.P., Hernndez D., Kie J., Conquest A., Waugh H.S., Wong R.C., Liang H.H., Hewitt A.W., Davidson K.C., Pbay A.We demonstrate that a combination of Noggin, Dickkopf-1, Insulin Growth Factor 1 and basic Fibroblast Growth Factor, promotes the differentiation of human pluripotent stem cells into retinal pigment epithelium (RPE) cells. We describe an efficient one-step approach that allows the generation of RPE cells from both human embryonic stem cells and human induced pluripotent stem cells within 4060days without the need for manual excision, floating aggregates or imbedded cysts. Compared to methods that rely on spontaneous differentiation, our protocol results in faster differentiation into RPE cells. This pro-retinal culture medium promotes the growth of functional RPE cells that exhibit key characteristics of the RPE including pigmentation, polygonal morphology, expression of mature RPE markers, electrophysiological membrane potential and the ability to phagocytose photoreceptor outer segments. This protocol can be adapted for feeder, feeder-free and serum-free conditions. This method thereby provides a rapid and simplified production of RPE cells for downstream applications such as disease modelling and drug screening.Publication link2015 K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with ProzacPort-a-Patch Publication in Science (2015) Authors: Dong Y.Y., Pike A.C., Mackenzie A., McClenaghan C., Aryal P., Dong L., Quigley A., Grieben M., Goubin S., Mukhopadhyay S., Ruda G.F., Clausen M.V., Cao L., Brennan P.E., Burgess-Brown N.A., Sansom M.S., Tucker S.J., Carpenter E.P.TREK-2 (KCNK10/K2P10), a two-pore domain potassium (K2P) channel, is gated by multiple stimuli such as stretch, fatty acids, and pH and by several drugs. However, the mechanisms that control channel gating are unclear. Here we present crystal structures of the human TREK-2 channel (up to 3.4 angstrom resolution) in two conformations and in complex with norfluoxetine, the active metabolite of fluoxetine (Prozac) and a state-dependent blocker of TREK channels. Norfluoxetine binds within intramembrane fenestrations found in only one of these two conformations. Channel activation by arachidonic acid and mechanical stretch involves conversion between these states through movement of the pore-lining helices. These results provide an explanation for TREK channel mechanosensitivity, regulation by diverse stimuli, and possible off-target effects of the serotonin reuptake inhibitor ProzacPublication link2015 The application of the Escherichia coli giant spheroplast for drug screening with automated planar patch clamp systemPort-a-Patch Publication in Biotechnology Reports (2015) Authors: Kikuchi K., Sugiura M., Nishizawa-Harada C., Kimura T.KV2.1, the voltage-gated ion channel, is ubiquitously expressed in variety of tissues and dysfunction of this ion channel is responsible for multiple diseases. Electrophysiological properties of ion channels are so far characterized with eukaryotic cells using the manual patch clamp which requires skilful operators and expensive equipments. In this research, we created a simple and sensitive drug screen method using bacterial giant spheroplasts and the automated patch clamp which does not require special skills. We expressed a eukaryotic voltage-gated ion channel KV2.1 in Escherichia coli using prokaryotic codon, and prepared giant spheroplasts large enough for the patch clamp. Human KV2.1 currents were successfully recorded from giant spheroplasts with the automated system, and KV2.1-expressed E. coli spheroplasts could steadily reacted to the doseresponse assay with TEA and 4-AP. Collectively, our results indicate for the first time that the bacterial giant spheroplast can be applied for practical pharmaceutical assay using the automated patch clamp.Publication link2014 L-Carnitine Reduces in Human Conjunctival Epithelial Cells Hypertonic- Induced Shrinkage through Interacting with TRPV1 ChannelsPort-a-Patch Publication in Cellular Physiology and Biochemistry (2014) Authors: Khajavi N., Reinach P.S., Skrzypski M., LudeA., Mergler S.Background/Aims: Ocular surface health depends on conjunctival epithelial (HCjE) layer integrity since it protects against pathogenic infiltration and contributes to tissue hydration maintenance. As the same increases in tear film hyperosmolarity described in dry eye disease can increase corneal epithelial transient receptor potential vanilloid type-1 (TRPV1) channel activity, we evaluated its involvement in mediating an osmoprotective effect by L-carnitine against such stress. Methods: Using siRNA gene silencing, Ca2+imaging, planar patch-clamping and relative cell volume measurements, we determined if the protective effects of this osmolyte stem from its interaction with TRPV1. Results: TRPV1 activation by capsaicin (CAP) and an increase in osmolarity to 450 mOsM both induced increases in Ca2+levels. In contrast, blocking TRPV1 activation with capsazepine (CPZ) fully reversed this response. Similarly, L-carnitine (1 mM) also reduced underlying whole-cell currents. In calcein-AM loaded cells, hypertonic-induced relative cell volume shrinkage was fully blocked during exposure to L-carnitine. On the other hand, in TRPV1 gene-silenced cells, this protective effect by L-carnitine was obviated.Conclusion:The described L-carnitine osmoprotective effect is elicited through suppression of hypertonic-induced TRPV1 activation leading to increases in L-carnitine uptake through a described Na+-dependent L-carnitine transporter.Publication link2014 Ultra-stable glass microcraters for on-chip patch clampingPort-a-Patch Publication in Joyal Society of Chemistry Advances (2014) Authors: Stava E., Shin H.C.,Yu M., Bhat A., Resto P.J., Seshadri A., Williams J.C., Blick R.H.We report on a single-step fabrication procedure of borosilicate glass micropores surrounded by a smooth microcrater. By inserting a thin air-gap between a borosilicate glass substrate and a reflective layer, we achieve dual-sided laser ablation of the device. The resultant crater provides a smoother, curved surface onto which cells settle during planar patch clamping. Gigaohm seals, which are more easily achievable on these devices as compared to conventional micropores, are achieved by patch clamping human embryonic kidney (HEK 293) cells. Further, the microcraters show enhanced mechanical stability of the planar patch clamped cells during perfusion. We integrate polydimethylsiloxane microfluidic devices with the microcraters and use passive pumping to perfuse the cells. We find that passive pumping increases the pressure within the device by 1.85 Pa. However, due to the enhanced stability of the microcrater, fluidic shearing reduces the seal resistance by only 6.8 M on average, which is less than one percent of the gigaohm seal resistance.Publication link2014 Improvement of Chloride Transport Defect by Gonadotropin-Releasing Hormone (GnRH) in Cystic Fibrosis Epithelial CellsPort-a-Patch Publication in PLoS One (2014) Authors: Benz N., Le Hir S., Norez C., Kerbiriou M., Calvez M.-L., Becq F., Trouv P. , Frec C.Cystic fibrosis (CF), the most common autosomal recessive disease in Caucasians, is due to mutations in the CFTR gene. F508del, the most frequent mutation in patients, impairs CFTR protein folding and biosynthesis. The F508del-CFTR protein is retained in the endoplasmic reticulum (ER) and its traffic to the plasma membrane is altered. Nevertheless, if it reaches the cell surface, it exhibits a Clchannel function despite a short half-life. Pharmacological treatments may target the F508del-CFTR defect directly by binding to the mutant protein or indirectly by altering cellular proteostasis, and promote its plasma membrane targeting and stability. We previously showed that annexine A5 (AnxA5) directly binds to F508del-CFTR and, when overexpressed, promotes its membrane stability, leading to the restoration of some Clchannel function in cells. Because Gonadotropin-Releasing Hormone (GnRH) increases AnxA5 expression in some cells, we tested it in CF cells. We showed that human epithelial cells express GnRH-receptors (GnRH-R) and that GnRH induces an AnxA5 overexpression and an increased Clchannel function in F508del-CFTR cells, due to an increased stability of the protein in the membranes. Beside the numerous physiological implications of the GnRH-R expression in epithelial cells, we propose that a topical use of GnRH is a potential treatment in CF.Publication link2014 KCNMA1 Encoded Cardiac BK Channels Afford Protection against Ischemia-Reperfusion InjuryPort-a-Patch Publication in PLoS One (2014) Authors: Soltysinska E., Bentzen B.H., Barthmes M., Hattel H., Thrush A.B., Harper M.E., Qvortrup K., Larsen F.J., Schiffer T.A., Losa-Reyna J., Straubinger J., Kniess A., Thomsen M.B., Brggemann A., Fenske S., Biel M., Ruth P., Wahl-Schott C., Boushel R.C., Olesen S.P., Lukowski R.Mitochondrial potassium channels have been implicated in myocardial protection mediated through pre-/postconditioning. Compounds that open the Ca2+- and voltage-activated potassium channel of big-conductance (BK) have a pre-conditioning-like effect on survival of cardiomyocytes after ischemia/reperfusion injury. Recently, mitochondrial BK channels (mitoBKs) in cardiomyocytes were implicated as infarct-limiting factors that derive directly from the KCNMA1 gene encoding for canonical BKs usually present at the plasma membrane of cells. However, some studies challenged these cardio-protective roles of mitoBKs. Herein, we present electrophysiological evidence for paxilline- and NS11021-sensitive BK-mediated currents of 190 pS conductance in mitoplasts from wild-type but not BK/cardiomyocytes. Transmission electron microscopy of BK/ventricular muscles fibres showed normal ultra-structures and matrix dimension, but oxidative phosphorylation capacities at normoxia and upon re-oxygenation after anoxia were significantly attenuated in BK/permeabilized cardiomyocytes. In the absence of BK, post-anoxic reactive oxygen species (ROS) production from cardiomyocyte mitochondria was elevated indicating that mitoBK fine-tune the oxidative state at hypoxia and re-oxygenation. Because ROS and the capacity of the myocardium for oxidative metabolism are important determinants of cellular survival, we tested BK/hearts for their response in anex-vivomodel of ischemia/reperfusion (I/R) injury. Infarct areas, coronary flow and heart rates were not different between wild-type and BK/hearts upon I/R injury in the absence of ischemic pre-conditioning (IP), but differed upon IP. While the area of infarction comprised 283% of the area at risk in wild-type, it was increased to 585% in BK/hearts suggesting that BK mediates the beneficial effects of IP. These findings suggest that cardiac BK channels are important for proper oxidative energy supply of cardiomyocytes at normoxia and upon re-oxygenation after prolonged anoxia and that IP might indeed favor survival of the myocardium upon I/R injury in a BK-dependent mode stemming from both mitochondrial post-anoxic ROS modulation and non-mitochondrial localizations.Publication link2014 Crystal structure of a human GABAA receptorPort-a-Patch Publication in Nature (2014) Authors: Miller P.S., Aricescu A.R.Type-A-aminobutyric acid receptors (GABAARs) are the principal mediators of rapid inhibitory synaptic transmission in the human brain. A decline in GABAAR signalling triggers hyperactive neurological disorders such as insomnia, anxiety and epilepsy. Here we present the first three-dimensional structure of a GABAAR, the human 3 homopentamer, at 3 resolution. This structure reveals architectural elements unique to eukaryotic Cys-loop receptors, explains the mechanistic consequences of multiple human disease mutations and shows an unexpected structural role for a conserved N-linked glycan. The receptor was crystallized bound to a previously unknown agonist, benzamidine, opening a new avenue for the rational design of GABAAR modulators. The channel region forms a closed gate at the base of the pore, representative of a desensitized state. These results offer new insights into the signalling mechanisms of pentameric ligand-gated ion channels and enhance current understanding of GABAergic neurotransmission.Publication link2014 High susceptibility to fatty liver disease in two-pore channel 2-deficient micePort-a-Patch Publication in Nature Communications (2014) Authors: Grimm C., Holdt L.M., Chen C.C., Hassan S., Mller C., Jrs S., Cuny H., Kissing S., Schrder B., Butz B., Northoff B., Castonguay J., Luber C.A., Moser M., Spahn S., Lllmann-Rauch R., Fendel C., Klugbauer N., Griesbeck O., Haas A., Mann M., Bracher F., Teupser D., Saftig P., Biel M., Wahl-Schott C.Endolysosomal organelles play a key role in trafficking, breakdown and receptor-mediated recycling of different macromolecules such as low-density lipoprotein (LDL)-cholesterol, epithelial growth factor (EGF) or transferrin. Here we examine the role of two-pore channel (TPC) 2, an endolysosomal cation channel, in these processes. Embryonic mouse fibroblasts and hepatocytes lacking TPC2 display a profound impairment of LDL-cholesterol and EGF/EGF-receptor trafficking. Mechanistically, both defects can be attributed to a dysfunction of the endolysosomal degradation pathway most likely on the level of late endosome to lysosome fusion. Importantly, endolysosomal acidification or lysosomal enzyme function are normal in TPC2-deficient cells. TPC2-deficient mice are highly susceptible to hepatic cholesterol overload and liver damage consistent with non-alcoholic fatty liver hepatitis. These findings indicate reduced metabolic reserve of hepatic cholesterol handling. Our results suggest that TPC2 plays a crucial role in trafficking in the endolysosomal degradation pathway and, thus, is potentially involved in the homoeostatic control of many macromolecules and cell metabolites.Publication link2014 A small molecule restores function to TRPML1 mutant isoforms responsible for mucolipidosis type IVPort-a-Patch Publication in Nature Communications (2014) Authors: Chen C.C., Keller M., Hess M., Schiffmann R., Urban N., Wolfgardt A., Schaefer M., Bracher F., Biel M., Wahl-Schott C., Grimm C.Mucolipidosis type IV (MLIV) is an autosomal recessive lysosomal storage disorder often characterized by severe neurodevelopmental abnormalities and neuro-retinal degeneration. Mutations in theTRPML1gene are causative for MLIV. We used lead optimization strategies to identifyand MLIV patient fibroblasts to testsmall-molecule activators for their potential to restore TRPML1 mutant channel function. Using the whole-lysosome planar patch-clamp technique, we found that activation of MLIV mutant isoforms by the endogenous ligand PI(3,5)P2is strongly reduced, while activity can be increased using synthetic ligands. We also found that the F465L mutation renders TRPML1 pH insensitive, while F408 impacts synthetic ligand binding. Trafficking defects and accumulation of zinc in lysosomes of MLIV mutant fibroblasts can be rescued by the small molecule treatment. Collectively, our data demonstrate that small molecules can be used to restore channel function and rescue disease associated abnormalities in patient cells expressing specific MLIV point mutations.Publication link2014 Calcium regulation by temperature-sensitive transient receptor potential channels in human uveal melanoma cellsPort-a-Patch Publication in Cellular Signalling (2014) Authors: Mergler S., Derckx R., Reinach P.S., Garreis F., Bhm A., Schmelzer L., Skosyrski S., Ramesh N., Abdelmessih S., Polat O.K., Khajavi N., Riechardt A.I.Uveal melanoma (UM) is both the most common and fatal intraocular cancer among adults worldwide. As with all types of neoplasia, changes in Ca2+channel regulation can contribute to the onset and progression of this pathological condition. Transient receptor potential channels (TRPs) and cannabinoid receptor type 1 (CB1) are two different types of Ca2+permeation pathways that can be dysregulated during neoplasia. We determined in malignant human UM and healthy uvea and four different UM cell lines whether there is gene and functional expression of TRP subtypes and CB1 since they could serve as drug targets to either prevent or inhibit initiation and progression of UM. RT-PCR, Ca2+transients, immunohistochemistry and planar patch-clamp analysis probed for their gene expression and functional activity, respectively. In UM cells, TRPV1 and TRPM8 gene expression was identified. Capsaicin (CAP), menthol or icilin induced Ca2+transients as well as changes in ion current behavior characteristic of TRPV1 and TRPM8 expression. Such effects were blocked with either La3+, capsazepine (CPZ) or BCTC. TRPA1 and CB1 are highly expressed in human uvea, but TRPA1 is not expressed in all UM cell lines. In UM cells, the CB1 agonist, WIN 55,212-2, induced Ca2+transients, which were suppressed by La3+and CPZ whereas CAP-induced Ca2+transients could also be suppressed by CB1 activation. Identification of functional TRPV1, TRPM8, TRPA1 and CB1 expression in these tissues may provide novel drug targets for treatment of this aggressive neoplastic disease.Publication link2013 Monoacylglycerols Activate TRPV1 A Link between Phospholipase C and TRPV1Port-a-Patch Publication in PLoS One (2013) Authors: Zygmunt P.M., Ermund A., Movahed P., Andersson D.A., Simonsen C., Jnsson B.A., Blomgren A., Birnir B., Bevan S., Eschalier A., Mallet C., Gomis A., Hgesttt E.D.Phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate generates diacylglycerol, inositol 1,4,5-trisphosphate and protons, all of which can regulate TRPV1 activity via different mechanisms. Here we explored the possibility that the diacylglycerol metabolites 2-arachidonoylglycerol and 1-arachidonoylglycerol, and not metabolites of these monoacylglycerols, activate TRPV1 and contribute to this signaling cascade. 2-Arachidonoylglycerol and 1-arachidonoylglycerol activated native TRPV1 on vascular sensory nerve fibers and heterologously expressed TRPV1 in whole cells and inside-out membrane patches. The monoacylglycerol lipase inhibitors methylarachidonoyl-fluorophosphonate and JZL184 prevented the metabolism of deuterium-labeled 2-arachidonoylglycerol and deuterium-labeled 1-arachidonoylglycerol in arterial homogenates, and enhanced TRPV1-mediated vasodilator responses to both monoacylglycerols. In mesenteric arteries from TRPV1 knock-out mice, vasodilator responses to 2-arachidonoylglycerol were minor. Bradykinin and adenosine triphosphate, ligands of phospholipase C-coupled membrane receptors, increased the content of 2-arachidonoylglycerol in dorsal root ganglia. In HEK293 cells expressing the phospholipase C-coupled histamine H1receptor, exposure to histamine stimulated the formation of 2-AG, and this effect was augmented in the presence of JZL184. These effects were prevented by the diacylglycerol lipase inhibitor tetrahydrolipstatin. Histamine induced large whole cell currents in HEK293 cells co-expressing TRPV1 and the histamine H1receptor, and the TRPV1 antagonist capsazepine abolished these currents. JZL184 increased the histamine-induced currents and tetrahydrolipstatin prevented this effect. The calcineurin inhibitor ciclosporin and the endogenous entourage compound palmitoylethanolamide potentiated the vasodilator response to 2-arachidonoylglycerol, disclosing TRPV1 activation of this monoacylglycerol at nanomolar concentrations. Furthermore, intracerebroventricular injection of JZL184 produced TRPV1-dependent antinociception in the mouse formalin test. Our results show that intact 2-arachidonoylglycerol and 1-arachidonoylglycerol are endogenous TRPV1 activators, contributing to phospholipase C-dependent TRPV1 channel activation and TRPV1-mediated antinociceptive signaling in the brain.Publication link2014 A potent and KV1.3-selective analogue of the scorpion toxin HsTX1 as a potential therapeutic for autoimmune diseasesPort-a-Patch Publication in Nature Scientific Reports (2014) Authors: Rashid M.H., Huq R., Tanner M.R., Chhabra S., Khoo K.K., Estrada R., Dhawan V., Chauhan S., Pennington M.W., Beeton C., Kuyucak S., Norton R.S.HsTX1 toxin, from the scorpion Heterometrus spinnifer, is a 34-residue, C-terminally amidated peptide cross-linked by four disulfide bridges. Here we describe new HsTX1 analogues with an Ala, Phe, Val or Abu substitution at position 14. Complexes of HsTX1 with the voltage-gated potassium channels KV1.3 and KV1.1 were created using docking and molecular dynamics simulations, then umbrella sampling simulations were performed to construct the potential of mean force (PMF) of the ligand and calculate the corresponding binding free energy for the most stable configuration. The PMF method predicted that the R14A mutation in HsTX1 would yield a > 2kcal/mol gain for the KV1.3/KV1.1 selectivity free energy relative to the wild-type peptide. Functional assays confirmed the predicted selectivity gain for HsTX1[R14A] and HsTX1[R14Abu], with an affinity for KV1.3 in the low picomolar range and a selectivity of more than 2,000-fold for KV1.3 over KV1.1. This remarkable potency and selectivity for KV1.3, which is significantly up-regulated in activated effector memory cells in humans, suggest that these analogues represent valuable leads in the development of therapeutics for autoimmune diseases.Publication link2013 Differential Effects of the Adrenoceptor Blockers Carvedilol and Metoprolol on SQT1 and SQT2Mutant ChannelsPort-a-Patch Publication in Journal of Cardiovascular Electrophysiology (2013) Authors: Bodi I., Franke G., Pantulu N.D., Wu K., Perez-Feliz S., Bode C., Zehender M., Zur Hausen A., Brunner M., Odening K.Background: N588K-KCNH2 and V307L-KCNQ1 mutations lead to a gain-of-function of IKrand IKsthus causing short-QT syndromes (SQT1, SQT2). Combined pharmacotherapies using K+-channel-blockers and -blockers are effective in SQTS. Since -blockers can block IKrand IKs, we aimed at determining carvedilol's and metoprolol's electrophysiological effects on N588K-KCNH2 and V307L-KCNQ1 channels. Methods Wild-type (WT)-KCNH2, WT-KCNQ1 and mutant N588K-KCNH2 and V307L-KCNQ1 channels were expressed in CHO-K1 or HEK-293T cells and IKsand IKrwere recorded at baseline and during -blocker exposure. Results Carvedilol (10 M) reduced IKstail in WT- and V307L-KCNQ1 by 36.5 5% and 18.6 9% (P 0.05). IC50values were 16.3 M (WT) and 46.1 M (V307L), indicating a 2.8-fold decrease in carvedilol's IKs-blocking potency in V307L-KCNQ1. Carvedilol's (1 M) inhibition of the IKrtail was attenuated in N588

overexpressing ion channels, and expect that this method will provide electrophysiological access to a broad range of intracellular ion channels.Publication link2010 TRPV channels mediate temperature-sensing in human corneal endothelial cellsPort-a-Patch Publication in Experimental Eye Research (2010) Authors: Mergler S., Valtink M., Coulson-Thomas V.J., Lindemann D., Reinach P.S., Engelmann K., Pleyer U.The physiology and transparency of the cornea are dependent on corneal endothelial function. The role of temperature sensitive ion channels in maintaining such activity is unknown. This study was undertaken to probe for the functional expression of such pathways in human corneal endothelial cells (HCEC). We used HCEC-12, an immortalized population derived from whole corneal endothelium, and two morphologically distinct clonal cell lines derived from HCEC-12 (HCEC-H9C1, HCEC-B4G12) to probe for gene expression and function of transient receptor potential (TRP) channels of the vanilloid (V) isoform subfamily (i.e. TRPV13) in these cell types. Expression of TRPV isotypes 1, 2 and 3 were detected by RT-PCR. Protein expression of TRPV1 in situ was confirmed by immunostaining of corneoscleral remnants after keratoplasty. TRPV13 functional activity was evident based on capsaicin-induced Ca2+transients and induction of these responses through rises in ambient temperature from 25C to over 40C. The currents underlying Ca2+transients were characterized with a novel high throughput patch-clamp system. The TRPV1 selective agonist, capsaicin (CAP) (1020M) increased non-selective cation whole-cell currents resulting in calcium increases that were fully blocked by either the TRPV1 antagonist capsazepine (CPZ) or removal of extracellular calcium. Similarly, heating from room temperature to over 40C increased the same currents resulting in calcium increases that were significantly reduced by the TRP channel blockers lanthanum chloride (La3+) (100M) and ruthenium-red (RuR) (10M), respectively. Moreover, application of the TRPV channel opener 2-aminoethoxydiphenyl borate (2-APB) (400M) led to a reversible increase in intracellular Ca2+indicating putative TRPV13 channel activity. Taken together, TRPV activity modulation by temperature underlies essential homeostatic mechanisms contributing to the support of corneal endothelial function under different ambient conditions.Publication link2009 T-type channel blocking properties and antiabsence activity of two imidazo[1,2-b]pyridazine derivatives structurally related to indomethacinPort-a-Patch Publication in Neuropharmacology (2009) Authors: Rimoli M.G., Russo E., Cataldi M., Citraro R., Ambrosino P., Melisi D., Curcio A., De Lucia S., Patrignani P., De Sarro G., Abignente E.It is presently unclear whether the antiseizure effects exerted by NSAIDs are totally dependent on COX inhibition or not. To clarify this point we investigated whether 7-methyl-2-phenylimidazo[1,2-b]pyridazine-3-carboxylic acid (DM1) and 6-methoxy-2-phenylimidazo[1,2-b]pyridazine-3-carboxylic acid (DM2), two imidazo[1,2-b]pyridazines structurally related to indomethacin (IDM) but ineffective in blocking COXs, retain IDM antiabsence activity. When administered by intraperitoneal injection in WAG/Rij rats, a rat strain which spontaneously develops SWDs, both DM1 and DM2 dose-dependently suppressed the occurrence of these seizures. Importantly, these compounds were both more potent in suppressing SWD occurrence than IDM. As T-type channel blockade is considered a mechanism of action common to many antiabsence drugs we explored by whole cell patch clamp electrophysiology in stably transfected HEK-293 the effect of DM1 and DM2 on CaV3.1 channels, the T-type channel subtype preferentially expressed in ventrobasal thalamic nuclei. Both these compounds dose-dependently suppressed the currents elicited by membrane depolarization in these cells. A similar T-type blocking effect was also observed when the cells were exposed to IDM. In conclusion, DM1 and DM2 whilst inactive on COXs, are potent antiabsence drugs. This suggests that compounds with structural features typical of NSAIDs may exert antiepileptic activity independently from COX inhibition and possibly by a direct interaction with T-type voltage-dependent Ca2+channels.Publication link2010 Characterization of two pore channel 2 (TPCN2) -mediated Ca2+ currents in isolated lysosomesPort-a-Patch Publication in The Journal of Biological Chemistry (2010) Authors: Schieder M., Rtzer K., Brggemann A., Biel M., Wahl-Schott C.A.Two-pore channels (TPCNs) have been proposed to form lysosomal Ca2+release channels that are activated by nicotinic acid adenine dinucleotide phosphate. Here, we employ a glass chip-based method to record for the first time nicotinic acid adenine dinucleotide phosphate -dependent currents through a two-pore channel (TPCN2) from intact lysosomes. We show that TPCN2 is a highly selective Ca2+channel that is regulated by intralysosomal pH. Using site-directed mutagenesis, we identify an amino acid residue in the putative pore region that is crucial for conferring high Ca2+selectivity. Our glass chip-based method will provide electrophysiological access not only to lysosomal TPCN channels but also to a broad range of other intracellular ion channels.Publication link2008 TRPA1 is a major oxidant sensor in murine airway sensory neuronsPort-a-Patch Publication in The Journal of Clinical Investigation (2008) Authors: Bessac B.F., Sivula M., von Hehn C.A., Escalera J., Cohn L., Jordt S.E.Sensory neurons in the airways are finely tuned to respond to reactive chemicals threatening airway function and integrity. Nasal trigeminal nerve endings are particularly sensitive to oxidants formed in polluted air and during oxidative stress as well as to chlorine, which is frequently released in industrial and domestic accidents. Oxidant activation of airway neurons induces respiratory depression, nasal obstruction, sneezing, cough, and pain. While normally protective, chemosensory airway reflexes can provoke severe complications in patients affected by inflammatory airway conditions like rhinitis and asthma. Here, we showed that both hypochlorite, the oxidizing mediator of chlorine, and hydrogen peroxide, a reactive oxygen species, activated Ca2+ influx and membrane currents in an oxidant-sensitive subpopulation of chemosensory neurons. These responses were absent in neurons from mice lacking TRPA1, an ion channel of the transient receptor potential (TRP) gene family. TRPA1 channels were strongly activated by hypochlorite and hydrogen peroxide in primary sensory neurons and heterologous cells. In tests of respiratory function, Trpa1/ mice displayed profound deficiencies in hypochlorite- and hydrogen peroxideinduced respiratory depression as well as decreased oxidant-induced pain behavior. Our results indicate that TRPA1 is an oxidant sensor in sensory neurons, initiating neuronal excitation and subsequent physiological responses in vitro and in vivo.Publication link2009 Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gasesPort-a-Patch Publication in FASEB (2009) Authors: Bessac B.F., Sivula M., von Hehn C.A., Caceres A.I., Escalera, J, Jordt S.The release of methyl isocyanate in Bhopal, India, caused the worst industrial accident in history. Exposures to industrial isocyanates induce lacrimation, pain, airway irritation, and edema. Similar responses are elicited by chemicals used as tear gases. Despite frequent exposures, the biological targets of isocyanates and tear gasesin vivohave not been identified, precluding the development of effective countermeasures. We use Ca2+imaging and electrophysiology to show that the noxious effects of isocyanates and those of all major tear gas agents are caused by activation of Ca2+influx and membrane currents in mustard oil-sensitive sensory neurons. These responses are mediated by transient receptor potential ankyrin 1 (TRPA1), an ion channel serving as a detector for reactive chemicals. In mice, genetic ablation or pharmacological inhibition of TRPA1 dramatically reduces isocyanate- and tear gas-induced nocifensive behavior after both ocular and cutaneous exposures. We conclude that isocyanates and tear gas agents target the same neuronal receptor, TRPA1. Treatment with TRPA1 antagonists may prevent and alleviate chemical irritation of the eyes, skin, and airways and reduce the adverse health effects of exposures to a wide range of toxic noxious chemicals.Publication link2008 Planar patch-clamp force microscopy on living cellsPort-a-Patch Publication in Ultramicroscopy (2008) Authors: Pamir E., George M., Fertig N., Benoit M.Here we report a new combination of the patch-clamp technique with the atomic force microscope (AFM). A planar patch-clamp chip microstructured from borosilicate glass was used as a support for mechanical probing of living cells. The setup not only allows for immobilizing even a non-adherent cell for measurements of its mechanical properties, but also for simultaneously measuring the electrophysiological properties of a single cell. As a proof of principle experiment we measured the voltage-induced membrane movement of HEK293 and Jurkat cells in the whole-cell voltage clamp configuration. The results of these measurements are in good agreement with previous studies. By using the planar patch-clamp chip for immobilization, the AFM not only can image non-adhering cells, but also gets easily access to an electrophysiologically controlled cellular probe at low vibrational noise.Publication link2008 Synthesis and biological evaluation of chalcones as inhibitors of the voltage-gated potassium channel KV1.3Port-a-Patch Publication in Bioorganic & Medicinal Chemistry Letters (2008) Authors: Cianci J., Baell J.B., Flynn B.L., Gable R.W., Mould J.A., Paul D., Harvey A.J.Chalcone derivatives of the natural product khellinone were synthesised and screened for bioactivity against the voltage-gated potassium channel KV1.3. X-ray crystallography was employed to investigate relationships between the structure and function of a selection of the reported chalcones.Publication link2008 High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiologyPort-a-Patch Publication in Nature Reviews Drug Discovery (2008) Authors: Dunlop J., Bowlby M., Peri R., Vasilyev D., Arias R.Ion channels represent highly attractive targets for drug discovery and are implicated in a diverse range of disorders, in particular in the central nervous and cardiovascular systems. Moreover, assessment of cardiac ion-channel activity of new chemical entities is now an integral component of drug discovery programmes to assess potential for cardiovascular side effects. Despite their attractiveness as drug discovery targets ion channels remain an under-exploited target class, which is in large part due to the labour-intensive and low-throughput nature of patch-clamp electrophysiology. This Review provides an update on the current state-of-the-art for the various automated electrophysiology platforms that are now available and critically evaluates their impact in terms of ion-channel screening, lead optimization and the assessment of cardiac ion-channel safety liability.Publication link2008 Planar patch clamp: Advances in electrophysiologyPort-a-Patch book chapter in "Potassium Channels" (2008) Authors: Brggemann A., Farre C., Haarmann C., Haythornthwaite A., Kreir M., Stoelzle S., George M., Fertig N.Ion channels have gained increased interest as therapeutic targets over recent years, since a growing number of human and animal diseases have been attributed to defects in ion channel function. Potassium channels are the largest and most diverse family of ion channels. Pharmaceutical agents such as Glibenclamide, an inhibitor of KATP channel activity which promotes insulin release, have been successfully sold on the market for many years. So far, only a small group of the known ion channels have been addressed as potential drug targets. The functional testing of drugs on these ion channels has always been the bottleneck in the development of these types of pharmaceutical compounds. New generations of automated patch clamp screening platforms allow a higher throughput for drug testing and widen this bottleneck. Due to their planar chip design not only is a higher throughput achieved, but new applications have also become possible. One of the advantages of planar patch clamp is the possibility of perfusing the intracellular side of the membrane during a patch clamp experiment in the whole-cell configuration. Furthermore, the extracellular membrane remains accessible for compound application during the experiment. Internal perfusion can be used not only for patch clamp experiments with cell membranes, but also for those with artificial lipid bilayers. In this chapter we describe how internal perfusion can be applied to potassium channels expressed in Jurkat cells, and to Gramicidin channels reconstituted in a lipid bilayer.Publication link2005 The Port-a-Patch: The smallest patch clamp set up for high quality electrophysiologyPort-a-Patch Publication in HEKA Impulse (2005) Authors: Brggemann A., George M., Klau M., Beckler M., Steindl J., Behrends J., Fertig N.In evaluating ion channel function, patch clamping, provides the highest information content but every electrophysiologist knows the draw back of the size and complexity of a patch clamp rig. We present here patch clamp recordings in the whole cell con- figuration performed with planar patch clamp chips, which are micro- structured from borosilicate glass substrate. The chips are used in the Port-a-Patch, a simplified and miniaturized patch clamp setup that enables automated patch clamp experiments on a single cell. The PatchMaker software performs the experiment by executing user-determined protocols for cell positioning and in communication with the PULSE software also protocols for electrical stimulation and current readout. In various electro-physiological experiments, the high quality of recordings and the versatility of the perfusion of the recorded cells are demonstrated.Publication link2007 Electrophysiological assessment of HERG blockade: Comparative study using automated and conventional patch clamp systemsPort-a-Patch Publication in Toxicological Letters (2007) Authors: Yook Y., Kim J., Cho H., Moon H., Kwak B.Publication link2004 Ion channel drug discovery and research: The automated Nano-Patch-Clamp technologyPort-a-Patch Publication in Current Drug Discovery Technologies (2004) Authors: Brggemann A., George M., Klau M., Beckler M., Steindl J., Behrends J.C., Fertig N.Unlike the genomics revolution, which was largely enabled by a single technological advance (high throughput sequencing), rapid advancement in proteomics will require a broader effort to increase the throughput of a number of key tools for functional analysis of different types of proteins. In the case of ion channels - a class of (membrane) proteins of great physiological importance and potential as drug targets- the lack of adequate assay technologies is felt particularly strongly. The available, indirect, high throughput screening methodsfor ion channels clearly generate insufficient information. The best technology to study ion channel function and screen for compound interaction is the patch clamp technique, but patch clamping suffers from low throughput, which is not acceptable for drug screening. A first step towards a solution is presented here. The nano patch clamp technology, which is based on a planar, microstructured glass chip, enables automatic whole cell patch clamp measurements. The Port-a-Patch is an automated electrophysiology workstation, which uses planar patch clamp chips. This approach enables high quality and high content ion channel and compound evaluation on a one-cell-at-a-time basis. The presented automation of the patch process and its scalability to an array format are the prerequisites for any higher throughput electrophysiology instruments.Publication link2005 Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticlesPort-a-Patch Publication in Nano Letters (2005) Authors: Kirchner C., Liedl T., Kudera S., Pellegrino T., Munoz J. A., Gaub H.E., Stoelzle S., Fertig N., Parak W.J.Cytotoxicity of CdSe and CdSe/ZnS nanoparticles has been investigated for different surface modifications such as coating with mercaptopropionic acid, silanization, and polymer coating. For all cases, quantitative values for the onset of cytotoxic effects in serum-free culture media are given. These values are correlated with microscope images in which the uptake of the particles by the cells has been investigated. Our data suggest that in addition to the release of toxic Cd2+ions from the particles also their surface chemistry, in particular their stability toward aggregation, plays an important role for cytotoxic effects. Additional patch clamp experiments investigate effects of the particles on currents through ion channels.Publication link2003 Simultaneous optical and electrical recording of single gramicidin channelsPort-a-Patch Publication in Biophysical Journal (2003) Authors: Borisenko V., Lougheed L., Hesse J., Fuereder-Kitzmueller E., Fertig N., Behrends, J.C., Woolley G.A., Schuetz G.J.We report here an approach for simultaneous fluorescence imaging and electrical recording of single ion channels in planar bilayer membranes. As a test case, fluorescently labeled (Cy3 and Cy5) gramicidin derivatives were imaged at the single-molecule level using far-field illumination and cooled CCD camera detection. Gramicidin monomers were observed to diffuse in the plane of the membrane with a diffusion coefficient of 3.3 x 10-8cm2s-1. Simultaneous electrical recording detected gramicidin homodimer (Cy3/Cy3, Cy5/Cy5) and heterodimer (Cy3/Cy5) channels. Heterodimer formation was observed optically by the appearance of a fluorescence resonance energy transfer (FRET) signal (irradiation of Cy3, detection of Cy5). The number of FRET signals was significantly smaller than the number of Cy3 signals (Cy3 monomers plus Cy3homodimers) as expected. The number of FRET signals increased with increasing channel activity. In numerous cases the appearance of a FRET signal was observed to correlate with a channel opening event detected electrically. The heterodimers also diffused in the plane of the membrane with a diffusion coefficient of 3.0 x 10-8cm2s-1. These experiments demonstrate the feasibility of simultaneous optical and electrical detection of structural changes in single ion channels as well as suggesting strategies for improving the reliability of such measurements.Publication link2012 Natural and artificial ion channels for biosensing platformsPort-a-Patch,Patchliner, SyncroPatch 96 ((a predecessor model of SyncroPatch 384PE) and Vesicle Prep ProPublication in Analytical and Bioanalytical Chemistry (2012) Authors: Steller L., Kreir M., Salzer R.The single-molecule selectivity and specificity of the binding process together with the expected intrinsic gain factor obtained when utilizing flow through a channel have attracted the attention of analytical chemists for two decades. Sensitive and selective ion channel biosensors for high-throughput screening are having an increasing impact on modern medical care, drug screening, environmental monitoring, food safety, and biowarefare control. Even virus antigens can be detected by ion channel biosensors. The study of ion channels and other transmembrane proteins is expected to lead to the development of new medications and therapies for a wide range of illnesses. From the first attempts to use membrane proteins as the receptive part of a sensor, ion channels have been engineered as chemical sensors. Several other types of peptidic or nonpeptidic channels have been investigated. Various gating mechanisms have been implemented in their pores. Three technical problems had to be solved to achieve practical biosensors based on ion channels: the fabrication of stable lipid bilayer membranes, the incorporation of a receptor into such a structure, and the marriage of the modified membrane to a transducer. The current status of these three areas of research, together with typical applications of ion-channel biosensors, are discussed in this review.Publication link2019 Role of HighThroughput Electrophysiology in Drug DiscoverySyncroPatch 384PE (a predecessor model of the SyncroPatch 384i),Patchliner and Port-a-Patch review article in Current Protocols in Pharmacology (2019) Authors: Liu C., Li T., Chen J.Due to their important physiological functions, ion channels are key therapeutic targets for a variety of disorders. However, electrophysiological assessment of ion channel activity is technically challenging and has been a bottleneck in the discovery of drugs that modulate channel function. To address this issue, automated patch clamp platforms have been developed with improved throughput and broader applications. An overview of the current status of highthroughput electrophysiology and its applications in drug discovery is provided.Publication link2020 Automated Patch Clamp in Drug Discovery: major breakthroughs and innovation in the last decadeSyncroPatch 384PE (a predecessor model of the SyncroPatch 384i instrument),Patchliner andPort-a-PatchPublication in Expert Opinion on Drug Discovery (2020) Authors: Obergrussberger A., Friis S., Brggemann A., Fertig N.Patch-clamp electrophysiology remains an important technique in studying ion channels; indeed, it is still considered the gold standard since it was first described by Neher and Sakmann in the 1970s [1]. Ion channels are integral membrane proteins which allow ion current flow across the cell membrane. They are involved in almost all physiological processes, and their malfunction underlies many disease states, making them important pharmacological targets. Conventional patch clamp is a very information-rich technique, but it requires skilled personnel to perform experiments, and typically, only one experiment can be performed at a time. In the late 1990s and early 2000s, the field of ion-channel research was revolutionized by the development of the automated patch-clamp (APC) technique. The most successful approach involved replacing the patch-clamp pipette with a planar substrate (for review, see [2]), making the experiments easier to perform and offering the option for recording multiple cells in parallel. In the last two decades, much has changed in the field of ion-channel drug discovery and APC, with increased throughput and enhanced simplicity. We summarize the main changes in the last decade and attempt to look into the future of whats to come.Publication link2011 State-of-the-art automated patch clamp devices: heat activation, action potentials, and high throughput in ion channel screeningPort-a-Patch,Patchliner and SyncroPatch 96 (a predecessor model of SyncroPatch 384PE) Publication in Frontiers in Pharmacology (2011) Authors: Stoelzle S., Obergrussberger A., Brggemann A., Haarmann C., George M., Kettenhofen R., Fertig N.Ion channels are essential in a wide range of cellular functions and their malfunction underlies many disease states making them important targets in drug discovery. The availability of standardized cell lines expressing ion channels of interest lead to the development of diverse automated patch clamp (APC) systems with high-throughput capabilities. These systems are now available for drug screening, but there are limitations in the application range. However, further development of existing devices and introduction of new systems widen the range of possible experiments and increase throughput. The addition of well controlled and fast solution exchange, temperature control and the availability of the current clamp mode are required to analyze standard cell lines and excitable cells such as stem cell-derived cardiomyocytes in a more physiologically relevant environment. Here we describe two systems with different areas of applications that meet the needs of drug discovery researchers and basic researchers alike. The here utilized medium throughput APC device is a planar patch clamp system capable of recording up to eight cells simultaneously. Features such as temperature control and recordings in the current clamp mode are described here. Standard cell lines and excitable cells such as stem cell-derived cardiomyocytes have been used in the voltage clamp and current clamp modes with the view to finding new drug candidates and safety testing methods in a more physiologically relevant environment. The high-throughput system used here is a planar patch clamp screening platform capable of recording from 96 cells in parallel and offers a throughput of 5000 data points per day. Full dose response curves can be acquired from individual cells reducing the cost per data point. The data provided reveals the suitability and relevance of both APC platforms for drug discovery, ion channel research, and safety testing.Publication link2012 HTS techniques for patch clamp-based ion channel screening economy and advancesPort-a-Patch, Patchliner and SyncroPatch 96 (a predecessor model of SyncroPatch 384PE) Publication in Expert Opinion on Drug Discovery (2012) Authors: Farre C. and Fertig N.Introduction: Ten years ago, the first publication appeared showing patch clamp recordings performed on a planar glass chip instead of using a conventional patch clamp pipette. Going planar proved to revolutionize ion channel drug screening as we know it, by allowing high quality measurements of ion channels and their effectors at a higher throughput and at the same time de-skilling the highly laborious technique. Over the years, platforms evolved in response to user requirements regarding experimental features, data handling plus storage, and suitable target diversity. Areas covered: This article gives a snapshot image of patch clamp-based ion channel screening with focus on platforms developed to meet requirements of high-throughput screening environments. The commercially available platforms are described, along with their benefits and drawbacks in ion channel drug screening. Expert opinion: Automated patch clamp (APC) platforms allow faster investigation of a larger number of ion channel active compounds or cell clones than previously possible. Since patch clamp is the only method allowing direct, real-time measurements of ion channel activity, APC holds the promise of picking up high quality leads, where they otherwise would have been overseen using indirect methods. In addition, drug candidate safety profiling can be performed earlier in the drug discovery process, avoiding late-phase compound withdrawal due to safety liability issues, which is highly costly and inefficient.Publication link2010 Renaissance of ion channel research and drug discovery by patch clamp automationPort-a-Patch, Patchliner and SyncroPatch 96 (a predecessor model of SyncroPatch 384PE) Publication in Future Medical Chemistry (2010) Authors: Farre C. and Fertig N.Ion channels are highly intriguing biophysical entities that play an incredibly subtle role in the concerted actions in which they are involved, and that also have a crucial impact on inter- and intra-cellular communication. They respond to numerous kinds of stimuli and play a decisive role in the vitality of all living organisms. Ion channels are involved in the function of the cardiovascular and nervous systems and their malfunction underlies numerous diseases and indications. For exactly these reasons, ion channels have for decades been, and are still, the subject of in-depth research into a very broad range of important therapeutic areas. As membrane-bound proteins they are highly druggable targets, being readily accessible to small molecules that are capable of fine tuning ion channel function by pharmacological modulation. Approximately 15% of the most successful drugs target ion channels, although ion channels have traditionally been difficult to screen due to a lack of adequate assays. Many of the marketed ion channel drugs were actually not discovered in rational drug-discovery programs, but rather empirically and by serendipity since the available ion channel-screening techniques typically confer a tradeoff between high content and high throughput.Publication link2015 Novel screening techniques for ion channel targeting drugsPatchliner, SyncroPatch 384PE(a predecessor model of SyncroPatch 384i) and CardioExcyte 96 Publication in Channels (2015) Authors: Obergrussberger A., Stlzle-Feix S., Becker N., Brggemann A., Fertig N., Mller C.Ion channels are integral membrane proteins that regulate the flux of ions across the cell membrane. They are involved in nearly all physiological processes, and malfunction of ion channels has been linked to many diseases. Until recently, high-throughput screening of ion channels was limited to indirect, e.g. fluorescence-based, readout technologies. In the past years, direct label-free biophysical readout technologies by means of electrophysiology have been developed. Planar patch-clamp electrophysiology provides a direct functional label-free readout of ion channel function in medium to high throughput. Further electrophysiology features, including temperature control and higher-throughput instruments, are continually being developed. Electrophysiological screening in a 384-well format has recently become possible. Advances in chip and microfluidic design, as well as in cell preparation and handling, have allowed challenging cell types to be studied by automated patch clamp. Assays measuring action potentials in stem cell-derived cardiomyocytes, relevant for cardiac safety screening, and neuronal cells, as well as a large number of different ion channels, including fast ligand-gated ion channels, have successfully been established by automated patch clamp. Impedance and multi-electrode array measurements are particularly suitable for studying cardiomyocytes and neuronal cells within their physiological network, and to address more complex physiological questions. This article discusses recent advances in electrophysiological technologies available for screening ion channel function and regulation.User meeting video15.10.2020 | Webinar: ICH S7B best practices considerations New Q&As and Benchmarking best practicesDr. Sonja Stoelzle-Feix (Nanion Technologies), This is an on-demand webinar from Nan]i[on and Friends 2020.The ICH E14/S7B Implementation Working Group released a draft version on August 28th 2020 on Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential Questions and Answers. This document is open for public consultation and comprises proposed revisions for some sections of the current Q&As for ICH E14. Furthermore, new Q&A for ICH S7B are included.This talk will focus on best practice outlines as depicted in the draft version, specifically on in vitro cardiac ion channel assays. The ultimate goal is to provide a more robust and reproducible evaluation of potency of drug block of cardiac ion channel current using patch clamp techniques and heterologous expression systems.Webinar28.07.2015 | Webinar: High Throughput and High Fidelity: Automated Patch Clamp in Screening and ResearchThe webinar covers the use of the Patchliner and the SyncroPatch 384/768PE for characterization of ion channels and screening of ion channel active compounds.The webinar covers the use of the Patchliner and the SyncroPatch 384/768PE for characterization of ion channels and screening of ion channel active compounds.Both automated patch clamp systems support high quality, giga-seal recordings, but differ in throughput capabilities and experimental features.The Patchliner, introduced in 2006, records eight cells in parallel, and is a highly appreciated research platform in industry and academia alike. Experimental features include individual pulse protocols, automated current clamp recordings, internal solution exchange, rapid perfusion, temperature control, and temperature jumps. In addition, scarce and expensive cells can readily be used because of optimized cell catch procedures requiring less than 500 cells/ml.The SyncroPatch 384/768PE , introduced in 2013, has been adopted by big pharma and academic core facilities around the globe. The SyncroPatch 384/768PE it fully compatible with high throughput screening standards and can be integrated into existing hardware and software. The system supports giga seal recording from up to 768 cells in parallel, excellent voltage-control of patch-clamped cells, fast solution exchange, and has been validated with a wide variety of ion channel targets. Today, the SyncroPatch 384/768PE is utilized for academic research and HTS ion channel drug discovery.Publication link2023 Automated Patch Clamp Screening of Amiloride and 5-N,N-Hexamethyleneamiloride Analogs Identifies 6-Iodoamiloride as a Potent Acid-Sensing Ion Channel InhibitorSyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument)and Patchliner Publication inMol. Pharmaceutics(2023) Authors: Finol-Urdaneta R.K., McArthur J.R., Aboelela A., Bujaroski R.S., Majed H., Rangel A., Adams D.J., Ranson M., Kelso M.J., Buckley B.J.Acid-sensing ion channels (ASICs) are transmembrane sensors of extracellular acidosis and potential drug targets in several disease indications, including neuropathic pain and cancer metastasis. The K+-sparing diuretic amiloride is a moderate nonspecific inhibitor of ASICs and has been widely used as a probe for elucidating ASIC function. In this work, we screened a library of 6-substituted and 5,6-disubstituted amiloride analogs using a custom-developed automated patch clamp protocol and identified 6-iodoamiloride as a potent ASIC1 inhibitor. Follow-up IC50determinations in tsA-201 cells confirmed higher ASIC1 inhibitory potency for 6-iodoamiloride94(hASIC194IC50= 88 nM, cf. amiloride11IC50= 1.7 M). A similar improvement in activity was observed in ASIC3-mediated currents from rat dorsal root ganglion neurons (rDRG single-concentration94IC50= 230 nM, cf.11IC50= 2.7 M). 6-Iodoamiloride represents the amiloride analog of choice for studying the effects of ASIC inhibition on cell physiology.Publication link2022 Development of ASIC1a ligand-gated ion channel drug screening assays across multiple automated patch clamp platformsSyncroPatch 384and Patchliner Publication in Frontiers in Molecular Neuroscience(2022) Authors: Ridley J., Manyweathers S., Tang R., Goetze T., Becker N., Rinke-Wei I., Kirby R., Obergrussberger A., Rogers M.Human acid-sensing ion channels (ASIC) are ligand-gated ionotropic receptors expressed widely in peripheral tissues as well as sensory and central neurons and implicated in detection of inflammation, tissue injury, and hypoxia-induced acidosis. This makes ASIC channels promising targets for drug discovery in oncology, pain and ischemia, and several modulators have progressed into clinical trials. We describe the use of hASIC1a as a case study for the development and validation of low, medium and high throughput automated patch clamp (APC) assays suitable for the screening and mechanistic profiling of new ligands for this important class of ligand-gated ion channel. Initial efforts to expand on previous manual patch work describing an endogenous hASIC1a response in HEK cells were thwarted by low current expression and unusual pharmacology, so subsequent work utilized stable hASIC1a CHO cell lines. Ligand-gated application protocols and screening assays on the Patchliner, QPatch 48, and SyncroPatch 384 were optimized and validated based on pH activation and nM-M potency of reference antagonists (e.g., Amiloride, Benzamil, Memantine, Mambalgin-3, A-317567, PcTx1). By optimizing single and stacked pipette tip applications available on each APC platform, stable pH-evoked currents during multiple ligand applications enabled cumulative EC50 and IC50 determinations with minimized receptor desensitization. Finally, we successfully demonstrated for the first time on an APC platform the ability to use current clamp to implement the historical technique of input resistance tracking to measure ligand-gated changes in membrane conductance on the Patchliner platform.Publication link2020 Reliable identification of cardiac liability in drug discovery using automated patch clamp: Benchmarking best practices and calibration standards for improved proarrhythmic assessmentPatchliner and SyncroPatch 384i (SyncroPatch 384PE a predecessor model) Publication in the Journal of Pharmacological and Toxicological Methods (2020) Authors: Brinkwirth N., Takasuna K., Masafumi D., Becker N., Obergrussberger A., Friis S., Furukawa Y., Hasegawa Y., Oka T., Ohtsuki A., Fertig N., Stoelzle-Feix S.Screening compounds for activity on the hERG channel using patch clamp is a crucial part of safety testing. Automated patch clamp (APC) is becoming widely accepted as an alternative to manual patch clamp in order to increase throughput whilst maintaining data quality. In order to standardize APC experiments, we have investigated the effects on IC50values under different conditions using several devices across multiple sites. Methods:APC instruments SyncroPatch 384i, SyncroPatch 384PE and Patchliner, were used to record hERG expressed in HEK or CHO cells. Up to 27 CiPA compounds were used to investigate effects of voltage protocol, incubation time, labware and time between compound preparation and experiment on IC50values.Publication link2021 Reliable identification of cardiac conduction abnormalities in drug discovery using automated patch clamp II: Best practices for NaV1.5 peak current in a high throughput screening environmentSyncroPatch 384 andPatchliner Publication in Journal of Pharmacological and Toxicological Methods (2021) Authors: Rotordam M.G., Obergrussberger A., Brinkwirth N., Takasuna K., Becker N., Horvtha A., Goetze T.A., Rapedius M., Furukawa H., Hasegawa Y., Oka T., Fertig N., Stoelzle-Feix SFor reliable identification of cardiac safety risk, compounds should be screened for activity on cardiac ion channels in addition to hERG, including NaV1.5 and CaV1.2. We identified different parameters that might affect IC50s of compounds on NaV1.5 peak and late currents recorded using automated patch clamp (APC) and suggest outlines for best practices.Publication link2020 Cross-site and cross-platform variability of automated patch clamp assessments of drug effects on human cardiac currents in recombinant cellsPatchliner andSyncroPatch 384PE (a predecessor model of the SyncroPatch 384i instrument) Publication in Nature Scientific Reports (2020) Authors: Kramer J., Himmel H.M., Lindqvist A., Stoelzle-Feix S., Chaudhary K.W., Li D., Bohme G.A., Bridgland-Taylor M., Hebeisen S., Fan J., Renganathan M., Imredy J., Humphries E.S.A, Brinkwirth N., Strassmaier T., Ohtsuki A., Danker T., Vanoye C., Poloncuk L., Fermini B., Pierson J.B. & Gintant G.Automated patch clamp (APC) instruments enable efficient evaluation of electrophysiologic effects of drugs on human cardiac currents in heterologous expression systems. Differences in experimental protocols, instruments, and dissimilar site procedures affect the variability of IC50 values characterizing drug block potency. This impacts the utility of APC platforms for assessing a drugs cardiac safety margin. We determined variability of APC data from multiple sites that measured blocking potency of 12 blinded drugs (with different levels of proarrhythmic risk) against four human cardiac currents (hERG [IKr], hCaV1.2 [L-Type ICa], peak hNaV1.5, [Peak INa], late hNaV1.5 [Late INa]) with recommended protocols (to minimize variance) using five APC platforms across 17 sites. IC50variability (25/75 percentiles) differed for drugs and currents (e.g., 10.4-fold for dofetilide block of hERG current and 4-fold for mexiletine block of hNaV1.5 current). Within-platform variance predominated for 4 of 12 hERG blocking drugs and 4 of 6 hNaV1.5 blocking drugs. hERG and hNaV1.5 block. Bland-Altman plots depicted varying agreement across APC platforms. A follow-up survey suggested multiple sources of experimental variability that could be further minimized by stricter adherence to standard protocols. Adoption of best practices would ensure less variable APC datasets and improved safety margins and proarrhythmic risk assessments.Publication link2020 Reengineering an Antiarrhythmic Drug Using Patient hiPSC Cardiomyocytes to Improve Therapeutic Potential and Reduce ToxicityPatchlinerandSyncroPatch 768PE (a predecessor model of the SyncroPatch 768i instrument) Publication in Cell Stem Cell (2020) Authors: McKeithan W. L., Feyen D.A.M., Bruyneel A.A.N., Okolotowicz K.J., Ryan D.A., Sampson K.J., Potet F., Savchenko A.,Gmez-Galeno J., Vu M., Serrano R., George Jr. A.L., Kass R.S., Cashman J.R., Mercola M.Modeling cardiac disorders with human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes is a new paradigm for preclinical testing of candidate therapeutics. However, disease-relevant physiological assays can be complex, and the use of hiPSC-cardiomyocyte models of congenital disease phenotypes for guiding large-scale screening and medicinal chemistry have not been shown. We report chemical refinement of the antiarrhythmic drug mexiletine via high-throughput screening of hiPSC-CMs derived from patients with the cardiac rhythm disorder long QT syndrome 3 (LQT3) carrying SCN5A sodium channel variants. Using iterative cycles of medicinal chemistry synthesis and testing, we identified drug analogs with increased potency and selectivity for inhibiting late sodium current across a panel of 7 LQT3 sodium channel variants and suppressing arrhythmic activity across multiple genetic and pharmacological hiPSC-CM models of LQT3 with diverse backgrounds. These mexiletine analogs can be exploited as mechanistic probes and for clinical development.Publication link2018 An update on the advancing high-throughput screening techniques for patch clamp-based ion channel screens: implications for drug discoverySyncroPatch 384/768PE(a predecessor model of SyncroPatch 384/768i) andPatchliner Publication in Expert Opinion on Drug Discovery Authors: Obergrussberger A., Goetze T.A., Brinkwirth N., Becker N., Friis S., Rapedius M., Haarmann C., Rinke-Wei I., Stlzle-Feix S., Brggemann A., George M., Fertig N.Introduction:Automated patch clamp (APC) devices have become commonplace in many industrial and academic labs. Their ease-of-use and flexibility have ensured that users can perform routine screening experiments and complex kinetic experiments on the same device without the need for months of training and experience. APC devices are being developed to increase throughput and flexibility. Areas covered:Experimental options such as temperature control, internal solution exchange and current clamp have been available on some APC devices for some time, and are being introduced on other devices. A comprehensive review of the literature pertaining to these features for the Patchliner, QPatch and Qube and data for these features for the SyncroPatch 384/768PE, is given. In addition, novel features such as dynamic clamp on the Patchliner and light stimulation of action potentials using channelrhodosin-2 is discussed. Expert opinion:APC devices will continue to play an important role in drug discovery. The instruments will be continually developed to meet the needs of HTS laboratories and for basic research. The use of stem cells and recordings in current clamp mode will increase, as will the development of complex addons such as dynamic clamp and optical stimulation on high throughput devices.Publication link2020 A systematic strategy for estimating hERG block potency and its implications in a new cardiac safety paradigmPatchliner andSyncroPatch 384PE (a predecessor model of the SyncroPatch 384i instrument) Publication in Toxicology and Applied Pharmacology (2020) Authors: Ridder B.J., Leishman D.J., Bridgland-Taylor M., Samieegohar M., Han X, Wu W.W., Randolph A., Tran P., Sheng J., Danker T., Lindqvist A., Konrad D., Hebeisen S., Polonchuk L., Gissinger E., Renganathan M., Koci B., Wei H., Fan J., Levesque P., Kwagh J., Imredy J., Zhai J., Rogers M., Humphries E., Kirby R., Stoelzle-Feix S., Brinkwirth N., Rotordam M.G., Becker N., Friis S., Rapedius M., Goetze T.A., Strassmaier T., Okeyo G., Kramer J., Kuryshev Y., Wu C., Himmel H., Mirams G.R., Strauss D.G., Bardenet R., Li Z.Highlights: A new regulatory paradigm promotes the integration of nonclinical and clinical data. Lack of uncertainty quantification hindered using hERG potency in the new paradigm. A systematic method was established to address this limitation. Analysis supports using different safety margin thresholds in different context. Abstract:- Introduction hERG block potency is widely used to calculate a drug's safety margin against its torsadogenic potential. Previous studies are confounded by use of different patch clamp electrophysiology protocols and a lack of stat

Patchliner.Tutorial video2018 Igor Tutorial for Patchliner Data AnalysisPresenter: Dr. Nadine Becker, Product Manager Patchliner, Nanion Technologies GmbH, GermanyThis Igor video tutorial for Patchliner data analysis demonstrates how concentration-response curves are quickly displayed and analyzed. We show how data are loaded and displayed, and the normalized data are fitted and averaged to obtain IC50 values. Please note that more details and features are described in our QuickStart Guide for Igor.User meeting video15.10.2020 | Webinar: Evaluation of possible proarrhythmic potency: variability of IC50 values of drugs under different conditions and in different platformsDr. Pter Orvos (University of Szeged), This is an on-demand webinar from Nan]i[on and Friends 2020.The conventional microelectrode technique and the manual patch clamp method offer direct, information-rich, and real-time in vitro technologies to study proarrhythmic effect of drugs and drug candidate compounds. Although providing excellent data quality, these tests are complicated, time consuming and expensive for the large numbers of compounds. Automated patch-clamp platforms are mainly used with stably expressing cell lines and suitable for rapid and high-quality pharmacological investigation of drug candidates. The Comprehensive in Vitro Proarrhythmia Assay (CiPA) was initiated to further improve these preclinical drug safety paradigms. However, some evidence indicates that the different proarrhythmic pharmacological assays result in contradictory outcomes raising serious questions regarding their predictability for in vivo situations including clinical settings. IC50 values may varied between platforms, therefore, aim of our study was to compare the effect of proarrhythmic compounds on hERG and IKr currents and on cardiac action potential. The hERG current was measured by using both automated and manual patch clamp methods on HEK293 cells. The native ion current (IKr) were recorded from rabbit ventricular myocytes by manual patch clamp technique.Dofetilide, cisapride, sotalol, terfenadine and verapamil were tested in hERG assay at both room temperature and 37C with Patchliner. All these compounds were more potent at physiological temperature and therefore, it is a desirable option to study hERG currents at physiological temperature. To evaluate the prognostic value of hERG assay these agents were subjected for further investigations. The IKr current blocking capability of the compounds was tested on rabbit ventricular myocytes with manual patch clamp method at 37C. The corresponding IC50 values of dofetilide, cisapride and verapamil were in good agreement with IC50 values obtained with Patchliner in hERG assays. As sotalol and terfenadine have stronger effect on IKr measured by manual patch clamp method compared with hERG automated patch clamp experiments, the effects of these drugs on hERG current using manual patch clamp technique were also investigated to study how the potency of these drugs are influenced by the experimental techniques themselves. In contrast with the hERG automated patch clamp assays, the effects of sotalol and terfenadine on hERG current were stronger measured by the manual patch-clamp technique.In conclusion, results obtained with automated patch-clamp equipment in HEK-hERG cells usually show a reasonable conformity with outcomes of IKr current experiments. The Patchliner system used in our study is well suited to perform safety pharmacological studies. Variability of IC50 values of drugs in different platforms observed in certain cases, which could have been caused by the lack of continuous flow of compound-containing solutions.Product videoPatchliner- 2021 (Product video demo)The Patchliner is a fully automated planar patch clamp instrument recording from up to 8 cells simultaneously.The Patchliner is a fully automated planar patch clamp instrument recording from up to 8 cells simultaneously. With its vast experimental freedom and gigaseal data quality, the Patchliner is one of the most versatile patch clamp instruments on the market.Product videoPatchliner- 2022 (Product video demo)The Patchliner is a fully automated planar patch clamp instrument recording from up to 8 cells simultaneously.The Patchliner is a fully automated planar patch clamp instrument recording from up to 8 cells simultaneously. With its vast experimental freedom and gigaseal data quality, the Patchliner is one of the most versatile patch clamp instruments on the market. The Patchliner is versatile yet robust, ideal for basic research of ion channel biophysics and mechanisms of action, and sophisticated assays including heat activation of TRP channels, activation of Ca2+-activated channels by internal exchange and fast external solution exchange with minimal exposure for ligand-gated ion channels such as nACh7. The Patchliner is also an excellent tool for routine assays such as safety screening of hERG or other cardiac ion channels in line with the CiPA initiative. Since its introduction in 2006, over 100 instruments have been installed worldwide, in academic labs (46%), pharmaceutical companies (34%) and CROs (20%). The Patchliner is appreciated because of routine high success rates (greater than 80% gigaseals), optimized assays including minimized cell consumption and proven use of primary cells and stem cells. Our dedicated team of electrophysiologists and engineers are committed to continuous in-house assay development, software and hardware advancements, ensuring fast and custom-tailored solutions for your assay demands.Poster PDF2022 Using single-chain variable fragments (scFv) to map the 3-subunit binding site on the pain-sensing sodium channel Nav1.7Patchliner Poster, Cambridge Ion Channel Forum 2022Product Sheet PDFPatchliner Product SheetFlyer PDFPatchliner Product Flyer Safety EditionPoster PDF2020 Automated Patch Clamp System Introducing Simulated Ik1 Into stem cell derived Cardiomycoytes Using Dynamic ClampPatchliner poster, 64th Annual Meeting of the Biophysical SocietyFlyer PDFPatchliner Product Flyer Dynamite8Flyer PDFPatchliner Product Flyer PatchControl HT Release 2021Application Note PDFTRPV3 Heat activation of TRPV3 on Nanions PatchlinerPatchliner application note: The transient receptor potential cation channel,subfamily V, member 3 (TRPV3), is a ligand-gated, nonselectivecation channel first described in 2002. Itexhibits 43% sequence identity to TRPV1. AlthoughTRPV3 has been detected immunologically in the CNSand suggested to be often co-localized with TRPV1, it isfound more robustly in keratinocytes in skin and, given itsthreshold for temperature activation of >34C, it hasbeen speculated that TRPV3 may act in co-operationwith sensory afferents to perceive warmth and signalelevated temperature. TRPV3 can also be activated bythe ligand 2-Aminoethoxydiphenyl borate (2-APB). TheTRPV3 channel, along with other TRPV channels, mayplay an important role in chronic pain and, therefore, isreceiving increasing attention as a potential therapeuticcandidate for the treatment of chronic pain.Here we present data collected on a 4-channel Patchlinerwith temperature control showing the potential use of thePatchliner to record TRPV3 currents activated by 2-APBor heat. As previously reported, TRPV3 currents sensitizeto repeated applications of 2-APB or heat, a phenomenonwe also observed. At low concentrations of 2-APB,the currents were primarily outwardly rectifying but athigher concentrations and with prolonged exposure theyoften became dual rectifying (data not shown). This isalso in good agreement with the literature. In contrast,the temperature-activated responses were always outwardlyrectifying with little inward current. The inward currentsactivated by 2-APB could be blocked by rutheniumred (RR) as expected.Flyer PDFPatchliner Product Flyer Cooling PlateApplication Note PDFTRPM8 TRPM8 activation by menthol and Eucalyptol performed on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Charles River.TRPM8 is a member of the transient receptor potentialchannel (TRP) family. TRPM8 is known to be a thermosensitivechannel, activated by cold temperatures(below ~25C) and ligands such as menthol, Eucalyptoland icilin. It belongs to the melastatin subfamily of TRPchannels and shows an outward rectification with arelatively high permeability for calcium ions andlittle selectivity between monovalent cations. Menthol,a secondary alcohol produced by the peppermintherb, Mentha piperita, is widely used in the food andpharmaceutical industries as a cooling/soothingcompound and odorant. It induces Ca2+influx in asubset of sensory neurons from dorsal root and trigeminalganglia, where the TRPM8 channel is specificallyexpressed.Here we present data of hTRPM8 collected on thePatchliner. Cells performed well on the Patchliner with a success rate of 89% for seal resistance >600 M.Channel activation by the agonists menthol andEucalyptol is shown.Application Note PDFTRPV1 Heat activation of TRPV1 on Nanions PatchlinerPatchliner application note: The transient receptor potential cation channel, subfamilyV, member 1 (TRPV1), is a ligand-gated, non-selectivecation channel widely expressed in the peripheraland central nervous system. The TRPV1 channel canbe activated by a number of physical and chemicalstimuli, including capsaicin (the active ingredient in chilipeppers), noxious heat (typically >42C) and low pH. TheTRPV1 channel is putatively involved in the perceptionand transmission of painful stimuli. Importantly, thischannel is proposed to underlie many chronic pain statesincluding inflammation, neuropathic pain and cancerpain, amongst others. These types of pain states arecurrently poorly managed by the pain medicationsavailable and this has led the pharmaceutical industryto seek novel targets for pain management, such asTRPV1. However, TRPV1 antagonists have so far failedin clinical trials due to an undesirable increase in corebody temperature resulting in hyperthermia. Fromthese studies, it is proposed that tonically active TRPV1channels are involved in maintaining normal bodytemperature and this could have significant influenceson drug design. Finding novel compounds with differingeffects on capsaicin activation and heat activation maybe crucial in the discovery of lead compounds for thetreatment of pain and other disease states.Here we present data collected on the 4-channelPatchliner with temperature control showing thepotential use of the Patchliner to record capsaicin andheat activation of TRPV1 channels and subsequent blockby ruthenium red (RR).Application Note PDFP2X3 Activation and inhibition of P2X3 channels recorded on the PatchlinerPatchliner application note: Cells were engineered and kindly provided by Axxam S.p.A., Milan.P2X receptors are ligand-gated ion channels that open in response to extracellular ATP. They are permeable to small monovalent cations, some having significant divalent or anion permeability. P2X receptors are found on many cell types including smooth muscle cells, sensory neurones, epithelia, bone and leukocytes. A role for P2X receptors has been suggested in transmission of thermal stimuli, chemosensory signalling, taste and pain. To date, 7 P2X receptor genes have been cloned and studied in heterologous expression systems. Functional receptors are trimeric, which can be homomeric or heteromeric. The P2X2and P2X3receptors can function either as homomers or as P2X2/3heteromers. When expressed together, a mixture of P2X2and P2X3homomers as well as P2X2/3heteromers are likely to exist, which may be distinguished through their biophysical and pharmacological properties. Both P2X3homomers and P2X2/3heteromeric receptors have been implicated in nociception and pain signalling and may be important therapeutic targets for analgesic drugs. The P2X3and P2X2/3receptor antagonist MK-7264 (gefapixant), has recently progressed to Phase III trials for refractory or unexplained chronic cough.Here we present data collected on the Patchliner showing activation and inhibition of P2X3currents expressed in CHO cells with rapid and brief application of ligand (using the stacked solution approach). -methylene ATP (-MeATP) activated P2X3 receptors in a concentrationdependent manner with an EC50 value similar to those in the literature. P2X3receptors could be repetitively activated by -MeATP and blocked by A-317491 with an IC50value in good agreement with the literature.Application Note PDFNeurons Stem cell-derived neurons recorded on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Cellular Dynamics.Cellular Dynamics International is developing iCellNeurons, human iPS cell-derived neurons. These neurons have been used on Nanions Patchliner, an automated patch clamp device for recording from up to 8 cells simultaneously. Cells were thawed and cultured as per the manufacturers instructions. After plating, cells developed neuronal outgrowth within 24 hours and could be kept in culture for 2 weeks. An investigation into voltage- and ligand-gated ion channels expressed in these cells was undertaken using the Patchliner. The first experiments are shown here and offer promising results for combining a cellular model for neurons and an increased throughput patch clamp device.Application Note PDFP2X2 / P2X3 P2X2/3 receptors recorded on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Evotec.P2X receptors are ligand-gated ion channels that open in response to extracellular ATP. They are permeable to small monovalent cations, some having significant divalent or anion permeability. P2X receptors are found on many cell types including smooth muscle cells, sensory neurones, epithelia, bone and leukocytes. A role for P2X receptors has been suggested in transmission of thermal stimuli, chemosensory signalling, taste and pain. To date, 7 P2X receptor genes have been cloned and studied in heterologous expression systems. Functional receptors are trimeric, which can be homomeric or heteromeric. The P2X2and P2X3receptors can function either as homomers or as P2X2/3heteromers. When expressed together, a mixture of P2X2and P2X3homomers as well as P2X2/3heteromers are likely to exist, which may be distinguished through their biophysical and pharmacological properties. P2X2/3receptors have been implicated in nociception and pain signalling and may be important therapeutic targets for analgesic drugs.Here we present data collected on a 4- or 8-channel Patchliner showing the potential use of the Patchliner to record P2X2/3currents activated by ATP. ATP activated P2X2/3receptors in a concentration-dependent manner with an EC50similar to those reported in the literature for a mixture of homomeric and heteromeric P2X2/3receptors. The currents desensitized slowly confirming that they are mediated by P2X2and P2X2/3receptors rather than P2X3receptors. P2X2/3receptors could be repetitively activated by ATP and blocked by suramin with an IC50in good agreement with the literature.Application Note PDFNaV1.8 Characterization of rNaV1.8 (ND7-23) on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Millipore.The NaV1.8 gene (originally named PN3 or SNS; gene symbol SCN10A) encodes a voltage-gated sodium (NaV) channel, selectively expressed in dorsal root ganglion (DRG) neurons. DRGs transmit peripheral stimuli to the central nervous system and are involved in nociception. Different NaVchannels play a key role in modulation of DRG action potentials. In particular, the fast upstroke of the action potential is mediated by NaVchannels. NaVchannels are in part characterized by their TTX-sensitivity (TTX-resistant [TTXr], TTX-sensitive [TTXs]). NaV1.8 is a TTXr channel. Compared with other NaVchannels, NaV1.8 has slow activation and inactivation kinetics and is opened at relatively high voltages. It is an interesting drug target for inflammatory and neuropathic pain, because modulation of NaV1.8 by inflammatory mediators seems to be a key mechanism of DRG nociceptor sensitization and activation. Interestingly, NaV1.8 has been reported to play an important role in the perception of cold pain. In this Application Note we present data recorded on the Patchliner characterizing ND7-23 cells (a rat DRG/mouse neuroblastoma hybrid) stably transfected with rat NaV1.8. All experiments were performed in the presence of 100 nM TTX to block the endogenous TTXs Na+current present in these cells. The NaV1.8 activation and inactivation properties and tetracaine sensitivity recorded on the Patchliner were consistent with those reported in the literature.Application Note PDFNeurons Peri.4U and Dopa.4U stem cell-derived neurons recorded on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Axiogenesis.Human induced pluripotent stem cell-derived neurons(hiPSC-neurons) may provide a viable cellular modelfor studying the mechanisms underlying neurologicaldiseases and drug development. Axiogenesis providesa number of hiPSC-neurons including dopaminergicneurons (Dopa.4U) and peripheral neurons (Peri.4U),amongst others. These neurons have been used onin-vitro systems such as multielectrode arrays (MEA),immunocytochemistry and calcium imaging. They arean interesting model for studying neurological diseasessuch as Parkinsons Disease, as well as for efficacy, drugdiscovery and toxicity studies.In this study, the Patchliner was used to record fromDopa.4U and Peri.4U neurons in voltage and currentclamp modes. The cell harvesting procedure wasoptimized (using papain) to ensure that the cells retainedproximal dendrites and initial axon segments in order tomaintain ion channel expression present in these regions.Due to their irregular shape (presence of processes),success rate (typically 20 - 50% for RSeal >200 M) waslower than other cell types which have a smooth, roundshape, e.g. standard cell lines. Voltage-gated Na+(NaV)and K+(KV) currents were recorded in both cell types.Action potentials (AP) were also recorded and block ofthe AP of Dopa.4U cells by lidocaine is shown.Application Note PDFKV1.3 Pharmacological analysis of heterologous and endogenous expressed KV1.3 channels in Sf21 insect cells and T-lymphocytesPatchliner application note: Cells were kindly provided by conoGenetix.The voltage-gated potassium channel KV1.3 is expressed in human naive, central memory T cells (TCM), and effector memory T cells (TEM). TEM cells are important mediators of a variety of chronic inflammatory autoimmune diseases including multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis, and vasculitis. Antigenic stimulation of TEM cells results in upregulated KV1.3 channel expression. It is proposed that a selective block of KV1.3 channels leads to an inhibition of functionality of TEM cells without affecting the function of naive and TCM cells. Thus, KV1.3 is a promising therapeutic target that could be used to efficiently medicate chronic autoimmune diseases without generating typical side effects associated with todays immunosuppressive therapies. In the laboratory of conoGenetix biosciences the insect cell line Spodoptera frugiperda (Sf21) is used in combination with a Baculovirus infection system for the heterologous expression of recombinant ion channels. Sf21 cells offer highly specific protein expression and correct posttranslational modifications resulting in functional channels with comparable electrophysiological parameters of ion channels in vivo. In this Application Note we compare the electrophysiological properties of the potassium selective ion channel KV1.3 expressed in Sf21 cells and endogenously expressed KV1.3 channels in TEM cells. For the pharmacological analysis the 4 - channel Nanion Patchliner and one of our innovative, KV1.3 specific, proprietary peptides was used.Application Note PDFNaV1.7 Pharmacology on hNaV1.7 performed on Nanions Patchliner at Vhalf Patchliner application note: Cells were kindly provided by Anaxon.The NaV1.7 gene (SCN9A) encodes a voltage-gated sodium (NaV) channel, primarily expressed in the peripheral nervous system and has been isolated from rat dorsal root ganglion (DRG) neurons, human medullary thyroid cancer cells (hNE-Na) and PC12 cells. Different NaVchannels play a key role in modulation of action potentials in the central and peripheral nervous systems. In particular, the fast upstroke of the action potential is mediated by NaVchannels.NaVchannels are in part characterized by their TTX-sensitivity (TTX-resistant [TTXr], TTX-sensitive [TTXs]). NaV1.7 is a TTXs channel and is sensitive to TTX in the nanomolar range. The role of hNaV1.7 has yet to be fully elucidated but is proposed to play an important role in nociception and pain sensing. NaV1.7 has been implicated to play a role in disease pain states, in particular inflammatory pain and hypersensitivity to heat following burn injury. Common to many of the voltage-gated ion channels, a number of compounds display a higher affinity for the inactivated state of the channel. For this reason, it is important to be able to reliably measure the effects of compounds at Vhalf of inactivation, the voltage at which 50% of the channels are inactivated. In this Application Note we present data using an 8-channel Patchliner characterizing CHO cells stably expressing hNaV1.7. The hNaV1.7 activation and inactivation properties are consistent with those reported in the literature. The potency of sodium channel blockers mexiletine, tetracaine, amitriptyline and lidocaine were compared using a holding potential of -120 mV vs the Vhalf of inactivation.Application Note PDFhERG Effect of temperature on erythromycin action on hERG currents recorded on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Millipore.The hERG gene encodes a potassium channel responsible for the repolarization of the IKrcurrent in cardiac cells (Sanguinetti et al, 1995). This channel is important in the repolarization of the cardiac action potential. Abnormalities in this channel can lead to long or short QT syndrome, leading to potentially fatal cardiac arrhythmia. Given the importance of this channel in maintaining cardiac function, and disturbances of channel activity by certain compounds such as anti-arrhythmias and anti-psychotics, it has become an important target in compound safety screening. Compounds can display different properties or different potencies at physiological temperature (35C) vs. room temperature (RT) and therefore, it is a desirable option to be able to study this channel electrophysiologically at elevated temperature. One such compound which has been shown to have an increase in potency at physiological temperature is erythromycin. Erythromycin is a macrolide antibiotic which can cause QT prolongation and cardiac arrhythmia. Erythromycin has been shown to block hERG channels at physiological temperature with an IC50of approximately 40 mM (Stanat et al, 2003; Duncan et al, 2005). However, at RT erythromycin is much less potent. At a concentration of 100 mM, erythromycin causes no significant block of hERG currents at RT but significantly blocks currents at physiological temperature (Guo et al, 2005). Here we present data collected on an 8-channel Patchliner with temperature control at RT and at 35C and the effect this has on the potency of erythromycin.Application Note PDFKCa3.1 Modulation of hKCa3.1 by internal Ca2+ performed on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Charles River.The intermediate-conductance calcium-activated K+channel, also known as KCa3.1, IKCa1 or SK4, is a member of the large family of potassium channels gated by calcium. It can be distinguished from the other calcium-activated K+channels by differences in channel conductance, calcium sensitivity, voltage dependence and pharmacological properties. The hKCa3.1 channel is encoded by the KCNN4 gene. It is primarily expressed in peripheral tissues, including those of the hematopoietic system, colon, lung, placenta, and pancreas and has been proposed to play an important role in a variety of physiological processes including volume regulation in erythrocytes, proliferation and differentiation of B- and T-lymphocytes, and tissue protection following spinal cord injury. Importantly, the hKCa3.1 channel is a promising therapeutic target for a variety of health disorders including sickle cell anaemia and immunological disorders. The Ca2+-binding protein, calmodulin (CaM), is required for the activation of hKCa3.1. The Ca2+-CaM complex is proposed to bind to an intracellular domain of the C terminus of all subunits in the tetramer, inducing conformational changes to open the channel. In this study the Patchliner was used to perform a biophysical and pharmacological characterization of hKCa3.1 channels expressed in CHO cells. hKCa3.1 was activated by exchanging the internal solution to a solution containing free-Ca2+and blocked by external application of non-selective (BaCl2) and selective (TRAM-34) inhibitors with an IC50value consistent with that reported in the literature.Application Note PDFGABAA (a1b2g2) Rapid agonist applications in precise intervals (GABAA) Patchliner application note: HEK-cells expressing GABAA-receptors were investigated with the Patchliner using a stacked application approach for rapid administration of compounds to the patch-clamped cells. The GABAAreceptor family is the most important class of inhibitory ion channels involved in synaptic transmission, and are selectively permeable to monovalent anions. They constitute an important therapeutic area for drugs affecting anxiety, sleep and muscle relaxation. As with most ligand gated ion channels, GABAAexhibit receptor desensitization, which is a common phenomenon for ligand gated ion channels. Desensitization can be either exposure time dependent or concentration dependent, or both. Desensitization and recovery kinetics varies from milliseconds to tens of minutes, all depending on receptor type and subunit composition. For rapidly desensitizing ion channels, it is important that compound application is fast, so that the entire ion channel population is exposed to maximum concentration before entering the desensitized state. Exposure time and application intervals are important factors affecting desensitization and recovery from desensitization, to minimize deletrious effects or receptor desensitization. In the experiments presented here, compounds were added to the cells with accurate timing. In addition, a method for brief compound application was developed, allowing 1 s pulses of compound to the patch clamped cells. In this way, receptor desensitization can be minimized, and because of the timed exposures, effects of desensitization.Application Note PDFGlycine receptors (GlyRa1) Modulators for glycine receptors investigated using the Patchliner Patchliner application note: Cells were kindly provided by AstraZeneca.The Patchliner was used to study the pharmacology of the hGlyR a1 receptor expressed in mouse fibroblast cell line (L-tk). The Patchliner allows for fast application of drugs (50 ms) with precisely controlled application intervals and wash times. These are important experimental parameters when investigating ligand gated ion channels and their effectors since many ligand gated ion channels rapidly desensitize. Kinetics and level of desensitization are determined by ligand concentration, exposure time, or both. Because of the Patchliner's rapid solution exchange combined with brief drug exposure, capabilities and timed application intervals, the deleterious effect caused by receptor desensitization can be minimized and corrected for. Here we show the use of Nanion's Patchliner for long (22 s) and short (1 s) application of glycine to patch clamped whole-cells. For the short applications of compound, a stacked solution application protocol was used. Specifically, two zones of solution were aspirated into the pipette used for administration. When applied to the cell, this results in exposure first to the agonist zone followed by a rapid wash out after 1 s of drug application. In all the protocols used, pre-incubation of modulators is possible, as well as washout in the presence of a modulator.Application Note PDFCFTR Effect of internal F- on activation of Cystic Fibrosis Transmembrane Conductance (CFTR) regulator by forskolin (report)Patchliner application report: The aim of this study was to investigate the effect of internal F-on the activation of the cystic fibrosis transmembrane conductance regulator (CFTR) by forskolin in the whole cell patch clamp configuration. Experiments were conducted with Nanions fully automated patch clamp device, the Patchliner.Application Note PDFGABAA (a1b2g2) Patchliner Investigating the pharmacology of GABAA receptors expressed in HEK293 cellsPatchliner application note: -amino butyric acid (GABA), -alanine and bicuculline were investigated on GABAA-receptors expressed in HEK293 cells. The GABAAreceptor family is the most important class of inhibitory ion channels involved in synaptic transmission. GABAAreceptors are selectively permeable to monovalent anions. They constitute an important therapeutic target for drugs affecting anxiety, sleep and muscle relaxation. In this study, the cells were exposed to compound for 30 s, followed by a 60 s wash step. Solution exchange around the cell was fast, in the order of 50 ms for saturating concentrations. Complete and rapid switching is important when investigating ligand gated ion channels, since the response is often very fast and most receptors desensitize. For rapidly desensitizing ion channels, fast compound application is crucial, so that the entire ion channel population is exposed to maximum concentration before entering the desensitized state. Desensitization and recovery kinetics vary from milliseconds to tens of minutes, depending on receptor type and subunit composition as well as exposure time and/ or compound concentration. Recordings were made in the whole-cell configuration, using the Patchliner (4 amplifier channels). Expected pharmacology was obtained for the investigated compounds.Application Note PDFCaV2.2 Characterization of CaV2.2 (HEK293) on Nanions PatchlinerPatchliner application note: The voltage gated N-type calcium channel (CaV2.2) is encoded by the gene CACNA1B. CaV2.2 is a high voltage activated calcium channel. CaV2.2 is found mainly in the brain, where it mediates neurotransmitter release at the synapse. The strong depolarization of neuronal action potentials causes the opening of the channel. Calcium can then enter the cell and initiates the fusion of the neurotransmitter vesicles with the membrane. CaV2.2 is inhibited by w-conotoxin, a neurotoxin of the fish hunting snail, with high specificity. CaV2.2 has been implicated in the transmission of pain. Pharmacological block of CaV2.2 by compounds based on w-conotoxin has been shown to be effective against strong chronical pain. The biophysical and pharmacological properties of the cells are presented in this Application Note.Application Note PDFCaV3.2 Characterization of CaV3.2 (HEK293) on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Millipore.The gene CACNA1H encodes the 1H subunit of the voltage-gated calcium channel CaV3.2. It belongs to the low voltage-activated T-type calcium channels. CaV3.2 displays the typical characteristics of the T-type channels: activation at low depolarization of the membrane and transient kinetics. T-type Ca2+channels are involved in diverse, mainly rhythmic processes like e.g. pacemaking and generation of thalamocortical rhythms in sleep or epilepsy. CaV3.2 is expressed in a wide variety of cells. Amongst others it has been found in kidney, smooth muscle, brain, adrenal and cardiac cells. It seems to be involved in contraction of smooth muscle and the secretion of the adrenal hormones aldosterone and cortisol. Pharmacological block of T-type channels may lead to new drugs for the treatment of hypertension and epilepsy. The biophysical and pharmacological properties of the cells are presented in this Application Note.Application Note PDFCardiomyocytes Voltage and current clamp recordings of Cellartis hiPS-CM on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Takara Bio Europe AB.Human induced pluripotent stem (iPS) cell-derived cardiomyocytes have the potential to provide the ultimate model system for identifying potential anti-arrythmic effects of drugs during routine safety screening. Takara Bio Europe AB is providing a human iPS cell-derived cardiomyocyte product line for use in testing the efficacy and safety of pharmaceutical therapies. The ability to characterize the ion channel profile of these cells and record action potentials at a reasonable throughput is essential to fully realise the potential of this kind of product line. Building on the success of recording stem cells on the Patchliner, CellartishiPS-CM, human iPS cell-derived cardiomyocytes, have now been characterized on the Patchliner in the voltage and current clamp mode. In this Application Note we present data using an 8-channel Patchliner. In the voltage clamp mode, voltage-dependent Na+(NaV), K+(KV) and Ca2+(CaV) channel currents were recorded. As expected, action potentials could be elicited in the current clamp mode. Furthermore, spontaneous action potentials could be recorded as well.Application Note PDFCardiomyocytes Voltage and current clamp recordings of Cor.4U human iPS cell-derived cardiomyocytes on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Axiogenesis.Although mouse embryonic stem (ES) cell-derived cardiomyocytes, e.g. Cor.Atcells from Axiogenesis, can provide a useful model for drug discovery and safety testing as an alternative to acutely dissociated rat or mouse cardiomyocytes, human induced pluripotent (iPS) cell-derived cardiomyocytes have the potential to provide the ultimate model system for identifying potential antiarrythmic effects of drugs during routine safety screening. Axiogenesis has now launched the Cor.4Uhuman iPS cell-derived cardiomyocyte product line for use in testing the efficacy and safety of pharmaceutical therapies. The ability to characterize the ion channel profile of these cells and reliably record action potentials at a reasonable throughput is essential to fully realise the potential of this kind of product line. Building on the success of Cor. Atmouse embryonic stem (ES) cell-derived cardiomyocytes on the Patchliner, Cor.4Uhuman iPS cell-derived cardiomyocytes have now been characterized on the Patchliner in the voltage and current clamp modes. In this Application Note we present data using an 8-channel Patchliner characterizing Cor.4Ucells. In the voltage clamp mode, voltage-dependent Na+(NaV), K+(KV) and hERG (an inward current using a high K+-containing external solution) channel currents were recorded (Fig. 1). When the Ca2+channel agonist BayK 8644 was used a voltage-gated Ca2+(CaV) current could be recorded. As expected, action potentials could be elicited in the current clamp mode. The effect of the compounds TTX and BayK 8644 on the action potentials evoked in Cor.4U cells is also shown.Application Note PDFASIC3 Characterization of hASIC3 (HEK) on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Millipore.Acid-sensing ion channels (ASICs) are ligand-gated ionchannels activated by protons and are members ofthe sodium-selective cation channels belonging to theepithelial sodium channel/degenerin (ENaC/DEG)family. They are highly sensitive to extracellular acidosis.In rodents, where they are mainly expressed inneurons of the peripheral nervous system, ASIC3 playsan important role as sensor of non-adaptive pain whichis correlated to tissue acidosis. However, the role of thehuman ASIC3 channel has not yet been elucidated.In contrast to other ASIC ion channels, ASIC3 shows asustained window current upon external acidification.Here we present data recorded on an 8-channelPatchliner. Current responses upon external acidificationas well as amiloride block of acidosis-induced currents ofhASIC3-expressing HEK293 cells are shown.Application Note PDFCardiomyocytes Recordings of action potentials inmouse ES cell-derived Cor.At cardiomyocytes on Nanions PatchlinerPatchliner application note: Cells were kindly provided byAxiogenesis.Cor.Atcardiomyocytes display typical cardiac ion channel activity and action potentials. Both the hERG blockers Quinidine and Cisapride, and the Na+-channel blocker Lidocaine, modulated the action potentials. The Quinidine and Lidocaine effects were reversible, the Cisapride effect was non-reversible. The results demonstrate the presence of an array of ion channels in Cor.Atcardiomyocytes which in conjunction are capable of generating action potentials. Hence these stem cell-derived cardiomyocytes are a suitable alternative to primary cardiomyocytes in drug screening and safety testing. In addition, these experiments demonstrate for the first time the suitability of a higher throughput planar patch clamp system, i.e. Nanions Patchliner, for recording action potentials. This is possible because of the flexibility of Nanions patch clamp systems allowing for a multitude of different experiments to be performed in both voltage and current clamp modes. Cells expressing multiple ion channels and therefore able to elicit action potentials, as opposed to cell lines overexpressing a single ion channel subtype, better represent the physiological system. Thus, the Patchliner in combination with Cor.Atcardiomyocytes form a powerful tool for ion channel research, drug screening and safety testing.Application Note PDFAcetylcholine Receptor Alpha 7 Human a7 nicotinic Acetylcholine Receptor on the Patchliner Patchliner application note: Cells were kindly provided by Galantos.The human a7 nicotinic acetylcholine receptor expressing cells (stable) from Galantos Pharma GmbH were tested on the Patchliner. Repeated stimulations with 100 M nicotine were tested to validate the system. In a run, six out of eight cells could be measured. All six cells showed characteristic a7 currents. Average peak currents for the shown experiment were 0.9 0.2 nA (n = 6) which is consistent with manual patch clamp results reported by Galantos Pharma.Application Note PDFAMPA receptor (GluA2) Activation, potentiation and inhibition of AMPA receptors on the PatchlinerPatchlinerapplication note:AMPA receptors are cation-permeable ionotropicglutamate receptors of the non-NMDA receptor subfamily.To date four subunits, GluA1-4, have been identifiedwhich are of similar size (approx. 900 kDa) andshare 68-73% amino acid sequence identity. The humanGluA2 subunit is encoded by the GRIA2 gene located onthe 4q32-33 chromosome. Each of the 4 subunits has fourdistinct domains: an extracellular amino acid terminaldomain (ATD); the extracellular ligand binding domain(LBD); the transmembrane domain (TMD) with 3 transmembranesegments (M1, M3 and M4) and 1 cytoplasmicfacing re-entrant loop (M2); and an intracellular carboxyterminaldomain. The functional receptor exists as atetramer, either as homomers or heteromers (GluA1 andGluA4). The vast majority of excitatory fast synaptictransmission in the mammalian central nervous system ismediated by AMPA receptors of differing subunitcombinations. It is well known that glutamate is aneurotoxin and it is proposed that overactivation ofionotropic glutamate receptors may underlie manyneurodegenerative disorders such as ischemicstroke, epilepsy, Parkinsons and dementia,amongst others. Enhancement of AMPA receptoractivation by, for example, BDNF, has beenproposed to have beneficial effects on learning andmemory and has potential therapeutic value in the treatmentof depression, Huntingtons and Parkinsons diseases.Here we present data collected on an 8-channel Patchlinershowing recordings of GluA2-mediated currents.Glutamate activated GluA2 receptors with an EC50similarto those reported in the literature. CNQX inhibited andLY404187 enhanced GluA2-mediated responses.Application Note PDFNaV1.5-Late INa-Late recorded from CHO cells and hiPSC-CMs on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Charles River. iCellCardiomyocytes2were kindly provided by Fujifilm Cellular Dynamics International.The voltage-gated Na+channel 1.5 (NaV1.5) is encoded by the SCN5A gene and is responsible for the rising phase of the cardiac action potential (AP). The NaV1.5 channel is comprised of a pore-forming subunit and auxillary subunits. When the cardiac cell membrane depolarizes, NaV1.5 opens for a short time allowing an influx of Na+ions resulting in the upstroke of the AP. During the AP, these channels can recover from inactivation and re-open resulting in a sustained current termed INa-Late. Although this current is substantially smaller than the peak Na+current (INa-Peak), it is active during the plateau phase and therefore contributes to AP morphology. There is a growing body of evidence that increased INa-Latecan have a pathophysiological role in acquired heart diseases such as myocardial ischemia and heart failure. INa-Lateis elevated in several pathological conditions which could result in Na+-overload in these cells. A number of loss or gain-of-function mutations in the SCN5A gene have been identified which lead to changes in the magnitude or duration of INa-Peakor INa-Lateresulting in fatal arrhythmias. INa-Lateis a potential drug target to treat cardiac disorders such as angina, heart failure and arrhythmia. It is also an important target in safety pharmacology as enhancement of INa-Lateis proarrhythmic.In this study the Patchliner was used to record INa-Latefrom CHO cells and hiPSC-CMs. INa-Latewas recorded using the voltage protocol specified by CiPA and activated using ATX-II. INa-Latecould be recorded from CHO cells stably expressing NaV1.5 and blocked by lidocaine. INa-Latecould also be detected in iCellCardiomyoctes2and blocked by ranolazine.Application Note PDFAcetylcholine Receptor Alpha 3 Beta 4 Nicotinic a3b4 receptors recorded on Nanions PatchlinerPatchliner application noteThe nicotinic acetylcholine receptor (nAChR) is a member of the ligand-gated ion channel superfamily which includes GABAA, 5HT3, NMDA and glycine receptors. It is a cation-permeable ion channel activated by the neurotransmitter acetylcholine and the natural alkaloid, nicotine. Neuronal nAChR are pentameric and functional channels are formed from a repertoire of nine (2 to 10) and three subunits (2 to 4). Most nAChR exist as heteromers with the stoichiometry 2 to 3, however some subunits function as homomers, these being 7 or 9. nAChR have been proposed to play a role in many neurological disorders such as Alzheimers Disease, Parkinsons, Tourettes Syndrome and depression. mRNA for the 3 and 4 subunits is found in the mammalian CNS and PNS, in particular autonomic ganglion cells and chromaffin cells. nAChR containing the 3 subunit are essential for mediating fast synaptic transmission in the autonomic nervous system and is essential for survival. Block of nicotinic 34 receptors by methadone has also been suggested to play a potential role in analgesia. Here we present data collected on a 4- or 8-channel Patchliner showing the potential use of the Patchliner to record nAChR 34 currents activated by nicotine. Nicotine activated 34 receptors in a concentration dependent manner with an EC50similar to those reported in the literature. Nicotinic 34 receptors could be repetitively activated by nicotine and blocked by mecamylamine, a ganglionic blocker with clinically relevant hypotensive actions, with an IC50in good agreement with the literature.Application Note PDFNaV1.5 Characterization of CreaCells hNaV1.5 (A-0822) on Nanions PatchlinerPatchliner application note: Cells were kindly provided by CreaCell.In this Application Note we present data characterizing hNaV1.5 overexpressing HEK293 cells. The data were collected with Nanions Patchliner. The performance of the cells was very good on the Patchliner. Current responses of an individual cell expressing the hNaV1.5 channel to an IV voltage protocol are shown as well as the corresponding current-voltage relationship. The average mean peak current density of the cells was -215.1 63 pA/pF at 0 mV (n=9).Application Note PDFNaV1.5 Pharmacology of hNaV1.5 recorded on Nanions PatchlinerPatchliner application note: Cells were kindly provided by Millipore.Voltage gated sodium channels (NaV) are important elements of action potential initiation and propagation in

in comparison to monotherapy. Here, we used human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to systematically investigate the effects of HCQ and AZM individually and in combination. The clinically observed QT prolongation caused by treatment with HCQ could be recapitulated in iPSC-CMs based on prolonged field potential duration (FPDc). Interestingly, HCQ-induced FPDc prolongation was strongly enhanced by combined treatment with AZM, although AZM alone slightly shortened FPDc in iPSC-CMs. Furthermore, combined treatment with AZM and HCQ leads to higher cardiotoxicity, more severe structural disarrangement, and more pronounced contractile and electrophysiological dysfunctions, compared to respective mono-treatments. First mechanistic insights underlying the synergistic effects of AZM and HCQ on iPSC-CM functionality are provided based on increased Cx43- and NaV1.5-protein levels. Taken together, our results highlight that combined treatment with HCQ and AZM strongly enhances the adverse effects on cardiomyocytes, providing mechanistic evidence for the high mortality in patients receiving HCQ/AZM combination treatment.Publication link2021 Effects of nicotinic acetylcholine receptoractivating alkaloids on anxietylike behavior in zebrafishPatchliner Publicationin Journal of Natural Medicines (2021) Authors: Alzualde A., Jaka O., Latino D.A.R.S., Alijevic O., Iturria I., Hurtado de Mendoza J., Pospisil P., Frentzel S., Peitsch M.C., Hoeng J., Koshibu K.Alkaloids are a structurally complex group of natural products that have a diverse range of biological activities and significant therapeutic applications. In this study, we examined the acute, anxiolytic-like effects of nicotinic acetylcholine receptor (nAChR)-activating alkaloids with reported neuropharmacological effects but whose effects on anxiety are less well understood. Because 42 nAChRs can regulate anxiety, we first demonstrated the functional activities of alkaloids on these receptors in vitro. Their effects on anxiety-like behavior in zebrafish were then examined using the zebrafish novel tank test (NTT). The NTT is a relatively high-throughput behavioral paradigm that takes advantage of the natural tendency of fish to dive down when stressed or anxious. We report for the first time that cotinine, anatabine, and methylanatabine may suppress this anxiety-driven zebrafish behavior after a single 20-min treatment. Effective concentrations of these alkaloids were well above the concentrations naturally found in plants and the concentrations needed to induce anxiolytic-like effect by nicotine. These alkaloids showed good receptor interactions at the 42 nAChR agonist site as demonstrated by in vitro binding and in silico docking model, although somewhat weaker than that for nicotine. Minimal or no significant effect of other compounds may have been due to low bioavailability of these compounds in the brain, which is supported by the in silico prediction of bloodbrain barrier permeability. Taken together, our findings indicate that nicotine, although not risk-free, is the most potent anxiolytic-like alkaloid tested in this study, and other natural alkaloids may regulate anxiety as well.Publication link2021 Discovery and Preclinical Characterization of Usmarapride (SUVN-D4010): A Potent, Selective 5-HT4 Receptor Partial Agonist for the Treatment of Cognitive Deficits Associated with Alzheimers DiseasePatchliner Publication in Journal of Medicinal Chemistry(2021) Authors: Nirogi R., Mohammed A. R., Shinde A. K., Gagginapally S. R., Kancharla D. M., Ravella S. R., Bogaraju N., Middekadi V. R., Subramanian R., Palacharla R. C., Benade V., Muddana N., Abraham R., Medapati R. B., Thentu J. B., Mekala V. R., Petlu S., Lingavarapu B. B., Yarra S., Kagita N., Goyal V. K., Pandey S. K., Jasti V.A series of oxadiazole derivatives were synthesized and evaluated as 5-hydroxytryptamine-4 receptor (5-HT4R) partial agonists for the treatment of cognitive deficits associated with Alzheimers disease. Starting from a reported 5-HT4R antagonist, a systematic structureactivity relationship was conducted, which led to the discovery of potent and selective 5-HT4R partial agonist 1-isopropyl-3-{5-[1-(3-methoxypropyl) piperidin-4-yl]-[1,3,4]oxadiazol-2-yl}-1H-indazole oxalate (Usmarapride, 12l). It showed balanced physicochemicalpharmacokinetic properties with robust nonclinical efficacy in cognition models. It also showed disease-modifying potential, as it increased neuroprotective soluble amyloid precursor protein alpha levels, and dose-dependent target engagement and correlation of efficacy with oral exposures. Phase 1 clinical studies have been completed and projected efficacious concentration was achieved without any major safety concerns. Phase 2 enabling long-term safety studies have been completed with no concerns for further development.Publication link2021 Dynamic Clamp in Electrophysiological Studies on Stem CellDerived CardiomyocytesWhy and How?PatchlinerReview Article in Journal of Cardiovascular Pharmacology (2021) Authors: Verkerk A.O., Wilders R.Human pluripotent stem cellderived cardiomyocytes (hPSC-CMs) are supposed to be a good human-based model, with virtually unlimited cell source, for studies on mechanisms underlying cardiac development and cardiac diseases, and for identification of drug targets. However, a major drawback of hPSC-CMs as a model system, especially for electrophysiological studies, is their depolarized state and associated spontaneous electrical activity. Various approaches are used to overcome this drawback, including the injection of synthetic inward rectifier potassium current (IK1), which is computed in real time, based on the recorded membrane potential(dynamic clamp). Such injection of an IK1-like current results in quiescent hPSC-CMs with a nondepolarized resting potential that show adult-like action potentials on stimulation, with functional availability of the most important ion channels involved in cardiac electrophysiology.These days, dynamic clamp has become a widely appreciated electrophysiological tool. However, setting up a dynamic clamp system can still be laborious and difficult, both because of the required hardware and the implementation of the dedicated software.In the present review, we first summarize the potential mechanisms underlying the depolarized state of hPSC-CMs and the functional consequences of this depolarized state. Next, we explain how an existing manual patch clamp setup can be extended with dynamic clamp. Finally, we shortly validate the extended setup with atrial-like and ventricular-like hPSC-CMs. We feel that dynamic clamp is a highly valuable tool in the field of cellular electrophysiological studies on hPSC-CMs and hope that our directions for setting up such dynamic clamp system may prove helpful.Publication link2021 Cell engineering method using fluorogenic oligonucleotide signaling probes and flow cytometryPatchlinerPublication in Biotechnology Letters (2021) Authors: Shekdar K., Langer J., Venkatachalan S., Schmid L., Anobile J., Shah P., Lancaster A., Babich O., Dedova O., Sawchuck D.Objective:Chromovert Technology is presented as a new cell engineering technology to detect and purify living cells based on gene expression.Methods:The technology utilizes fluorogenic oligonucleotide signaling probes and flow cytometry to detect and isolate individual living cells expressing one or more transfected or endogenously-expressed genes.Results:Results for production of cell lines expressing a diversity of ion channel and membrane proteins are presented, including heteromultimeric epithelial sodium channel (-ENaC), sodium voltage-gated ion channel 1.7 (NaV1.7-12), four unique -aminobutyric acid A (GABAA) receptor ion channel subunit combinations 132s, 232s, 332s and 532s, cystic fibrosis conductance regulator (CFTR), CFTR-508 and two G-protein coupled receptors (GPCRs) without reliance on leader sequences and/or chaperones. In addition, three novel plasmid-encoded sequences used to introduce 3 untranslated RNA sequence tags in mRNA expression products and differentially-detectable fluorogenic probes directed to each are described. The tags and corresponding fluorogenic signaling probes streamline the process by enabling the multiplexed detection and isolation of cells expressing one or more genes without the need for gene-specific probes.Publication link2021 Discovery and Characterization of the Potent and Highly Selective 1,7-Naphthyridine-Based Inhibitors BAY-091 and BAY-297 of the Kinase PIP4K2APatchliner Publication in Journal of Medicinal Chemistry(2021) Authors: Wortmann L., Bruer N., Holton S.J., Irlbacher H., Weiske J., Lechner C., Meier R., Karn J., Siberg C.B., Ptter V., Christ C.D., ter Laak A., Lienau P., Lesche R., Nicke B., Cheung S-H., Bauser M., Haegebarth A., von Nussbaum F., Mumberg D., Lemos C.PIP4K2A is an insufficiently studied type II lipid kinase that catalyzes the conversion of phosphatidylinositol-5-phosphate (PI5P) into phosphatidylinositol 4,5-bisphosphate (PI4,5P2). The involvement of PIP4K2A/B in cancer has been suggested, particularly in the context of p53 mutant/null tumors. PIP4K2A/B depletion has been shown to induce tumor growth inhibition, possibly due to hyperactivation of AKT and reactive oxygen species-mediated apoptosis. Herein, we report the identification of the novel potent and highly selective inhibitors BAY-091 and BAY-297 of the kinase PIP4K2A by high-throughput screening and subsequent structure-based optimization. Cellular target engagement of BAY-091 and BAY-297 was demonstrated using cellular thermal shift assay technology. However, inhibition of PIP4K2A with BAY-091 or BAY-297 did not translate into the hypothesized mode of action and antiproliferative activity in p53-deficient tumor cells. Therefore, BAY-091 and BAY-297 serve as valuable chemical probes to study PIP4K2A signaling and its involvement in pathophysiological conditions such as cancer.Publication link2021 BAY-8400: A Novel Potent and Selective DNA-PK Inhibitor which Shows Synergistic Efficacy in Combination with Targeted Alpha TherapiesPatchliner Publication in Journal of Medicinal Chemistry (2021) Authors: Berger M., Wortmann L., Buchgraber P.,Lcking U., Zitzmann-Kolbe S., Wengner A.M., Bader B.,Bmer U., Briem H., Eis K., Rehwinkel H., Bartels F., Moosmayer D., Eberspcher U., Lienau P., Hammer S., Schatz C.A., Wang Q., Wang Q., Mumberg D., Nising C.F., Siemeister G.Eukaryotes have evolved two major pathways to repair potentially lethal DNA double-strand breaks. Homologous recombination represents a precise, DNA-template-based mechanism available during the S and G2 cell cycle phase, whereas non-homologous end joining, which requires DNA-dependent protein kinase (DNA-PK), allows for fast, cell cycle-independent but less accurate DNA repair. Here, we report the discovery of BAY-8400, a novel selective inhibitor of DNA-PK. Starting from a triazoloquinoxaline, which had been identified as a hit from a screen for ataxia telangiectasia and Rad3-related protein (ATR) inhibitors with inhibitory activity against ATR, ATM, and DNA-PK, lead optimization efforts focusing on potency and selectivity led to the discovery of BAY-8400. In in vitro studies, BAY-8400 showed synergistic activity of DNA-PK inhibition with DNA damage-inducing targeted alpha therapy. Combination of PSMA-targeted thorium-227 conjugate BAY 2315497 treatment of human prostate tumor-bearing mice with BAY-8400 oral treatment increased antitumor efficacy, as compared to PSMA-targeted thorium-227 conjugate monotherapy.Publication link2021 Blebbistatin protects iPSC-CMs from hypercontraction and facilitates automated patch-clamp based electrophysiological studyPatchliner Publication in Stem Cell Research (2021) Authors: Li W.,LuoX.,UlbrichtY.,GuanK.Recently, there have been great advances in cardiovascular channelopathy modeling and drug safety pharmacology using human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). The automated patch-clamp (APC) technique overcomes the disadvantages of the manual patch-clamp (MPC) technique, which is labor intensive and gives low output. However, the application of the APC platform is still limited in iPSC-CM based research, due to the difficulty in maintaining the high quality of single iPSC-CMs during dissociation and recording. In this study, we improved the method for single iPSC-CM preparation by applying 2.5M blebbistatin (BB, an excitationcontraction coupling uncoupler) throughout APC procedures (dissociation, filtration, storage, and recording). Under non-BB buffered condition, iPSC-CMs in suspension showed a severe bleb-like morphology. However, BB-supplement led to significant improvements in morphology andINarecording, and we even obtained several CMs that showed spontaneous action potentials with typical morphology. Furthermore, APC faithfully recapitulated the single-cell electrophysiological phenotypes of iPSC-CMs derived from Brugada syndrome patients, as detected with MPC. Our study indicates that APC is capable of replacing MPC in the modeling of cardiac channelopathies using human iPSC-CMs by providing high-quality data with higher throughput.Publication link2021 A model-guided pipeline for drug cardiotoxicity screening with human stem-cell derived cardiomyocytesPatchliner Pre-Print Publication in bioRxiv (2021) Authors: Clark A. P., Wie S., Krogh-Madsen T., Christini D. J.New therapeutic compounds go through a preclinical drug cardiotoxicity screening process that is overly conservative and provides limited mechanistic insight, leading to the misclassification of potentially beneficial drugs as proarrhythmic. There is a need to develop a screening paradigm that maintains this high sensitivity, while ensuring non-cardiotoxic compounds pass this phase of the drug approval process. In this study, we develop an in vitro-in silico pipeline using human induced stem-cell derived cardiomyocytes (iPSC-CMs) to address this problem. The pipeline includes a model-guided optimization that produces a voltage-clamp (VC) protocol to determine drug block of seven cardiac ion channels. Such VC data, along with action potential (AP) recordings, were acquired from iPSC-CMs before and after treatment with a control solution or a low-, intermediate-, or high-risk drug. We identified significant AP prolongation (a proarrhythmia indicator) in two high-risk drugs and, from the VC data, determined strong ion channel blocks that led to the AP changes. The VC data also uncovered an undocumented funny current (If) block by quinine, which we confirmed with experiments using a HEK-293 expression line. We present a new approach to cardiotoxicity screening that simultaneously evaluates proarrhythmia risk (e.g. AP prolongation) and mechanism (e.g. channel block) from iPSC-CMs.Publication link2021 Antiarrhythmic Hit to Lead Refinement in a Dish Using Patient-Derived iPSC CardiomyocytesPatchliner Publicationin Journal of Medicinal Chemistry Authors: Cashman J.R., Ryan D., McKeithan W.L., Okolotowicz K., Gomez-Galeno J., Johnson M., Sampson K.J., Kass R.S, Pezhouman A, Karagueuzian H.S., Mercola M.,Ventricular cardiac arrhythmia (VA) arises in acquired or congenital heart disease. Long QT syndrome type-3 (LQT3) is a congenital form of VA caused by cardiac sodium channel (INaL) SCN5A mutations that prolongs cardiac action potential (AP) and enhances INaLcurrent. Mexiletine inhibits INaLand shortens the QT interval in LQT3 patients. Above therapeutic doses, mexiletine prolongs the cardiac AP. We explored structure-activity relationships (SAR) for AP shortening and prolongation using dynamic medicinal chemistry and AP kinetics in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Using patient-derived LQT3 and healthy hiPSC-CMs, we resolved distinct SAR for AP shortening and prolongation effects in mexiletine analogues and synthesized new analogues with enhanced potency and selectivity for INaL. This resulted in compounds with decreased AP prolongation effects, increased metabolic stability, increased INaLselectivity, and decreased avidity for the potassium channel. This study highlights using hiPSC-CMs to guide medicinal chemistry and "drug development in a dish".Publication link2020 Thrombospondin-1/CD47 signaling modulates transmembrane cation conductance, survival, and deformability of human red blood cellsPatchliner Publication in Cell Communication and Signaling (2020) Authors: Bissinger R., Petkova-Kirova P., Mykhailova O., Oldenborg PA., Novikova E., Donkor D.A., Dietz T., Bhuyan A.A.M., Sheffield W.P., Grau M., Artunc F., Kaestner L., Acker J.P., Qadri S.M.Thrombospondin-1 (TSP-1), a Ca2+-binding trimeric glycoprotein secreted by multiple cell types, has been implicated in the pathophysiology of several clinical conditions. Signaling involving TSP-1, through its cognate receptor CD47, orchestrates a wide array of cellular functions including cytoskeletal organization, migration, cell-cell interaction, cell proliferation, autophagy, and apoptosis. In the present study, we investigated the impact of TSP-1/CD47 signaling on Ca2+dynamics, survival, and deformability of human red blood cells (RBCs).Publication link2020 Utilising Automated Electrophysiological Platform in Epilepsy ResearchPatchliner Chapter in Patch Clamp Electrophysiology (2020) Authors: Milligan C.J., Pachernegg S.Chapter 7:Utilising Automated Electrophysiological Platform in Epilepsy ResearchAbstract:Genetic mutations have long been implicated in epilepsy, particularly in genes that encode ion channels and neurotransmitter receptors. Among some of those identified are voltage-gated sodium, potassium and calcium channels, and ligand-gated gamma-aminobutyric acid (GABA), neuronal nicotinic acetylcholine (CHRN), and glutamate receptors, making them key therapeutic targets. In this chapter we discuss the use of automated electrophysiological technologies to examine the impact of gene defects in two potassium channels associated with different epilepsy syndromes. The hKCNC1 gene encodes the voltage-gated potassium channel hKV3.1, and mutations in this gene cause progressive myoclonus epilepsy (PME) and ataxia due to a potassium channel mutation (MEAK). The hKCNT1 gene encodes the weakly voltage-dependent sodium-activated potassium channel hKCNT1, and mutations in this gene cause a wide spectrum of seizure disorders, including severe autosomal dominant sleep-related hypermotor epilepsy (ADSHE) and epilepsy of infancy with migrating focal seizures (EIMFS), both conditions associated with drug-resistance. Importantly, both of these potassium channels play vital roles in regulating neuronal excitability.Since its discovery in the late nineteen seventies, the patch-clamp technique has been regarded as the bench-mark technology for exploring ion channel characteristics. In more recent times, innovations in automated patch-clamp technologies, of which there are many, are enabling the study of ion channels with much greater productivity that manual systems are capable of. Here we describe aspects of Nanion NPC-16 Patchliner, examining the effects of temperature on stably and transiently transfected mammalian cells, the latter of which for most automated systems on the market is quite challenging. Remarkable breakthroughs in the development of other automated electrophysiological technologies, such as multielectrode arrays that support extracellular signal recordings, provide additional features to examine network activity in the area of ion channel research, particularly epilepsy. Both of these automated technologies enable the acquisition of consistent, robust, and reproducible data. Numerous systems have been developed with very similar capabilities, however, not all the systems on the market are adapted to work with primary cells, particularly neurons that can be problematic.This chapter also showcases methods that demonstrate the versatility of Nanion NPC-16 Patchliner and the Multi Channel Systems (MCS) multielectrode array (MEA) assay for acutely dissociated murine primary cortical neurons, enabling the study of potassium channel mutations implicated in severe refractory epilepsies.Publication link2020 Targeting different binding sites in the CFTR structures allows to synergistically potentiate channel activityPatchliner Publication in European Journal of Medicinal Chemistry (2020) Authors: Froux L., Elbahnsi A., Boucherle B., Billet A., Baatallah N., Hoffmann B., Alliot J., Zelli R., Zeinyeh W., Haudecoeur R., Chevalier B., Fortun A., Mirval S., Simard C., Lehn P., Mornon J-P., Hinzpeter A., Becq F., Callebaut I., Dcout J-L.Recent evidence shows that combination of correctors and potentiators, such as the drug ivacaftor (VX-770), can significantly restore the functional expression of mutated Cystic Fibrosis Transmembrane conductance Regulator (CFTR), an anion channel which is mutated in cystic fibrosis (CF). The success of these combinatorial therapies highlights the necessity of identifying a broad panel of specific binding mode modulators, occupying several distinct binding sites at structural level. Here, we identified two small molecules, SBC040 and SBC219, which are two efficient cAMP-independent potentiators, acting at low concentration of forskolin with EC50close to 1M and in a synergic way with the drug VX-770 on several CFTR mutants of classes II and III. Molecular dynamics simulations suggested potential SBC binding sites at the vicinity of ATP-binding sites, distinct from those currently proposed for VX-770, outlining SBC molecules as members of a new family of potentiators.Publication link2020 The electrophysiological effect of cannabidiol on hERG current and in guinea-pig and rabbit cardiac preparationsPatchliner Publication in Nature Scientific Reports (2020) Authors: Orvos P., Pszti B., Topal L., Gazdag P., Prorok J., Polyk A., Kiss T., Tth-Molnr E., Csupor-Lffler B., Bajtel A., Varr A., Hohmann J., Virg L., Csupor D.Cannabis use is associated with cardiovascular adverse effects ranging from arrhythmias to sudden cardiac death. The exact mechanism of action behind these activities is unknown. The aim of our work was to study the effect of cannabidiol (CBD), tetrahydrocannabinol and 11-nor-9-carboxy-tetrahydrocannabinol on cellular cardiac electrophysiological properties including ECG parameters, action potentials, hERG and IKrion channels in HEK cell line and in rabbit and guinea pig cardiac preparations. CBD increased action potential duration in rabbit and guinea pig right ventricular papillary muscle at lower concentrations (1M, 2.5M and 5M) but did not significantly change it at 10M. CBD at high concentration (10M) decreased inward late sodium and L-type calcium currents as well. CBD inhibited hERG potassium channels with an IC50value of 2.07M at room temperature and delayed rectifier potassium current with 6.5M at 37C, respectively. The frequency corrected QT interval (QTc) was significantly lengthened in anaesthetized guinea pig without significantly changing other ECG parameters. Although the IC50value of CBD was higher than literary Cmaxvalues after CBD smoking and oral intake, our results raise the possibility that hERG and potassium channel inhibition might have a role in the possible proarrhythmic adverse effects of cannabinoids in situations where metabolism of CBD impaired and/or the repolarization reserve is weakened.Publication link2020 Screening Technologies for Inward Rectifier Potassium Channels: Discovery of New Blockers and ActivatorsPatchliner Publication in SLAS DISCOVERY (2020) Authors: Walsh, K. B.K+ channels play a critical role in maintaining the normal electrical activity of excitable cells by setting the cell resting membrane potential and by determining the shape and duration of the action potential. In nonexcitable cells, K+ channels establish electrochemical gradients necessary for maintaining salt and volume homeostasis of body fluids. Inward rectifier K+ (Kir) channels typically conduct larger inward currents than outward currents, resulting in an inwardly rectifying current versus voltage relationship. This property of inward rectification results from the voltage-dependent block of the channels by intracellular polyvalent cations and makes these channels uniquely designed for maintaining the resting potential near the K+ equilibrium potential (EK). The Kir family of channels consist of seven subfamilies of channels (Kir1.x through Kir7.x) that include the classic inward rectifier (Kir2.x) channel, the G-protein-gated inward rectifier K+ (GIRK) (Kir3.x), and the adenosine triphosphate (ATP)-sensitive (KATP) (Kir 6.x) channels as well as the renal Kir1.1 (ROMK), Kir4.1, and Kir7.1 channels. These channels not only function to regulate electrical/electrolyte transport activity, but also serve as effector molecules for G-protein-coupled receptors (GPCRs) and as molecular sensors for cell metabolism. Of significance, Kir channels represent promising pharmacological targets for treating a number of clinical conditions, including cardiac arrhythmias, anxiety, chronic pain, and hypertension. This review provides a brief background on the structure, function, and pharmacology of Kir channels and then focuses on describing and evaluating current high-throughput screening (HTS) technologies, such as membrane potential-sensitive fluorescent dye assays, ion flux measurements, and automated patch clamp systems used for Kir channel drug discovery.Publication link2020 Structural basis of the potency and selectivity of Urotoxin, a potent KV1 blocker from scorpion venomPatchliner Publication in Biochemical Pharmacology (2020) Authors: Luna-Ramirez K., Csoti A., McArthur J.R., Chin Y.K.Y., Anangi R., del Carmen Najera R., Possani L.D., King G.F., Panyi G., Yu H., Adams D.J., Finol-Urdaneta R.K.Urotoxin (-KTx 6), a peptide from venom of the Australian scorpionUrodacus yaschenkoi, is the most potent inhibitor of KV1.2 described to date (IC50= 160 pM). The native peptide also inhibits KV1.1, KV1.3 and KCa3.1 with nanomolar affinity but its low abundance in venom precluded further studies of its actions. Here we produced recombinant Urotoxin (rUro) and characterized the molecular determinants of KV1 channel inhibition. The 3D structure of rUro determined using NMR spectroscopy revealed a canonical cysteine-stabilised / (CS/) fold. Functional assessment of rUro using patch-clamp electrophysiology revealed the importance of C-terminal amidation for potency against KV1.11.3 and KV1.5. Neutralization of the putative pore-blocking K25 residue in rUro by mutation to Ala resulted in a major decrease in rUro potency against all KV channels tested, without perturbing the toxins structure. Reciprocal mutations in the pore of Uro-sensitive KV1.2 and Uro-resistant KV1.5 channels revealed a direct interaction between Urotoxin and the KV channel pore. Our experimental work supports postulating a mechanism of action in which occlusion of the permeation pathway by the K25 residue in Urotoxin is the basis of its KV1 inhibitory activity. Docking analysis was consistent with occlusion of the pore by K25 and the requirement of a small, non-charged amino acid in the KV1 channel vestibule to facilitate toxin-channel interactions. Finally, computational studies revealed key interactions between the amidated C-terminus of Urotoxin and a conserved Asp residue in the turret of KV1 channels, offering a potential rationale for potency differences between native and recombinant Urotoxin.Publication link2020 N-alkylisatin-based microtubule destabilizers bind to the colchicine site on tubulin and retain efficacy in drug resistant acute lymphoblastic leukemia cell lines with less in vitro neurotoxicityPatchliner Publication in Cancer Cell International (2020) Authors: Keenan B., Finol-Urdaneta R.K., Hope A., Bremner J.B., Kavallaris M., Lucena-Agell D., ngela Oliva M., Daz J.F & Vine K.L.Drug resistance and chemotherapy-induced peripheral neuropathy continue to be significant problems in the successful treatment of acute lymphoblastic leukemia (ALL). 5,7-Dibromo-N-alkylisatins, a class of potent microtubule destabilizers, are a promising alternative to traditionally used antimitotics with previous demonstrated efficacy against solid tumours in vivo and ability to overcome P-glycoprotein (P-gp) mediated drug resistance in lymphoma and sarcoma cell lines in vitro. In this study, three di-brominated N-alkylisatins were assessed for their ability to retain potency in vincristine (VCR) and 2-methoxyestradiol (2ME2) resistant ALL cell lines. For the first time, in vitro neurotoxicity was also investigated in order to establish their suitability as candidate drugs for future use in ALL treatment.Publication link2020 Pharmacological and genetic characterisation of the canine P2X4 receptorPatchliner Publication in British Journal of Pharmacology (2020) Authors: Sophocleous R.A., Berg, T., FinolUrdaneta R.K., Sluyter V., Keshiya S., Bell L., Curtis S.J., Curtis B.L., Seavers A., Bartlett R., Dowton M., Stokes L., Ooi L., Sluyter R.Background and PurposeThe P2X4 receptor is an emerging therapeutic target for the treatment of chronic pain and cardiovascular disease. Dogs are wellrecognised natural models of human disease but information regarding P2X4 in dogs is absent. To aid the development and validation of P2X4targeting therapeutics, this study aimed to characterise and compare canine and human P2X4.Experimental ApproachGenomic DNA was extracted from whole blood samples from 101 randomly selected dogs and sequenced across theP2RX4gene to identify potential missense variants. Recombinant canine and human P2X4 tagged with Emerald GFP were expressed in 1321N1 and HEK293 cells and analysed by immunoblotting and confocal microscopy. P2X4 pharmacology was characterised using nucleotideinduced Fura2 AM measurements of intracellular Ca2+ responses and known P2X4 antagonists in 1321N1 and HEK293 cells. P2X4mediated inward currents in HEK293 cells were assessed by automated patch clamp.Key ResultsNoP2RX4missense variants were identified in any canine samples. Canine and human P2X4 were localised primarily to lysosomal compartments. ATP was identified as the primary agonist of canine P2X4 with near identical efficacy and potency at human P2X4. 2(3)O(4benzoyl)benzoyl ATP (BzATP), but not ADP, was identified as a partial agonist with reduced potency for dog P2X4 compared to the human orthologue. Five antagonists inhibited canine P2X4, with BX430 displaying reduced sensitivity and potency against canine P2X4.Conclusion and ImplicationsP2X4 is highly conserved across dog pedigrees and displays a similar expression pattern and pharmacological profile to human P2X4, providing support for validation and use of therapeutics designed for P2X4related disease onset and management in dogs and humans.Publication link2020 Discovery and Development of SUVN-911: A Novel, Potent, Selective, and Orally Active Neuronal Nicotinic Acetylcholine 42 Receptor Antagonist for the Treatment of DepressionPatchliner Publication in Journal of Medicinal Chemistry (2020) Authors: Nirogi R., Mohammed A.R., Shinde A.K., Ravella S.R., Bogaraju N., Subramanian R., Mekala V.R., Palacharla R.C., Muddana N., Thentu J.B., Bhyrapuneni G., Abraham R., Jasti V.A series of chemical optimizations guided by in vitro affinity at the 42 receptor in combination with selectivity against the 34 receptor, pharmacokinetic evaluation, and in vivo efficacy in a forced swim test resulted in identification of 3-(6-chloropyridine-3-yloxymethyl)-2-azabicyclo[3.1.0]hexane hydrochloride (9h, SUVN-911) as a clinical candidate. Compound 9h is a potent 42 receptor ligand with a Ki value of 1.5 nM. It showed >10 M binding affinity toward the ganglionic 34 receptor apart from showing selectivity over 70 other targets. It is orally bioavailable and showed good brain penetration in rats. Marked antidepressant activity and dose-dependent receptor occupancy in rats support its potential therapeutic utility in the treatment of depression. It does not affect the locomotor activity at doses several folds higher than its efficacy dose. It is devoid of cardiovascular and gastrointestinal side effects. Successful long-term safety studies in animals and phase-1 evaluation in healthy humans for safety, tolerability, and pharmacokinetics paved the way for its further development.Publication link2020 Disease Phenotypes and Mechanisms of iPSC-Derived Cardiomyocytes From Brugada Syndrome Patients With a Loss-of-Function SCN5A MutationPatchliner Publication in Frontiers in Cell and Developmental Biology (2020) Authors: Li W., Stauske M., Luo X., Wagner S., Vollrath M., Mehnert C.S., Schubert M., Cyganek L., Chen S., Hasheminasab S.M., Wulf G., El-Armouche A., Maier L.S., Hasenfuss G., Guan K.Brugada syndrome (BrS) is one of the major causes of sudden cardiac death in young people, while the underlying mechanisms are not completely understood. Here, we investigated the pathophysiological phenotypes and mechanisms using induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CMs) from two BrS patients (BrS-CMs) carrying a heterozygousSCN5Amutation p.S1812X. Compared to CMs derived from healthy controls (Ctrl-CMs), BrS-CMs displayed a 50% reduction ofINadensity, a 69.5% reduction of NaV1.5 expression, and the impaired localization of NaV1.5 and connexin 43 (Cx43) at the cell surface. BrS-CMs exhibited reduced action potential (AP) upstroke velocity and conduction slowing. TheItoin BrS-CMs was significantly augmented, and theICaLwindow current probability was increased. Our data indicate that the electrophysiological mechanisms underlying arrhythmia in BrS-CMs may involve both depolarization and repolarization disorders. Cilostazol and milrinone showed dramatic inhibitions ofItoin BrS-CMs and alleviated the arrhythmic activity, suggesting their therapeutic potential for BrS patients.Publication link2020 Assessment of the Effects of Online Linear Leak Current Compensation at Different Pacing Frequencies in a Dynamic Action Potential Clamp SystemPatchliner Publication in Computing in Cardiology (2020) Authors: Fabbri A., Prins A., de Boer T.P.Dynamic action potential (AP) clamp (dAPC) is an electrophysiology technique that allows one to study in real time the effects of a biological current included in a computational AP model. During an experiment, the seal resistance between the cell membrane and the pipette is finite and a leak current (Ileak) occurs. Its reduction is crucial to properly assess the effect of a drug. Our work aims to quantify the impact of Ileak on a ventricular AP model and to evaluate the benefits of an online compensation. We carried out the experiments using a prototype Nanion Patchliner Dynamite8 in dAPC mode, running the ten Tusscher human ventricular AP model. We use a passive model cell (Cm = 19.8pF, Rseal = 500MOhm) and online compensate the leak current adopting a linear model. Vmax, RMP and AP D20,50,90 are measured at several degrees of compensation (within 0 and 100%), at different pacing frequencies (0.5, 1, 2 Hz), and compared with the AP model in the open loop condition (i.e. with no connection between the model cell and the AP model). Ileak decreases Vmax, depolarizes RMP (up to +6.1mV at 1 Hz) and prolongs AP D both during the plateau and the late repolarization; a full compensation of Ileak brings the AP biomarkers close to the open loop condition. With this test, we show that online compensation of Ileak is beneficial for proper assessment of AP biomarker. The correction off RMP is key, as it affects the following phases of the AP.Publication link2020 Automated Dynamic Clamp for Simulation of IK1 in Human Induced Pluripotent Stem CellDerived Cardiomyocytes in Real Time Using Patchliner Dynamite8Patchliner and Dynamite8Publication in Current Protocols in Pharmacology (2020) Authors: Becker N., Horvth A., De Boer T., Fabbri A., Grad C., Fertig N., George M., Obergrussberger A.Current in vitro assays typically poorly predict cardiac liability as they focus on single ion channels overexpressed in cell lines. Human induced pluripotent stem cellderived cardiomyocytes (hiPSCCMs), on the other hand, provide a unique opportunity for drug testing on human cardiomyocytes using highthroughput systems. However, these cells can differ from adult cardiomyocytes in their ion channel expression and, therefore, electrophysiologic properties. One of the main challenges of hiPSCCMs is the physiologic expression of ion channels such as the inward rectifiers (e.g., Kir2.12.3), which conduct the cardiac inward rectifier potassium current (IK1). IK1is one of the primary contributors in maintaining a stable resting membrane potential in cardiac cells, which is essential for excitability. This is only expressed in low levels, or sometimes not at all, in hiPSCCMs as shown by patch clamp studies. Dynamic clamp is a method of electronically introducing ion currents (e.g., IK1) into cells to compensate for the lack of endogenous expression, thus offering the potential to record more stable action potentials in hiPSCCMs. In this article, we describe the method of using hiPSCCMs on an automated patch clamp device (Patchliner) coupled with the automated dynamic clamp addon (Dynamite8). We describe protocols for optimized cell handling and harvesting for use on the Patchliner and the steps required for automated execution of experiments and data analysis in dynamic clamp mode. 2019 by John Wiley & Sons, Inc.Basic Protocol:Recording action potential pharmacology from human induced pluripotent stem cellderived cardiomyocytes in automated patch clamp combined with dynamic clamp to introduce simulated IK1and compensate seal resistanceSupport Protocol 1:Cardiomyocyte plating and cultureSupport Protocol 2:Cell harvesting and dissociationAlternate Protocol:Recording action potential pharmacology at physiologic temperaturesPublication link2020 A P2RX7 single nucleotide polymorphism haplotype promotes exon 7 and 8 skipping and disrupts receptor functionPatchliner Publication in The Faseb Journal (2020) Authors: Skarratt K.K., Gu B.J., Lovelace M.D., Milligan C.J., Stokes L., Glover R., Petrou S, Wiley J.S., Fuller S.J.P2X7 is an ATPgated membrane ion channel that is expressed by multiple cell types. Brief exposure to ATP induces the opening of a nonselective cation channel; while repeated or prolonged exposure induces formation of a transmembrane pore. This process may be partially regulated by alternative splicing of fulllengthP2RX7ApremRNA, producing isoforms that delete or retain functional domains. Here, we report cloning and expression of a novelP2RX7splice variant,P2RX7L, that is, characterized by skipping of exons 7 and 8. In HEK 293 cells, expression ofP2RX7Lproduces a protein isoform, P2X7L, that forms a heteromer with P2X7A. A haplotype defined by six single nucleotide polymorphisms (SNPs) (rs208307, rs2208306, rs36144485, rs208308, rs208309, and rs373655596) promotes allelespecific alternative splicing, increasing mRNA levels ofP2RX7Land another isoform,P2RX7E, which in addition has a truncated Cterminus. Skipping of exons 7 and 8 is predicted to delete critical amino acids in the ATPbinding site. P2X7Ltransfected HEK 293 cells have phagocytic but not channel, pore, or membraneblebbing function, and doubletransfected P2X7L and P2X7A cells have reduced pore function. Heteromeric receptor complexes of P2X7A and P2X7L are predicted to have reduced numbers of ATPbinding sites, which potentially alters receptor function compared to homomeric P2X7A complexes.Publication link2020 An electrophysiological characterization of naturally occurring tobacco alkaloids and their action on human 42 and 7 nicotinic acetylcholine receptorsPatchliner Publication in Phytochemistry (2020) Authors: Alijevic O., McHugh D., Rufener L., Mazurov A., Hoeng J., Peitsch M.Nicotinic acetylcholine receptor (nAChR) subtype-selective pharmacological profiles of tobacco alkaloids are essential for understanding the physiological effects of tobacco products. In this study, automated electrophysiology was used to functionally characterize the effects of distinct groups of tobacco alkaloids on human 42 and 7 nAChRs. We found that, in tobacco alkaloids, pyridine as a hydrogen bond acceptor and a basic nitrogen atom at a distance of 47 are pharmacophoric elements necessary for molecular recognition by 42 and 7 nAChRs with various degrees of selectivity, potency, and efficacy. While four alkaloidsnicotine, nornicotine, anabasine and R-anatabinepotently activated 42, they were also weak agonists of 7 nAChRs. Nicotine was the most potent agonist of 42, while anabasine elicited the highest activation of 7. None of the tobacco alkaloids enhanced nAChR activity elicited by the endogenous ligand acetylcholine; therefore, none was considered to be a positive allosteric modulator (PAM) of either 42 or 7 nAChRs. In contrast, we identified tobacco alkaloids, such as the tryptophan metabolite 6-hydroxykynurenic acid, that decreased the activity of both 42 and 7 nAChRs. Our study identified a class of alkaloids with positive and negative effects against human 42 and 7 nAChRs. It also revealed human 42 to be the principal receptor for sensing the most abundant alkaloids in tobacco leaves.Publication link2019 Red Blood Cell Membrane Conductance in Hereditary Haemolytic AnaemiasPatchliner Publication in Frontiers in Physiology (2019) Authors: Petkova-Kirova P., Hertz L., Danielczok J., Huisjes R., Makhro A., Bogdanova A., del Mar Ma-Pereira M., Vives Corrons J.-L., van Wijk R., Kaestner L.Congenital haemolytic anaemias are inherited disorders caused by red blood cell membrane and cytoskeletal protein defects, deviant hemoglobin synthesis and met

pre-treatment with sodium nitroprusside (SNP), an effect significantly augmented by additional removal of extracellular Ca2+. A 3 hours treatment with 0.1 M Ca2+ionophore ionomycin triggered annexin-V-binding and cell shrinkage, effects fully reversed by removal of extracellular Ca2+. Conclusions: TFP induces eryptosis and decreases protein S-nitrosylation, effects blunted by nitroprusside. The effect of nitroprusside is attenuated in the presence of extracellular Ca2+.Publication link2018 A Hybrid Model for Safety Pharmacology on an Automated Patch Clamp Platform: Using Dynamic Clamp to Join iPSC-Derived Cardiomyocytes and Simulations of IK1 Ion Channels in Real-TimePatchliner Publication in Frontiers in Physiology Authors: Goversen B., Becker., N., Stlzle-Feix S.,Obergrussberger A., Vos M.A., van Veen T.A.B., Fertig N., de Boer T.P.An important aspect of the Comprehensive In Vitro Proarrhythmia Assay (CiPA) proposal is the use of human stem cell-derived cardiomyocytes and the confirmation of their predictive power in drug safety assays. The benefits of this cell source are clear; drugs can be tested in vitro on human cardiomyocytes, with patient-specific genotypes if needed, and differentiation efficiencies are generally excellent, resulting in a virtually limitless supply of cardiomyocytes. There are, however, several challenges that will have to be surmounted before successful establishment of hSC-CMs as an all-round predictive model for drug safety assays. An important factor is the relative electrophysiological immaturity of hSC-CMs, which limits arrhythmic responses to unsafe drugs that are pro-arrhythmic in humans. Potentially, immaturity may be improved functionally by creation of hybrid models, in which the dynamic clamp technique joins simulations of lacking cardiac ion channels (e.g., IK1) with hSC-CMs in real-time during patch clamp experiments. This approach has been used successfully in manual patch clamp experiments, but throughput is low. In this study, we combined dynamic clamp with automated patch clamp of iPSC-CMs in current clamp mode, and demonstrate that IK1conductance can be added to iPSC-CMs on an automated patch clamp platform, resulting in an improved electrophysiological maturity.Publication link2017 Myoclonus Epilepsy and Ataxia due to KCNC1 Mutation: Analysis of 20 Cases and K+ Channel PropertiesPatchliner Publication in Annals in Neurobiology (2017) Authors: Oliver K.L., Franceschetti S., Milligan C.J., Muona M., Mandelstam S.A., Canafoglia L., Boguszewska-Chachulska A.M., Korczyn A.D.,Bisulli F., Di BoNaVentura C., Ragona F., Michelucci R., Ben-Zeev B., Straussberg R., Panzica F., Massano J., Friedman D., Crespel A., Engelsen B.A., Andermann F., Andermann E., Spodar K., Lasek-Bal A., Riguzzi P.,Pasini E., Tinuper P., Licchetta L., Gardella E., Lindenau M., Wulf A., Mller R.S.Objective:To comprehensively describe the new syndrome of myoclonus epilepsy and ataxia due to potassium channel mutation (MEAK), including cellular electrophysiological characterization of observed clinical improvement with fever.Methods:We analyzed clinical, electroclinical, and neuroimaging data for 20 patients with MEAK due to recurrent KCNC1 p.R320H mutation. In vitro electrophysiological studies were conducted using whole cell patch-clamp to explore biophysical properties of wild-type and mutant KV3.1 channels.Results:Symptoms began at between 3 and 15 years of age (median=9.5), with progressively severe myoclonus and rare tonicclonic seizures. Ataxia was present early, but quickly became overshadowed by myoclonus; 10 patients were wheelchair-bound by their late teenage years. Mild cognitive decline occurred in half. Early death was not observed. Electroencephalogram (EEG) showed generalized spike and polyspike wave discharges, with documented photosensitivity in most. Polygraphic EEGelectromyographic studies demonstrated a cortical origin for myoclonus and striking coactivation of agonist and antagonist muscles. Magnetic resonance imaging revealed symmetrical cerebellar atrophy, which appeared progressive, and a prominent corpus callosum. Unexpectedly, transient clinical improvement with fever was noted in 6 patients. To explore this, we performed high-temperature in vitro recordings. At elevated temperatures, there was a robust leftward shift in activation of wild-type KV3.1, increasing channel availability.Interpretation:MEAK has a relatively homogeneous presentation, resembling UnverrichtLundborg disease, despite the genetic and biological basis being quite different. A remarkable improvement with fever may be explained by the temperature-dependent leftward shift in activation of wild-type KV3.1 subunitcontaining channels, which would counter the loss of function observed for mutant channels, highlighting KCNC1 as a potential target for precision therapeutics.Publication link2017 Purinergic receptors P2RX4 and P2RX7 in familial multiple sclerosisPatchliner Publication in Human Mutation (2017) Authors: Sadovnick A.D., Gu B.J., Traboulsee A.L., Bernales C.Q., Encarnacion M., Yee I.M., Criscuoli M.G., Huang X., Ou A., Milligan C.J., Petrou S, Wiley J.S, Vilario-Gell C.Genetic variants in the purinergic receptorsP2RX4andP2RX7have been shown to affect susceptibility to multiple sclerosis (MS). In this study, we set out to evaluate whether rare coding variants of major effect could also be identified in these purinergic receptors. Sequencing analysis ofP2RX4andP2RX7in 193 MS patients and 100 controls led to the identification of a rare three variant haplotype (P2RX7rs140915863:C>T [p.T205M],P2RX7rs201921967:A>G [p.N361S], andP2RX4rs765866317:G>A [p.G135S]) segregating with disease in a multi-incident family with six family members diagnosed with MS (logarithm of odds = 3.07). Functional analysis of this haplotype in HEK293 cells revealed impaired P2X7 surface expression (P 0.01), resulting in over 95% inhibition of adenosine triphosphate (ATP)-induced pore function (P 0.001) and a marked reduction in phagocytic ability (P 0.05). In addition, transfected cells showed 40% increased peak ATP-induced inward current (P 0.01), and a greater Ca2+response to the P2X4 135S variant compared with wild type (P 0.0001). Our study nominates rare genetic variants inP2RX4andP2RX7as major genetic contributors to disease, further supporting a role for these purinergic receptors in MS and the disruption of transmembrane cation channels leading to impairment of phagocytosis as the pathological mechanisms of disease.Publication link2017 Identification of Na+/K+-ATPase inhibition-independent proarrhythmic ionic mechanisms of cardiac glycosidesPatchlinerPublication in Nature Scientific Reports (2017) Authors: Koh C.H., Wu J., Chung Y.Y., Liu Z., Zhang R.R., Chong K., Korzh V., Ting S., Oh S., Shim W., Tian H.Y., Wei H.The current study explored the Na+/K+-ATPase (NKA) inhibition-independent proarrhythmic mechanisms of cardiac glycosides (CGs) which are well-known NKA inhibitors. With the cytosolic Ca2+chelated by EGTA and BAPTA or extracellular Ca2+replaced by Ba2+, effects of bufadienolides (bufalin (BF) and cinobufagin (CBG)) and cardenolides (ouabain (Oua) and pecilocerin A (PEA)) on the L-type calcium current (ICa,L) were recorded in heterologous expression CaV1.2-CHO cells and human embryonic stem cell-derived cardiomyocytes (hESC-CMs). BF and CBG demonstrated a concentration-dependent (0.1 to100M) I Ca,Linhibition (maximal 50%) without and with the NKA activity blocked by 10M Oua. BF significantly shortened the action potential duration at 1.0M and shortened the extracellular field potential duration at 0.01~1.0M. On the other hand, BF and CBG at 100M demonstrated a strong inhibition (40%) of the rapidly activating component of the delayed rectifier K+current (IKr) in heterologous expression HEK293 cells and prolonged the APD of the heart of day-3 Zebrafish larva with disrupted rhythmic contractions. Moreover, hESC-CMs treated with BF (10nM) for 24hours showed moderate yet significant prolongation in APD90. In conclusion, our data indicate that CGs particularly bufadienolides possess cytosolic [Ca2+]i- and NKA inhibition-independent proarrhythmic potential through ICa,Land IKrinhibitions.Publication link2017 L-Type Calcium Channel Inhibition Contributes to the Proarrhythmic Effects of Aconitine in Human CardiomyocytesPatchliner Publication in PLoS ONE (2017) Authors: Wu J., Wang X., Chung Y.Y. Koh C.H. Liu Z., Guo H., Yuan Q., Wang C., Su S., Wei H.Aconitine (ACO) is well-known for causing lethal ventricular tachyarrhythmias. While cardiac Na+channel opening during repolarization has long been documented in animal cardiac myocytes, the cellular effects and mechanism of ACO in human remain unexplored. This study aimed to assess the proarrhythmic effects of ACO in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). ACO concentration-dependently (0.3 ~ 3.0 M) shortened the action potentials (AP) durations (APD) in ventricular-like hiPSC-CMs by > 40% and induced delayed after-depolarization. Laser-scanning confocal calcium imaging analysis showed that ACO decreased the duration and amplitude of [Ca2+]itransients and increased in the beating frequencies by over 60%. Moreover, ACO was found to markedly reduce the L-type calcium channel (LTCC) currents (ICa,L) in hiPSC-CMs associated with a positive-shift of activation and a negative shift of inactivation. ACO failed to alter the peak and late Na+currents (INa) in hiPSC-CMs while it drastically increased the late INain Guinea-pig ventricular myocytes associated with enhanced activation/delayed inactivation of INaat -55 mV~ -85 mV. Further, the effects of ACO on ICa,L, INaand the rapid delayed rectifier potassium current (IKr) were validated in heterologous expression systems by automated voltage-clamping assays and a moderate suppression of IKrwas observed in addition to concentration-dependent ICa,Linhibition. Lastly, increased beating frequency, decreased Ca2+wave and shortened field potential duration were recorded from hiPSC-CMs by microelectrode arrays assay. In summary, our data demonstrated that LTCC inhibition could play a main role in the proarrhythmic action of ACO in human cardiomyocytes.Publication link2017 Discovery of benzimidazole derivatives as modulators of mitochondrial function: A potential treatment for Alzheimers diseasePatchliner Publication in PLoS ONE (2017) Authors: Kim T., Yang H.Y., Park B.G., Jung S.Y., Park J.H., Park K.D., Min S.J., Tae J., Yang H., Cho S., Cho S.J., Song H., Mook-Jung I., Lee J., Pae A.N.In this study, we designed a library of compounds based on the structures of well-known ligands of the 18kDa translocator protein (TSPO), one of the putative components of the mPTP. We performed diverse mitochondrial functional assays to assess their ability to restore cells from A-induced toxicity invitro and invivo. Among tested compounds, compound 25 effectively improved cognitive function in animal models of AD. Given the excellent invitro and invivo activity and a favorable pharmacokinetic profile of compound 25, we believe that it can serve as a promising lead compound for a potential treatment option for AD.Publication link2017 Gardos Channelopathy: a variant of hereditary Stomatocytosis with complex molecular regulationPatchliner Publication in Scientific Reports (2017) Authors: Fermo E., Bogdanova A., etkova-Kirova P., Zaninoni A., Marcello A.P., Makhro A., Hnggi P., Hertz L., Danielczok J., Vercellati C., Mirra N., Zanella A., Cortelezzi A., Barcellini W., Kaestner L., Bianchi P.The Gardos channel is a Ca2+sensitive, K+selective channel present in several tissues including RBCs, where it is involved in cell volume regulation. Recently, mutations at two different aminoacid residues inKCNN4have been reported in patients with hereditary xerocytosis. We identified by whole exome sequencing a new family with two members affected by chronic hemolytic anemia carrying mutation R352H in theKCNN4gene. No additional mutations in genes encoding for RBCs cytoskeletal, membrane or channel proteins were detected. We performed functional studies on patients RBCs to evaluate the effects of R352H mutation on the cellular properties and eventually on the clinical phenotype. Gardos channel hyperactivation was demonstrated in circulating erythrocytes and erythroblasts differentiatedex-vivofrom peripheral CD34+ cells. Pathological alterations in the function of multiple ion transport systems were observed, suggesting the presence of compensatory effects ultimately preventing cellular dehydration in patients RBCs; moreover, flow cytometry and confocal fluorescence live-cell imaging showed Ca2+overload in the RBCs of both patients and hypersensitivity of Ca2+uptake by RBCs to swelling. Altogether these findings suggest that the Gardos channelopathy is a complex pathology, to some extent different from the common hereditary xerocytosis.Publication link2017 Correlation between human ether-a-go-go related gene channel inhibition and action potential prolongationPatchlinerPublication in British Journal of Pharmacology (2017) Authors: Saxena P., HortigonVinagre M.P., Beyl S.,Baburin I., Andranovits S., Iqbal S.M., Costa A., IJzerman A.P., Kgler P., Timin E., Smith G.L., Hering S.BACKGROUND AND PURPOSE:Human ether-a-go-go-related gene (hERG; KV 11.1) channel inhibition is a widely accepted predictor of cardiac arrhythmia. hERG channel inhibition alone is often insufficient to predict pro-arrhythmic drug effects. This study used a library of dofetilide derivatives to investigate the relationship between standard measures of hERG current block in an expression system and changes in action potential duration (APD) in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). The interference from accompanying block of CaV1.2 and NaV1.5 channels was investigated along with an in silico AP model.EXPERIMENTAL APPROACH:Drug-induced changes in APD were assessed in hiPSC-CMs using voltage-sensitive dyes. The IC50values for dofetilide and 13 derivatives on hERG current were estimated in an HEK293 expression system. The relative potency of each drug on APD was estimated by calculating the dose (D150) required to prolong the APD at 90% (APD90) repolarization by 50%.KEY RESULTS:The D150 in hiPSC-CMs was linearly correlated with IC50of hERG current. In silico simulations supported this finding. Three derivatives inhibited hERG without prolonging APD, and these compounds also inhibited CaV1.2 and/or NaV1.5 in a channel state-dependent manner. Adding CaV 1.2 and NaV 1.2 block to the in silico model recapitulated the direction but not the extent of the APD change.CONCLUSIONS AND IMPLICATIONS:Potency of hERG current inhibition correlates linearly with an index of APD in hiPSC-CMs. The compounds that do not correlate have additional effects including concomitant block of CaV1.2 and/or NaV1.5 channels. In silico simulations of hiPSC-CMs APs confirm the principle of the multiple ion channel effects.Publication link2017 Development of Automated Patch Clamp Technique to Investigate CFTR Chloride Channel FunctionPatchliner Publication in Frontiers in Pharmacology (2017) Authors: Billet A.,Froux L., Hanrahan J.W., Becq F.The chloride (Cl-) channel cystic fibrosis transmembrane conductance regulator (CFTR) is defective in cystic fibrosis (CF), and mutation of its encoding gene leads to various defects such as retention of the misfolded protein in the endoplasmic reticulum, reduced stability at the plasma membrane, abnormal channel gating with low open probability, and thermal instability, which leads to inactivation of the channel at physiological temperature. Pharmacotherapy is one major therapeutic approach in the CF field and needs sensible and fast tools to identify promising compounds. The high throughput screening assays available are often fast and sensible techniques but with lack of specificity. Few works used automated patch clamp (APC) for CFTR recording, and none have compared conventional and planar techniques and demonstrated their capabilities for different types of experiments. In this study, we evaluated the use of planar parallel APC technique for pharmacological search of CFTR-trafficking correctors and CFTR function modulators. Using optimized conditions, we recorded both wt- and corrected F508del-CFTR Cl-currents with automated whole-cell patch clamp and compared the data to results obtained with conventional manual whole-cell patch clamp. We found no significant difference in patch clamp parameters such as cell capacitance and series resistance between automated and manual patch clamp. Also, the results showed good similarities of CFTR currents recording between the two methods. We showed that similar stimulation protocols could be used in both manual and automatic techniques allowing precise control of temperature, classicI/Vrelationship, and monitoring of current stability in time. In conclusion, parallel patch-clamp recording allows rapid and efficient investigation of CFTR currents with a variety of tests available and could be considered as new tool for medium throughput screening in CF pharmacotherapy.Publication link2016 The potassium channels TASK2 and TREK1 regulate functional differentiation of murine skeletal muscle cellsPatchliner Publication in American Journal of Physiology - Cell Physiology (2016) Authors: Afzali A.M., Ruck T., Herrmann A.M., Iking J., Sommer C., Kleinschnitz C., Preue C., Stenzel W., Budde T., Wiendl H., Bittner S., Meuth S.G.Two-pore domain potassium (K2P) channels influence basic cellular parameters such as resting membrane potential, cellular excitability, or intracellular Ca2+-concentration [Ca2+]iWhile the physiological importance of K2Pchannels in different organ systems (e.g., heart, central nervous system, or immune system) has become increasingly clear over the last decade, their expression profile and functional role in skeletal muscle cells (SkMC) remain largely unknown. The mouse SkMC cell line C2C12, wild-type mouse muscle tissue, and primary mouse muscle cells (PMMs) were analyzed using quantitative PCR, Western blotting, and immunohistochemical stainings as well as functional analysis including patch-clamp measurements and Ca2+ imaging. Mouse SkMC express TWIK-related acid-sensitive K+channel (TASK) 2, TWIK-related K+channel (TREK) 1, TREK2, and TWIK-related arachidonic acid stimulated K+channel (TRAAK). Except TASK2 all mentioned channels were upregulated in vitro during differentiation from myoblasts to myotubes. TASK2 and TREK1 were also functionally expressed and upregulated in PMMs isolated from mouse muscle tissue. Inhibition of TASK2 and TREK1 during differentiation revealed a morphological impairment of myoblast fusion accompanied by a downregulation of maturation markers. TASK2 and TREK1 blockade led to a decreased K+outward current and a decrease of ACh-dependent Ca2+influx in C2C12 cells as potential underlying mechanisms. K2P-channel expression was also detected in human muscle tissue by immunohistochemistry pointing towards possible relevance for human muscle cell maturation and function. In conclusion, our findings for the first time demonstrate the functional expression of TASK2 and TREK1 in muscle cells with implications for differentiation processes warranting further investigations in physiologic and pathophysiologic scenarios.Publication link2017 14-3-3 proteins regulate K2P5.1 surface expression on T lymphocytesPatchliner Publication in Traffic (2017) Authors: Fernndez-Orth J., Ehling P., Ruck T., Pankratz S., Hofmann M.,Landgraf P., Dieterich D.C., SmallaK.-H., Khne T., Seebohm G., Budde T., Wiendl H., Bittner S., Meuth S.G.K2P5.1 channels (also called TASK-2 orKcnk5) have already been shown to be relevant in the pathophysiology of autoimmune disease because they are known to be upregulated on peripheral and central T lymphocytes of multiple sclerosis (MS) patients. Moreover, overexpression of K2P5.1 channels in vitro provokes enhanced T-cell effector functions. However, the molecular mechanisms regulating intracellular K2P5.1 channel trafficking are unknown so far. Thus, the aim of the study is to elucidate the trafficking of K2P5.1 channels on T lymphocytes. Using mass spectrometry analysis, we have identified 14-3-3 proteins as novel binding partners of K2P5.1 channels. We show that a non-classical 14-3-3 consensus motif (R-X-X-pT/S-x) at the channel's C-terminus allows the binding between K2P5.1 and 14-3-3. The mutant K2P5.1/S266A diminishes the protein-protein interaction and reduces the amplitude of membrane currents. Application of a non-peptidic 14-3-3 inhibitor (BV02) significantly reduces the number of wild-type channels in the plasma membrane, whereas the drug has no effect on the trafficking of the mutated channel. Furthermore, blocker application reduces T-cell effector functions. Taken together, we demonstrate that 14-3-3 interacts with K2P5.1 and plays an important role in channel trafficking.Publication link2016 Myrsinane, Premyrsinane, and Cyclomyrsinane Diterpenes fromEuphorbia falcata as Potassium Ion Channel Inhibitors with Selective G Protein-Activated Inwardly Rectifying Ion Channel (GIRK) Blocking EffectsPatchliner Publication in Journal of Natural Products (2016) Authors: Vasas A., Forgo P., Orvos P., Tlosi L., Csorba A., Pinke G., Hohmann J.GIRK channels are activated by a large number of G protein-coupled receptors and regulate the electrical activity of neurons, cardiac atrial myocytes, and -pancreatic cells. Abnormalities in GIRK channel function have been implicated in the pathophysiology of neuropathic pain, drug addiction, and cardiac arrhythmias. In the heart, GIRK channels are selectively expressed in the atrium, and their activation inhibits pacemaker activity, thereby slowing the heart rate. In the present study, 19 new diterpenes, falcatins AS, and the known euphorprolitherin Dwere isolated fromEuphorbia falcata. The compounds were assayed on stable transfected HEK-hERG (KV11.1) and HEK-GIRK1/4 (Kir3.1 and Kir3.4) cells. Blocking activity on GIRK channels was exerted by 13 compounds (6183% at 10 M), and, among them, five possessed low potency on the hERG channel (420% at 10 M). These selective activities suggest that myrsinane-related diterpenes are potential lead compounds for the treatment of atrial fibrillation.Publication link2016 The antiepileptic medications carbamazepine and phenytoin inhibit native sodium currents in murine osteoblastsPatchliner Publication in Epilepsia (2016) Authors: Petty S.J., Milligan C.J., Todaro M., Richards K.L., Kularathna P.K., Pagel C.N., French C.R., Hill-Yardin E.L., O'Brien T.J., Wark J.D., Mackie E.J., Petrou S.Objective:Fracture risk is a serious comorbidity in epilepsy and may relate to the use of antiepileptic drugs (AEDs). Many AEDs inhibit ion channel function, and the expression of these channels in osteoblasts raises the question of whether altered bone signaling increases bone fragility. We aimed to confirm the expression of voltage-gated sodium (NaV) channels in mouse osteoblasts, and to investigate the action of carbamazepine and phenytoin on NaVchannels.Methods:Immunocytochemistry was performed on primary calvarial osteoblasts extracted from neonatal C57BL/6J mice and additional RNA sequencing (RNASeq) was included to confirm expression of NaV. Whole-cell patch-clamp recordings were made to identify the native currents expressed and to assess the actions of carbamazepine (50 m) or phenytoin (50 m).Results:NaVexpression was demonstrated with immunocytochemistry, RNA sequencing, and functionally, with demonstration of robust tetrodotoxin-sensitive and voltage-activated inward currents. Application of carbamazepine or phenytoin resulted in significant inhibition of current amplitude for carbamazepine (31.6 5.9%, n = 9; p 0.001), and for phenytoin (35.5 6.9%, n = 7; p 0.001).Significance:Mouse osteoblasts express NaV, and native NaVcurrents are blocked by carbamazepine and phenytoin, supporting our hypothesis that AEDs can directly influence osteoblast function and potentially affect bone strength.Publication link2016 Human EAG channels are directly modulated by PIP2 as revealed by electrophysiological and optical interference investigationsPatchliner Publication in Scientific Reports (2016) Authors: Han B., He K., Cai C., Tang Y., Yang L., Heinemann S.H., Hoshi T., Hou S.Voltage-gated ether go-go(EAG) K+channels are expressed in various types of cancer cells and also in the central nervous system. Aberrant overactivation of human EAG1 (hEAG1) channels is associated with cancer and neuronal disorders such as Zimmermann-Laband and Temple-Baraitser syndromes. Although hEAG1 channels are recognized as potential therapeutic targets, regulation of their functional properties is only poorly understood. Here, we show that the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2) is a potent inhibitory gating modifier of hEAG1 channels. PIP2inhibits the channel activity by directly binding to a short N-terminal segment of the channel important for Ca2+/calmodulin (CaM) binding as evidenced by bio-layer interferometry measurements. Conversely, depletion of endogenous PIP2either by serotonin-induced phospholipase C (PLC) activation or by a rapamycin-induced translocation system enhances the channel activity at physiological membrane potentials, suggesting that PIP2exerts a tonic inhibitory influence. Our study, combining electrophysiological and direct binding assays, demonstrates that hEAG1 channels are subject to potent inhibitory modulation by multiple phospholipids and suggests that manipulations of the PIP2signaling pathway may represent a strategy to treat hEAG1 channel-associated diseases.Publication link2016 Human T cells in silico: Modelling their electrophysiological behaviour in health and diseasePatchliner Publication in Journal of Theoretical Biology (2016) Authors: Ehling P., Meuth P., Eichinger P., Hermann A.M., Bittner S., Pawlowski M., Pankratz S., Herty M., Budde T., Meuth S.G.Although various types of ion channels are known to have an impact on human T cell effector functions, their exact mechanisms of influence are still poorly understood. The patch clamp technique is a well-established method for the investigation of ion channels in neurons and T cells. However, small cell sizes and limited selectivity of pharmacological blockers restrict the value of this experimental approach. Building a realistic T cell computer model therefore can help to overcome these kinds of limitations as well as reduce the overall experimental effort. The computer model introduced here was fed off ion channel parameters from literature and new experimental data. It is capable of simulating the electrophysiological behaviour of resting and activated human CD4+T cells under basal conditions and during extracellular acidification. The latter allows for the very first time to assess the electrophysiological consequences of tissue acidosis accompanying most forms of inflammation.Publication link2015 The two-pore domain K2P channel TASK2 drives human NK-cell proliferation and cytolytic functionPatchliner Publication in European Journal of Immunology (2015) Authors: Schulte-Mecklenbeck A., Bittner S., Ehling P.,Dring F., Wischmeyer E., Breuer J., Herrmann A.M., Wiendl H., Meuth S.G., Gross C.C.Natural killer (NK) cells are a subset of cytotoxic lymphocytes that recognize and kill tumor and virusinfected cells without prior stimulation. Killing of target cells is a multistep process including adhesion to target cells, formation of an immunological synapse, and polarization and release of cytolytic granules. The role of distinct potassium channels in this orchestrated process is still poorly understood. The current study reveals that in addition to the voltagegated KV1.3 and the calciumactivated KCa3.1 channels, human NK cells also express the twopore domain K2P channel TASK2 (TWIKrelated acidsensitive potassium channel). Expression ofTask2varies among NKcell subsets and depends on their differentiation and activation state. Despite its different expression in TASK2highCD56brightCD16and TASK2lowCD56dimCD16+NK cells, TASK2 is involved in cytokineinduced proliferation and cytolytic function of both subsets. TASK2 is crucial for leukocyte functional antigen (LFA1) mediated adhesion of both resting and cytokineactivated NK cells to target cells, an early step in killing of target cells. With regard to the following mechanism, TASK2 plays a role in release of cytotoxic granules by resting, but not IL15induced NK cells. Taken together, our data exhibit twopore potassium channels as important players in NKcell activation and effector function.Publication link2016 Automated Electrophysiological and Pharmacological Evaluation of Human Pluripotent Stem Cell-Derived CardiomyocytesPatchliner Publication in Stem Cells and Development (2016) Authors: Rajamohan D., Kalra S., Hoang M.D., George V., Staniforth A., Russell H., Yang X., Denning C.Automated planar patch clamp systems are widely used in drug evaluation studies because of their ability to provide accurate, reliable, and reproducible data in a high-throughput manner. Typically, CHO and HEK tumorigenic cell lines overexpressing single ion channels are used since they can be harvested as high-density, homogenous, single-cell suspensions. While human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) are physiologically more relevant, these cells are fragile, have complex culture requirements, are inherently heterogeneous, and are expensive to produce, which has restricted their use on automated patch clamp (APC) devices. Here, we used high efficiency differentiation protocols to produce cardiomyocytes from six different hPSC lines for analysis on the Patchliner (Nanion Technologies GmbH) APC platform. We developed a two-step cell preparation protocol that yielded cell catch rates and whole-cell breakthroughs of 80%, with 40% of these cells allowing electrical activity to be recorded. The protocol permitted formation of long-lasting (>15min), high quality seals (>2G) in both voltage- and current-clamp modes. This enabled density of sodium, calcium, and potassium currents to be evaluated, along with doseresponse curves to their respective channel inhibitors, tetrodotoxin, nifedipine, and E-4031. Thus, we show the feasibility of using the Patchliner platform for automated evaluation of the electrophysiology and pharmacology of hPSC-CMs, which will enable considerable increase in throughput for reliable and efficient drug evaluation.Publication link2014 Physiological and genetic analysis of multiple sodium channel variants in a model of genetic absence epilepsyPatchliner Publication in Neurobiology of Disease (2014) Authors: Oliva M.K., McGarr T.C., Beyer B.J., Gazina E., Kaplan D.I., Cordeiro L., Thomas E., Dib-Hajj S.D., Waxman S.G., Frankel W.N., Petrou S.In excitatory neurons, SCN2A (NaV1.2) and SCN8A (NaV1.6) sodium channels are enriched at the axon initial segment. NaV1.6 is implicated in several mouse models of absence epilepsy, including a missense mutation identified in a chemical mutagenesis screen (Scn8aV929F). Here, we confirmed the prior suggestion thatScn8aV929Fexhibits a striking genetic background-dependent difference in phenotypic severity, observing that spike-wave discharge (SWD) incidence and severity are significantly diminished whenScn8aV929Fis fully placed onto the C57BL/6J strain compared with C3H. Examination of sequence differences in NaVsubunits between these two inbred strains suggested NaV1.2V752Fas a potential source of this modifier effect. Recognising that the spatial co-localisation of the NaVchannels at the axon initial segment (AIS) provides a plausible mechanism for functional interaction, we tested this idea by undertaking biophysical characterisation of the variant NaVchannels and by computer modelling. NaV1.2V752Ffunctional analysis revealed an overall gain-of-function and for NaV1.6V929Frevealed an overall loss-of-function. A biophysically realistic computer model was used to test the idea that interaction between these variant channels at the AIS contributes to the strain background effect. Surprisingly this modelling showed that neuronal excitability is dominated by the properties of NaV1.2V752Fdue to functional silencing of NaV1.6V929Fsuggesting that these variants do not directly interact. Consequent genetic mapping of the major strain modifier to Chr 7, and not Chr 2 whereScn2amaps, supported this biophysical prediction. While a NaV1.6V929Floss of function clearly underlies absence seizures in this mouse model, the strain background effect is apparently not due to an otherwise temptingScn2avariant, highlighting the value of combining physiology and genetics to inform and direct each other when interrogating genetic complex traits such as absence epilepsy.Publication link2015 Scalable Electrophysiological Investigation of iPS Cell-Derived Cardiomyocytes Obtained by a Lentiviral Purification StrategyPatchliner Publication in Journal of Clinical Medicine (2015) Authors: Friedrichs S., Malan D., Voss Y., Sasse P.Disease-specific induced pluripotent stem (iPS) cells can be generated from patients and differentiated into functional cardiomyocytes for characterization of the disease and for drug screening. In order to obtain pure cardiomyocytes for automated electrophysiological investigation, we here report a novel non-clonal purification strategy by using lentiviral gene transfer of a puromycin resistance gene under the control of a cardiac-specific promoter. We have applied this method to our previous reported wild-type and long QT syndrome 3 (LQTS 3)-specific mouse iPS cells and obtained a pure cardiomyocyte population. These cells were investigated by action potential analysis with manual and automatic planar patch clamp technologies, as well as by recording extracellular field potentials using a microelectrode array system. Action potentials and field potentials showed the characteristic prolongation at low heart rates in LQTS 3-specific, but not in wild-type iPS cell-derived cardiomyocytes. Hence, LQTS 3-specific cardiomyocytes can be purified from iPS cells with a lentiviral strategy, maintain the hallmarks of the LQTS 3 disease and can be used for automated electrophysiological characterization and drug screeningPublication link2013 Red cell investigations: Art and artefactsPatchliner Publication in Blood reviews (2013) Authors: Minetti G.,Ege S.,Mrsdorf D.,Steffen P.,Makhro A.,Achilli C.,Ciana A.,Wang J.,Bouyer G.,Bernhardt I.,Wagner C.,Thomas S.,Bogdanova A.,Kaestner L.Red blood cell research is important for both, the clinical haematology, such as transfusion medicine or anaemia investigations, and the basic research fields like exploring general membrane physiology or rheology. Investigations of red blood cells include a wide spectrum of methodologies ranging from population measurements with a billion cells evaluated simultaneously to single-cell approaches. All methods have a potential for pitfalls, and the comparison of data achieved by different technical approaches requires a consistent set of standards. Here, we give an overview of common mistakes using the most popular methodologies in red blood cell research and how to avoid them. Additionally, we propose a number of standards that we believe will allow for data comparison between the different techniques and different labs. We consider biochemical analysis, flux measurements, flow cytometry, patch-clamp measurements and dynamic fluorescence imaging as well as emerging single-cell techniques, such as the use of optical tweezers and atomic force microscopy.Publication link2014 Multi-Generational Pharmacophore Modeling for Ligands to the Cholane Steroid-Recognition Site in the 1 Modulatory Subunit of the BK(Ca) ChannelPatchliner Publication in Journal of Molecular Graphics and Modelling (2014) Authors: McMillan J.E., Bukiya A.N., Terrell C.L., Patil S.A., Miller D.D., Dopico A.M., Parrilla A.L.Large conductance, voltage- and Ca2+-gated K+(BKCa) channels play a critical role in smooth muscle contractility and thus represent an emerging therapeutic target for drug development to treat vascular disease, gastrointestinal, bladder and uterine disorders. Several compounds are known to target the ubiquitously expressed BKCachannel-forming subunit. In contrast, just a few are known to target the BKCamodulatory 1subunit, which is highly expressed in smooth muscle and scarce in most other tissues. Lack of available high-resolution structural data makes structure-based pharmacophore modeling of 1subunit-dependent BKCachannel activators a major challenge. Following recent discoveries of novel BKCachannel activators that act via 1subunit recognition, we performed ligand-based pharmacophore modeling that led to the successful creation and fine-tuning of a pharmacophore over several generations. Initial models were developed using physiologically active cholane steroids (bile acids) as template. However, as more compounds that act on BKCa1have been discovered, our model has been refined to improve accuracy. Database searching with our best-performing model has uncovered several novel compounds as candidate BKCa1subunit ligands. Eight of the identified compounds were experimentally screened and two proved to be activators of recombinant BKCa1complexes. One of these activators, sobetirome, differs substantially in structure from any previously reported activator.Publication link2013 Establishment of a Secondary Screening Assay for P/Q-Type Calcium Channel BlockersPatchliner Publication in Combinatorial Chemistry & High Throughput Screening (2013) Authors: Hermann D., Mezler M., Swensen A., Bruehl C., Obergruberger A., Wicke K., Schoemaker H., Gross G., Draguhn A., Nimmrich V.Development of calcium channel blockers is attractive, but has in the past been hampered by lack of high throughput electrophysiological technology. This limitation has been overcome by the implementation of automated patch clamp systems that allow identification of state-dependent compounds, which preferentially target pathologically overactive channels.We recently presented a fluorescence-based high-throughput screen for P/Q-type calcium channels followed by automated electrophysiology. Here, we provide a detailed description of the development of the secondary screen, and show the full analysis of the inactivation kinetics of the recombinant P/Q channel that served as a basis for the automated patch clamp protocol. Increasing the length of pre-depolarization shifted the inactivation to more hyperpolarized potentials. No steadystate inactivation was reached up to pre-depolarization durations of 3 min, while stability of the recordings progressively declined. As a compromise, a 3s pre-depolarization protocol was proposed for functional screening. In order to validate the electrophysiological screening, we compared kinetics and pharmacology of recombinant P/Q-type channels between automated and manual patch clamp measurements. Channel activation was similar under both conditions. By contrast, inactivation occurred at more hyperpolarized potentials in the automated system. Therefore, P/Q-type calcium channel inactivation is sensitive to the applied technological platform and needs to be adjusted when performing automated patch clamp recordings.Our results indicate that a thorough analysis of the inactivation kinetics is mandatory, when establishing an electrophysiological screening protocol for calcium channel blockers. As some data obtained by automated recordings may not be identical to manual patch clamp analysis, we recommend a proper initial validation of the screening assay and if necessary a posthoc adjustment of automated patch clamp values. The protocol presented here supports hit-to-lead and lead optimization efforts during the development of novel P/Q-type calcium channel blockers, and may be valuable for the generation of assays in other ion channel programsPublication link2013 Minimized cell usage for stem cell-derived and primary cells on an automated patch clamp systemPatchliner Publication in Journal of Pharmacological and Toxicological Methods (2013) Authors: Becker N., Stoelzle S., Gpel S., Guinot D., Mumm P., Haarmann C., Malan D., Bohlen H., Kossolov E., Kettenhofen R., George M., Fertig N., Brggemann A.IntroductionChip-based automated patch clamp systems are widely used in drug development and safety pharmacology, allowing for high quality, high throughput screening at standardized experimental conditions. The merits of automation generally come at the cost of large amounts of cells needed, since cells are not targeted individually, but randomly positioned onto the chip aperture from cells in suspension. While cell usage is of little concern when using standard cell lines such as CHO or HEK cells, it becomes a crucial constraint with cells of limited availability, such as primary or otherwise rare and expensive cells, like induced pluripotent stem (IPS) cell-derived cardiomyocytes or neurons.MethodsWe established application protocols for CHO cells, IPS cell-derived neurons (iCellNeurons, Cellular Dynamics International), cardiomyocytes (Cor.4U, Axiogenesis) and pancreatic islet cells, minimizing cell usage for automated patch clamp recordings on Nanion's Patchliner. Use of 5l cell suspension per well for densities between 55,000cells/ml and 400,000cells/ml depending on cell type resulted in good cell capture.ResultsWe present a new cell application procedure optimized for the Patchliner achieving>80% success rates for using as little as 300 to 2000 cells per well depending on cell type. We demonstrate that this protocol works for standard cell lines, as well as for stem cell-derived neurons and cardiomyocytes, and for primary pancreatic islet cells. We present recordings for these cell types, demonstrating that high data quality is not compromised by altered cell application.DiscussionOur new cell application procedure achieves high success rates with unprecedentedly low cell numbers. Compared to other standard automated patch clamp systems we reduced the average amount of cells needed by more than 150 times. Reduced cell usage crucially improves cost efficiency for expensive cells and opens up automated patch clamp for primary cells of limited availability.Publication link2012 TRPA1 Agonist Activity of Probenecid Desensitizes Channel Responses: Consequences for ScreeningPatchliner Publication inASSAY and Drug Development Technologies (2012) Authors: McClenaghan C., Zeng F., Verkuyl J.M.The transient receptor potential channel subtype A member 1 (TRPA1) is a nonselective cation channel widely viewed as having therapeutic potential, particularly for pain-related indications.

cell proliferation as well as migration, and cell surface localization studies revealed TRPC1 alone, as well as with STIM1. The data suggest a complex situation in which there is not only plasma membranespanning STIM1 that is important for cell migration and TRPC1-independent store-operated cationic current but also TRPC1-STIM1 interaction, a TRPC1dependent component of store-operated current, and STIM1-independent TRPC1 linked to cell proliferation.Publication link2008 Production of a specific extracellular inhibitor of TRPM3 channelsPatchliner Publication inBritish Journal of Pharmacology (2008) Authors: Naylor J., Milligan C.J., Zeng F., Jones C., Beech D.J.Background and purpose: Isoform-specific ion channel blockers are useful for target validation in drug discovery and can provide the basis for new therapeutic agents and aid in determination of physiological functions of ion channels. The aim of this study was to generate a specific blocker of human TRPM3 channels as a tool to help investigations of this member of the TRP cationic channel family. Experimental approach: A polyclonal antibody (TM3E3) was made to a conserved peptide of the third extracellular (E3) loop of TRPM3 and tested for binding and functional effect. Studies of channel activity were made by whole-cell planar patch-clamp and fura-2 intracellular Ca2+measurement. Key results: Ionic current mediated by TRPM3 was inhibited partially by TM3E3 over a period of 510 min. Ca2+entry in TRPM3-expressing cells was also partially inhibited by TM3E3 in a peptide-specific manner and independently of the type of agonist used to activate TRPM3. TM3E3 had no effect on TRPC5, TRPV4, TRPM2 or an endogenous ATP response. Conclusions and implications: The data show the successful development of a specific TRPM3 inhibitor and give further confidence in E3 targeting as an approach to producing isoform-specific ion channel blockers.Publication link2020 Structure and mechanism of bactericidal mammalian perforin-2, an ancient agent of innate immunityOrbit mini andVesicle Prep Pro Publication in Science Advances (2020) Authors: Ni T., Jiao F., Yu X., Aden S., Ginger L., Williams S.I., Bai F., Prak V., Karia D., Stansfeld P., Zhang P., Munson G., Anderluh G.,Scheuring S., Gilbert R.J.C.Perforin-2 (MPEG1) is thought to enable the killing of invading microbes engulfed by macrophages and other phagocytes, forming pores in their membranes. Loss of perforin-2 renders individual phagocytes and whole organisms significantly more susceptible to bacterial pathogens. Here, we reveal the mechanism of perforin-2 activation and activity using atomic structures of pre-pore and pore assemblies, high-speed atomic force microscopy, and functional assays. Perforin-2 forms a pre-pore assembly in which its pore-forming domain points in the opposite direction to its membrane-targeting domain. Acidification then triggers pore formation, via a 180 conformational change. This novel and unexpected mechanism prevents premature bactericidal attack and may have played a key role in the evolution of all perforin family proteins.Publication link2022 An oomycete NLP cytolysin forms transient small pores in lipid membranesOrbit mini andVesicle Prep Pro Publication in Science Advances (2022) Authors: Pirc K., Clifton L.A., Yilmaz N., Saltalmacchiamo A., Mally M., Snoj T.,nidari N., Srnko M.,BoriekJ., Parkkila P., Albert I., Podobnik M., Numata K.,Nrnberger T., Viitala T., Derganac J., Magistrato A., Lakey J.H., Anderluh G.Microbial plant pathogens secrete a range of effector proteins that damage host plants and consequently constrain global food production. Necrosis and ethylene-inducing peptide 1like proteins (NLPs) are produced by numerous phytopathogenic microbes that cause important crop diseases. Many NLPs are cytolytic, causing cell death and tissue necrosis by disrupting the plant plasma membrane. Here, we reveal the unique molecular mechanism underlying the membrane damage induced by the cytotoxic model NLP. This membrane disruption is a multistep process that includes electrostatic-driven, plant-specific lipid recognition, shallow membrane binding, protein aggregation, and transient pore formation. The NLP-induced damage is not caused by membrane reorganization or large-scale defects but by small membrane ruptures. This distinct mechanism of lipid membrane disruption is highly adapted to effectively damage plant cells.Publication link2017 Engineering a pH responsive pore forming proteinOrbit mini and Vesicle Prep Pro Publication in Scientific Reports (2017) Authors: Kisovec M., Rezelj S., Knap P., Cajnko M.M., Caserman S., Flaker A., nidari N., Repi M., Mavri J., Ruan Y., Scheuring S., Podobnik M., Anderluh G.Listeriolysin O (LLO) is a cytolysin capable of forming pores in cholesterol-rich lipid membranes of host cells. It is conveniently suited for engineering a pH-governed responsiveness, due to a pH sensor identified in its structure that was shown before to affect its stability. Here we introduced a new level of control of its hemolytic activity by making a variant with hemolytic activity that was pH-dependent. Based on detailed structural analysis coupled with molecular dynamics and mutational analysis, we found that the bulky side chain of Tyr406 allosterically affects the pH sensor. Molecular dynamics simulation further suggested which other amino acid residues may also allosterically influence the pH-sensor. LLO was engineered to the point where it can, in a pH-regulated manner, perforate artificial and cellular membranes. The single mutant Tyr406Ala bound to membranes and oligomerized similarly to the wild-type LLO, however, the final membrane insertion step was pH-affected by the introduced mutation. We show that the mutant toxin can be activated at the surface of artificial membranes or living cells by a single wash with slightly acidic pH buffer. Y406A mutant has a high potential in development of novel nanobiotechnological applications such as controlled release of substances or as a sensor of environmental pH.Webinar29.06.2021 | Webinar: Channelopathies resolved via cryo-EM and X-ray crystallography: an investigation of muscle excitation-contraction couplingSpeakers: Dr. Conrad Weichbrodt (Senior Scientist / Product Manager Orbit family; Nanion Technologies)Dr. Filip Van Petegem (Professor, Biochemistry and Molecular Biology - University of British Columbia)Muscle contraction requires a tight communication between Ca2+-permeable ion channels located in the plasma membrane (L-type calcium channels, CaVs) and in the Sarcoplasmic Reticulum (Ryanodine Receptors, RyRs). In cardiac muscle, Ca2+ entering through CaV1.2 can stimulate the nearby RyR2 isoform, through a process of Ca2+-induced Ca2+ release. In skeletal muscle, however, the coupling is thought to occur mechanically, with voltage-dependent conformational changes in CaV1.1 being transmitted to RyR1, either through direct or indirect interactions. Mutations in RyR1 (as well as CaV1.1) can give rise to malignant hyperthermia (MH), a potentially lethal condition typically triggered by volatile anaesthetics. Other mutations cause central core disease (CCD), whereas some are linked to both MH and CCD. Similarly, mutations in RyR2 are linked to a form of stress-induced cardiac arrhythmia, known as CPVT (catecholaminergic polymorphic ventricular tachycardia). Our lab has been solving crystal structures of isolated RyR domains in WT and disease mutant forms, which show that many mutations cause conformational changes. More recently, we solved cryo-EM structures of RyR1 carrying the founding mutation (R615C) that links RyR1 to malignant hyperthermia. The structures show how many mutations can destabilize the closed state, leading to facilitated channel opening.In addition, RyRs are heavily regulated by kinases (PKA, CaMKII) that can also facilitate channel opening. Excessive phosphorylation of RyRs has been linked to a range of acquired disorders, including atrial fibrillation and arrhythmias during heart failure. We elucidated how PKA recognizes the cardiac RyR2 via an unusual interface and show that phosphorylation may induce the formation of extra secondary structure elements.Finally, I will discuss our efforts to understand the mechanical coupling between CaV1.1 and RyR1 in skeletal muscle. STAC3 is a small protein recently shown to be essential for the coupling. We found this protein to interact with a cytosolic loop of CaV1.1 and found that several mutations in STAC3, linked to myopathy, directly affect this interaction and the mechanical coupling.Webinar17.09.2020 | Webinar: Electrophysiological investigation of integral membrane proteins using the Orbit miniSpeakers: Dr. Conrad Weichbrodt (Senior Scientist / Product Manager Orbit family; Nanion Technologies)A free standing lipid bilayer separating two aqueous compartments represents a fundamental prerequisite for the investigation of electrophysiological features of membrane spanning proteins like ion channels, porins or certain membrane active toxins. The convenient and reproducible preparation of these model bilayers and the often tedious workflow of conducting such an experiment on classic one channel setups, however, still remain an obstacle for easy and fast data generation.Here we present Nanions Orbit mini device which is explicitly designed to meet the special requirements of experiments on artificial lipid bilayers: use of Ioneras MECA (micro electrode cavity array) chip technology combined with state of the art low noise amplifiers (Elements S.R.L.) enable the fully parallel low-noise recording of four separate lipid bilayers at bandwidths up to 100 kHz. Todays webinar consists of a general introduction of the system and a brief overview of it features, applications and optional add ons. We then demonstrate how to actually perform an experiment on the device showcasing translocation of Polyethylene glycol (PEG) polymers through the well established and commercially available porin alpha-Hemolysin.Webinar28.06.2018 | Webinar: Artificial Lipid Bilayers in focus: Hand-held DNA-sequencing and biosensing with nanoporesSpeakers: Dr. Conrad Weichbrodt, Nanion Technologies GermanyWebinar28.06.2018 | Webinar: Artificial Lipid Bilayers in focus: Hand-held DNA-sequencing and biosensing with nanoporesSpeaker: Prof. Dr. Stefan Howorka, University College London, Department of ChemistryPortable DNA sequencing and biosensing can advance research, bedside-diagnostics, and homeland security. I describe how label-free sensing is achieved with atom-scale designed membrane nanopores. In this strategy, nanopores act as electronic sensors that detect when individual molecules pass the pores nanoscale hole. The temporary blockages cause changes in ionic pore current. The approach has helped pioneer portable DNA sequencing with protein pores(1) to discriminate individual bases. More recently, synthetic pores have been built by folding DNA strands into defined channels(2). The DNA nanopores are relevant as they overcome the narrow size range of protein pores and thereby accommodate folded protein analytes. The DNA nanostructures are also easier to rationally design than proteins(3) and thereby enable new applications, also in synthetic biology(4).(1) Nature 2014 516 250;(2) Nat. Nanotechnol. 2016 11 152;(3) Nat. Nanotechnol. 2017 12 619;(4) Science 2016 352 890; Nat. Chem. 2017 9 611Webinar27.01.2016 | Webinar: Instant bilayers just add protein.Speakers: Dr. Gerhard Baaken, Ionera GmbH and Dr. Mohamed Kreir, Application Scientist, Nanion TechnologiesThis webinar covers the use of the lipid bilayer platforms from Nanion: the Orbit16 and the Orbit mini for characterization of membrane proteins like ion channels, bacterial porins and biological nanopores. Both bilayer systems support high quality low noise recordings, but differ in throughput capabilities and experimental features. The Orbit16, introduced in 2012 is a device for efficient formation of 16 lipid bilayers simultaneously, allowing for parallel bilayer-reconstitution of ion channels and nanoporesWebinar27.01.2016 | Webinar: Instant bilayers just add protein.This webinar covers the use of the lipid bilayer platforms from Nanion: the Orbit16 and the Orbit mini for characterization of membrane proteins like ion channels, bacterial porins and biological nanopores. Both bilayer systems support high quality low noise recordings, but differ in throughput capabilities and experimental features. The Orbit16, introduced in 2012 is a device for efficient formation of 16 lipid bilayers simultaneously, allowing for parallel bilayer-reconstitution of ion channels and nanopores.Lipid bilayer arrays are automatically formed by remotely actuated painting. Orbit 16 has proven to be a versatile device for research and scientific applications which is reflected by numerous publication in high rank journals such as Science, Nature, Nano Letters, ACS Nano etc. The Orbit mini, introduced in 2015, is a smaller system, allowing low noise, high bandwidth recordings from 4 bilayers simultaneously. Orbit mini supports temperature-controlled experiments, allowing both active cooling and heating, a benefit when investigating thermo-sensitive channels such as TRPA1 or TRPV1, which will be shown in this webinar. Micro Electrode Cavity Array chips (MECA chips, Ionera Technologies GmbH, Freiburg) are the core of both devices. The MECA chips have successfully been validated with a wide variety of different ion channels and pores including KcsA, gramicidin, -hemolysin, Kv- and Nav-channels etc. In this webinar the basics of the MECA technology will be explained and its applications will be shown in detail. Join this webinar and see how these outstanding platforms can add up to your work: Orbit 16 and Orbit mini!Application Note PDFTRPV1 Temperature activation of TRPV1 reconstituted into planar lipid bilayers and recorded using the Orbit mini platformOrbit mini application note: The transient receptor potential cation channel, subfamily V, member 1 (TRPV1), is a ligand-gated, non-selective cation channel widely expressed in the peripheral and central nervous system. The TRPV1 channel can be activated by a number of physical and chemical stimuli, including capsaicin (the active ingredient in chili peppers), noxious heat (typically >42C) and low pH. The TRPV1 channel is putatively involved in the perception and transmission of painful stimuli. Importantly, this channel is proposed to underlie many chronic pain states including inflammation, neuropathic pain and cancer pain, amongst others (1). These types of pain states are currently poorly managed by the pain medications available and this has led the pharmaceutical industry to seek novel targets for pain management, such as TRPV1. However, TRPV1 antagonists have so far failed in clinical trials due to an undesirable increase in core body temperature (2) resulting in hyperthermia. From these studies, it is proposed that tonically active TRPV1 channels are involved in maintaining normal body temperature and this could have significant influences on drug design. Finding novel compounds with differing effects on capsaicin activation and heat activation may be crucial in the discovery of lead compounds for the treatment of pain and other disease states.Here we present data collected on the 4-channel Orbit mini with temperature control showing the potential use of the Orbit mini to record heat activation of single channel TRPV1.Tutorial video2015 Orbit mini Tutorial VideoShort introduction on the Orbit mini"The functionality of the Orbit mini is just incredible! Learning to use it takes only a few moments. Setting up an experiment, painting 4 individual bilayers and getting data so quickly, makes me feel almost sad. As I cannot deny the notion wasting a lot of time as PhD student working with different technology"Tom Gtze, Nanion TechnologiesProduct Sheet PDFOrbit mini Product SheetTutorial video2015 Orbit mini Membrane Preparation Tutorial VideoShort introduction how to prepare membranes on the Orbit miniSimultaneous recording from four lipid bilayersFlyer PDFOrbit mini Product Flyer OPTO- INVFlyer PDFOrbit mini Product Flyer Temperature ControlPublication link2022 Triggered Assembly of a DNA-Based Membrane ChannelOrbit Mini Publication in American Chemical Society(2022) Authors: Lanphere C., Ciccone J., Dorey A., Hagleitner-Erturul N., Knyazev D., Haider S., Howorka S.Chemistry is in a powerful position to synthetically replicate biomolecular structures. Adding functional complexity is key to increase the biomimetics value for science and technology yet is difficult to achieve with poorly controlled building materials. Here, we use defined DNA blocks to rationally design a triggerable synthetic nanopore that integrates multiple functions of biological membrane proteins. Soluble triggers bind via molecular recognition to the nanopore components changing their structure and membrane position, which controls the assembly into a defined channel for efficient transmembrane cargo transport. Using ensemble, single-molecule, and simulation analysis, our activatable pore provides insight into the kinetics and structural dynamics of DNA assembly at the membrane interface. The triggered channel advances functional DNA nanotechnology and synthetic biology and will guide the design of controlled nanodevices for sensing, cell biological research, and drug delivery.Flyer PDFOrbit mini Quickstart Guide Getting StartedPublication link2022 Photoactivation of a Mechanosensitive ChannelOrbit mini Publication in Frontiers in Molecular Biosciences(2022) Authors: Crea F., Vorkas A., Redlich A., Cruz R., Shi C., Trauner D., Lange A., Schlesinger R., Heberle J.Optogenetics in the conventional sense, i.e. the use of engineered proteins that gain their light sensitivity from naturally abundant chromophores, represents an exciting means to trigger and control biological activity by light. As an alternate approach, photopharmacology controls biological activity with the help of synthetic photoswitches. Here, we used an azobenzene-derived lipid analogue to optically activate the transmembrane mechanosensitive channel MscL which responds to changes in the lateral pressure of the lipid bilayer. In this work, MscL has been reconstituted in nanodiscs, which provide a native-like environment to the protein and a physical constraint to membrane expansion. We characterized this photomechanical system by FTIR spectroscopy and assigned the vibrational bands of the light-induced FTIR difference spectra of thetransandcisstates of the azobenzene photolipid by DFT calculations. Differences in the amide I range indicated reversible conformational changes in MscL as a direct consequence of light switching. With the mediation of nanodiscs, we inserted the transmembrane protein in a free standing photoswitchable lipid bilayer, where electrophysiological recordings confirmed that the ion channel could be set to one of its sub-conducting states upon light illumination. In conclusion, a novel approach is presented to photoactivate and control cellular processes as complex and intricate as gravitropism and turgor sensing in plants, contractility of the heart, as well as sensing pain, hearing, and touch in animals.Publication link2022 Pore-forming moss protein bryoporin is structurally and mechanistically related to actinoporins from evolutionarily distant cnidariansOrbit mini Publication inJournal of Biological Chemistry (2022) Authors: olincG.,vigeljT.,OmersaN.,SnojT.,PircK., ZnidariN., Yamaji-Hasegawa A.,Kobayashi T.,Anderluh G.,PodobnikM.Pore-forming proteins perforate lipid membranes and consequently affect their integrity and cell fitness. Therefore, it is not surprising that many of these proteins from bacteria, fungi, or certain animals act as toxins. While pore-forming proteins have also been found in plants, there is little information on their molecular structure and mode of action. Bryoporin is a protein from the moss Physcomitrium patens, and its corresponding gene was found to be upregulated by various abiotic stresses, especially dehydration, as well as upon fungal infection. Based on the amino acid sequence, it was suggested that bryoporin was related to the actinoporin family of pore-forming proteins, originally discovered in sea anemones. Here, we provide the first detailed structural and functional analysis of this plant cytolysin. The crystal structure of the monomeric bryoporin is highly similar to those of actinoporins. Our cryo-EM analysis of its pores showed an actinoporin-like octameric structure, thereby revealing a close kinship of proteins from evolutionarily distant organisms. This was further confirmed by our observation of bryoporins preferential binding to and formation of pores in membranes containing animal sphingolipids, such as sphingomyelin and ceramide phosphoethanolamine; however, its binding affinity was weaker than that of actinoporin equinatoxin II. We determined bryoporin did not bind to major sphingolipids found in fungi or plants, and its membrane-binding and pore-forming activity were enhanced by various sterols. Our results suggest that bryoporin could represent a part of the moss defense arsenal, acting as a pore-forming toxin against membranes of potential animal pathogens, parasites, or predators.Publication link2022 Integrative Study of the Structural and Dynamical Properties of a KirBac3.1 Mutant: Functional Implication of a Highly Conserved Tryptophan in the Transmembrane DomainOrbit Mini Publication in Molecular Biophysics (2022) Authors: Fagnen C., Bannwarth L., Oubella I., Zuniga D., Haouz A., Forest E., Scala R., Bendahhou S., De Zorzi R., Perahia D., Vnien-Bryan C.ATP-sensitive potassium (K-ATP) channels are ubiquitously expressed on the plasma membrane of cells in several organs, including the heart, pancreas, and brain, and they govern a wide range of physiological processes. In pancreatic -cells, K-ATP channels composed of Kir6.2 and SUR1 play a key role in coupling blood glucose and insulin secretion. A tryptophan residue located at the cytosolic end of the transmembrane helix is highly conserved in eukaryote and prokaryote Kir channels. Any mutation on this amino acid causes a gain of function and neonatal diabetes mellitus. In this study, we have investigated the effect of mutation on this highly conserved residue on a KirBac channel (prokaryotic homolog of mammalian Kir6.2). We provide the crystal structure of the mutant KirBac3.1 W46R (equivalent to W68R in Kir6.2) and its conformational flexibility properties using HDX-MS. In addition, the detailed dynamical view of the mutant during the gating was investigated using the in silico method. Finally, functional assays have been performed. A comparison of important structural determinants for the gating mechanism between the wild type KirBac and the mutant W46R suggests interesting structural and dynamical clues and a mechanism of action of the mutation that leads to the gain of function.Publication link2022 Opening of glutamate receptor channel to subconductance levelsOrbit Mini Publication in Nature(2022) Authors: Yelshanskaya M.V., Patel D.S., Kottke C.M., Kurnikova M.G., & Sobolevsky A.I.Ionotropic glutamate receptors (iGluRs) are tetrameric ligand-gated ion channels that open their pores in response to binding of the agonist glutamate. An ionic current through a single iGluR channel shows up to four discrete conductance levels (O1O4). Higher conductance levels have been associated with an increased number of agonist molecules bound to four individual ligand-binding domains (LBDs). Here we determine structures of a synaptic complex of AMPA-subtype iGluR and the auxiliary subunit 2 in non-desensitizing conditions with various occupancy of the LBDs by glutamate. We show that glutamate binds to LBDs of subunits B and D only after it is already bound to at least the same number of LBDs that belong to subunits A and C. Our structures combined with single-channel recordings, molecular dynamics simulations and machine-learning analysis suggest that channel opening requires agonist binding to at least two LBDs. Conversely, agonist binding to all four LBDs does not guarantee maximal channel conductance and favours subconductance states O1 and O2, with O3 and O4 being rare and not captured structurally. The lack of subunit independence and low efficiency coupling of glutamate binding to channel opening underlie the gating of synaptic complexes to submaximal conductance levels, which provide a potential for upregulation of synaptic activity.Publication link2022 Cryoelectron microscopy unveils unique structural features of the human Kir2.1 channelOrbit Mini Publication in Science Advances (2022) Authors: Fernandes C., Zuniga D., Fagnen C., Kugler V., Scala R., Phau-Arnaudet G., Wagner R., Perahia D., Bendahhou S., Vnien-Bryan C.We present the first structure of the human Kir2.1 channel containing both transmembrane domain (TMD) and cytoplasmic domain (CTD). Kir2.1 channels are strongly inward-rectifying potassium channels that play a key role in maintaining resting membrane potential. Their gating is modulated by phosphatidylinositol 4,5-bisphosphate (PIP2). Genetically inherited defects in Kir2.1 channels are responsible for several rare human diseases, including Andersens syndrome. The structural analysis (cryoelectron microscopy), surface plasmon resonance, and electrophysiological experiments revealed a well-connected network of interactions between the PIP2-binding site and the G-loop through residues R312 and H221. In addition, molecular dynamics simulations and normal mode analysis showed the intrinsic tendency of the CTD to tether to the TMD and a movement of the secondary anionic binding site to the membrane even without PIP2. Our results revealed structural features unique to human Kir2.1 and provided insights into the connection between G-loop and gating and the pathological mechanisms associated with this channel.Publication link2022 Emerging enterococcus pore-forming toxins with MHC/HLA-I as receptorsOrbit Mini Publication in Cell (2022) Authors: XiongX.,SonghaiTianS.,Yang P.,Lebreton F.,Bao H.,ShengK.,Yin L.,Chen P., Zhang J.,Qi W.,Ruan J.,Wu H.,Chen H.,Breault D.T.,Wu H.,Earl A.M.,Gilmore M.S.,AbrahamJ.,Dong M.Enterococci are a part of human microbiota and a leading cause of multidrug resistant infections. Here, we identify a family of Enterococcus pore-forming toxins (Epxs) in E. faecalis, E. faecium, and E. hirae strains isolated across the globe. Structural studies reveal that Epxs form a branch of -barrel pore-forming toxins with a -barrel protrusion (designated the top domain) sitting atop the cap domain. Through a genome-wide CRISPR-Cas9 screen, we identify human leukocyte antigen class I (HLA-I) complex as a receptor for two members (Epx2 and Epx3), which preferentially recognize human HLA-I and homologous MHC-I of equine, bovine, and porcine, but not murine, origin. Interferon exposure, which stimulates MHC-I expression, sensitizes human cells and intestinal organoids to Epx2 and Epx3 toxicity. Co-culture with Epx2-harboring E. faecium damages human peripheral blood mononuclear cells and intestinal organoids, and this toxicity is neutralized by an Epx2 antibody, demonstrating the toxin-mediated virulence of Epx-carrying Enterococcus.Publication link2022 A chip-based array for high-resolution fluorescence characterization of free-standing horizontal lipid membranes under voltage clampOrbit mini OPTO-INV Publication in Lab on a Chip (2022) Authors: Ensslen T., Behrends J.C.Optical techniques, such as fluorescence microscopy, are of great value in characterizing the structural dynamics of membranes and membrane proteins. A particular challenge is to combine high-resolution optical measurements with high-resolution voltage clamp electrical recordings providing direct information on e.g. single ion channel gating and/or membrane capacitance. Here, we report on a novel chip-based array device which facilitates optical access with water or oil-immersion objectives of high numerical aperture to horizontal free-standing lipid membranes while controlling membrane voltage and recording currents using micropatterned Ag/AgCl-electrodes. We demonstrate both wide-field and confocal imaging, as well as time-resolved single photon counting on free-standing membranes spanning sub-picoliter CaVities are demonstrated while electrical signals, including single channel activity, are simultaneously acquired. This optically addressable microelectrode CaVity array will allow combined electrical-optical studies of membranes and membrane proteins to be performed as a routine experiment.Publication link2022 Circularized fluorescent nanodiscs for probing proteinlipid interactionsOrbit mini Publication inCommunications Biology(2022) Authors: Ren Q., Zhang S., Bao H.Proteinlipid interactions are vital for numerous transmembrane signaling pathways. However, simple tools to characterize these interactions remain scarce and are much needed to advance our understanding of signal transduction across lipid bilayers. To tackle this challenge, we herein engineer nanodisc as a robust fluorescent sensor for reporting membrane biochemical reactions. We circularize nanodiscs via split GFP and thereby create an intensity-based fluorescent sensor (isenND) for detecting membrane binding and remodeling events. We show that isenND responds robustly and specifically to the action of a diverse array of membrane-interacting proteins and peptides, ranging from synaptotagmin and synuclein involved in neurotransmission to viral fusion peptides of HIV-1 and SARS-CoV-2. Together, isenND can serve as a versatile biochemical reagent useful for basic and translational research of membrane biology.Publication link2021 Saccharomyces cerevisiae as a superior host for overproduction of prokaryotic integral membrane proteinsOrbit Mini Publication in Current Research in Structural Biology (2021) Authors: Spruce Preisler S., Wiuf A.D., Friss M., Kjaergaard L., Hurd M., Ramos Becares E., Nurup C.N., Bjoerkskov F.B., Szathmary Z., Gourson P.E., Calloe K., Klaerke D.A., Gotfryd K., Pedersen P.A.Integral membrane proteins (IMPs) constitute ~30% of all proteins encoded by the genome of any organism and Escherichia coli remains the first-choice host for recombinant production of prokaryotic IMPs. However, the expression levels of prokaryotic IMPs delivered by this bacterium are often low and overproduced targets often accumulate in inclusion bodies. The targets are therefore often discarded to avoid an additional and inconvenient refolding step in the purification protocol. Here we compared expression of five prokaryotic (bacterial and archaeal) IMP families in E. coli and Saccharomyces cerevisiae. We demonstrate that our S. cerevisiae-based production platform is superior in expression of four investigated IMPs, overall being able to deliver high quantities of active target proteins. Surprisingly, in case of the family of zinc transporters (Zrt/Irt-like proteins, ZIPs), S. cerevisiae rescued protein expression that was undetectable in E. coli. We also demonstrate the effect of localization of the fusion tag on expression yield and sample quality in detergent micelles. Lastly, we present a road map to achieve the most efficient expression of prokaryotic IMPs in our yeast platform. Our findings demonstrate the great potential of S. cerevisiae as host for high-throughput recombinant overproduction of bacterial and archaeal IMPs for downstream biophysical characterization.Publication link2021 Structure and desensitization of AMPA receptor complexes with type II TARP 5 and GSG1LOrbit Mini Publication in Molecular Cell (2021) Authors: Klykov O., Gangwar S. P., Yelshanskaya M. V., Laura Yen., Sobolevsky A. I.AMPA receptors (AMPARs) mediate the majority of excitatory neurotransmission. Their surface expression, trafficking, gating, and pharmacology are regulated by auxiliary subunits. Of the two types of TARP auxiliary subunits, type I TARPs assume activating roles, while type II TARPs serve suppressive functions. We present cryo-EM structures of GluA2 AMPAR in complex with type II TARP 5, which reduces steady-state currents, increases single-channel conductance, and slows recovery from desensitization. Regulation of AMPAR function depends on its ligand-binding domain (LBD) interaction with the 5 head domain. GluA2-5 complex shows maximum stoichiometry of two TARPs per AMPAR tetramer, being different from type I TARPs but reminiscent of the auxiliary subunit GSG1L. Desensitization of both GluA2-GSG1L and GluA2-5 complexes is accompanied by rupture of LBD dimer interface, while GluA2-5 but not GluA2-GSG1L LBD dimers remain two-fold symmetric. Different structural architectures and desensitization mechanisms of complexes with auxiliary subunits endow AMPARs with broad functional capabilities.Publication link2021 Pathological conformations of disease mutant Ryanodine Receptors revealed by cryo-EMOrbit mini Publication in NatureCommunications(2021) Authors: Woll K.A., Haji-Ghassemi O., Van Petegem F.Ryanodine Receptors (RyRs) are massive channels that release Ca2+ from the endoplasmic and sarcoplasmic reticulum. Hundreds of mutations are linked to malignant hyperthermia (MH), myopathies, and arrhythmias. Here, we explore the first MH mutation identified in humans by providing cryo-EM snapshots of the pig homolog, R615C, showing that it affects an interface between three solenoid regions. We also show the impact of apo-calmodulin (apoCaM) and how it can induce opening by bending of the bridging solenoid, mediated by its N-terminal lobe. For R615C RyR1, apoCaM binding abolishes a pathological intermediate conformation, distributing the population to a mixture of open and closed channels, both different from the structure without apoCaM. Comparisons show that the mutation primarily affects the closed state, inducing partial movements linked to channel activation. This shows that disease mutations can cause distinct pathological conformations of the RyR and facilitate channel opening by disrupting interactions between different solenoid regions.Publication link2021 Rapid and multiplex preparation of engineered Mycobacterium smegmatis porin A (MspA) nanopores for single molecule sensing and sequencingOrbit Mini Publicationin Chemical Science (2021) Authors: Yan S., Wang L., Du X., Zhang S., Wang S., Cao J., Zhang J., Jia W., Wang Y., Zhang P., Chen H-Y., Huang S.Acknowledging its unique conical lumen structure, Mycobacterium smegmatis porin A (MspA) was the first type of nanopore that has successfully sequenced DNA. Recent developments of nanopore single molecule chemistry have also suggested MspA to be an optimum single molecule reactor. However, further investigations with this approach require heavy mutagenesis which is labor intensive and requires high end instruments for purifications. We here demonstrate an efficient and economic protocol which performs rapid and multiplex preparation of a variety of MspA mutants. The prepared MspA mutants were demonstrated in operations such as nanopore insertion, sequencing, optical single channel recording (oSCR), nanopore single molecule chemistry and nanopore rectification. The performance is no different from that of pores however prepared by other means. The time of all human operations and the cost for a single batch of preparation have been minimized to 40 min and 0.4$, respectively. This method is extremely useful in the screening of new MspA mutants, which has an urgent requirement in further investigations of new MspA nanoreactors. Its low cost and simplicity also enable efficient preparations of MspA nanopores for both industrial manufacturing and academic research.Publication link2020 Small Molecule Permeation Across Membrane Channels: Chemical Modification to Quantify Transport Across OmpFOrbit mini Publication in Angewandte Chemie (2020) Authors: Wang J., Bafna J.A., Bhamidimarri S.P., Winterhalter M.Biological channels facilitate the exchange of small molecules across membranes, but surprisingly there is a lack of general tools for the identification and quantification of transport (i.e., translocation and binding). Analyzing the ion current fluctuation of a typical channel with its constriction region in the middle does not allow a direct conclusion on successful transport. For this, we created an additional barrier acting as a molecular counter at the exit of the channel. To identify permeation, we mainly read the molecule residence time in the channel lumen as the indicator whether the molecule reached the exit of the channel. As an example, here we use the well-studied porin, OmpF, an outer membrane channel fromE. coli. Inspection of the channel structure suggests that aspartic acid at position 181 is located below the constriction region (CR) and we subsequently mutated this residue to cysteine, where else cysteine free and functionalized it by covalent binding with 2-sulfonatoethyl methanethiosulfonate (MTSES) or the larger glutathione (GLT) blockers. Using the dwell time as the signal for transport, we found that both mono-arginine and tri-arginine permeation process is prolonged by 20% and 50% respectively through OmpFE181CMTSES, while the larger sized blocker modification OmpFE181CGLT drastically decreased the permeation of mono-arginine by 9-fold and even blocked the pathway of the tri-arginine. In case of the hepta-arginine as substrate, both chemical modifications led to an identical blocked pattern observed by the dwell time of ion current fluctuation of the OmpFwt. As an instance for antibiotic permeation, we analyzed norfloxacin, a fluoroquinolone antimicrobial agent. The modulation of the interaction dwell time suggests possible successful permeation of norfloxacin across OmpFwt. This approach may discriminate blockages from translocation events for a wide range of substrates. A potential application could be screening for scaffolds to improve the permeability of antibiotics.Publication link2021 ATP and large signaling metabolites flux through caspase-activated Pannexin 1 channelsOrbit mini Publication in eLife(2021) Authors: Narahari A.K., Kreutzberger A JB., Gaete P.S., Chiu Yu-Hsin, Leonhardt S.A., Medina C.B., Jin X., Oleniacz P.W., Kiessling V., Barrett P.Q., Ravichandran K.S., Yeager M., Contreras J.E., Tamm L.K., Bayliss D.A.Pannexin 1 (Panx1) is a membrane channel implicated in numerous physiological and pathophysiological processes via its ability to support release of ATP and other cellular metabolites for local intercellular signaling. However, to date, there has been no direct demonstration of large molecule permeation via the Panx1 channel itself, and thus the permselectivity of Panx1 for different molecules remains unknown. To address this, we expressed, purified, and reconstituted Panx1 into proteoliposomes and demonstrated that channel activation by caspase cleavage yields a dye-permeable pore that favors flux of anionic, large-molecule permeants (up to ~1 kDa). Large cationic molecules can also permeate the channel, albeit at a much lower rate. We further show that Panx1 channels provide a molecular pathway for flux of ATP and other anionic (glutamate) and cationic signaling metabolites (spermidine). These results verify large molecule permeation directly through caspase-activated Panx1 channels that can support their many physiological roles.Publication link2020 Cryo-EM structure of human Cx31.3/GJC3 connexin hemichannelOrbit mini Publication in Science Advances (2020) Authors: Lee H.J., Jeong H., Hyun J., Ryu B., Park K., Lim H.H., Yoo J., Woo J.S.Connexin family proteins assemble into hexameric channels called hemichannels/connexons, which function as transmembrane channels or dock together to form gap junction intercellular channels (GJIChs). We determinedthe cryoelectron microscopy structures of human connexin 31.3 (Cx31.3)/GJC3 hemichannels in the presenceand absence of calcium ions and with a hearing-loss mutation R15G at 2.3-, 2.5-, and 2.6- resolutions, respectively. Compared with available structures of GJICh in open conformation, Cx31.3 hemichannel shows substantial structural changes of highly conserved regions in the connexin family, including opening of calcium ionbinding tunnels, reorganization of salt-bridge networks, exposure of lipid-binding sites, and collocation of amino-terminal helices at the cytoplasmic entrance. We also found that the hemichannel has a pore with a diameter of ~8 and selectively transports chloride ions. Our study provides structural insights into the permeant selectivity of Cx31.3 hemichannel.Publication link2020 New Structural insights into Kir channel gating from molecular simulations, HDX-MS and functional studiesOrbit mini Publication in Nature Scientific Reports (2020) Authors: Fagnen C., Bannwarth L., Oubella I., Forest E., De Zorzi R., de Araujo A., Mhoumadi Y., Bendahhou S., Perahia D., Vnien-Bryan C.Inward rectifer potassium (Kir) channels play diverse and important roles in shaping action potentials in biological membranes. An increasing number of diseases are now known to be directly associated with abnormal Kir function. However, the gating of Kir still remains unknown. To increase our understanding of its gating mechanism, a dynamical view of the entire channel is essential. Here the gating activation was studied using a recent developped in silico method, MDeNM, which combines normal mode analysis and molecular dynamics simulations that showed for the very frst time the importance of interrelated collective and localized conformational movements. In particular, we highlighted the role played by concerted movements of the diferent regions throughout the entire protein, such as the cytoplasmic and transmembrane domains and the slide helices. In addition, the HDX-MS analysis achieved in these studies provided a comprehensive and detailed view of the dynamics associated with open/closed transition of the Kir channel in coherence with the theoretical results. MDeNM gives access to the probability of the diferent opening states that are in agreement with our electrophysiological experiments. The investigations presented in this article are important to remedy dysfunctional channels and are of interest for designing new pharmacological compounds.Publication link2020 Aerolysin nanopores decode digital information stored in tailored macromolecular analytesOrbit mini Publication in Science Advances (2020) Authors: Cao C., Krapp L.F., Al Ouahabi A.,Knig N.F., Cirauqui N., Radenovic A., Lutz J.F. Dal Peraro M.Digital data storage is a growing need for our society and finding alternative solutions than those based on silicon or magnetic tapes is a challenge in the era of big data. The recent development of polymers that can store information at the molecular level has opened up new opportunities for ultrahigh density data storage, long-term archival, anticounterfeiting systems, and molecular cryptography. However, synthetic informational polymers are so far only deciphered by tandem mass spectrometry. In comparison, nanopore technology can be faster, cheaper, nondestructive and provide detection at the single-molecule level; moreover, it can be massively parallelized and miniaturized in portable devices. Here, we demonstrate the ability of engineered aerolysin nanopores to accurately read, with single-bit resolution, the digital information encoded in tailored informational polymers alone and in mixed samples, without compromising information density. These findings open promising possibilities to develop writing-reading technologies to process digital data using a biological-inspired platform.Publication link2020 Computational Modeling of Ion Transport in Bulk and through a Nanopore Using the Drude Polarizable Force FieldOrbit mini Publication in Journal of Chemical Information and Modeling (2020) Authors: Prajapati J.D., Mele C., Alphan Aksoyoglu M.A., Winterhalter M., Kleinekathfer U.In the past two decades, molecular dynamics simulations have become the method of choice for elucidating the transport mechanisms of ions through various membrane channels. Often, these simulations heavily rely on classical nonpolarizable force fields (FFs), which lack electronic polarizability in the treatment of the electrostatics. The recent advancements in the Drude polarizable

and pandemics. Here, we present the use of eukaryotic cell-free systems for the rapid response to novel zoonotic diseases represented by SARS-CoV-2. Non-structural, structural and accessory proteins encoded by SARS-CoV-2 were synthesized by cell-free protein synthesis in a fast and efficient manner. The inhibitory effect of the non-structural protein 1 on protein synthesis could be shownin vitro. Structural proteins were quantitatively detected by commercial antibodies, therefore facilitating cell-free systems for the validation of available antibodies. The cytotoxic envelope protein was characterized in electrophysiological planar lipid bilayer measurements. Hence, our study demonstrates the potential of eukaryotic cell-free systems as a rapid response mechanism for the synthesis, functional characterization and antibody validation against a viral pathogen.Publication link2022 In Vitro Biophysical Characterization of Candidalysin: A Fungal Peptide ToxinOrbit 16 Publication inFrontiers inMethods in Molecular Biology(2022), vol 2542 Authors: Lee S., Kichik N., Hepworth O., Richardson J., Naglik J.In 2016, the first peptide toxin in any human fungal pathogen was identified. It was discovered in Candida albicans and was named candidalysin. Candidalysin is an amphipathic cationic peptide that damages cell membranes. Like most lytic peptides, candidalysin shows alpha-helical secondary structure. As the helicity and the membrane lytic activity of candidalysin are key factors for pathogenicity, here we describe in vitro approaches to monitor both its membrane-lytic function and the secondary structure. First, membrane permeabilization activity of candidalysin is measured in real time by direct electrical recording. Second, the secondary structure and helicity of candidalysin are determined by circular dichroism spectroscopy. These biophysical methods provide a means to characterize the activity and physical properties of candidalysin in vitro and will be useful in determining the structural and functional features of candidalysin and other similar cationic membrane-active peptides.Publication link2022 Resolving Isomeric Posttranslational Modifications Using a Biological Nanopore as a Sensor of Molecular ShapeOrbit 16 (a predecessor model of the Orbit 16 TC instrument) Publication in the Journal of the American Chemical Society(2022) Authors: Ensslen T., Sarthak K., Aksimentiev A., Behrends J.C.The chemical nature and precise position of posttranslational modifications (PTMs) in proteins or peptides are crucial for various severe diseases, such as cancer. State-of-the-art PTM diagnosis is based on elaborate and costly mass-spectrometry or immunoassay-based approaches, which are limited in selectivity and specificity. Here, we demonstrate the use of a protein nanopore to differentiate peptidesderived from human histone H4 proteinof identical mass according to the positions of acetylated and methylated lysine residues. Unlike sequencing by stepwise threading, our method detects PTMs and their positions by sensing the shape of a fully entrapped peptide, thus eliminating the need for controlled translocation. Molecular dynamics simulations show that the sensitivity to molecular shape derives from a highly nonuniform electric field along the pore. This molecular shape-sensing principle offers a path to versatile, label-free, and high-throughput characterizations of protein isoforms.Publication link2022 A Two-Step Calibration Method for Evaluation High Bandwidth Electrochemical InstrumentOrbit 16 Publication in Journal of Electroanalytical Chemistry(2022) Authors: Zhang L.L., Zhong C.B., Li J.G., Niu H.Y., Ying Y.L., Long Y.T.Single-entity electrochemistry allows the ultrasensitive detection of individual entities by incorporating high bandwidth electrochemical instruments. However, differences among instruments and shielding setups cause large deviations in the recorded signals, which leads to remarkable measurement errors in practical applications. Here, we developed a two-step method to calibrate instruments differences for achieving an accurate single-entity analysis. Two coefficients C1 and C2 were calculated with standard measurement through the model resistors of 1.00 G. C1 obtained from the slope value of the current-voltage (I-V) curve is used to calibrate the shifting of the current statistic distribution. Then, C2, assessed from the standard deviation (STD) noises, is employed to calibrate the full-width half maximum of current statistic distributions. After applying in the model single-molecule experiments of Poly(dA)4 detection with aerolysin nanopores, we showed the effective calibration of measurement differences among four high bandwidth electrochemical instruments. Therefore, this method is generally applicable to all kinds of single-entity electrochemical measurements to reduce measurement errors from instrument differences.Publication link2022 Candidalysins Are a New Family of Cytolytic Fungal Peptide ToxinsOrbit 16 Publication in mBio(2022) Authors: Richardson J. P., Brown R., Kichik N., Lee S., Priest E., Mogavero S., Maufrais C., Wickramasinghe D. N., Tsavou A., Kotowicz N. K., Hepworth O. W., Gallego-Corts A., Ponde N. O., Ho J., Moyes D. L., Wilson D., DEnfert C., Hube B., Naglik J. R.Candidalysin is the first cytolytic peptide toxin identified in any human fungal pathogen. Candidalysin is secreted by Candida albicans and is critical for driving infection and host immune responses in several model systems. However, Candida infections are also caused by non-C. albicans species. Here, we identify and characterize orthologs of C. albicans candidalysin in C. dubliniensis and C. tropicalis. The candidalysins have different amino acid sequences, are amphipathic, and adopt a predominantly -helical secondary structure in solution. Comparative functional analysis demonstrates that each candidalysin causes epithelial damage and calcium influx and activates intracellular signaling pathways and cytokine secretion. Importantly, C. dubliniensis and C. tropicalis candidalysins have higher damaging and activation potential than C. albicans candidalysin and exhibit more rapid membrane binding and disruption, although both fungal species cause less damage to epithelial cells than C. albicans. This study identifies the first family of peptide cytolysins in human-pathogenic fungi.Publication link2020 Dynamic interaction of fluoroquinolones with magnesium ions monitored using bacterial outer membrane nanoporesOrbit 16 Publication in Chemical Science (2020) Authors: Wang J., Prajapati J.D., Kleinekatfer U., Winterhalter M.Divalent ions are known to have a severe effect on the translocation of several antibiotic molecules into (pathogenic) bacteria. In the present study we have investigated the effect of divalent ions on the permeability of norfloxacin across the major outer membrane channels fromE. coli(OmpF and OmpC) andE. aerogenes(Omp35 and Omp36) at the single channel level. To understand the rate limiting steps in permeation, we reconstituted single porins into planar lipid bilayers and analyzed the ion current fluctuations caused in the presence of norfloxacin. Moreover, to obtain an atomistic view, we complemented the experiments with millisecond-long free energy calculations based on temperature-accelerated Brownian dynamics simulations to identify the most probable permeation pathways of the antibiotics through the respective pores. Both, the experimental analysis and the computational modelling, suggest that norfloxacin is able to permeate through the larger porins,i.e., OmpF, OmpC, and Omp35, whereas it only binds to the slightly narrower porin Omp36. Moreover, divalent ions can bind to negatively charged residues inside the porin, reversing the ion selectivity of the pores. In addition, the divalent ions can chelate with the fluoroquinolone molecules and alter their physicochemical properties. The results suggest that the conjugation with either pores or molecules must break when the antibiotic molecules pass the lumen of the porin, with the conjugation to the antibiotic being more stable than that to the respective pore. In general, the permeation or binding process of fluoroquinolones in porins occurs irrespective of the presence of divalent ions, but the presence of divalent ions can vary the kinetics significantly. Thus, a detailed investigation of the interplay of divalent ions with antibiotics and pores is of key importance in developing new antimicrobial drugs.Publication link2020 Electrical recognition of the twenty proteinogenic amino acids using an aerolysin nanoporeOrbit 16 Publication in Nature Biotechnology (2020) Authors: Ouldali H., Sarthak K., Ensslen T., Piguet F., Manivet P., Pelta J., Behrends J.C., Aksimentiev A., Oukhaled A.Efforts to sequence single protein molecules in nanopores have been hampered by the lack of techniques with sufficient sensitivity to discern the subtle molecular differences among all twenty amino acids. Here we report ionic current detection of all twenty proteinogenic amino acids in an aerolysin nanopore with the help of a short polycationic carrier. Application of molecular dynamics simulations revealed that the aerolysin nanopore has a built-in single-molecule trap that fully confines a polycationic carrier-bound amino acid inside the sensing region of the aerolysin. This structural feature means that each amino acid spends sufficient time in the pore for sensitive measurement of the excluded volume of the amino acid. We show that distinct current blockades in wild-type aerolysin can be used to identify 13 of the 20 natural amino acids. Furthermore, we show that chemical modifications, instrumentation advances and nanopore engineering offer a route toward identification of the remaining seven amino acids. These findings may pave the way to nanopore protein sequencing.Publication link2019 Activity of the Gramicidin A Ion Channel in a Lipid Membrane with Switchable Physical PropertiesOrbit 16 Publication in Langmuir (2019) Authors: Reiter R., Zaitseva E., Baaken G., Halimeh I., Behrends J.C., Zumbuehl ALipid bilayer membranes formed from the artificial 1,3-diamidophospholipid Pad-PC-Pad have the remarkable property that their hydrophobic thickness can be modified in situ: the particular arrangement of the fatty acid chains in Pad-PC-Pad allows them to fully interdigitate below 37 C, substantially thinning the membrane with respect to the noninterdigitated state. Two stimuli, traversing the main phase transition temperature of the lipid or addition of cholesterol, have previously been shown to disable the interdigitated state. Both manipulations cause an increase in hydrophobic thickness of about 25 due to enhanced conformational entropy of the lipids. Here, we characterize the interdigitated state using electrophysiological recordings from free-standing lipid-membranes formed on micro structured electrode CaVity arrays. Compared to standard membranes made from 1,2-diphytanoyl-sn-glycero-3-phosphocholin (DPhPC), pure Pad-PC-Pad membranes at room temperature had lowered electroporation threshold and higher capacitance. Ion channel formation by the peptide Gramicidin A was clearly facilitated in pure Pad-PC-Pad membranes at room temperature, with activity occurring at significantly lower peptide concentrations and channel dwell times increased by 2 orders of magnitude with respect to DPhPC-membranes. Both elevation of temperature beyond the phase transition and addition of cholesterol reduced channel dwell times, as expected if the reduced membrane thickness stabilized channel formation due to decreased hydrophobic mismatch.Publication link2019 Real-time monitoring -lactam/-lactamase inhibitor (BL/BLI) mixture towards the bacteria porin pathway at single molecule levelOrbit 16 Publication in Analytical and Bioanalytical Chemistry (2019) Authors: Wang J., Fertig N., Ying Y.L.Multidrug-resistant bacteria are a great concern and a problem that must be addressed. Extended-spectrum -lactamases are a common defence mechanism of bacteria to make -lactam (BL) antibiotics ineffective. -Lactamase inhibitors (BLIs) are consequently designed and are often clinically prescribed with a BL antibiotic to hinder degradation. Current studies focusing on how BL antibiotics or BLIs interact solely with the bacterial outer membrane nanopores (porins) on reaching the periplasmic side using a nanopore-based sensing technique. In electrochemical studies, the bias voltage allows real-time monitoring of BL antibiotics, BLIs and their mixture through the porin pathway at the single-molecule level. Here we consider the most abundant membrane protein from Escherichia coli (i.e. OmpF), purify and reconstitute the membrane protein in an artificial lipid bilayer and then study its ex vivo electrochemical behaviour. We show the piperacillin/tazobactam mixture interacts with OmpF, whereas the substrate interacts under the maximum bandwidth. The power spectrum analysis of the ionic current trace demonstrates the ampicillin/sulbactram mixture requires more energy than ampicillin alone to pass through the porin pathway. Our results demonstrate that clinically relevant combinations (e.g. piperacillin/tazobactam and ampicillin/sulbactam) interact more strongly with OmpF than either the BL antibiotic or the BLI alone. We suggest a quick and relatively cheap screening method to test the ability of BL antibiotics/BLIs to cross the bacterial cellular membrane.Publication link2018 The Multifaceted Antibacterial Mechanisms of the Pioneering Peptide Antibiotics Tyrocidine and Gramicidin SOrbit 16 Publication in American Society for Microbiology (2018) Authors: Wenzel M., Rautenbach M., Vosloo J.A., Siersma T., Aisenbrey C.H.M., Zaitseva E., Laubscher W.E., van Rensburg W., Behrends J.C., Bechinger B., Hamoen L.W.Cyclic -sheet decapeptides from the tyrocidine group and the homologous gramicidin S were the first commercially used antibiotics, yet it remains unclear exactly how they kill bacteria. We investigated their mode of action using a bacterial cytological profiling approach. Tyrocidines form defined ion-conducting pores, induce lipid phase separation, and strongly reduce membrane fluidity, resulting in delocalization of a broad range of peripheral and integral membrane proteins. Interestingly, they also cause DNA damage and interfere with DNA-binding proteins. Despite sharing 50% sequence identity with tyrocidines, gramicidin S causes only mild lipid demixing with minor effects on membrane fluidity and permeability. Gramicidin S delocalizes peripheral membrane proteins involved in cell division and cell envelope synthesis but does not affect integral membrane proteins or DNA. Our results shed a new light on the multifaceted antibacterial mechanisms of these antibiotics and explain why resistance to them is virtually nonexistent. Importance:Cyclic -sheet decapeptides, such as tyrocidines and gramicidin S, were among the first antibiotics in clinical application. Although they have been used for such a long time, there is virtually no resistance to them, which has led to a renewed interest in this peptide class. Both tyrocidines and gramicidin S are thought to disrupt the bacterial membrane. However, this knowledge is mainly derived from in vitro studies, and there is surprisingly little knowledge about how these long-established antibiotics kill bacteria. Our results shed new light on the antibacterial mechanism of -sheet peptide antibiotics and explain why they are still so effective and why there is so little resistance to them.Publication link2019 A comparison of ion channel current blockades caused by individual poly(ethylene glycol) molecules and polyoxometalate nanoclustersOrbit 16 Publication in The European Physical Journal E (2019) Authors: Wang H., Kasianowicz J.J., Robertson J.W.F., Poster D.L., Ettedgui J.Proteinaceous nanometer-scale pores have been used to detect and physically characterize many different types of analytes at the single-molecule limit. The method is based on the ability to measure the transient reduction in the ionic channel conductance caused by molecules that partition into the pore. The distribution of blockade depth amplitudes and residence times of the analytes in the pore are used to physically and chemically characterize them. Here we compare the current blockade events caused by flexible linear polymers of ethylene glycol (PEGs) and structurally well-defined tungsten polyoxymetallate nanoparticles in the nanopores formed by Staphylococcus aureus -hemolysin and Aeromonas hydrophila aerolysin. Surprisingly, the variance in the ionic current blockade depth values for the relatively rigid metallic nanoparticles is much greater than that for the flexible PEGs, possibly because of multiple charged states of the polyoxymetallate clusters.Publication link2018 Getting drugs into Gram-negative bacteria: Rational rules for permeation through general porinsOrbit 16 Publication in ACS Infectious Diseases (2018) Authors: Acosta-Gutierrez S., Ferrara L., Pathania M., Masi M., Wang J., Bodrenko I., Zahn M., Winterhalter M., Stavenger R.A., Pages J.-M., Naismith J.H., van den Berg B., Page M., Ceccarelli M.Small, hydrophilic molecules, including most important antibiotics in clinical use, cross the Gram-negative outer membrane through the water-filled channels provided by porins. We have determined the X-ray crystal structures of the principal general porins from three species of Enterobacteriaceae, namely Enterobacter aerogenes, Enterobacter cloacae and Klebsiella pneumoniae and determined their antibiotic permeabilities as well as those of the orthologues from Escherichia coli. Starting from the structure of the porins and molecules we propose a physical mechanism underlying transport and condense it in a computationally efficient scoring function. The scoring function shows good agreement with in-vitro penetration data and will enable the screening of virtual databases to identify molecules with optimal permeability through porins and help to guide the optimization of antibiotics with poor permeation.Publication link2018 Size-dependent interaction of a 3-arm star poly(ethylene glycol) with two biological nanoporesOrbit 16 Publication in The European Physical Journal E (2018) Authors: Talarimoghari M., Baaken G., Hanselmann R., Behrends J.C.We use two pore-forming proteins, alpha-hemolysin and aerolysin, to compare the polymer size-dependence of ionic current block by two types of ethyleneglycol polymers: 1) linear and 2) 3-arm star poly(ethylene glycol), both applied as a polydisperse mixture of average mass 1kDa under high salt conditions. The results demonstrate that monomer size sensitivity, as known for linear PEGs, is conserved for the star polymers with only subtle differences in the dependence of the residual conductance on monomer number. To explain this absence of a dominant effect of polymer architecture, we propose that PEG adsorbs to the inner pore wall in a collapsed, salted-out state, likely due to the effect of hydrophobic residues in the pore wall on the availability of water for hydration.Publication link2018 Cellfree production of pore forming toxins: Functional analysis of thermostable direct hemolysin from Vibrio parahaemolyticusOrbit 16 Publication in Engineering in Life Sciences (2018) Authors: Dondapati S.K., Wstenhagen D.A., Strauch E., Kubick S.The pore forming characteristic of TDH1 and TDH2 variants of thermostable direct hemolysin (TDH), a major toxin involved in the pathogenesis of Vibrio parahaemolyticus, was studied on a planar lipid bilayer painted over individual picoliter CaVities containing microelectrodes assembled in a multiarray. Both proteins formed pores upon insertion into the lipid bilayer which was shown as a shift in the conductance from the baseline current. TDH2 protein was able to produce stable currents and the currents were influenced by external factors like concentration, type of salt and voltage. The pore currents were influenced and showed a detectable response in the presence of polymers which makes them suitable for biotechnology applications.Publication link2018 Defined Bilayer Interactions of DNA Nanopores Revealed with a Nuclease-Based Nanoprobe StrategyOrbit 16 Publication in ACS Nano (2018) Authors: Burns J.R., Howorka S.DNA nanopores are a recent class of bilayer-puncturing nanodevices that can help advance biosensing, synthetic biology, and nanofluidics. Here, we create archetypal lipid-anchored DNA nanopores and characterize them with a nanoprobe-based approach to gain essential information about their interactions with bilayers. The strategy determines the molecular accessibility of DNA pores with a nuclease and can thus distinguish between the nanopores' membrane-adhering and membrane-spanning states. The analysis reveals, for example, that pores interact with bilayers in two steps whereby fast initial membrane tethering is followed by slower reorientation to the puncturing state. Tethering occurs for pores with one anchor, while puncturing requires multiple anchors. Both low and high-curvature membranes are good substrates for tethering, but efficient insertion proceeds only for high-curvature bilayers of the examined lipid composition. This is likely due to the localized lipid misalignments and the associated lower energetic barrier for pore permeation. Our study advances the fields of DNA nanotechnology and nanopores by overcoming the considerable experimental hurdle of efficient membrane insertion. It also provides mechanistic insights to aid the design of advanced nanopores, and offers a useful route to probe bilayer orientation of DNA nanostructures.Publication link2017 Stability and dynamics of membrane-spanning DNA nanoporesOrbit 16 Publication in Nature Communications (2017) Authors: Maingi V., Burns J.R., Uusitalo J.J., Howorka S., Marrink S.J., Sansom M.S.P.Recently developed DNA-based analogues of membrane proteins have advanced synthetic biology. A fundamental question is how hydrophilic nanostructures reside in the hydrophobic environment of the membrane. Here, we use multiscale molecular dynamics (MD) simulations to explore the structure, stability and dynamics of an archetypical DNA nanotube inserted via a ring of membrane anchors into a phospholipid bilayer. Coarse-grained MD reveals that the lipids reorganize locally to interact closely with the membrane-spanning section of the DNA tube. Steered simulations along the bilayer normal establish the metastable nature of the inserted pore, yielding a force profile with barriers for membrane exit due to the membrane anchors. Atomistic, equilibrium simulations at two salt concentrations confirm the close packing of lipid around of the stably inserted DNA pore and its cation selectivity, while revealing localized structural fluctuations. The wide-ranging and detailed insight informs the design of next-generation DNA pores for synthetic biology or biomedicine.Publication link2017 Validation of ADAM10 metalloprotease as a Bacillus thuringiensis Cry3Aa toxin functional receptor in Colorado potato beetle (Leptinotarsa decemlineata)Orbit 16 Publication in Insect Molecular Biology (2017) Authors: Ruiz-Arroyo V.M., Garca-Robles I., Ochoa-Campuzano C., Goig G.A., Zaitseva E., Baaken G., Martnez-Ramrez A.C., Rausell C., Real M.D.Bacillus thuringiensisparasporal crystal proteins (Cry proteins) are insecticidal pore-forming toxins that bind to specific receptor molecules on the brush border membrane of susceptible insect midgut cells to exert their toxic action. In the Colorado potato beetle (CPB), a coleopteran pest, we previously proposed that interaction of Cry3Aa toxin with a CPB ADAM10 metalloprotease is an essential part of the mode of action of this toxin. Here, we annotated the gene sequence encoding an ADAM10 metalloprotease protein (CPB-ADAM10) in the CPB genome sequencing project, and using RNA interference gene silencing we demonstrated that CPB-ADAM10 is a Cry3Aa toxin functional receptor in CPB. Cry3Aa toxicity was significantly lower in CPB-ADAM10 silenced larvae andin vitrotoxin pore-forming ability was greatly diminished in lipid planar bilayers fused with CPB brush border membrane vesicles (BBMVs) prepared from CPB-ADAM10 silenced larvae. In accordance with our previous data that indicated this toxin was a substrate of ADAM10 in CPB, Cry3Aa toxin membrane-associated proteolysis was altered when CPB BBMVs lacked ADAM10. The functional validation of CPB-ADAM10 as a Cry3Aa toxin receptor in CPB expands the already recognized role of ADAM10 as a pathogenicity determinant of pore-forming toxins in humans to an invertebrate species.Publication link2016 Probing driving forces in aerolysin and -hemolysin biological nanopores: electrophoresis versus electroosmosisOrbit 16 Publication in Nanoscale (2016) Authors: Boukhet M., Piguet F., Ouldali H., Pastoriza-Gallego M.,Pelta J., Oukhaled A.The transport of macromolecules through nanopores is involved in many biological functions and is today at the basis of promising technological applications. Nevertheless the interpretation of the dynamics of the macromolecule/nanopore interaction is still misunderstood and under debate. At the nanoscale, inside biomimetic channels under an external applied voltage, electrophoresis, which is the electric force acting on electrically charged molecules, and electroosmotic flow (EOF), which is the fluid transport associated with ions, contribute to the direction and magnitude of the molecular transport. In order to decipher the contribution of the electrophoresis and electroosmotic flow, we explored the interaction of small, rigid, neutral molecules (cyclodextrins) and flexible, non-ionic polymers (poly(ethylene glycol), PEG) that can coordinate cations under appropriate experimental conditions, with two biological nanopores: aerolysin (AeL) and -hemolysin (aHL). We performed experiments using two electrolytes with different ionic hydration (KCl and LiCl). Regardless of the nature of the nanopore and of the electrolyte, cyclodextrins behaved as neutral analytes. The dominant driving force was attributed to EOF, acting in the direction of the anion flow and stronger in LiCl than in KCl. The same qualitative behaviour was observed for PEGs in LiCl. In contrast, in KCl, PEGs behaved as positively charged polyelectrolytes through both AeL and aHL. Our results are in agreement with theoretical predictions about the injection of polymers inside a confined geometry (ESI). We believe our results to be of significant importance for better control of the dynamics of analytes of different nature through biological nanopores.Publication link2017 High-yield production of difficult-to-express proteins in a continuous exchange cell-free system based on CHO cell lysatesOrbit 16 Publication in Scientific Reports (2017) Authors: Thoring L., Dondapati S.K., Stech M., Wstenhagen D.A., Kubick S.Cell-free protein synthesis (CFPS) represents a promising technology for efficient protein production targeting especially so called difficult-to-express proteins whose synthesis is challenging in conventional in vivo protein production platforms. Chinese hamster ovary (CHO) cells are one of the most prominent and safety approved cell lines for industrial protein production. In this study we demonstrated the ability to produce high yields of various protein types including membrane proteins and single chain variable fragments (scFv) in a continuous exchange cell-free (CECF) system based on CHO cell lysate that contains endogenous microsomal structures. We showed significant improvement of protein yield compared to batch formatted reactions and proved biological activity of synthesized proteins using various analysis technologies. Optimized CECF reaction conditions led to membrane protein yields up to 980g/ml, which is the highest protein yield reached in a microsome containing eukaryotic cell-free system presented so far.Publication link2016 A biomimetic DNA-made channel for the ligand-controlled and selective transport of small-molecule cargo through a biological membraneOrbit 16 Publication in Nature Nanotechnology (2016) Authors: Burns J.R., Seifert A., Fertig N, Howorka S.Biological ion channels are molecular gatekeepers that control transport across cell membranes. Recreating the functional principle of such systems and extending it beyond physiological ionic cargo is both scientifically exciting and technologically relevant to sensing or drug release. However, fabricating synthetic channels with a predictable structure remains a significant challenge. Here, we use DNA as a building materialto create an atomistically determined molecular valve that can control when and which cargo is transported across a bilayer. The valve, which is made from seven concatenated DNA strands, can bind a specific ligand and, in response, undergo a nanomechanical change to open up the membrane-spanning channel. It is also able to distinguish with high selectivity the transport of small organic molecules that differ by the presence of a positively or negatively charged group. The DNA device could be used for controlled drug release and the building of synthetic cell-like or logic ionic networksPublication link2016 Alamethicin Supramolecular Organization in Lipid Membranes from 19F Solid-State NMROrbit 16 Publication in Biophysical Journal (2016) Authors: Salnikov E.S., Raya J., De Zotti M., Zaitseva E., Peggion C., Ballano G., Toniolo C., Raap J., Bechinger B.Alamethicins (ALMs) are antimicrobial peptides of fungal origin. Their sequences are rich in hydrophobic amino acids and strongly interact with lipid membranes, where they cause a well-defined increase in conductivity. Therefore, the peptides are thought to form transmembrane helical bundles in which the more hydrophilic residues line a water-filled pore. Whereas the peptide has been well characterized in terms of secondary structure, membrane topology, and interactions, much fewer data are available regarding the quaternary arrangement of the helices within lipid bilayers. A new, to our knowledge, fluorine-labeled ALM derivative was prepared and characterized when reconstituted into phospholipid bilayers. As a partof these studies, C19F3-labeled compounds were characterized and calibrated for the first time, to our knowledge, for19F solid-state NMR distance and oligomerization measurements by centerband-only detection of exchange (CODEX) experiments, which opens up a large range of potential labeling schemes. The19F-19F CODEX solid-state NMR experiments performed with ALM in POPC lipid bilayers and at peptide/lipid ratios of 1:13 are in excellent agreement with molecular-dynamics calculations of dynamic pentameric assemblies. When the peptide/lipid ratio was lowered to 1:30, ALM was found in the dimeric form, indicating that the supramolecular organization is tuned by equilibria that can be shifted by changes in environmental conditions.Publication link2014 Structural and mechanistic insights into the bacterial amyloid secretion channel CsgGOrbit 16 Publication in Nature (2014) Authors: Goyal P., Krasteva P.V., Van GervenN., GubelliniF., Van den BroeckI., Troupiotis-TsalakiA., Jonckheere W., Phau-ArnaudetG., Pinkner J.S., ChapmanM.R., Hultgren S.J., Howorka S., FronzesR., Remaut H.Curli are functional amyloid fibres that constitute the major protein component of the extracellular matrix in pellicle biofilms formed by Bacteroidetes and Proteobacteria (predominantly of theandclasses). They provide a fitness advantage in pathogenic strains and induce a strong pro-inflammatory response during bacteraemia. Curli formation requires a dedicated protein secretion machinery comprising the outer membrane lipoprotein CsgG and two soluble accessory proteins, CsgE and CsgF. Here we report the X-ray structure ofEscherichia coliCsgG in a non-lipidated, soluble form as well as in its native membrane-extracted conformation. CsgG forms an oligomeric transport complex composed of nine anticodon-binding-domain-like units that give rise to a 36-stranded -barrel that traverses the bilayer and is connected to a cage-like vestibule in the periplasm. The transmembrane and periplasmic domains are separated by a 0.9-nm channel constriction composed of three stacked concentric phenylalanine, asparagine and tyrosine rings that may guide the extended polypeptide substrate through the secretion pore. The specificity factor CsgE forms a nonameric adaptor that binds and closes off the periplasmic face of the secretion channel, creating a 24,0003pre-constriction chamber. Our structural, functional and electrophysiological analyses imply that CsgG is an ungated, non-selective protein secretion channel that is expected to employ a diffusion-based, entropy-driven transport mechanism.Publication link2015 Automated Formation of Lipid Membrane Microarrays for Ionic Single-Molecule Sensing with Protein NanoporesOrbit 16 Publication in Small (2015) Authors: Del Rio Martinez J.M., Zaitseva E., Petersen S., Baaken G., Behrends J.C.Efficient use of membrane protein nanopores in ionic single-molecule sensing requires technology for the reliable formation of suspended molecular membranes densely arrayed in formats that allow high-resolution electrical recording. Here, automated formation of bimolecular lipid layers is shown using a simple process where a poly(tetrafluoroethylene)-coated magnetic bar is remotely actuated to perform a turning motion, thereby spreading phospholipid in organic solvent on a nonpolar surface containing a 1 mm24 4 array of apertures with embedded microelectrodes (microelectrode CaVity array). Parallel and high-resolution single-molecule detection by single nanopores is demonstrated on the resulting bilayer arrays, which are shown to form by a classical but very rapid self-assembly process. The technique provides a robust and scalable solution for the problem of reliable, automated formation of multiple independent lipid bilayers in a dense microarray format, while preserving the favorable electrical properties of the microelectrode CaVity array.Publication link2013 Self-Assembled DNA Nanopores That Span Lipid BilayersOrbit 16 Publication in Nano Letters (2013) Authors: Burns J.R., Stulz E., Howorka S.DNA nanotechnology excels at rationally designing bottom-up structures that can functionally replicate naturally occurring proteins. Here we describe the design and generation of a stable DNA-based nanopore that structurally mimics the amphiphilic nature of protein pores and inserts into bilayers to support a steady transmembrane flow of ions. The pore carries an outer hydrophobic belt comprised of small chemical alkyl groups which mask the negatively charged oligonucleotide backbone. This modification overcomes the otherwise inherent energetic mismatch to the hydrophobic environment of the membrane. By merging the fields of nanopores and DNA nanotechnology, we expect that the small membrane-spanning DNA pore will help open up the design of entirely new molecular devices for a broad range of applications including sensing, electric circuits, catalysis, and research into nanofluidics and controlled transmembrane transport.Publication link2014 Generation of chip based microelectrochemical cell arrays for long-term and high-resolution recording of ionic currents through ion channel proteinsOrbit 16 Publication in Sensors and Actuators B: Chemical (2014) Authors: Zheng T., Baaken G., Vellinger M., Behrends J.C., Rhe J.High throughput and a long life-time of the devices are two crucial challenges in planar chip technology for electrophysiological measurements of ionic current recording through ion channel proteins. In this paper, we present a wafer-scale process for the generation of novel arrays of microelectrochemical cells for long-term and high-resolution current recording. At the bottom of each of the cells, which have typically diameters of around 60 m and volumes of around 30 pL, a nanocrystalline silver/silver chloride secondary electrode is generated for ionic current recording. The top of the cell is closed by a lid containing a small (616 m) opening which connects liquid in the chamber to a contacting liquid on the outside. The processes necessary for manufacturing such a chip through photolithography and wafer-scale bonding have been developed, the resulting structures were characterized and the procedures were optimized. Combining a large surface area of the electrode with a in relation to the cell size relatively large amount of silver/silver chloride allows for the recording of DC ionic currents for prolonged periods of time. First measurements were performed where the electrochemical cells were closed by model membranes containing single ion channel proteins. The currents generated by ions passing through these ion channels are reported. These measurements demonstrate the usefulness of the microelectrochemical cell array for long time ionic current recordings at for these type of measurements relatively high current levels.Publication link2011 Nanopore-based single-molecule mass spectrometry on a lipid membrane microarrayOrbit 16 Publication in Journal of the American Chemical Society Nano (2011) Authors: Baaken G., Ankri N., Schuler A.K., Rhe J., Behrends C.We report on parallel high-resolution electrical single-molecule analysis on a chip-based nanopore microarray. Lipid bilayers of 20 m diameter containing single alpha-hemolysin pores were formed on arrays of subpicoliter CaVities containing individual microelectrodes (microelectrode CaVity array, MECA), and ion conductance-based single molecule mass spectrometry was performed on mixtures of poly(ethylene glycol) molecules of different length. We thereby demonstrate the function of the MECA device as a chip-based platform for array-format nanopore recordings with a resolution at least equal to that of established single microbilayer supports. We conclude that devices based on MECAs may enable more widespread analytical use of nanopores by providing the high throughput and ease of operation of a high-density array format while maintaining or exceeding the precision of state-of-the-art microbilayer recordings.Publication link2012 Synthetic Lipid Membrane Channels Formed by Designed DNA NanostructuresOrbit 16 Publication in Science (2012) Authors: Langecker M., ArnautV., Martin T.G., ListJ., RennerS., Mayer M., Dietz H., Simmel F.C.We created nanometer-scale transmembrane channels in lipid bilayers using self-assembled DNA-based nanostructures. Scaffolded DNA origami was used to create a stem that penetrates and spans a lipid membrane, and a barrel-shaped cap that adheres to the membrane in part via 26 cholesterol moieties. In single-channel electrophysiological measurements, we find similarities to the response of natural ion channels, such as conductances on the order of 1 nS and channel gating. More pronounced gating was seen for mutations in which a single DNA strand of the stem protruded into the channel. In single-molecule translocation experiments, we highlight one of many potential applications of the synthetic channels, namely as single DNA molecule sensing devices.Publication link2008 Planar microelectrode-CaVity array for high-resolution and parallel electrical recording of membrane ionic currentsOrbit 16 Publication in Lab on a Chip (2008) Authors: Baaken G., Sondermann M., Schlemmer C., Rhe J., Behrends J.C.Increasing the throughput and resolution of electrical recording of currents through ion conducting channels and pores is an important technical challenge both for the functional analysis of ion channel proteins and for the application of nanoscale pores in single molecule analytical tasks. We present a novel design based on sub-picoliter-CaVities arrayed in a polymer substrate and endowed with individual planar microelectrodes that allows low-noise and parallel electrical recording from ion channels and pores. Resolution of voltage-dependent current transitions of alamethicin channels as well as polyethylene-glycol-induced blocking events of alpha-hemolysin nanopores on the submillisecond time scale is demonstrated using this device.Poster PDF2021 Drug Effects on human Na+/Ca2+ Exchanger and Implications for Drug DevelopmentFLEXcyte 96, SURFE2R N1 and SURFE2R 96SE poster, Biophysical Society Meeting 2021Publication link2022 Hybrid Cell Analysis System to Assess Structural and Contractile Changes of Human iPSC-Derived Cardiomyocytes for Preclinical Cardiac Risk EvaluationFLEXcyte 96 and CardioExcyte 96 Publication in JoVe (2022) Authors: Lickiss B., Gossmann M., Linder P., Thomas U., Dragicevic E., Lemme M., George M., Fertig N., Stlzle-Feix S.Cardiac contractility assessment is of immense importance for the development of new therapeutics and their safe transition into clinical stages. While human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) hold promise to serve as a human-relevant model in preclinical phases of drug discovery and safety pharmacology, their maturity is still controversial in the scientific community and under constant development. We present a hybrid contractility and impedance/extracellular field potential (EFP) technology, adding significant pro-maturation features to an industry-standard 96-well platform. The impedance/EFP system monitors cellular functionality in real-time. Besides the beat rate of contractile cells, the electrical impedance spectroscopy readouts detect compound-induced morphological changes like cell density and integrity of the cellular monolayer. In the other component of the hybrid cell analysis system, the cells are cultured on bio-compliant membranes that mimic the mechanical environment of real heart tissue. This physiological environment supports the maturation of hiPSC-CMs in vitro, leading to more adult-like contractile responses including positive inotropic effects after treatment with isoproterenol, S-Bay K8644, or omecamtiv mecarbil. Parameters such as the amplitude of contraction force (mN/mm2) and beat duration also reveal downstream effects of compounds with influence on electrophysiological properties and calcium handling. The hybrid system provides the ideal tool for holistic cell analysis, allowing preclinical cardiac risk assessment beyond the current perspectives of human-relevant cell-based assays.Webinar27.01.2021 | Webinar: Thinking outside the cardiac boxSpeaker: Dr. Elena Dragicevic (Senior Sales and Alliance Manager; Nanion Technologies)Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are attractive due to their unlimited availability and human origin, making them a promising tool for cardiac research and safety pharmacology. However, they can show an immature phenotype such as lower inward rectifier potassium current (IK1), atypical expression pattern of ion channels, divergent response to pharmacological agents and contractile behaviour compared to adult CMs. Thus, their detailed characterization and optimized recording environments are essential.We aimed to characterize and modulate electrophysiological and contractile properties of hiPSC-CMs using automated dynamic clamp and contraction measurements on flexible substrate.Here, we recorded iCell Cardiomyocytes2 in voltage and current clamp using a combined automated patch clamp (APC) and dynamic clamp device (Patchliner Dynamite8), and contractility recordings were made using the FLEXcyte 96. During the APC recordings simulated IK1 and seal compensation were applied to up to 8 hiPSC-CMs simultaneously, while the contractility recordings were conducted in 96-well plates. We have tested various compounds targeting cardiac ion channels and recorded their effects on action potential duration (APD), sodium, calcium and potassium currents, as well as their effect on the contraction capabilities of these cells. Additionally, different levels of static and cyclic pressure were applied to the cell monolayers with the aim to induce membrane deflection for reproducibility test of Frank-Starling mechanism and to imitate the physiological stretching experienced by CMs in the beating human heart during systolic and diastolic phases, respectively.Seal compensation and virtual IK1 in hiPSC-CMs resulted in more stable and longer APs with low APD variability. Consequently, the dynamic clamp approach enabled reliable calcium, sodium and potassium channel pharmacology on action potentials of these cells. Culturing conditions that support contractility, i.e. flexible membrane substrates, demonstrate Ca2+ channel pharmacology equivalent to that expected from adult CMs while applied mechanical stimulation resulted in functional changes of hiPSC-CMs physiology.Presentation PDF2020 Mechanobiology of in vitro assays: Tackling prevailing challanges in pre-clinical drug developmentPresenter: Dr. Matthias Gossmannco-founder and CEO at InnoVitro, Germany, Source:The 64th Annual Meeting of the Biophysical Society, San Diego, CA (USA), Feb 15-19. 2020Abstract:Common systems for the quantification of cellular contraction rely on animal-based models, complex experimental setups or indirect approaches. Integ

repolarization from pre-addition values and arrhythmia after drug application and thus can provide predictive cardiac electrophysiology data. The baseline electrophysiological parameters vary between iPS cells from different sources, therefore positive and negative control recordings are recommendedUser meeting video15.10.2020 | Webinar: Heart slice culture system reliably demonstrates clinical drug-related cardiotoxicitySpeakers: Dr. Tamer M. Mohamed (University of Louisville), This is an on-demand webinar from Nan]i[on and Friends 2020.The limited availability of human heart tissue and its complex cell composition are major limiting factors for the reliable testing of drug efficacy and toxicity. Recently, we developed functional human and pig heart slice biomimetic culture systems that preserve the viability and functionality of 300 m heart slices for up to 6 days. Here, we tested the reliability of this culture system for testing the cardiotoxicity of anti-cancer drugs. We tested three anti-cancer drugs (doxorubicin, trastuzumab, and sunitinib) with known different mechanisms of cardiotoxicity at three concentrations and assessed the effect of these drugs on heart slice viability, structure, function and gene expression. Slices incubated with any of these drugs for 48 h showed diminished in viability as well as loss of cardiomyocyte structure and function. Mechanistically, RNA sequencing of doxorubicin-treated tissues demonstrated a significant downregulation of cardiac genes and upregulation of oxidative stress responses. Trastuzumab treatment downregulated cardiac muscle contraction-related genes consistent with its clinically known effect on cardiomyocytes. Interestingly, sunitinib treatment resulted in significant downregulation of angiogenesis-related genes, in line with its mechanism of action. Similar to hiPS-derived-cardiomyocytes, heart slices recapitulated the expected toxicity of doxorubicin and trastuzumab, however, slices were superior in detecting sunitinib cardiotoxicity and mechanism in the clinically relevant concentration range of 0.11 M. These results indicate that heart slice culture models have the potential to become a reliable platform for testing and elucidating mechanisms of drug cardiotoxicity.Webinar13.10.2020 | Webinar: Impedance measurements and Calcium imaging to investigate the role of store operated Ca2+ entry in GPCR-mediated endothelial barrier regulationSpeakers:Dr. Judith Stolwijk (University Hospital Regensburg; Germany)This is an on-demand webinar from Nan]i[on and Friends 2020.The endothelium of our blood vessels is under the control of various G protein-coupled receptors (GPCRs), regulating endothelial barrier function, vascular tone, angiogenesis and inflammation. Some of these GPCRs signal via Gq,11 protein, which activates Ca2+-release from the IP3-sensitive internal stores of the endoplasmic reticulum (ER). Calcium store depletion can subsequently activate so-called store-operated Ca2+ entry (SOCE) via the ER-resident Ca2+-sensor stromal-interacting molecule 1 (STIM1) and Orai1 Ca2+ entry channels.We used Impedance measurements and ratiometric Calcium imaging to investigate the role of calcium entry via Orai1 channels downstream of barrier-regulating GPCRs in response to their agonists Thrombin, Histamine and Sphingosine-1-Phosphate (S1P) in human endothelial cells.Product Sheet PDFCardioExcyte 96 Product SheetTutorial video2020 CardioExcyte 96 Tutorial VideoShort introduction of the CardioExcyte 96 (tutorial video, 2 minutes)2017 HTS in Cardiac SafetyPresenter: Dr. Sonja Stlzle-Feix, Nanion Technologies GmbH, Source: Satellite symposium during SPS, Berlin, September 22. 2017Presentation PDF2020 Validation of a impedance-based phenotypic screening assay able to detect multiple mechanisms of chronic cardiotoxicity in human stem cell-derived cardiomyocytesPresenter: Dr. Marc Rogers, CSO at Metrion Biosciences, United KingdomSource: The 64th Annual Meeting of the Biophysical Society, San Diego, CA (USA) Feb 15-19. 2020 About: Metrion Biosciences is a UK-based Contract Research Organization (CRO) focussed on delivering a range of high quality ion channel screening and drug discovery services. We provide sophisticated electrophysiology assays to support medicinal chemistry optimisation programmes, CiPA-compliant cardiac safety.Poster PDF2019 High content in vitro cell monitoring effects of adjuvant chemotherapy in breast cancer and cancer treatment-related cardiomyopathyPoster PDF2021 Cell Monitoring using Multi-Frequency Impedance Recordings for Label-free and Time-resolved Cell Response AnalysisPoster PDF2018 Assessment of Drug Effects on Cardiomyocyte Function: Comprehensive In Vitro Proarrhythmia Assay (CiPA) ResultsPoster PDF2018 Investigations into idiosyncratic drug-induced hepatotoxicity and chronic proliferation of cancer cells using a label-free methodFlyer PDFCardioExcyte 96 Flyer PacingFlyer PDFCardioExcyte 96 Flyer SOLFlyer PDFCardioExcyte 96 Application AreasFlyer PDFCardioExcyte 96 / FLEXcyte 96 Product Flyer PlatesApplication Note PDFHepatocytes Toxicity analysis in expanded upcyte liver cells on Nanions CardioExcyte 96CardioExcyte 96 Application Note: Cells were kindly provided by upcyte technologiesDrug induced hepatic toxicity is one of the main reasonsfor regulatory actions and market withdrawals in the last50 years [1]. In Europe and the USA, the major causeof acute liver failure is indeed Drug Induced Liver Injury(DILI) [2]. Current existing in vitro models employed topredict DILI mostly focus on hepatocytes. Other cell typesof the liver, such as liver sinusoidal endothelial cells (LSECs),are often overlooked. It is thus questionable whether,hepatotoxicity can be sufficiently predicted by analyzinghepatocytes only. LSECs are highly specialized endothelialcells forming the hepatic sinusoidal wall. Besides their highendocytic potential, LSECs have been demonstrated tomarkedly contribute to liver homeostasis, immunity, andmay partially explain unexpected hepatotoxicity ofselected drug candidates.Several reports in the literature have highlighted a highsensitivity of LSECs towards hepatotoxic drugs [3]. Ithas been suggested that LSECs act as an early directtarget for acetaminophen (paracetamol) inducedtoxicity, causing early swelling and loss of uptake activityand fenestrations before effects on hepatocytes areobserved [4]. LSECs are further important cellular targetsduring sinusoidal obstruction syndrome (a distinctiveand potentially fatal form of hepatic injury that occurspredominantly, if not only, after drug or toxin exposure).The use of primary LSECs for comprehensive in vitro studiesis compromised by poor cell yields, rapid dedifferentiation,contamination with other endothelial cells, and limitedproliferation after isolation [5]. In this study upcyte LSECshave been developed as a complementary tool topredict hepatotoxicity, uptake and drug interactions. Inaddition to conventional toxicity readouts such as ATPlevels, upcyte LSECs have been successfully tested usingan impedance based system.Application Note PDFhiPSC-CM Conduction velocity with the 2-electrode layout of the CardioExcyte 96CardioExcyte 96 Application Note: Cardiosight-S hiPSC-CMs kindly provided by NexelCardiac conduction is the process by which electrical excitation spreads through the heart, triggering individual myocytes to contract synchronously. Slowed cardiac conduction velocity (CV) is associated with an increased risk of re-entrant excitation, leading to a pre-disposition to life-threatening arrhythmias1.CV is determined by the ion channel properties of cardiac myocytes and by their interconnections. It is strictly dependent on the maximum upstroke velocity of an action potential, which is determined by the sodium current2. In addition, gap junctions play a key role because they ultimately determine how much depolarizing sodium current passes from excited to non-excited regions of the network. Uncoupling of gap junctions causes discontinuities leading to slower CV. Defective intercellular coupling between the cardiomyocytes results in increased subthreshold depolarization, which slowly inactivates the voltage-gated sodium channels, further reducing the sodium current and excitability3.In the case of collagenous scar tissue the uncoupling of myocytemyocyte connections and subsequent coupling of myocytes with fibroblasts impairs the electrical conduction. In fact, collagen deposition results in electrically isolated fibers of viable myocardium, discontinuing the conduction path and globally reducing the action potential propagation velocity and consequently promoting the onset of re-entrant arrhythmias4. Since CV plays a pivotal role in cardiovascular diseases, it is essential to investigate the effect of a new compound on the cardiac CV.In this study, we have investigated the effect of the sodium channel blocker lidocaine (30 and 100 M) on CV in hiPSC-CMs (NEXEL Cardiosight-S) using the CardioExcyte 96.Application Note PDFHepatocytes Investigating DILI using MetaHeps cells on Nanions CardioExcyte 96CardioExcyte 96 Application Note: Cells were kindly provided by Metaheps GmbH.Drug Induced Liver Injury (DILI) is the major cause of acute liver failure in the USA and Europe and is one of the main reasons for regulatory actions and market withdrawals. Indeed, hepatic toxicity has accounted for 15 of the 47 drugs withdrawn from the market in the period 1975 - 2007. DILI is classified as intrinsic or dosedependent, acetaminophen (paracetamol) being the most important example of this class, or idiosyncratic which is unpredictable and not directly dependent on dose. A number of factors contribute to an individuals susceptibility to develop idiosyncratic DILI including age, sex, alcohol consumption, drug interactions and genetic variability. Although improvements have been made to cellular and animal models to predict intrinsic (dose-dependent) DILI, it is almost impossible to predict idiosyncratic DILI. Withdrawal of compounds at a late stage (or postmarketing) due to idiosyncratic DILI is costly and can lead to incorrect withdrawal of potentially useful compounds. Monocyte-derived hepatocyte-like (MH) cells have been developed as a tool to investigate longterm hepatotoxicity, metabolism and drug interactions. Furthermore, patient-derived MH cells could provide a tool for diagnosis or exclusion of idiosyncratic DILI and provide the causative agent in polymedicated patients.In this study, MH cells (MetaHeps) were used on the CardioExcyte 96 and changes in impedance, and therefore confluency, were used as a measure of toxicity. Intrinsic (dose-dependent) effects of paracetamol could be identified consistent with other methods of liver injury detection. Therefore, the CardioExcyte 96 in combination with patient-specific MH cells provides a novel tool for investigating intrinsic and idiosyncratic DILI.Application Note PDFCardiotoxicity Assessing cardiotoxic risk of anti-cancer agents on Nanions CardioExcyte 96CardioExcyte 96 Application Note:Cells were kindly provided by Ncardia and experiments performed by Oliver Reinhardt and Dr. Frauke Alves from the University of Gttingen.New anticancer agents have led to higher life expectancy for patients surviving cancer. However, treatment related morbidity factors such as cardiac toxicity have become important issues for long-term cancer survivors. Cardiotoxic side effects such as arrhythmia, thromboembolism and myocardial ischemia are common with anti-cancer drugs such as the anthracyclines. This has led to the need for a sub-speciality of medicine, cardio-oncology or oncocardiology, to promote cardiovascular health whilst providing the best therapy to fight cancer. It is important to be able to assess the cardiotoxic risk of new and existing cancer therapies in order to facilitate effective cardiovascular health during chemotherapy. In addition, advances in human stem cell derived cardiomyocytes (hiPSC-CMs) and, indeed, patient-derived hiPSC-CMs offers new possibilities for personalized medicine, being able to assess a patients risk of developing cardiovascular complications based on their own cells, thus taking into account their own genetic factors. Using the measurement of electrical impedance coupled with human stem cell-derived cardiomyocytes (hSC-CMs) we could confirm the cardiotoxic effects of paclitaxel, also known as Taxol, a microtuble stabilizing drug approved for the treatment of breast, ovarian and lung cancer. In addition, we investigated different combinations of cylophosphamide (CP), doxorubicin (DOX) and 5-Fluorouracil (5F) and found that any combination which included DOX was highly cardiotoxic.Application Note PDFCytoSwitch Inducing cell death using a photostatin on the CardioExcyte 96 and SOLCardioExcyte 96 and SOL Application Note:Cells were kindly provided by LMU Munich/CytoSwitch.Microtubules are important for cell support, acting as a kind of internal scaffold providing both shapeand an organized structure. Another important role of microtubules is the formation of the mitotic spindle which is critical in cell division. For this reason,compounds which control microtubule dynamics such as Taxol and Vinca alkaloids are important as anti-cancer therapeutics and biological research tools. Other compounds e.g. combretastin A-4 (CA4), have been shown to be powerful inhibitors of tubule polymerization. Until now, the use of such compounds in both biological research and as anti-cancer agentshas been limited because of their non-specificity; their bioactivity cannot be spatially or temporally directed,e.g. against particular tissues or cells. As an anti-cancer agent, CA4 was taken into Phase III clinical trials but was discontinued due to the cardio- or neurotoxic side effects at high doses as healthy organs were affected. The use of light to control the activity of microtubule inhibitors may offer a solution to combat the specificity problem by controlling the activation of the compound using visible light in a spatial, temporal and reversible fashion. The CytoSwitch startup project at the LMU Munich is developing photostatins as anti-cancer agents with reduced side-effects. Furthermore, CytoSwitch will commercialize photostatins as research tools for biological research.Application Note PDFCardiomyocytes Impedance and EFP recordings of Cor.4U cells using Nanions CardioExcyte 96CardioExcyte 96 Application Note:Cells were kindly provided byNcardia.Cardiomyocytes derived from human inducedpluripotent stem cells (hiPSCs) are gaining interest in cardiac safety screening. Given their relative abundance, availability, ease of use and standardized production, they are likely to provide a viable alternative to acutely isolated cardiomyocytes to assess the pro-arrythmic potentials of drug candidates. Although automated patch clamp can provide excellent information about the effects of compounds on cardiac ion channels and possible effects on the cardiac action potential, other techniques, such as impedance, also provide crucial and complementary information about complex physiological parameters such as beat rate, amplitude and duration. The CardioExcyte 96 is a new hybrid screening tool combining impedance (cell contractility) and extracellular field potential (EFP) recordings. These measurements are non-invasive, label-free and have a temporal resolution of 1 ms. The recordings are made from cells within a network thus providing a physiologically relevant environment for measuring drug-induced changes in beat parameters. This hybrid technology addresses the lack of easy-to-use high-throughput screening for in-vitro assays, and permits the reliable investigation of short- and long-term pharmacological effects. Here we present data recorded on the CardioExcyte 96 using Cor.4U hiPSCs from Axiogenesis. The effects of the compounds blebbistatin, E4031 and nifedipine on the impedance and EFP signals are shown.Application Note PDFCardiomyocytes Impedance and EFP recordings of iCell Cardiomyocytes on the CardioExcyte 96CardioExcyte 96 Application Note: Cells were kindly provided by Cellular Dynamics.Cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) are gaining interest in cardiac safety screening. Given their recapitulation of native behavior, availability, ease of use and standardized production, they are likely to provide a viable alternative to acutely isolated cardiomyocytes to assess the pro-arrhythmic potentials of drug candidates. Although automated patch clamp can provide excellent information about the effects of compounds on cardiac ion channels and possible effects on the cardiac action potential, other outputs such as extracellular field potential (EFP) and impedance, also provide crucial and complementary information about complex physiological parameters such as beat rate, amplitude and duration. The CardioExcyte 96 is a new hybrid screening tool combining impedance (cell contractility) and EFP recordings. These measurements are non-invasive, label free and have a temporal resolution of 1 ms. The recordings are made from cells within a network thus providing a physiologically relevant environment for measuring drug-induced changes in contractileparameters. This hybrid technology is a high-throughput screening tool which permits the reliable investigation of short- and long-term pharmacological effects.Here we present data recorded on the CardioExcyte 96 using iCellCardiomyocytes2 from Cellular Dynamics International (CDI). When handled according to the manufactures instructions, impedance and EFP signals were stable 4 days afterplating and compound effects could be robustly measured and analyzed.Publication link2022 The potential of remdesivir to affect function, metabolism and proliferation of cardiac and kidney cells in vitroCardioExcyte 96 Publication inOrgan Toxicity and Mechanisms(2022) Authors: Merches K., Breunig L., Fender J., Brand T., Btz V., Idel S., Kollipara L., Reinders Y., Sickmann A., Mally A., & Lorenz K.Remdesivir is a prodrug of a nucleoside analog and the first antiviral therapeutic approved for coroNaVirus disease. Recent cardiac safety concerns and reports on remdesivir-related acute kidney injury call for a better characterization of remdesivir toxicity and understanding of the underlying mechanisms. Here, we performed an in vitro toxicity assessment of remdesivir around clinically relevant concentrations (Cmax 9 M) using H9c2 rat cardiomyoblasts, neonatal mouse cardiomyocytes (NMCM), rat NRK-52E and human RPTEC/TERT1 cells as cell models for the assessment of cardiotoxicity or nephrotoxicity, respectively. Due to the known potential of nucleoside analogs for the induction of mitochondrial toxicity, we assessed mitochondrial function in response to remdesivir treatment, early proteomic changes in NMCM and RPTEC/TERT1 cells and the contractile function of NMCM. Short-term treatments (24 h) of H9c2 and NRK-52E cells with remdesivir adversely affected cell viability by inhibition of proliferation as determined by significantly decreased 3H-thymidine uptake. Mitochondrial toxicity of remdesivir (1.63.1 M) in cardiac cells was evident by a significant decrease in oxygen consumption, a collapse of mitochondrial membrane potential and an increase in lactate secretion after a 2448-h treatment. This was supported by early proteomic changes of respiratory chain proteins and intermediate filaments that are typically involved in mitochondrial reorganization. Functionally, an impedance-based analysis showed that remdesivir (6.25 M) affected the beat rate and contractility of NMCM. In conclusion, we identified adverse effects of remdesivir in cardiac and kidney cells at clinically relevant concentrations, suggesting a careful evaluation of therapeutic use in patients at risk for cardiovascular or kidney disease.Application Note PDFCardiomyocytes Impedance and EFP recordings of Pluricyte Cardiomyocytes on the CardioExcyte 96CardioExcyte 96 Application Note: Cells were kindly provided by Ncardia.Cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) are gaining interest in cardiac safety screening. Given their recapitulation of native behavior, availability, ease of use and standardized production, they are likely to provide a viable alternative to acutely isolated cardiomyocytes to assess the pro-arrhythmic potentials of drug candidates. Although automated patch clamp can provide excellent information about the effects of compounds on cardiac ion channels and possible effects on the cardiac action potential, other outputs such as extracellular field potential (EFP) and impedance, also provide crucial and complementary information about complex physiological parameters such as beat rate, amplitude and duration. The CardioExcyte 96 is a new hybrid screening tool combining impedance (cell contractility) and EFP recordings. These measurements are non-invasive, label-free and have a temporal resolution of 1 ms. The recordings are made from cells within a network thus providing a physiologically relevant environment for measuring drug-induced changes in contractileparameters. This hybrid technology is an easy-to-use screening tool which permits the reliable investigation of short- and long-term pharmacological effects. Here we present data recorded on the CardioExcyte 96 using Pluricyte Cardiomyocytes from Ncardia. The effects of the CaVblocker, nifedipine, hERG blockers, dofetilide and E4031, and NaVblocker, mexiletine, on EFP and impedance parameters are shown.Publication link2022 NRF-2/HO-1 Pathway-Mediated SHOX2 Activation Is a Key Switch for Heart Rate Acceleration by Yixin-Fumai GranulesCardioExcyte 96 Publication in Oxidative Medicine and Cellular Longevity(2022) Authors: Zhang H., Chen C., Liu Y., Ren L., Qi J., Yang Y., Chen W., Yao Y., Cai X., Liu Z., Hao M., Li L., Deng Z., Sun M., Lu Y., Chen K., Hou P.Population aging has led to increased sick sinus syndrome (SSS) incidence; however, no effective and safe medical therapy has been reported thus far. Yixin-Fumai granules (YXFMs), a Chinese medicine granule designed for bradyarrhythmia treatment, can effectively increase SSS patients heart rate. Senescence-induced sinoatrial node (SAN) degeneration is an important part of SSS pathogenesis, and older people often show high levels of oxidative stress; reactive oxygen species (ROS) accumulation in the SAN causes abnormal SAN pacing or conduction functions. The current study observed the protective effects of YXFMs on senescent SAN and explored the relationship between the NRF-2/HO-1 pathway, SHOX2, and T-type calcium channels. We selected naturally senescent C57BL/6 mice with bradycardia to simulate SSS; electrocardiography, Massons trichrome staining, and DHE staining were used to assess SAN function and tissue damage. Immunofluorescence staining and Western blotting were used to assay related proteins. In vitro, we treated human-induced pluripotent stem cell-derived atrial myocytes (hiPSC-AMs) and mouse atrial myocyte-derived cell line HL-1 with D-galactose to simulate senescent SAN-pacemaker cells. CardioExcyte96 was used to evaluate the pulsatile function of the hiPSC-AMs, and the mechanism was verified by DCFH-DA, immunofluorescence staining, RT-qPCR, and Western blotting. The results demonstrated that YXFMs effectively inhibited senescence-induced SAN hypofunction, and this effect possibly originated from sCaVenging of ROS and promotion of NRF-2, SHOX2, and T-type calcium channel expression. In vitro experiment results indicated that ML385, si-SHOX2, LDN193189, and Mibefradil reversed YXFMs effects. Moreover, we, for the first time, found that ROS accumulation may hinder SHOX2 expression; YXFMs can activate SHOX2 through the NRF-2/HO-1 pathway-mediated ROS sCaVenging and then regulate CACNA1G through the SHOX2/BMP4/GATA4/NKX2-5 axis, improve T-type calcium channel function, and ameliorate the SAN dysfunction. Finally, through network pharmacology and molecular docking, we screened for the most stable YXFMs compound that docks to NRF-2, laying the foundation for future studies.Publication link2022 The G213D variant in NaV1.5 alters sodium current and causes an arrhythmogenic phenotype resulting in a multifocal ectopic Purkinje-related premature contraction phenotype in human-induced pluripotent stem cell-derived cardiomyocytesCardioExcyte 96 Publication in EP Europace(2022) Authors: Calloe K., Geryk M., Freude K., Treat J.A., Vold V.A., Frederiksen H.R.S., Broendberg A.K., Frederiksen T.C., Jensen H.K., Cordeiro J.M.Variants in SCN5A encoding NaV1.5 are associated with cardiac arrhythmias. We aimed to determine the mechanism by which c.638G>A in SCNA5 resulting in p.Gly213Asp (G213D) in NaV1.5 altered Na+ channel function and how flecainide corrected the defect in a family with multifocal ectopic Purkinje-related premature contractions (MEPPC)-like syndrome. Methods and results Five patients carrying the G213D variant were treated with flecainide. Gating pore currents were evaluated in Xenopus laevis oocytes. The 638G>A SCN5A variant was introduced to human-induced pluripotent stem cell (hiPSC) by CRISPRCas9 gene editing and subsequently differentiated to cardiomyocytes (hiPSC-CM). Action potentials and sodium currents were measured in the absence and presence of flecainide. Ca2+ transients were measured by confocal microscopy. The five patients exhibited premature atrial and ventricular contractions which were suppressed by flecainide treatment. G213D induced gating pore current at potentials negative to 50mV. Voltage-clamp analysis in hiPSC-CM revealed the activation threshold of INa was shifted in the hyperpolarizing direction resulting in a larger INa window current. The G213D hiPSC-CMs had faster beating rates compared with wild-type and frequently showed Ca2+ waves and alternans. Flecainide applied to G213D hiPSC-CMs decreased window current by shifting the steady-state inactivation curve and slowed the beating rate. Conclusion The G213D variant in NaV1.5 induced gating pore currents and increased window current. The changes in INa resulted in a faster beating rate and Ca2+ transient dysfunction. Flecainide decreased window current and inhibited INa, which is likely responsible for the therapeutic effectiveness of flecainide in MEPPC patients carrying the G213D variant.Publication link2022 How Carvedilol activates 2-adrenoceptorsCardioExcyte 96 Publication inNature Communications(2022) Authors: Benkel T., Zimmermann M., Zeiner J., Bravo S., Merten N., Jun Yu Lim V., Fabienne Matthees E., Drube J., Miess-Tanneberg E., Malan D., Szpakowska M., Monteleone S., Grimes J., Koszegi Z., Lanoisele Y., OBrien S., Pavlaki N., Dobberstein N., Inoue A., Nikolaev V., Calebiro D., Chevign A., Sasse P., Schulz S., Hoffmann C., Kolb P., Waldhoer M., Simon K., Gomeza J., Kostenis E.Carvedilol is among the most effective -blockers for improving survival after myocardial infarction. Yet the mechanisms by which carvedilol achieves this superior clinical profile are still unclear. Beyond blockade of 1-adrenoceptors, arrestin-biased signalling via 2-adrenoceptors is a molecular mechanism proposed to explain the survival benefits. Here, we offer an alternative mechanism to rationalize carvedilols cellular signalling. Using primary and immortalized cells genome-edited by CRISPR/Cas9 to lack either G proteins or arrestins; and combining biological, biochemical, and signalling assays with molecular dynamics simulations, we demonstrate that G proteins drive all detectable carvedilol signalling through 2ARs. Because a clear understanding of how drugs act is imperative to data interpretation in basic and clinical research, to the stratification of clinical trials or to the monitoring of drug effects on the target pathway, the mechanistic insight gained here provides a foundation for the rational development of signalling prototypes that target the -adrenoceptor system.Publication link2022 Hypoglycemia-Exacerbated Mitochondrial Connexin 43 Accumulation Aggravates Cardiac Dysfunction in Diabetic CardiomyopathyCardioExcyte 96 prePublication inFrontiers in Cardiovascular Medicine(2022) Authors: Wei X., Chang A.C.H., Chang H., Xu S., Xue Y., Zhang Y., Lei M., Chang A.C.Y., Zhang Q.Diabetic cardiomyopathy (DCM) is a complex multifaceted disease responsible for elevated heart failure (HF) morbidity and mortality in patients with diabetes mellitus (DM). Patients with DCM exhibit subclinical diastolic dysfunction, progression toward systolic impairment, and abnormal electrophysiology. Hypoglycemia events that occur spontaneously or due to excess insulin administration threaten the lives of patients with DMwith the increased risk of sudden death. However, the molecular underpinnings of this fatal disease remain to be elucidated.Publication link2021 Towards engineering heart tissues from bioprinted cardiac spheroidsCardioExcyte 96 Publicationin IOP Science (2021) Authors: Polonchuk L.,Surija L.,Lee M.H.,Sharma P,Ming C.L.C.,Richter F.,Ben-Sefer E.,Rad M.A.,Sarmast H.M.S.,Shamery W.A.,Tran H.A.,Vettori L.,Haeusermann F.,Filipe E.C.,Rnjak-Kovacina J.,Cox T.,Tipper J.,Kabakova I.,andGentile C.Currentin vivoandin vitromodels fail to accurately recapitulate the human heart microenvironment for biomedical applications. This study explores the use of cardiac spheroids (CSs) to biofabricate novel and advanced physiological cardiac models forin vitrotesting. CSs were created from human cardiac myocytes, fibroblasts and endothelial cells, mixed within optimal alginate/gelatin (Al/Ge) hydrogels and then bioprinted on a microelectrode plate for drug testing. Bioprinted CSs maintained their structure and viability for at least 30 days after printing. Vascular endothelial growth factor (VEGF) promoted endothelial cell branching from CSs within hydrogels. Alginate/gelatin-based hydrogels enabled spheroids fusion, which was further facilitated by addition of VEGF. Bioprinted CSs contracted spontaneously and under stimulation, allowing to record contractile and electrical signals on the microelectrode plates for industrial applications. Taken together, our findings indicate for the first time that bioprinted CSs within Al/Ge hydrogels can be used to biofabricate durable, viable and functional human heart tissues for long termin vitrotesting.Publication link2021 Transient Cell Cycle Induction in Cardiomyocytes to Treat Ischemic Heart FailureCardioExcyte 96 prePublication in Research Square(2021) Authors: Abouleisa R., Ou Q., Tang X-L., Solanki M., Guo Y., Nong Y., Mcnally L., Lorkiewicz P., Kassem K., Ahern B., Choudhary K., Thomas R., Huang Y., Juhardeen H., Siddique A., Ifthikar Z., Salama A-B., Hammad S., Elbaz A., Ivey K., Satin J., Hill B., Srivastava D., Bolli R., Mohamed T.The regenerative capacity of the heart after myocardial infarction (MI) is limited. Our previous study showed that ectopic introduction of Cdk1/CyclinB1 and Cdk4/CyclinD1 complexes (4F) promotes cardiomyocyte proliferation in 15-20% of infected cardiomyocytes in vitro and in vivo and improves cardiac function after MI. Here, we aim to identify the necessary reprogramming stages during the forced cardiomyocyte proliferation with 4F on a single cell basis. Also, we aim to start the first preclinical testing to introduce 4F gene therapy as a candidate for the treatment of ischemia-induced heart failure. Temporal bulk and single-cell RNAseq and further biochemical validations of mature hiPS-CMs treated with either LacZ or 4F adenoviruses revealed full cell cycle reprogramming in 15% of the cardiomyocyte population after 48 h post-infection with 4F, which was associated with sarcomere disassembly and metabolic reprogramming. Transient overexpression of 4F, specifically in cardiomyocytes, was achieved using a polycistronic non-integrating lentivirus (NIL) encoding the 4F; each is driven by a TNNT2 promoter (TNNT2-4F-NIL). TNNT2-4F-NIL or control virus was injected intramyocardially one week after MI in rats or pigs. TNNT2-4F-NIL treated animals showed significant improvement in left ventricular ejection fraction and scar size compared with the control virus treated animals four weeks post-injection. In conclusion, the present study provides a mechanistic demonstration of the process of forced cardiomyocyte proliferation and advances the clinical feasibility of this approach by minimizing the oncogenic potential of the cell cycle factors using a novel transient and cardiomyocyte-specific viral construct.Publication link2021 Sex-Specific Control of Human Heart Maturation by the Progesterone ReceptorCardioExcyte 96 Publication in Circulation (2021) Authors: Sim C.B., Phipson B., Zienabb N., Rafehi H., Mills R.J., Watt K.I., Abu-Bonsrah K.D., Kalathur R.K.R., Voges H.K., Dinh D.T., ter Huurne M.,Vivien C.J., Kaspi A., Kaipanaickal H., Hidalgo A., Delbridge L.M.D., Robker R.L., Gregorevic P., dos Remedios C.G., Lal S., Piers A.T., Konstantinov I.E., Elliott D.A., El-Osta A., Oshlack A., Hudson J.E., Porrello E.R.Background: Despite in-depth knowledge of the molecular mechanisms controlling embryonicheart development, little is known about the signals governing postnatal maturation of the humanheart. Methods: Single nucleus RNA-sequencing (snRNA-seq) of 54,140 nuclei from 9 human donors was used to profile transcriptional changes in diverse cardiac cell types during maturation from fetal stages to adulthood. Bulk RNA-sequencing and the assay for transposase-accessible chromatin using sequencing (ATAC-seq) were used to further validate transcriptional changes and to profile alterations in the chromatin accessibility landscape in purified cardiomyocyte nuclei from 21 human donors. Functional validation studies of sex steroids implicated in cardiac maturation were performed in human pluripotent stem cell-derived cardiac organoids and mice. Results: Our data identify the progesterone receptor as a key mediator of sex-dependent transcriptional programs during cardiomyocyte maturation. Functional validation studies in human cardiac organoids and mice demonstrate the progesterone receptor drives sex-specificmetabolic programs and maturation of cardiac contractile properties. Conclusions: These data provide a blueprint for understanding human heart maturation in bothsexes and reveal an important role for the progesterone receptor in human heart development.Publication link2021 Targeting of CAT and VCAM1 as Novel Therapeutic Targets for DMD CardiomyopathyCardioExcyte 96 Publication in Cell & Development Biology (2021) Authors: Li B., Xiong W., Liang W-M., Chiou J-S., Lin Y-J., Chang A.C.Y.Duchenne muscular dystrophy (DMD) related cardiomyopathy is the leading cause of early mortality in DMD patients. There is an urgent need to gain a better understanding of the disease molecular pathogenesis and develop effective therapies to prevent the onset of heart failure. In the present study, we used DMD human induced pluripotent stem cells (DMD-hiPSCs) derived cardiomyocytes (CMs) as a platform to explore the active compounds in commonly used Chinese herbal medicine (CHM) herbs. Single CHM herb (DaH, ZK, and CQZ) reduced cell beating rate, decreased cellular ROS accumulation, and improved structure of DMD hiPSC-CMs. Cross-comparison of transcriptomic profiling data and active compound library identified nine active chemicals targeting ROS neutralizing Catalase (CAT) and structural protein vascular cell adhesion molecule 1 (VCAM1). Treatment with Quecetin, Kaempferol, and Vitamin C, targeting CAT, conferred ROS protection and improved contraction; treatment with Hesperidin and Allicin, targeting VCAM1, induced structure enhancement via induction of focal adhesion. Lastly, overexpression of CAT or VCAM1 in DMD hiPSC-CMs reconstituted efficacious effects and conferred increase in cardiomyocyte function. Together, our results provide a new insight in treating DMD cardiomyopathy via targeting of CAT and VCAM1, and serves as an example of translating Bed to Bench back to Bed using a muti-omics approach.Publication link2021 Long noncoding RNA VENTHEART is required for cardiomyocyte specification and functionCardioExcyte 96pre-print Publication in bioRxiv(2021) Authors: Dashi A., Tan W.L.W., Anene-Nzelu C.G., Pan B., Ilmari A.M., Tiang Z., Hartman R.J.G., Stenzig J., Wei H., Gao Bin C., Ackers-Johnson M.A., Lim B., Walentinsson A., Iyer V.V., Jonsson M.K.B., Foo R.S.Long noncoding RNAs (lncRNAs) control cardiac gene expression during heart development and disease. It is accordingly plausible for the same lncRNA to regulate both cardiac development, as well as play a role in adult heart disease progression. lncRNA regulators of early cardiomyocyte (CM) lineage commitment have been identified and characterised, however those controlling later CM specification remain unknown.Publication link2021 Overlap Arrhythmia Syndromes Resulting from Multiple Genetic Variations Studied in Human Induced Pluripotent Stem Cell-Derived CardiomyocytesCardioExcyte 96 Publicationin International Journal of Molecular Sciences (2021) Authors: Treat J.A., Pfeiffer R., Barajas-Martinez H., Goodrow R.J., Bot C., Haedo R.J., Knox R., Cordeiro J.M.Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are usedfor genetic models of cardiac diseases. We report an arrhythmia syndrome consisting of EarlyRepolarization Syndrome (ERS) and Short QT Syndrome (SQTS). The index patient (MMRL1215)developed arrhythmia-mediated syncope after electrocution and was found to carry six mutations.Functional alterations resulting from these mutations were examined in patient-derived hiPSC-CMs.Electrophysiological recordings were made in hiPSC-CMs from MMRL1215 and healthy controls.ECG analysis of the index patient showed slurring of the QRS complex and QTc = 326 ms. Actionpotential (AP) recordings from MMRL1215 myocytes showed slower spontaneous activity and APduration was shorter. Field potential recordings from MMRL1215 hiPSC-CMs lack a pseudo QRScomplex suggesting reduced inward current(s). Voltage clamp analysis of ICashowed no difference inthe magnitude of current. Measurements of INareveal a 60% reduction in INadensity in MMRL1215hiPSC-CMs. Steady inactivation and recovery of INawas unaffected. mRNA analysis revealedANK2 and SCN5A are significantly reduced in hiPSC-CM derived from MMRL1215, consistent withelectrophysiological recordings. The polygenic cause of ERS/SQTS phenotype is likely due to a lossof INadue to a mutation inPKP2coupled with and a gain of function in IK,ATPdue to a mutationinABCC9.Publication link2021 Cardiac Safety of Kinase Inhibitors Improving Understanding and Prediction of Liabilities in Drug Discovery Using Human Stem Cell-Derived ModelsCardioExcyte 96 prePublication inFrontiers in Cardiovascular Medicine(2021) Authors: Ziegler R., Husermann F.,Kirchner S., Polonchuk LMany small molecule kinase inhibitors (SMKIs) used to fight cancer have been associated with cardiotoxicity in the clinic. Therefore, preventing their failure in clinical development is a priority for preclinical discovery. Our study focused on the integration and concurrent measurement of ATP, apoptosis dynamics and functional cardiac indexes in human stem cell-derived cardiomyocytes (hSC-CMs) to provide further insights into molecular determinants of compromised cardiac function. Ten out of the fourteen tested SMKIs resulted in a biologically relevant decrease in either beating rate or base impedance (cell number index), illustrating cardiotoxicity as one of the major safety liabilities of SMKIs, in particular of those involved in the PI3KAKT pathway. Pearson's correlation analysis indicated a good correlation between the different read-outs of functional importance. Therefore, measurement of ATP concentrations and apoptosisin vitrocould provide important insight into mechanisms of cardiotoxicity. Detailed investigation of the cellular signals facilitated multi-parameter evaluation allowing integrative assessment of cardiomyocyte behavior. The resulting correlation can be used as a tool to highlight changes in cardiac function and potentially to categorize drugs based on their mechanisms of action.Publication link2021 Hypoglycemia Induced Mitochondrial Connexin-43 Accumulation Aggravates Diabetic CardiomyopathyCardioExcyte 96 pre-Publication in Research Square(2021) Authors: Wei X., Chang A.C.H., Chang H., Xu S., Xue Y., Zhang Y., Lei M., Chang A.C.Y., Zhang Q.Background:Diabetic cardiomyopathy (DCM) is a complex multifaceted disease responsible for elevated hospitalization and mortality in patients with diabetes mellitus (DM). DCM patients exhibit subclinical diastolic dysfunction, progression towards systolic impairment, and abnormal electrophysiology. Hypoglycemia events that occur spontaneously or due to excess insulin administration threaten the lives of DM patients with the increased risk of sudden death. However, the molecular underpinnings of hypoglycemia-aggravated DCM remain to be elucidated. Methods and Results:Here we used the established streptozotocin-induced type 1 diabetic cardiomyopathy (T1 DCM) murine model to investigate how hypoglycemia aggravates DCM progression. We showed that chronic hyper- or hypoglycemic challenges dampened cardiac diastolic function in vivo as well as myocardial contractility and calcium handling in isolated cardiomyocytes. Similar contractile defects were recapitulated using neonatal mouse ventricular myocytes (NMVMs) under glucose fluctuation challenges. Using immunoprecipitation mass spectrometry, we identified and validated that hypoglycemia challenge activates the MEK/ERK and PI3K/Akt pathways which results in Cx43 phosphorylation by Src protein in cardiomyocytes. Cx43 dissociation and accumulation at mitochondrial inner membrane was confirmed both in human and murine cardiomyocytes. To determine causality, we overexpressed a mitoch

region of Pln and Ank2. Significant upregulation of PLN and ANK2 at the mRNA and protein levels was observed in the myocardium of sunitinib-treated mice. Cardiac-specific overexpression of miR-146a prevented the deteriorate effect of SNT on calcium transients, thereby alleviating the decreased contractility of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). SiRNA knockdown of PLN or ANK2 prevented sunitinib-induced suppression of contractility in hiPSC-CMs. Therefore, our in vivo and in vitro results showed that sunitinib downregulated miR-146a, which contributes to cardiac contractile dysfunction by regulating the downstream targets PLN and ANK2, and that upregulation of miR-146a alleviated the inhibitory effect of SNT on cardiac contractility. Thus, miR-146a could be a useful protective agent against sunitinib-induced cardiac dysfunction.Publication link2019 Label-Free Monitoring of Cells in vitroCardioExcyte 96 book chapter in Label-Free Monitoring of Cells in vitro in Bioanalytical Reviews - Springer (2019) Authors: Stolwijk J.A., Wegener J.This book is dedicated to label-free, non-invasive monitoring of cell-based assays and it comprises the most widely applied techniques. Each approach is described and critically evaluated by an expert in the field such that researchers get an overview on what is possible and where the limitations are. The book provides the theoretical basis for each technique as well as the most successful and exciting applications. Label-free bioanalytical techniques have been known for a long time as valuable tools to monitor adsorption processes at the solid-liquid interface in general and biomolecular interaction analysis (BIA) in particular. The underlying concepts have been progressively transferred to the analysis of cell-based assays. The strength of these approaches is implicitly given with the name 'label-free': the readout is independent of any label, reagent or additive that contaminates the system under study and potentially affects its properties. Thus, label-free techniques provide an unbiased analytical perspective in the sense that the sample is not manipulated by additives but pure. They are commonly based on physical principles and read changes in integral physical properties of the sample like refractive index, conductivity, capacitance or elastic modulus to mention just a few. Even though it is not implied in the name, label-free approaches usually monitor the cells under study non-invasively meaning that the amplitude of the signal (e.g. electric field strength, mechanical elongation) that is used for the measurement is too low to interfere or affect. In contrast to label-based analytical techniques that are commonly restricted to a single reading at a predefined time point, label-free approaches allow for a continuous observation so that the dynamics of the biological system or reaction become accessible.Publication link2018 The opioid oxycodone usedependently inhibits the cardiac sodium channel NaV1.5CardioExcyte 96 Publication in British Journal of Pharmacology (2018) Authors: Meents J.E., Juhasz K., Stlzle-Feix S., Peuckmann-Post V., Rolke R. Lampert A.BACKGROUND AND PURPOSE: Oxycodone is a potent semi-synthetic opioid that is commonly used for the treatment of severe acute and chronic pain. However, treatment with oxycodone can lead to cardiac electrical changes, such as long-QT syndrome, potentially inducing sudden cardiac arrest. Here, we investigate whether the cardiac side effects of oxycodone can be explained by modulation of the cardiac sodium channel NaV1.5. EXPERIMENTAL APPROACH: Heterologously expressed NaV1.5, NaV1.7 or NaV1.8 were used for whole-cell patch-clamp electrophysiology. A variety of voltage-clamp protocols was used to test the effect of oxycodone on different channel gating modalities. Human stem cell-derived cardiomyocytes were used to measure the effect of oxycodone on cardiomyocyte beating. KEY RESULTS: Oxycodone concentration-dependently and use-dependently inhibits NaV1.5 with an IC50of 483.2 M. In addition, oxycodone slows recovery of NaV1.5 from fast inactivation and increases slow inactivation. At high concentrations, these effects lead to a reduced beat rate in cardiomyocytes and to arrhythmia. In contrast, no effects could be observed on NaV1.7 or NaV1.8. CONCLUSION AND IMPLICATIONS: Oxycodone leads to an accumulation of NaV1.5 in inactivated states with a slow time course. While the concentrations needed to elicit cardiac arrhythmia in vitro are comparably high, some patients under long-term treatment with oxycodone as well as drug abusers and addicts might suffer from severe cardiac side effects induced by the slow effects of oxycodone on NaV1.5.Publication link2019 An integrated characterization of contractile, electrophysiological, and structural cardiotoxicity of Sophora tonkinensis Gapnep. in human pluripotent stem cell-derived cardiomyocytesCardioExcyte 96 Publication in Stem Cell Research & Therapy (2019)Authors: Wang R., Wang M., Wang S., Yang K., Zhou P., Xie X., Cheng Q., Ye J., Sun G., Sun X.Background Cardiotoxicity remains an important concern in drug discovery and clinical medication. Meanwhile, Sophora tonkinensis Gapnep. (S. tonkinensis) held great value in the clinical application of traditional Chinese medicine, but cardiotoxic effects were reported, with matrine, oxymatrine, cytisine, and sophocarpine being the primary toxic components. Methods In this study, impedance and extracellular field potential (EFP) of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were recorded using the cardio non-labeled cell function analysis and culture system (CardioExcyte 96). The effects of matrine, oxymatrine, cytisine, and sophocarpine (2, 10, 50M) on cell viability; level of lactate dehydrogenase (LDH), creatine kinase MB isoenzyme (CK-MB), and cardiac troponin I (CTn-I); antioxidant activities; production of reactive oxygen species (ROS) and malondialdehyde (MDA); and disruption of intracellular calcium homeostasis were also added into the integrated assessment. Results The results showed that matrine and sophocarpine dose-dependently affected both impedance and EFP, while oxymatrine and cytisine altered impedance significantly. Our study also indicated that cardiotoxicity of matrine, oxymatrine, cytisine, and sophocarpine was related to the disruption of calcium homeostasis and oxidative stress. Four alkaloids of S. tonkinensis showed significant cardiotoxicity with dose dependence and structural cardiotoxicity synchronized with functional changes of cardiomyocytes. Conclusions This finding may provide guidance for clinical meditation management. Furthermore, this study introduced an efficient and reliable approach, which offers alternative options for evaluating the cardiotoxicity of the listed drugs and novel drug candidates.Publication link2018 Safety Pharmacology Methods and Models in an Evolving Regulatory EnvironmentCardioExcyte 96 CiPA Editorial in Journal of Pharmacological and Toxicological Methods (2018) Authors: Pugsley M.K., Harter M.L., de Korte T., Connaughton C., Authier S., Curtis M.J.This editorial prefaces the annual themed issue on safety pharmacology (SP) methods published in the Journal of Pharmacological and Toxicological Methods (JPTM). We highlight here the content derived from the recent 2016 Safety Pharmacology Society (SPS), Canadian Society of Pharmacology and Therapeutics (CSPT), and Japanese Safety Pharmacology Society (JSPS) joint meeting held in Vancouver, B.C., Canada. This issue of JPTM continues the tradition of providing a publication summary of articles primarily presented at the joint meeting with direct bearing on the discipline of SP. As the regulatory landscape is expected to evolve with revision announced for the existing guidance document on non-clinical proarrhythmia risk assessment (ICHS7B) there is also imminent inception of the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative. Thus, the field of SP is dynamically progressing with characterization and implementation of numerous alternative non-clinical safety models. Novel method development and refinement in all areas of the discipline are reflected in the content.Publication link2018 Simplified footprint-free Cas9/CRISPR editing of cardiac-associated genes in human pluripotent stem cellsCardioExcyte 96 Publication in Stem Cells and Development Authors: Kondrashov A., Hoang M.D., Smith J., Bhagwan J., Duncan G., Mosqueira D., Munoz M., Vo N.T.N., Denning C.Modelling disease with hPSCs is hindered because the impact on cell phenotype from genetic variability between individuals can be greater than from the pathogenic mutation. While footprint-free Cas9/CRISPR editing solves this issue, existing approaches are inefficient or lengthy. Here, a simplified PiggyBac strategy shortened hPSC editing by 2 weeks and required one round of clonal expansion and genotyping rather than two, with similar efficiencies to the longer conventional process. Success was shown across 4 cardiac-associated loci (ADRB2, GRK5, RYR2, ACTC1) by genomic cleavage and editing efficiencies of 8-93% and 8-67%, respectively, including mono- and/or bi-allelic events. Pluripotency was retained, as was differentiation into high purity cardiomyocytes (CMs; 88-99%). Using the GRK5 isogenic lines as an exemplar, chronic stimulation with the b-adrenoceptor agonist, isoprenaline, reduced beat rate in hPSC-CMs expressing GRK5-Q41 but not GRK5-L41; this was reversed by the b-blocker, propranolol. This simplified, footprint-free approach will be useful for mechanistic studies.Publication link2018 Cross-site comparison of excitation-contraction coupling using impedance and field potential recordings in hiPSC cardiomyocytesCardioExcyte 96 Publication in Journal of Pharmacological and Toxicological Methods (2018) Authors: Bot C.T., Juhasz K., Haeusermann F., Polonchuk L., Traebert M., Stlzle-Feix S.Introduction:Since 2005 the S7B and E14 guidances from ICH and FDA have been in place to assess a potential drug candidate's ability to cause long QT syndrome. To refine these guidelines, the FDA proposed the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, where the assessment of drug effects on cardiac repolarization was one subject of investigation. Within the myocyte validation study, effects of pharmaceutical compounds on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were assessed and this article will focus on the evaluation of the proarrhythmic potential of 23 blinded drugs in four hiPSC-CM cell lines.Methods:Experiments were performed on the CardioExcyte 96 at different sites. A combined readout of contractility (via impedance) and electrophysiology endpoints (field potentials) was performed.Results:Our data demonstrates that hERG blockers such as dofetilide and further high risk categorized compounds prolong the field potential duration. Arrhythmia were detected in both impedance as well as field potential recordings. Intermediate risk compounds induced arrhythmia in almost all cases at the highest dose. In the case of low risk compounds, either a decrease in FPDmax was observed, or not a significant change from pre-addition control values.Discussion:With exceptions, hiPSC-CMs are sensitive and exhibit at least 10% delayed or shortened repolarization from pre-addition values and arrhythmia after drug application and thus can provide predictive cardiac electrophysiology data. The baseline electrophysiological parameters vary between iPS cells from different sources, therefore positive and negative control recordings are recommended.Publication link2018 Hypertrophic cardiomyopathy-linked mutation in troponin T causes myofibrillar disarray and pro-arrhythmic action potential changes in human iPSC cardiomyocytesCardioExcyte 96 Publication in Journal of Molecular and Cellular CardiologyAuthors: Wang L., Kim K., Parikh S., Cadar A.G., Bersell K.R., He H., Pinto J.R., Kryshtal D.O., Knollmann B.C.Background Mutations in cardiac troponin T (TnT) are linked to increased risk of ventricular arrhythmia and sudden death despite causing little to no cardiac hypertrophy. Studies in mice suggest that the hypertrophic cardiomyopathy (HCM)-associated TnT-I79N mutation increases myofilament Ca sensitivity and is arrhythmogenic, but whether findings from mice translate to human cardiomyocyte electrophysiology is not known.ObjectivesTo study the effects of the TnT-I79N mutation in human cardiomyocytes. Methods Using CRISPR/Cas9, the TnT-I79N mutation was introduced into human induced pluripotent stem cells (hiPSCs). We then used the matrigel mattress method to generate single rod-shaped cardiomyocytes (CMs) and studied contractility, Ca handling and electrophysiology. Results Compared to isogenic control hiPSC-CMs, TnT-I79N hiPSC-CMs exhibited sarcomere disorganization, increased systolic function and impaired relaxation. The Ca-dependence of contractility was leftward shifted in mutation containing cardiomyocytes, demonstrating increased myofilament Ca sensitivity. In voltage-clamped hiPSC-CMs, TnT-I79N reduced intracellular Ca transients by enhancing cytosolic Ca buffering. These changes in Ca handling resulted in beat-to-beat instability and triangulation of the cardiac action potential, which are predictors of arrhythmia risk. The myofilament Ca sensitizer EMD57033 produced similar action potential triangulation in control hiPSC-CMs. Conclusions The TnT-I79N hiPSC-CM model not only reproduces key cellular features of TnT-linked HCM such as myofilament disarray, hypercontractility and diastolic dysfunction, but also suggests that this TnT mutation causes pro-arrhythmic changes of the human ventricular action potential.Publication link2017 Frequency-Dependent Multi-Well Cardiotoxicity Screening Enabled by Optogenetic StimulationCardioExcyte 96 Publication in International Journal of Molecular Sciences Authors: Rehnelt S., Malan D., Juhasz K., Wolters B., Doerr L., Beckler M., Kettenhofen R., Bohlen H., Bruegmann T., Sasse P.Side effects on cardiac ion channels causing lethal arrhythmias are one major reason for drug withdrawals from the market. Field potential (FP) recording from cardiomyocytes, is a well-suited tool to assess such cardiotoxic effects of drug candidates in preclinical drug development, but it is currently limited to the spontaneous beating of the cardiomyocytes and manual analysis. Herein, we present a novel optogenetic cardiotoxicity screening system suited for the parallel automated frequency-dependent analysis of drug effects on FP recorded from human-induced pluripotent stem cell-derived cardiomyocytes. For the expression of the light-sensitive cation channel Channelrhodopsin-2, we optimised protocols using virus transduction or transient mRNA transfection. Optical stimulation was performed with a new light-emitting diode lid for a 96-well FP recording system. This enabled reliable pacing at physiologically relevant heart rates and robust recording of FP. Thereby we detected rate-dependent effects of drugs on Na+, Ca2+and K+channel function indicated by FP prolongation, FP shortening and the slowing of the FP downstroke component, as well as generation of afterdepolarisations. Taken together, we present a scalable approach for preclinical frequency-dependent screening of drug effects on cardiac electrophysiology. Importantly, we show that the recording and analysis can be fully automated and the technology is readily available using commercial products.Publication link2017 Structural and electrophysiological dysfunctions due to increased endoplasmic reticulum stress in a long-term pacing model using human induced pluripotent stem cell-derived ventricular cardiomyocytesCardioExcyte 96 Publication in Stem Cell Research and Therapy (2017)Authors: Cui C., Geng L., Shi J., Zhu Y., Yang G., Wang Z., Wang J., Chen M.Background: Long-term ventricular pacing has deleterious effects and becomes more significant when cumulative percent ventricular pacing (Cum%VP) exceeds 40% of time. However, cellular disturbances and pathways by which pacing leads to myocardial disorders are not well understood. Attempts to resolve these questions have been hampered by difficulties in obtaining human cardiac tissue and the inability to build a longer-lasting (lasting longer than weeks) pacing model in vitro. Methods: Human induced pluripotent stem cell-derived ventricular cardiomyocytes (VCMs) were cultured in the presence of electrical stimulation for 2weeks. Quantitative structural and electrophysiological analyses were used to define the functional disturbances of pacing. Results: Compared to controls, paced VCMs exhibited a remarkable reduction in the contractile protein expression, an increased apoptosis ratio and electrophysiological remodelling in a Cum%VP-dependent manner. Investigation of the protein expression levels revealed that long-term pacing universally activated both ER stress and downstream calpain. Moreover, the inhibition of calpain attenuated the adverse effects on the structural remodelling and increased the ICa, Lin paced VCMs. Conclusions: The results demonstrated that pacing VCMs for 2weeks in vitro led to a series of structural and electrophysiological dysfunctions. The increased ER stress and downstream calpain could be a central mechanism underlying the disease pathogenesis. This finding could represent a new therapeutic target in the management of long-term pacing patients.Publication link2017 An impedance-based approach using human iPSC-derived cardiomyocytes significantly improves in vitro prediction of in vivo cardiotox liabilitiesCardioExcyte 96 Publication in Toxicology and Applied Pharmacology (2017)Authors: Authors:Current in vitro approaches to cardiac safety testing typically focus on mechanistic ion channel testing to predict in vivo proarrhythmic potential. Outside of the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, structural and functional cardiotoxicity related to chronic dosing effects are of great concern as these effects can impact compound attrition. Development and implementation of an in vitro cardiotoxicity screening platform that effectively identifies these liabilities early in the discovery process should reduce costly attrition and decrease preclinical development time. Impedence platforms have the potential to accurately identify structural and functional cardiotoxicity and have sufficient throughput to be included in a multi-parametric optimization approach. Human induced pluripotent stem cell cardiomyocytes (hIPSC-CMs) have demonstrated utility in cardiac safety and toxicity screening. The work described here leverages these advantages to assess the predictive value of data generated by two impedance platforms. The response of hIPSC-CMs to compounds with known or predicted cardiac functional or structural toxicity was determined. The compounds elicited cardiac activities and/or effects on macro impedance often associated with overt structural or cellular toxicity, detachment, or hypertrophy. These assays correctly predicted in vivo cardiotox findings for 81% of the compounds tested and did not identify false positives. In addition, internal or literature Cmaxvalues from in vivo studies correlated within 4 fold of the in vitro observations. The work presented here demonstrates the predictive power of impedance platforms with hIPSC-CMs and provides a means toward accelerating lead candidate selection by assessing preclinical cardiac safety earlier in the drug discovery process.Publication link2017 Combined Impedance and Extracellular Field Potential Recordings from Human Stem Cell-Derived CardiomyocytesCardioExcyte 96 book chapter in Stem Cell-Derived Models in Toxicology (2017) Authors: Obergrussberger A., Thomas U., Stlzle-Feix S., Becker N., Juhasz K, Doerr L., Beckler M., George M., Fertig N.Measurement of contractility using impedance is a novel method for gaining information about a drug candidates potential to disturb cardiac cell contraction. The impedance signal is recorded from a monolayer of cardiac cells, most commonly derived from human-induced pluripotent stem cells (hiPSCs), which are becoming an attractive model for safety testing, especially in the light of the Comprehensive In Vitro Proarrhythmia Assay (CiPA) initiative introduced in 2013. The goal of this initiative is, in part, to standardize assays, targets, and cell types but also to evaluate the potential of new technologies, in this context, such as impedance. The CardioExcyte 96 is a hybrid system that combines the impedance readout (a measure of cell contractility) with extracellular field potential (EFP) recordings. This chapter focuses on cell handling of hiPSC cardiomyocytes (CMs) and the short- and long-term investigation into pharmacological effects of a wide range of pharmacological agents, including flecainide, nifedipine, isoproterenol, and E4031 using the CardioExcyte 96.Publication link2016 Identification of Drug-Drug Interactions In Vitro: A Case Study Evaluating the Effects of Sofosbuvir and Amiodarone on hiPSC-Derived CardiomyocytesCardioExcyte 96 Publication in Toxicological Sciences (2016)Authors: Millard DC, Strock CJ, Carlson CB, Aoyama N, Juhasz K, Goetze TA, Stoelzle-Feix S, Becker N, Fertig N, January CT, Anson BD, Ross JD.Drugdrug interactions pose a difficult drug safety problem, given the increasing number of individuals taking multiple medications and the relative complexity of assessing the potential for interactions. For example, sofosbuvir-based drug treatments have significantly advanced care for hepatitis C virus-infected patients, yet recent reports suggest interactions with amiodarone may cause severe symptomatic bradycardia and thus limit an otherwise extremely effective treatment. Here, we evaluated the ability of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) to recapitulate the interaction between sofosbuvir and amiodarone in vitro, and more generally assessed the feasibility of hiPSC-CMs as a model system for drugdrug interactions. Sofosbuvir alone had negligible effects on cardiomyocyte electrophysiology, whereas the sofosbuvir-amiodarone combination produced dose-dependent effects beyond that of amiodarone alone. By comparison, GS-331007, the primary circulating metabolite of sofosbuvir, had no effect alone or in combination with amiodarone. Further mechanistic studies revealed that the sofosbuvir-amiodarone combination disrupted intracellular calcium (Ca2+) handling and cellular electrophysiology at pharmacologically relevant concentrations, and mechanical activity at supra-pharmacological (30x Cmax) concentrations. These effects were independent of the common mechanisms of direct ion channel block and P-glycoprotein activity. These results support hiPSC-CMs as a comprehensive, yet scalable model system for the identification and evaluation of cardioactive pharmacodynamic drugdrug interactions.Publication link2016 Safety pharmacology studies using EFP and impedanceCardioExcyte 96 Publication in Journal of Pharmacological and Toxicological Methods (2016) Authors: Obergrussberger A., Juhasz K., Thomas U., Stlzle-Feix S., Becker N., Drr L., Beckler M., Bot C., George M., Fertig N.Introduction While extracellular field potential (EFP) recordings using multi-electrode arrays (MEAs) are a well-established technique for monitoring changes in cardiac and neuronal function, impedance is a relatively unexploited technology. The combination of EFP, impedance and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) has important implications for safety pharmacology as functional information about contraction and field potentials can be gleaned from human cardiomyocytes in a beating monolayer. The main objectives of this study were to demonstrate, using a range of different compounds, that drug effects on contraction and electrophysiology can be detected using a beating monolayer of hiPSC-CMs on the CardioExcyte 96. Methods hiPSC-CMs were grown as a monolayer on NSP-96 plates for the CardioExcyte 96 (Nanion Technologies) and recordings were made in combined EFP and impedance mode at physiological temperature. The effect of the hERG blockers, E4031 and dofetilide, hERG trafficking inhibitor, pentamidine, -adrenergic receptor agonist, isoproterenol, and calcium channel blocker, nifedipine, was tested on the EFP and impedance signals. Results Combined impedance and EFP measurements were made from hiPSC-CMs using the CardioExcyte 96 (Nanion Technologies). E4031 and dofetilide, known to cause arrhythmia and Torsades de Pointes (TdP) in humans, decreased beat rate in impedance and EFP modes. Early afterdepolarization (EAD)-like events, an in vitro marker of TdP, could also be detected using this system. Isoproterenol and nifedipine caused an increase in beat rate. A long-term study (over 30 h) of pentamidine, a hERG trafficking inhibitor, showed a concentration and time-dependent effect of pentamidine. Discussion In the light of the new Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative to improve guidelines and standardize assays and protocols, the use of EFP and impedance measurements from hiPSCs may become critical in determining the proarrhythmic risk of potential drug candidates. The combination of EFP offering information about cardiac electrophysiology, and impedance, providing information about contractility from the same area of a synchronously beating monolayer of human cardiomyocytes in a 96-well plate format has important implications for future cardiac safety testing.Publication link2021 Ultrasound mediated cellular deflection results in cellular depolarizationBuffer Solution Publicationin Advanced Science (2021) Authors: Vasan A., Orosco J., Magara U., Duque M., Weiss C., Tufail Y., Chalasani S.H., Friend J.Advanced Science (2021)Existing methods to stimulate neural activity include electrical optical and chemical techniques. They have enabled the development of novel therapies that are used in clinical settings, inaddition to helping understand aspects of neural function and disease mechanisms. Despite their beneficial impact, these approaches are fundamentally limited. Electrical stimulation is invasive, requiring direct contact with the target of interest. Inserting electrodes into the brain may lead to inflammation,bleeding, cell death, and local cytokine concentration increases in microglia that precipitate astrocyteformation around the electrodes that, in turn, reduce long-term effectiveness. In addition, it may have non-specific effects depending on the electric field generated by the electrodes and the stimulation parameters used. Transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (tMS)are new and non-invasive, yet they have poor spatial resolution on the order of 1 cm. Furthermore,approaches combining genetic tools with light or small molecules achieve cellular specificity. Optogenetics,which involves the use of light and genetically encoded membrane proteins, has enabled elucidation ofcellular circuits in animal models. However, it remains an invasive technique and applications are limited bythe depth of penetration of light in tissue. In contrast, chemogenetics, using small molecule sensitive designerreceptors, is limited by poor temporal resolution and is unfortunately impractical for many neural applications that require millisecond response timesProduct Sheet PDFBuffer Solutions Product SheetPublication link2023 Synthesis and Pharmacological Characterization of a Difluorinated Analogue of Reduced Haloperidol as a Sigma-1 Receptor LigandSyncroPatch 384 Publication in ACS Chem. Neurosci. (2023) Authors: Gao R-D, Taylor M., McInnis T., Chen Z., Gori S.S., LaPorte H.M., Siegler M.A., Neisewander J.L., Mach, R.H., Singh M., Slusher B.S., Rais R., Luedtke R.R., Tsukamoto T.Reduced haloperidol (1) was previously reported to act as a potent sigma-1 receptor (S1R) ligand with substantially lower affinity to the dopamine D2 receptor (D2R) compared to haloperidol. It was also found to facilitate brain-derived neurotrophic factor (BDNF) secretion from astrocytic glial cell lines in a sigma-1 receptor (S1R)-dependent manner. Although an increase in BDNF secretion may have beneficial effects in some neurological conditions, the therapeutic utility of reduced haloperidol (1) is limited because it can be oxidized back to haloperidol in the body, a potent dopamine D2 receptor antagonist associated with well-documented adverse effects. A difluorinated analogue of reduced haloperidol, ()-4-(4-chlorophenyl)-1-(3,3-difluoro-4-(4-fluorophenyl)-4-hydroxybutyl)piperidin-4-ol (2), was synthesized in an attempt to minimize the oxidation. Compound ()-2was found to exhibit high affinity to S1R and facilitate BDNF release from mouse brain astrocytes. It was also confirmed that compound2cannot be oxidized back to the corresponding haloperidol analogue in liver microsomes. Furthermore, compound2was distributed to the brain following intraperitoneal administration in mice and reversed the learning deficits in active avoidance tasks. These findings suggest that compound2could serve as a promising S1R ligand with therapeutic potential for the treatment of cognitive impairments.Publication link2023 The Unusual Aggregation and Fusion Activity of the Antimicrobial Peptide W-BP100 in Anionic VesiclesVesicle Prep Pro Publication in Membranes (2023) Authors: Ferreira A.R., Ferreira M., Nunes C. Reis S., Teixeira C., Gomes P., Gameiro P.Cationic antimicrobial peptides (CAMPs) offer a promising strategy to counteract bacterial resistance, mostly due to their membrane-targeting activity. W-BP100 is a potent broad-spectrum cecropin-melittin CAMP bearing a single N-terminal Trp, which was previously found to improve its antibacterial activity. W-BP100 has high affinity toward anionic membranes, inducing membrane saturation at low peptide-to-lipid (P/L) ratios and membrane permeabilization, with the unique property of promoting the aggregation of anionic vesicles only at specific P/L ratios. Herein, we aimed to investigate this unusual behavior of W-BP100 by studying its aggregation and fusion properties with negatively-charged large (LUVs) or giant (GUVs) unilamellar vesicles using biophysical tools. Circular dichroism (CD) showed that W-BP100 adopted an -helical conformation in anionic LUVs, neutralizing its surface charge at the aggregation P/L ratio. Its fusion activity, assessed by Frster resonance energy transfer (FRET) using steady-state fluorescence spectroscopy, occurred mainly at the membrane saturation/aggregation P/L ratio. Confocal microscopy studies confirmed that W-BP100 displays aggregation and detergent-like effects at a critical P/L ratio, above which it induces the formation of new lipid aggregates. Our data suggest that W-BP100 promotes the aggregation and fusion of anionic vesicles at specific P/L ratios, being able to reshape the morphology of GUVs into new lipid structures.Publication link2023 Missense mutations in PIEZO1, encoding the Piezo1 mechanosensor protein, define the Er red blood cell antigensSyncroPatch 384PE (a predecessor model of the SyncroPatch 384 instrument) Publication in Blood(2023) Authors: Crew V., Tilley L., Satchwell T., AlSubhi S., Jones B., Spring F., Walser P., Freire C., Murciano N., Rotordam M., Woestmann S., Hamed M., Alradwan R., AlKhrousey M., Skidmore I., Lewis S., Hussain S., Jackson J., Latham T., Kilby M., Lester W., Becker N., Rapedius M., Toye A., Thornton N.Despite the identification of the high incidence red cell antigen Era nearly 40 years ago, the molecular background of this antigen, together with the other two members of the Er blood group collection, has yet to be elucidated. Whole exome and Sanger sequencing of individuals with serologically defined Er alloantibodies identified several missense mutations within the PIEZO1 gene, encoding amino acid substitutions within the extracellular domain of the Piezo1 mechanosensor ion channel. Confirmation of Piezo1 as the carrier molecule for the Er blood group antigens was demonstrated using immunoprecipitation, CRISPR/Cas9-mediated gene knockout and expression studies in an erythroblast cell line. We report the molecular bases of five Er blood group antigens: the recognised Era, Erb and Er3 antigens; and two novel high incidence Er antigens, described here as Er4 and Er5, establishing a new blood group system. Anti-Er4 and anti-Er5 are implicated in severe hemolytic disease of the fetus and newborn (HDFN). Demonstration of Piezo1, present at just a few hundred copies on the surface of the red blood cell, as the site of a new blood group system highlights the potential antigenicity of even low abundance membrane proteins and contributes to our understanding of the in vivo characteristics of this important and widely studied protein in transfusion biology and beyond.Publication link2023 Design, synthesis, and evaluation of novel tetrazoles featuring isoxazole moiety as highly selective antifungal agentsSyncroPatch 384 Publication in Eur. J. Med. Chem (2023) Authors: Ni T., Chi X., Xie F., Li L., Wu H., Hao Y., Wang X., Zhang D., Jiang Y.In an effort to develop novelazoleantifungalswith potent activity and high selectivity, a series of (2R,3R)-3-((3-substitutied-phenyl-isoxazol-5-yl)methoxy)-2-(2,4-difluorophenyl)-1-(1H-tetrazol-1-yl)butan-2-ol derivatives were designed and synthesized based on our previously work. All compounds exhibited moderate to excellentin vitroantifungal activities againstCandida albicansSC5314 andCryptococcus neoformansH99, but inactive againstAspergillus fumigatus7544. Among them, the most active compound10hdisplayed outstanding antifungal activity against fluconazole-resistantC. albicans103,C. glabrata537 andC. auris922 withMICvalues of 0.008g/mL. In addition, compound10hwas superior to FLC in inhibiting the filamentation of FLC-resistantC. albicans103. Notably, compound10hshowed no inhibition of humanCYP3A4with IC50values of >100M, low cytotoxicity at 32g/mL and low hERG inhibition with IC50values of 6.22M, suggesting a low risk of drug-drug interactions and good safety profiles. Furthermore, compound10hexhibited excellent PK profiles and showed remarkablein vivoefficacy in a mouse model ofC. albicansandC. neoformansinfection. Taken together, compound10hwill be further investigated as a promising leadantifungal agent.Publication link2023 Establishment and validation of a torsade de pointes prediction model based on human iPSCderived cardiomyocytesCardioExcyte 96Publication in Exp. & Ther. Med.(2023) Authors: Pan D., Li B., Wang S.Druginduced cardiotoxicity is one of the main causes of drug failure, which leads to subsequent withdrawal from pharmaceutical development. Therefore, identifying the potential toxic candidate in the early stages of drug development is important. Human induced pluripotent stem cellderived cardiomyocytes (hiPSCCMs) are a useful tool for assessing candidate compounds for arrhythmias. However, a suitable model using hiPSCCMs to predict the risk of torsade de pointes (TdP) has not been fully established. The present study aimed to establish a predictive TdP model based on hiPSCCMs. In the current study, 28 compounds recommended by the ComprehensiveinvitroProarrhythmia Assay (CiPA) were used as training set and models were established in different risk groups, high and intermediaterisk versus lowrisk groups. Subsequently, six endpoints of electrophysiological responses were used as potential model predictors. Accuracy, sensitivity and area under the curve (AUC) were used as evaluation indices of the models and seven compounds with known TdP risk were used to verify model differentiation and calibration. The results showed that among the seven models, the AUC of logistic regression and AdaBoost model was higher and had little difference in both training and test sets, which indicated that the discriminative ability and model stability was good and excellent, respectively. Therefore, these two models were taken as submodels, similar weight was configured and a new TdP risk prediction model was constructed using a soft voting strategy. The classification accuracy, sensitivity and AUC of the new model were 0.93, 0.95 and 0.92 on the training set, respectively and all 1.00 on the test set, which indicated good discrimination ability on both training and test sets. The risk threshold was defined as 0.50 and the consistency between the predicted and observed results were 92.8 and 100% on the training and test sets, respectively. Overall, the present study established a risk prediction model for TdP based on hiPSCCMs which could be an effective predictive tool for compoundinduced arrhythmias.Publication link2023 A bioengineered probiotic for the oral delivery of a peptide Kv1.3 channel blocker to treat rheumatoid arthritisPort-a-Patch Publication in PNAS (2023) Authors: Wanga Y., Zhu D., Ortiz-Velez L.C., Perry J.L., Pennington M.W., Hyser J.M., Britton R.A., Beeton C.SignificanceNew therapeutics that combine efficacy with limited side effects and can be delivered noninvasively are needed to adequately treat patients with rheumatoid arthritis (RA) and other autoimmune diseases. Kv1.3 channel-expressing CCR7effector memory T (TEM) lymphocytes are significant players in the pathogenesis of multiple autoimmune diseases, and blocking Kv1.3 reduces disease severity in rat models of RA and patients with plaque psoriasis. However, peptide therapeutics require repeated injections, reducing patient compliance. We used a bioengineeredLactobacillus reuterias an oral delivery method of a Kv1.3 blocker for immunomodulation in rat models of atopic dermatitis and RA. This study demonstrates a novel approach for the noninvasive delivery of peptide-based therapeutics for the oral treatment of chronic inflammatory diseases.AbstractEngineered microbes for the delivery of biologics are a promising avenue for the treatment of various conditions such as chronic inflammatory disorders and metabolic disease. In this study, we developed a genetically engineered probiotic delivery system that delivers a peptide to the intestinal tract with high efficacy. We constructed an inducible system in the probioticLactobacillus reuterito secrete the Kv1.3 potassium blocker ShK-235 (LrS235). We show that LrS235 culture supernatants block Kv1.3 currents and preferentially inhibit human T effector memory (TEM) lymphocyte proliferation invitro. A single oral gavage of healthy rats with LrS235 resulted in sufficient functional ShK-235 in the circulation to reduce inflammation in a delayed-type hypersensitivity model of atopic dermatitis mediated by TEMcells. Furthermore, the daily oral gavage of LrS235 dramatically reduced clinical signs of disease and joint inflammation in rats with a model of rheumatoid arthritis without eliciting immunogenicity against ShK-235. This work demonstrates the efficacy of using the probioticL. reuterias a novel oral delivery platform for the peptide ShK-235 and provides an efficacious strategy to deliver other biologics with great translational potential.Publication link2023 Stable expression of human Nav1.5 for high-throughput cardiac safety assessmentPatchliner Publication in Molecular and Cellular Toxicology (2023) Authors: Choi J., Kim R-E., Cho Y-Y., Choi J-S.BackgroundCardiac safety of new drugs is essential for public health. Nav1.5 is the cardiac sodium channel responsible for action potentials in cardiomyocytes.ObjectiveFor high-throughput cardiotoxicity assays in the development of antiarrhythmic drugs, we have established an electrophysiologically validated stable HEK293 cell line expressing human Nav1.5 (hNav1.5). To validate the cell line, we examined the effects of lidocaine, an antiarrhythmic agent, and compared its effects using conventional and automated patch-clamp systems.ResultsWe isolated three stable cell lines originating from a single clonal cell and selected one stable cell line that produced a minimum 5 nA of hNav1.5 currents without any change in biophysical properties compared to the current from the transiently expressed hNav1.5. We further compared the pharmacological effects of lidocaine on this cell line using the conventional patch-clamp and automated patch-clamp systems. Lidocaine blocked hNav1.5 currents in a concentration- and voltage-dependent manner. The IC50values at holding potentials of 90mV, near the resting membrane potential of cardiomyocytes, and 120mV were 18.42.6M and 775.637.1M, respectively. In the automated patch-clamp system, the IC50values at holding potentials of 90mV and 120mV were 17.92.0M and 578.774.3M, respectively, indicating no difference between the systems. In both systems, lidocaine caused significant shifts toward hyperpolarization in the steady-state inactivation curves by~20mV and induced slower recovery from inactivation and stronger use-dependent inhibition.ConclusionThe new HEK293 cell line stably expressing hNav1.5 channels produced a current that could be tested using both conventional and automated patch-clamp systems with similar results. This current would be strong enough to evaluate cardiac safety, thus allowing the use of the automated patch-clamp system for drug screening and functional kinetic studies to reveal the mechanism of drug action.Unlike carrier proteins, channel proteins form hydrophilic pores across membranes. One class of channel proteins found in virtually all animals forms gap junctions between two adjacent cells; each plasma membrane contributes equally to the formation of the channel, which connects the cytoplasm of the two cells. These channels are discussed in Chapter 19 and will not be considered further here. Both gap junctions and porins, the channel-forming proteins of the outer membranes of bacteria, mitochondria, and chloroplasts (discussed in Chapter 10) have relatively large and permissive pores, which would be disastrous if they directly connected the inside of a cell to an extracellular space. Indeed, many bacterial toxins do exactly that to kill other cells (discussed in Chapter 25).In contrast, most channel proteins in the plasma membrane of animal and plant cells that connect the cytosol to the cell exterior necessarily have narrow, highly selective pores that can open and close. Because these proteins are concerned specifically with inorganic ion transport, they are referred to as ion channels. For transport efficiency, channels have an advantage over carriers in that up to 100 million ions can pass through one open channel each seconda rate 105 times greater than the fastest rate of transport mediated by any known carrier protein. However, channels cannot be coupled to an energy source to perform active transport, so the transport that they mediate is always passive (downhill). Thus, the function of ion channels is to allow specific inorganic ionsprimarily Na+, K+, Ca2+, or Cl-to diffuse rapidly down their electrochemical gradients across the lipid bilayer. As we shall see, the ability to control ion fluxes through these channels is essential for many cell functions. Nerve cells (neurons), in particular, have made a specialty of using ion channels, and we shall consider how they use a diversity of such channels for receiving, conducting, and transmitting signals.Two important properties distinguish ion channels from simple aqueous pores. First, they show ion selectivity, permitting some inorganic ions to pass, but not others. This suggests that their pores must be narrow enough in places to force permeating ions into intimate contact with the walls of the channel so that only ions of appropriate size and charge can pass. The permeating ions have to shed most or all of their associated water molec

cell than inside (see Table 11-1, p. 616), but its influx is opposed by the membrane potential. In fact, for many neurons, the equilibrium potential for Cl- is close to the resting potentialor even more negative. For this reason, opening Cl- channels tend to buffer the membrane potential; as the membrane starts to depolarize, more negatively charged Cl- ions enter the cell and counteract the effect. Thus, the opening of Cl- channels makes it more difficult to depolarize the membrane and hence to excite the cell. The opening of K+ channels has a similar effect. The importance of inhibitory neurotransmitters is demonstrated by the effects of toxins that block their action: strychnine, for example, by binding to glycine receptors and blocking the action of glycine, causes muscle spasms, convulsions, and death.However, not all chemical signaling in the nervous system operates through ligand-gated ion channels. Many of the signaling molecules that are secreted by nerve terminals, including a large variety of neuropeptides, bind to receptors that regulate ion channels only indirectly. These so-called G-protein-linked receptors and enzyme-linked receptors are discussed in detail in Chapter 15. Whereas signaling mediated by excitatory and inhibitory neurotransmitters binding to transmitter-gated ion channels is generally immediate, simple, and brief, signaling mediated by ligands binding to G-protein-linked receptors and enzyme-linked receptors tends to be far slower and more complex, and longer lasting in its consequences.The best-studied example of a transmitter-gated ion channel is the acetylcholine receptor of skeletal muscle cells. This channel is opened transiently by acetylcholine released from the nerve terminal at a neuromuscular junctionthe specialized chemical synapse between a motor neuron and a skeletal muscle cell (Figure 11-34). This synapse has been intensively investigated because it is readily accessible to electrophysiological study, unlike most of the synapses in the central nervous system.The acetylcholine receptor has a special place in the history of ion channels. It was the first ion channel to be purified, the first to have its complete amino acid sequence determined, the first to be functionally reconstituted in synthetic lipid bilayers, and the first for which the electrical signal of a single open channel was recorded. Its gene was also the first ion channel gene to be cloned and sequenced, and it is the only ligand-gated channel whose three-dimensional structure has been determined, albeit at moderate resolution. There were at least two reasons for the rapid progress in purifying and characterizing this receptor. First, an unusually rich source of the acetylcholine receptors exists in the electrical organs of electric fish and rays (these organs are modified muscles designed to deliver a large electrical shock to prey). Second, certain neurotoxins (such as -bungarotoxin) in the venom of certain snakes bind with high affinity (Ka = 109 liters/mole) and specificity to the receptor and can therefore be used to purify it by affinity chromatography. Fluorescent or radiolabeled -bungarotoxin can also be used to localize and count acetylcholine receptors. In this way, it has been shown that the receptors are densely packed in the muscle cell plasma membrane at a neuromuscular junction (about 20,000 such receptors per m2), with relatively few receptors elsewhere in the same membrane.The acetylcholine receptor of skeletal muscle is composed of five transmembrane polypeptides, two of one kind and three others, encoded by four separate genes. The four genes are strikingly similar in sequence, implying that they evolved from a single ancestral gene. The two identical polypeptides in the pentamer each have binding sites for acetylcholine. When two acetylcholine molecules bind to the pentameric complex, they induce a conformational change that opens the channel. With ligand bound, the channel still flickers between open and closed states, but now has a 90% probability of being in the open state. This state continues until the concentration of acetylcholine is lowered sufficiently by hydrolysis by a specific enzyme (acetylcholinesterase) located in the neuromuscular junction. Once freed of its bound neurotransmitter, the acetylcholine receptor reverts to its initial resting state. If the presence of acetylcholine persists for a prolonged time as a result of excessive nerve stimulation, the channel inactivates (Figure 11-35).The general shape of the acetylcholine receptor and the likely arrangement of its subunits have been determined by electron microscopy (Figure 11-36). The five subunits are arranged in a ring, forming a water-filled transmembrane channel that consists of a narrow pore through the lipid bilayer, which widens into vestibules at both ends. Clusters of negatively charged amino acids at either end of the pore help to exclude negative ions and encourage any positive ion of diameter less than 0.65 nm to pass through. The normal traffic consists chiefly of Na+ and K+, together with some Ca2+. Thus, unlike voltage-gated cation channels, such as the K+ channel discussed earlier, there is little selectivity among cations, and the relative contributions of the different cations to the current through the channel depend chiefly on their concentrations and on the electrochemical driving forces. When the muscle cell membrane is at its resting potential, the net driving force for K+ is near zero, since the voltage gradient nearly balances the K+ concentration gradient across the membrane (see Panel 11-2, p. 634). For Na+, in contrast, the voltage gradient and the concentration gradient both act in the same direction to drive the ion into the cell. (The same is true for Ca2+, but the extracellular concentration of Ca2+ is so much lower than that of Na+ that Ca2+ makes only a small contribution to the total inward current.) Therefore, the opening of the acetylcholine receptor channels leads to a large net influx of Na+ (a peak rate of about 30,000 ions per channel each millisecond). This influx causes a membrane depolarization that signals the muscle to contract, as discussed below.The ion channels that open directly in response to the neurotransmitters acetylcholine, serotonin, GABA, and glycine contain subunits that are structurally similar, suggesting that they are evolutionarily related and probably form transmembrane pores in the same way, even though their neurotransmitter-binding specificities and ion selectivities are distinct. These channels seem to have a similar overall structure, in each case formed by homologous polypeptide subunits, which probably assemble as a pentamer resembling the acetylcholine receptor. Glutamate-gated ion channels are constructed from a distinct family of subunits and are thought to form tetramers, like the K+ channels discussed earlier.For each class of transmitter-gated ion channels, alternative forms of each type of subunit exist, either encoded by distinct genes or generated by alternative RNA splicing of the same gene product. These combine in different variations to form an extremely diverse set of distinct channel subtypes, with different ligand affinities, different channel conductances, different rates of opening and closing, and different sensitivities to drugs and toxins. Vertebrate neurons, for example, have acetylcholine-gated ion channels that differ from those of muscle cells in that they are usually formed from two subunits of one type and three of another; but there are at least nine genes coding for different versions of the first type of subunit and at least three coding for different versions of the second, with further diversity due to alternative RNA splicing. Subsets of acetylcholine-sensitive neurons performing different functions in the brain are characterized by different combinations of these subunits. This, in principle and already to some extent in practice, makes it possible to design drugs targeted against narrowly defined groups of neurons or synapses, thereby influencing particular brain functions specifically.Indeed, transmitter-gated ion channels have for a long time been important targets for drugs. A surgeon, for example, can make muscles relax for the duration of an operation by blocking the acetylcholine receptors on skeletal muscle cells with curare, a drug from a plant that was originally used by South American Indians to poison arrows. Most drugs used in the treatment of insomnia, anxiety, depression, and schizophrenia exert their effects at chemical synapses, and many of these act by binding to transmitter-gated channels. Both barbiturates and tranquilizers, such as Valium and Librium, for example, bind to GABA receptors, potentiating the inhibitory action of GABA by allowing lower concentrations of this neurotransmitter to open Cl- channels. The new molecular biology of ion channels, by revealing both their diversity and the details of their structure, holds out the hope of designing a new generation of psychoactive drugs that will act still more selectively to alleviate the miseries of mental illness.In addition to ion channels, many other components of the synaptic signaling machinery are potential targets for psychoactive drugs. As discussed earlier, many neurotransmitters are cleared from the synaptic cleft after release by Na+ -driven carriers. The inhibition of such a carrier prolongs the effect of the transmitter and thereby strengthens synaptic transmission. Many antidepressant drugs, including Prozac, for example, act by inhibiting the uptake of serotonin; others inhibit the uptake of both serotonin and norepinephrine.Ion channels are the basic molecular components from which neuronal devices for signaling and computation are built. To provide a glimpse of how sophisticated the functions of these devices can be, we consider several examples that demonstrate how groups of ion channels work together in synaptic communication between electrically excitable cells.The importance of ion channels to electrically excitable cells can be illustrated by following the process whereby a nerve impulse stimulates a muscle cell to contract. This apparently simple response requires the sequential activation of at least five different sets of ion channels, all within a few milliseconds (Figure 11-37).1.The process is initiated when the nerve impulse reaches the nerve terminal and depolarizes the plasma membrane of the terminal. The depolarization transiently opens voltage-gated Ca2+ channels in this membrane. As the Ca2+ concentration outside cells is more than 1000 times greater than the free Ca2+ concentration inside, Ca2+ flows into the nerve terminal. The increase in Ca2+ concentration in the cytosol of the nerve terminal triggers the localized release of acetylcholine into the synaptic cleft.2.The released acetylcholine binds to acetylcholine receptors in the muscle cell plasma membrane, transiently opening the cation channels associated with them. The resulting influx of Na+ causes a localized membrane depolarization.3.The local depolarization of the muscle cell plasma membrane opens voltage-gated Na+ channels in this membrane, allowing more Na+ to enter, which further depolarizes the membrane. This, in turn, opens neighboring voltage-gated Na+ channels and results in a self-propagating depolarization (an action potential) that spreads to involve the entire plasma membrane (see Figure 11-28).4.The generalized depolarization of the muscle cell plasma membrane activates voltage-gated Ca2+ channels in specialized regions (the transverse [T] tubulesdiscussed in Chapter 16) of this membrane.5.This, in turn, causes Ca2+release channels in an adjacent region of the sarcoplasmic reticulum membrane to open transiently and release the Ca2+ stored in the sarcoplasmic reticulum into the cytosol. It is the sudden increase in the cytosolic Ca2+ concentration that causes the myofibrils in the muscle cell to contract. It is not certain how the activation of the voltage-gated Ca2+ channels in the T-tubule membrane leads to the opening of the Ca2+ release channels in the sarcoplasmic reticulum membrane. The two membranes are closely apposed, however, with the two types of channels joined together in a specialized structure (see Figure 16-74). It is possible, therefore, that a voltage-induced change in the conformation of the plasma membrane Ca2+ channel directly opens the Ca2+ release channel in the sarcoplasmic reticulum through a mechanical coupling.Whereas the activation of muscle contraction by a motor neuron is complex, an even more sophisticated interplay of ion channels is required for a neuron to integrate a large number of input signals at synapses and compute an appropriate output, as we now discuss.In the central nervous system, a single neuron can receive inputs from thousands of other neurons, and can in turn synapse on many thousands of other cells. Several thousand nerve terminals, for example, make synapses on an average motor neuron in the spinal cord; its cell body and dendrites are almost completely covered with them (Figure 11-38). Some of these synapses transmit signals from the brain or spinal cord; others bring sensory information from muscles or from the skin. The motor neuron must combine the information received from all these sources and react either by firing action potentials along its axon or by remaining quiet.Of the many synapses on a neuron, some tend to excite it, others to inhibit it. Neurotransmitter released at an excitatory synapse causes a small depolarization in the postsynaptic membrane called an excitatory postsynaptic potential (excitatory PSP), while neurotransmitter released at an inhibitory synapse generally causes a small hyperpolarization called an inhibitory PSP. The membrane of the dendrites and cell body of most neurons contains a relatively low density of voltage-gated Na+ channels, and an individual excitatory PSP is generally too small to trigger an action potential. Instead, each incoming signal is reflected in a local PSP of graded magnitude, which decreases with distance from the site of the synapse. If signals arrive simultaneously at several synapses in the same region of the dendritic tree, the total PSP in that neighborhood will be roughly the sum of the individual PSPs, with inhibitory PSPs making a negative contribution to the total. The PSPs from each neighborhood spread passively and converge on the cell body. Because the cell body is small compared with the dendritic tree, its membrane potential is roughly uniform and is a composite of the effects of all the signals impinging on the cell, weighted according to the distances of the synapses from the cell body. The combined PSP of the cell body thus represents a spatial summation of all the stimuli being received. If excitatory inputs predominate, the combined PSP is a depolarization; if inhibitory inputs predominate, it is usually a hyperpolarization.Whereas spatial summation combines the effects of signals received at different sites on the membrane, temporal summation combines the effects of signals received at different times. If an action potential arrives at a synapse and triggers neurotransmitter release before a previous PSP at the synapse has decayed completely, the second PSP adds to the remaining tail of the first. If many action potentials arrive in quick succession, each PSP adds to the tail of the preceding PSP, building up to a large sustained average PSP whose magnitude reflects the rate of firing of the presynaptic neuron (Figure 11-39). This is the essence of temporal summation: it translates the frequency of incoming signals into the magnitude of a net PSP.Temporal and spatial summation together provide the means by which the rates of firing of many presynaptic neurons jointly control the membrane potential in the body of a single postsynaptic cell. The final step in the neuronal computation made by the postsynaptic cell is the generation of an output, usually in the form of action potentials, to relay a signal to other cells. The output signal reflects the magnitude of the PSP in the cell body. While the PSP is a continuously graded variable, however, action potentials are always all-or-nothing and uniform in size. The only variable in signaling by action potentials is the time interval between one action potential and the next. For long-distance transmission, the magnitude of the PSP is therefore translated, or encoded, into the frequency of firing of action potentials (Figure 11-40). This encoding is achieved by a special set of gated ion channels that are present at high density at the base of the axon, adjacent to the cell body, in a region known as the axon hillock (see Figure 11-38).We have seen that the intensity of stimulation received by a neuron is determined for long-distance transmission by the frequency of action potentials that the neuron fires: the stronger the stimulation, the higher the frequency of action potentials. Action potentials are initiated at the axon hillock, a unique region of each neuron where voltage-gated Na+ channels are plentiful. But to perform its special function of encoding, the membrane of the axon hillock also contains at least four other classes of ion channelsthree selective for K+ and one selective for Ca2+. The three varieties of K+ channels have different properties; we shall refer to them as the delayed, the early, and the Ca2+-activated K+channels.To understand the need for multiple types of channels, consider first what would happen if the only voltage-gated ion channels present in the nerve cell were the Na+ channels. Below a certain threshold level of synaptic stimulation, the depolarization of the axon hillock membrane would be insufficient to trigger an action potential. With gradually increasing stimulation, the threshold would be crossed, the Na+ channels would open, and an action potential would fire. The action potential would be terminated in the usual way by inactivation of the Na+ channels. Before another action potential could fire, these channels would have to recover from their inactivation. But that would require a return of the membrane voltage to a very negative value, which would not occur as long as the strong depolarizing stimulus (from PSPs) was maintained. An additional channel type is needed, therefore, to repolarize the membrane after each action potential to prepare the cell to fire again.This task is performed by the delayed K+ channels discussed previously in relation to the propagation of the action potential (see p. 639). They are voltage-gated, but because of their slower kinetics they open only during the falling phase of the action potential, when the Na+ channels are inactive. Their opening permits an efflux of K+ that drives the membrane back toward the K+ equilibrium potential, which is so negative that the Na+ channels rapidly recover from their inactivated state. Repolarization of the membrane also causes the delayed K+ channels to close. The axon hillock is now reset so that the depolarizing stimulus from synaptic inputs can fire another action potential. In this way, sustained stimulation of the dendrites and cell body leads to repetitive firing of the axon.Repetitive firing in itself, however, is not enough. The frequency of the firing has to reflect the intensity of the stimulation, and a simple system of Na+ channels and delayed K+ channels is inadequate for this purpose. Below a certain threshold level of steady stimulation, the cell will not fire at all; above that threshold level, it will abruptly begin to fire at a relatively rapid rate. The early K+channels solve the problem. These, too, are voltage-gated and open when the membrane is depolarized, but their specific voltage sensitivity and kinetics of inactivation are such that they act to reduce the rate of firing at levels of stimulation that are only just above the threshold required for firing. Thus, they remove the discontinuity in the relationship between the firing rate and the intensity of stimulation. The result is a firing rate that is proportional to the strength of the depolarizing stimulus over a very broad range (see Figure 11-40).The process of encoding is usually further modulated by the two other types of ion channels in the axon hillock that were mentioned at the outset, voltage-gated Ca2+ channels and Ca2+ -activated K+ channels. They act together to decrease the response of the cell to an unchanging, prolonged stimulationa process called adaptation. These Ca2+ channels are similar to the Ca2+ channels that mediate the release of neurotransmitter from presynaptic axon terminals; they open when an action potential fires, transiently allowing Ca2+ into the axon hillock.The Ca2+-activated K+ channel is both structurally and functionally different from any of the channel types described earlier. It opens in response to a raised concentration of Ca2+ at the cytoplasmic face of the nerve cell membrane. Suppose that a strong depolarizing stimulus is applied for a long time, triggering a long train of action potentials. Each action potential permits a brief influx of Ca2+ through the voltage-gated Ca2+ channels, so that the intracellular Ca2+ concentration gradually builds up to a level high enough to open the Ca2+ -activated K+ channels. Because the resulting increased permeability of the membrane to K+ makes the membrane harder to depolarize, it increases the delay between one action potential and the next. In this way, a neuron that is stimulated continuously for a prolonged period becomes gradually less responsive to the constant stimulus.Such adaptation, which can also occur by other mechanisms, allows a neuronindeed, the nervous system generallyto react sensitively to change, even against a high background level of steady stimulation. It is one of the strategies that help us, for example, to feel a light touch on the shoulder and yet ignore the constant pressure of our clothing. We discuss adaptation as a general feature in cell signaling processes in more detail in Chapter 15.Other neurons do different computations, reacting to their synaptic inputs in myriad ways, reflecting the different assortments of members of the various ion channel families that reside in their membranes. There are, for example, at least five known types of voltage-gated Ca2+ channels in the vertebrate nervous system and at least four known types of voltage-gated K+ channels. The multiplicity of genes evidently allows for a host of different types of neurons, whose electrical behavior is specifically tuned to the particular tasks that they must perform.One of the crucial properties of the nervous system is its ability to learn and remember, which seems to depend largely on long-term changes in specific synapses. We end this chapter by considering a remarkable type of ion channel that is thought to have a special role in some forms of learning and memory. It is located at many synapses in the central nervous system, where it is gated by both voltage and the excitatory neurotransmitter glutamate. It is also the site of action of the psychoactive drug phencyclidine, or angel dust.Practically all animals can learn, but mammals seem to learn exceptionally well (or so we like to think). In a mammal's brain, the region called the hippocampus has a special role in learning. When it is destroyed on both sides of the brain, the ability to form new memories is largely lost, although previous long-established memories remain. Correspondingly, some synapses in the hippocampus show marked functional alterations with repeated use: whereas occasional single action potentials in the presynaptic cells leave no lasting trace, a short burst of repetitive firing causes long-term potentiation (LTP), such that subsequent single action potentials in the presynaptic cells evoke a greatly enhanced response in the postsynaptic cells. The effect lasts hours, days, or weeks, according to the number and intensity of the bursts of repetitive firing. Only the synapses that were activated exhibit LTP; synapses that have remained quiet on the same postsynaptic cell are not affected. However, while the cell is receiving a burst of repetitive stimulation via one set of synapses, if a single action potential is delivered at another synapse on its surface, that latter synapse also will undergo LTP, even though a single action potential delivered there at another time would leave no such lasting trace.The underlying rule in such synapses seems to be that LTP occurs on any occasion when a presynaptic cell fires (once or more) at a time when the postsynaptic membrane is strongly depolarized (either through recent repetitive firing of the same presynaptic cell or by other means). This rule reflects the behavior of a particular class of ion channels in the postsynaptic membrane. Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system, and glutamate-gated ion channels are the most common of all transmitter-gated channels in the brain. In the hippocampus, as elsewhere, most of the depolarizing current responsible for excitatory PSPs is carried by glutamate-gated ion channels that operate in the standard way. But the current has, in addition, a second and more intriguing component, which is mediated by a separate subclass of glutamate-gated ion channels known as NMDA receptors, so named because they are selectively activated by the artificial glutamate analog N-methyl-D-aspartate. The NMDA-receptor channels are doubly gated, opening only when two conditions are satisfied simultaneously: glutamate must be bound to the receptor, and the membrane must be strongly depolarized. The second condition is required for releasing the Mg2+ that normally blocks the resting channel. This means that NMDA receptors are normally only activated when conventional glutamate-gated ion channels are activated as well and depolarize the membrane. The NMDA receptors are critical for LTP. When they are selectively blocked with a specific inhibitor, LTP does not occur, even though ordinary synaptic transmission continues. An animal treated with this inhibitor exhibits specific deficits in its learning abilities but behaves almost normally otherwise.How do NMDA receptors mediate such a remarkable effect? The answer is that these channels, when open, are highly permeable to Ca2+, which acts as an intracellular mediator in the postsynaptic cell, triggering a cascade of changes that are responsible for LTP. Thus, LTP is prevented when Ca2+ levels are held artificially low in the postsynaptic cell by injecting the Ca2+ chelator EGTA into it and can be induced by artificially raising intracellular Ca2+ levels. Among the long-term changes that increase the sensitivity of the postsynaptic cell to glutamate is the insertion of new conventional glutamate receptors into the plasma membrane (Figure 11-41). Evidence also indicates that changes can occur in the presynaptic cell as well, so that it releases more glutamate than normal when it is activated subsequently.There is evidence that NMDA receptors have an important role in learning and related phenomena in other parts of the brain, as well as in the hippocampus. In Chapter 21 we see, moreover, that NMDA receptors have a crucial role in adjusting the anatomical pattern of synaptic connections in the light of experience during the development of the nervous system.Thus, neurotransmitters released at synapses, besides relaying transient electrical signals, can also alter concentrations of intracellular mediators that bring about lasting changes in the efficacy of synaptic transmission. However, it is still uncertain how these changes endure for weeks, months, or a lifetime in the face of the normal turnover of cell constituents.Some of the ion channel families that we have discussed are summarized in Table 11-2.Ion channels form aqueous pores across the lipid bilayer and allow inorganic ions of appropriate size and charge to cross the membrane down their electrochemical gradients at rates about 1000 times greater than those achieved by any known carrier. The channels are gated and usually open transiently in response to a specific perturbation in the membrane, such as a change in membrane potential (voltage-gated channels) or the binding of a neurotransmitter (transmitter-gated channels).K+ -selective leak channels have an important role in determining the resting membrane potential across the plasma membrane in most animal cells. Voltage-gated cation channels are responsible for the generation of self-amplifying action potentials in electrically excitable cells, such as neurons and skeletal muscle cells. Transmitter-gated ion channels convert chemical signals to electrical signals at chemical synapses. Excitatory neurotransmitters, such as acetylcholine and glutamate, open transmitter-gated cation channels and thereby depolarize the postsynaptic membrane toward the threshold level for firing an action potential. Inhibitory neurotransmitters, such as GABA and glycine, open transmitter-gated Cl-or K+ channels and thereby suppress firing by keeping the postsynaptic membrane polarized. A subclass of glutamate-gated ion channels, called NMDA-receptor channels, are highly permeable to Ca2+, which can trigger the long-term changes in synapses that are thought to be involved in some forms of learning and memory.Ion channels work together in complex ways to control the behavior of electrically excitable cells. A typical neuron, for example, receives thousands of excitatory and inhibitory inputs, which combine by spatial and temporal summation to produce a postsynaptic potential (PSP) in the cell body. The magnitude of the PSP is translated into the rate of firing of action potentials by a mixture of cation channels in the membrane of the axon hillock.

Ion channels. Ion channels types. What structures in the cell membrane function as ion channels. Ion channels in cell membrane. Channels in cell membrane. Types of channels in cell membrane.