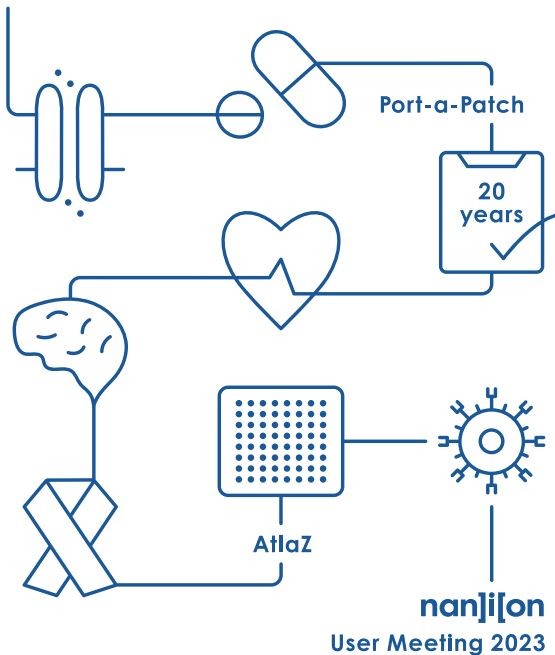


Nanon User Meeting 2023

Accelerate
your research



October, 24-25th 2023
Nanon HQ & via Zoom

Nanion User Meeting 2023

October 24-25th. Munich, Germany

October, 24th 2023

Niels Fertig

Nanion Technologies
CEO,



13:00 h - What is new at Nanion?

For over 20 years, Nanion Technologies has been providing diverse solutions for electrophysiologists worldwide. We aim to successfully implement innovative technologies in the fields of ion channel automated patch clamp (APC) electrophysiology, monitoring of cell viability and contraction, as well as electrogenic transporters, with various throughput capabilities. This year, our User Meeting in HQ will start with an introduction by Niels Fertig (CEO, Nanion) who will guide you through the highlights of the company in the past year.

Artem Kondratskyi

Nanion Technologies,
Scientific Solution Manager



13:10 h - Ion Channel Research: Quo Vadis

The "Ion Channels" field has evolved rapidly over the last few decades. Here, we will examine the global research output in this field and highlight key research topics and trends. We aim to provide a bird's-eye view of ion channel research, enabling a better understanding of the evolution of the field as a whole.

Session 1: (13:20 - 14:35 h) (B)old and New Tools for Drug Discovery

Chairperson: Marc Rogers

Michael Dudek

Technical University Munich
Group leader



13:20 h - Killing dynamics of human cytotoxic CD8 T cells

In the last decade immunotherapy, particularly T-cell therapy with chimeric antigen receptor (CAR) revolutionized the field of cancer treatments since this "living drug" uses genetically modified T cells from the same patient to find and kill cancer cells. In vitro assays are key for evaluating the potency of a treatment and also to understand the interactions between immune (effector) cells and cancer (target) cells. Here, the AtlaZ platform was used to quantitatively investigate cell behavior and viability. The studies provide mechanistic insights into the tight balance of the functionality of CD8 T cells in organs like the liver and how this balance is out-of control during diseases.

Christine Beeton

Baylor College of Medicine
Professor



13:40 h - Using the Port-a-Patch to assess the efficacy of a bacteria- based oral drug delivery for the treatment of autoimmune diseases

The pathogenesis role of T lymphocytes in rheumatoid arthritis (RA) has been ex-

tensively studied. Whereas CCR7⁺ naïve and central memory T (T_{CM}) cells are the predominant T lymphocyte populations in the circulation and lymphoid organs, most T cells in the synovium and synovial fluid of patients with RA are CCR7⁻ effector memory T (T_{EM}) cells, making them a desirable therapeutic target for RA. At rest, human and rat T lymphocytes express low levels of two K⁺ channels, K_v1.3 and K_{Ca}3.1, that regulate plasma membrane potential and the homeostasis of Ca²⁺, a crucial second messenger in T cell activation. Upon activation, CCR7⁻ T_{EM} cells upregulate K_v1.3 while naïve and T_{CM} cells upregulate K_{Ca}3.1. Thus, T_{EM} cells are exquisitely sensitive to inhibition by K_v1.3 blockers. On the contrary, naïve and T_{CM} cells rely on K_{Ca}3.1 and escape K_v1.3 blockers. ShK-186 is a potent and selective peptide blocker of K_v1.3 that has been extensively tested in rats, non-human primates, healthy volunteers, and patients with a T_{EM} cell-mediated autoimmune disease in a Phase 1A/B clinical trial. The *in vivo* safety and efficacy of ShK-186 were demonstrated, like for other biologics, after injections. The delivery of K_v1.3-blocking peptides via the buccal mucosa showed that a transmucosal route of delivery is feasible, albeit with low efficacy. We bioengineered the probiotic *Limosilactobacillus reuteri* to generate LrS235 that secretes ShK-235. The secreted peptide is functional in blocking K_v1.3 channels and suppressing the activation of T_{EM} lymphocytes *in vitro*. It crosses from the lumen of the GI tract into the circulation after the oral gavage of healthy rats and displays good bioavailability and pharmacokinetics. Treatment with LrS235 effectively reduces disease severity in a rat model of RA, including joint inflammation, car-

tilage destruction, and bone damage, without immunogenicity.

Samuel Fountain

University of East Anglia
Professor of Pharmacology



14:10 h - Developing new therapies targeting P2X receptors

P2X receptors are a family of ligand-gated non-selective cation channels activated by extracellular ATP and found in amoeba and man. Mammalian genomes encode 7 P2X receptor (P2X1-7) subunits which assemble as trimers, with binding sites for ATP located between subunits. There has been a resurgent interest in the development of therapies targeting P2X receptor following the approval of Gefapixant, a new medicine targeting P2X3 receptors. This talk will focus on some of our recent advances in understanding the molecular action of small molecules that allosterically modulate P2X receptors, and the development of drugs targeting P2X receptors. I will focus on the P2X4 receptor and discuss work to understand the molecular action of both negative and positive allosteric modulators and their potential as new therapeutic routes. P2X4 receptor activity is associated with inflammation, neuropathic pain, pulmonary secretion, vascular tone.

14:35 - 15:50 h

**Extended coffee break:
Instrument demos & SyncroPatch
384 Workshop**

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Session 2: (15:50 - 17:25 h) Advances in cardiovascular research and stem cells

Chairperson: Will Seibertz

David Beech

University of Leeds
Professor



15:50 h - PIEZO1 force sensing ion channel

PIEZO1 forms calcium-permeable non-selective cation channels that are sensitive to activation by mechanical forces. Emerging concepts for the channels and potential new research directions will be discussed. We identified importance of PIEZO1 in cardiovascular biology, showing its sensitivity to the physiological force of fluid shear stress and roles in embryonic vascular maturation, elevated blood pressure of whole body physical activity, capillary density in skeletal muscle and physical exercise performance. We found that endothelial cells disable intrinsic inactivation in the channels via SMPD3 catalytic activity and ceramide, enabling sustained functions. We developed molecular dynamics simulations of the channels in endothelial membranes to begin to understand how the channels work at the molecular level and interact with lipids. We identified downstream signalling to calpain, nitric oxide production via NOS3, NOTCH1 via ADAM10, cell apoptosis via NOS3 and its signalling to pericyte thrombospondin-2, and integration with cell adhesion molecules PECAM1 and CADH5 for force dependent remodelling of cell junctions. We found functions also in cardiac fibroblasts whe-

re the channels regulate inflammation and fibrosis via p38, interleukin-6 and tenascin c. Through medicinal chemistry studies of the Yoda1 small-molecule, we found new modulators with different properties and improved efficacy, potency and solubility. In studies with human genetics collaborators, we identified missense mutations associated with Generalised Lymphatic Dysplasia that disrupt channel function, showing partial normalisation of such channels by novel PIEZO1 agonists. Collaboration with Nanion enabled automated 384-well "mechano-patch-clamp" for quantifying PIEZO1 activities and sensitivities to small-molecule agonists and antagonists. PIEZO1 importance is thus emerging and opportunities are arising for new pharmacology with potential therapeutic value.

Alessandro Cerchiara

University of Bari
PhD candidate



16:15 h - My first time with Patchli- ner: from mexiletine selectivity to human myofiber precursors biophy- sical characterization

Ion channels play key roles in several tissues, especially in skeletal muscle. Efficient and reliable pre-clinical tests are pivotal to characterize biophysical properties of tissues and find novel drugs for diseases caused by ion channels mutations (e.g., $\text{Na}_v1.4$ for myotonia, $\text{Na}_v1.7$ for erythromelalgia). Automatic patch-clamp could be a successful and pivotal tool for reproducible and efficient screening of different drugs, such

as antimyotonic/analgesic agents. Our aim was to use the Patchliner to assess the channel isoform selectivity of mexiletine-like compounds, as well as study the electrophysiological asset of immortalized human skeletal muscle cells (SkMc) from healthy donors (WT). Briefly my first time with Patchliner allowed me to rapidly advance with the pharmacological characterization of mexiletine and its analogue on different Na_v channel types of interest for therapeutic and/or side effects and, for the first time, to record ion currents from myocytes, confirming Patchliner as an outstanding tool for academic research

Björn Knollmann
Vanderbilt University
Professor of Medicine



16:35 h - Optical pacing and machine learning for improved electrophysiological characterization of human iPSC cardiomyocyte disease models using CardioExcyte96

Human induced pluripotent stem cell (hiPSC) derived cardiomyocytes (CM) are increasingly used for heart disease modeling, drug discovery and safety testing. However, rapid assessment of electrophysiological phenotypes in a 96-well format remains challenging. Here we report our experiences using optical pacing and machine learning approaches to improve fidelity and throughput of measuring and analyzing extracellular field potential (EFP) measurements from hiPSC-CM monolayers with the CardioExcyte 96 (Nanon) system.

Short break: (17:00 - 17:10 h)

Bettina Lickiss
innoVitro
Scientific Communications
Manager



17:10 h - Overcoming cardiac risk related obstacles with cell monitoring technologies

Over the last decade, human-based preclinical cardiac risk assessment with cell-based assay systems has evolved as important alternative to animal models. However, questions related to commonly employed hiPSC-derived cell types need to be addressed comprehensively to underpin the full potential of human cell-based assay systems. Here, the FLEXcyte 96 and AtlaZ technologies are applied for addressing such obstacles in a label-free and high throughput manner.

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Niels Voigt

UMG Göttingen
Professor



17:25 h - Enlightening mechanisms of cardiac arrhythmias using iPSC-derived cardiomyocytes

Atrial fibrillation (AF) is the most common clinically reported arrhythmia. It is associated with electrophysiological and structural alterations that promote the worsening of the disease. The limited longevity of human cardiac samples ex vivo has historically restricted mechanistic investigation into these remodeling processes and therefore hampers the development of new antiarrhythmic therapies. We aim to assess if classical AF-associated remodeling events can be elicited in atrial subtype-specific induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) and atrial engineered human myocardium (EHM).

To allow for long-term optical tachypacing at 3 Hz (TP), atrial iPSC-CM were generated from cells expressing the light-gated ion-channel f-Chrimson (CRISPR-Cas9 technique). Membrane currents were measured using the Nanion SyncroPatch 384 automated patch-clamp device. Action potentials (AP) were acquired from EHM using the sharp microelectrode impaling technique. 24 hour TP-stimulated atrial iPSC-CM displayed classical hallmarks of AF-associated remodelling including decreased L-type Ca^{2+} current ($I_{\text{Ca,L}}$) and impaired activation of acetylcholine-activated inward-rectifier K^{+} current ($I_{\text{K,ACh}}$). This resulted in action potenti-

al shortening and an absent response to the M-receptor agonist carbachol in EHM subjected to TP. After 7 day TP, increased amplitude of inward-rectifier K^{+} current (I_{K1}) was observed in atrial iPSC-CM, in addition to hyperpolarization of the resting membrane potential in EHM. This unique optogenetic study represents the first in vitro model to investigate the time-dependence of cellular AF-associated remodeling over clinically relevant (7 day) time scales. Such a model has immense potential for drug development studies and personalized approaches for antiarrhythmic therapy.

Poster session and beer:
(17:50 - 18:45 h)

19:00 h Nanion User Meeting Dinner

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Session 3: (09:00 - 10:20 h)
Automated Patch Clamp at its best

Chairperson: Markus Rapedius

David Dalrymple

SB Drug Discovery
Director of Business
Development



09:00 h - A multi-platform approach for TMEM175 drug discovery

TMEM175 is a novel, constitutively active lysosomal channel involved in regulating lysosomal pH and autophagy. Mutations in this gene have been shown to impair normal lysosomal and mitochondrial function, and as a result, increase aggregation of insoluble proteins such as phosphorylated α -synuclein, a hallmark of Parkinson's Disease. Such

protein aggregation can lead to cell toxicity and death, resulting in the degenerative physical and cognitive symptoms typical of PD. Consequently, there is significant potential for TMEM175 to play a key role in the treatment of Parkinson's disease. By increasing the activity of this channel, it may be possible to enhance the efficiency of the cellular recycling process, leading to increased breakdown of toxic aggregates such as α -synuclein. However, the lack of specific pharmacological tools has hampered further investigation into the exact role of TMEM175 in normal lysosomal function and its role in such pathological processes.

Although TMEM175 is commonly reported as a potassium "leak" channel, under normal lysosomal pH (4.5-5.0) TMEM175 has been shown to be much more permeable to protons than to potassium or sodium. To facilitate the detection of TMEM175-induced proton conductance, we have successfully developed an automated electrophysiology assay to measure proton-activated, proton conductance using plasma membrane localized TMEM175. By introducing a proton gradient across the membrane, we successfully detected large inward proton currents which increased in relation to the lowering of the extracellular pH. This proton conductance was modulated by known TMEM175 activators and blocked by the reference inhibitor 4-AP. In addition, SSM-based electrophysiology has been utilized to develop assays to measure TMEM175 activity in its natural lysosomal environment.

The assays presented can be used to identify and characterize novel phar-

macological tools for TMEM175 drug discovery and should help to further investigate into the role of this exciting target in normal physiology and its involvement in neurodegenerative diseases.

Sahar Avazzadeh

Relevium Medical
Principal scientist



09:20 h - Osteoarthritis Pain: Can Biopolymer Therapeutics Be the Answer?

Knee osteoarthritis (KOA) is the 4th leading cause of disability affecting over 250 million people globally. The prevalence of KOA is rapidly rising due to ageing and obesity, with an expected Knee osteoarthritis (KOA) is the 4th leading cause of disability affecting over 250 million people globally. The prevalence of KOA is rapidly rising due to ageing and obesity, with an expected 40% increase by 2040. These patients consider pain as the most traumatic element of the disease, and most difficult to control. Additionally, KOA pain is consistently linked to a reduction in all quality of life aspects, including mental health, sleep quality, and daily activities. All current and pipeline pain treatments are failing due to poor efficacy, short duration, and/or harmful side effects including dependency in the case of opioids.

The most promising advancements in alleviating KOA pain come from emerging drug categories centred around the established TRPV1 pathway. This pathway focuses on targeting the TRPV1 receptor which is exclusive to pain-sensing nerves, thus giving long-lasting and

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selective pain relief. Although TRPV1 therapeutics are EMA/FDA for neuropathic pain, the clinical uptake of these effective and safe molecules remains limited due to challenges in pharmacokinetic and pharmacodynamic (PK/PD) properties in-vivo. Optimisation of these PK/PD properties remains the single biggest barrier to clinical adoption. Relevium Medical has responded to this urgent medical need by developing RM-010. RM-010 features the development of a novel injectable biopolymer tailored to use with TRPV1 molecules. Selective chemistry is used to stabilise and solubilise the TRPV1 molecule within the biopolymer, modifying release to selectively ablate pain-sensing nerves in a controlled manner with optimised PK/PD properties.

Ralf Hausmann

RTW Aachen

Lecturer in Pharmacology



09:40 h - Drug screening reveals inhibitors of sodium channels and their variants

Background & Aims: The voltage gated sodium channel $\text{Na}_v1.7$ plays a key role in the propagation of action potentials in nociceptive neurons. Paroxysmal extreme pain disorder (PEPD) is an autosomal dominant pain syndrome characterized by severe pain attacks and skin redness already in infancy. It is caused by gain-of-function mutations of the voltage-dependent sodium channel $\text{Na}_v1.7$ (SCN9A). We have biophysically characterized a previously unknown SCN9A mutation (M1628K) of a patient diagnosed with PEPD. This mutation

was not described yet and resides next to the previously described PEPD mutant M1627K. We screened ~350 active pharmaceutical ingredients (APIs) of approved drugs to identify potential therapeutics to treat the hyper-functional phenotype of the M1628K mutation.

Methods: Stable or transiently transfected cell lines expressing either the wild type (WT) channel $\text{Na}_v1.7$, $\text{Na}_v1.7/\text{M1628K}$ or $\text{Na}_v1.7/\text{M1627K}$ were characterized by manual patch-clamp recordings and screened with 354 drugs by automated patch clamp (APC) electrophysiology using the SyncroPatch 384i (Nanion Technologies GmbH). Drug screening analyzed the effect of the drugs on the peak currents and voltage dependence of activation and fast inactivation at a test concentration of 20 μM . For hits a concentration response curve was determined.

Results: Electrophysiological characterization of the mutation revealed typical PEPD gain-of-function changes. Current recordings on cells expressing the M1628K mutation revealed a depolarization of the voltage dependence of fast inactivation (M1628K: $V_{50} = -81.61 \pm 4.84$ mV, $n=31$; WT: $V_{50} = -97.96 \pm 11.82$ mV, $n=36$) with unchanged voltage dependence of activation (M1628K: $V_{50} = -32.70 \pm 6.07$ mV, $n=20$; WT: $V_{50} = -33.27 \pm 5.24$ mV, $n=21$). Interestingly, the M1628K mutation differs in time to peak and current decay from the previously described PEPD mutation M1627K.

Drug (API) screening revealed a previously unknown effect on $\text{Na}_v1.7$ for several approved drugs used in everyday clinical practice. In addition, the mutant M1628K and the wild type showed differences in individual drug susceptibility. These drugs were included in a subse-

quent concentration response analysis with agents like Butamben, Diltiazem and Memantine.

Conclusions: The newly described PEPD mutation Na_v1.7/M1628K shows a shift in inactivation similar to the previously described mutations, but differs concerning time to peak and current decay. In upcoming experiments, we aim to elaborate on the different biophysical effects with homology modeling and mutagenesis studies.

Using APC for a high throughput screening, already approved drugs were identified that inhibited Nav1.7-mediated responses. Although most compounds show similar inhibitory effects on Na_v1.7WT and the M1628K mutation, individual compounds showed a moderate difference in their inhibitory effects with preference either for the WT or the mutant, so that variant-specific pharmacotherapy may seem feasible in the future. Our findings may help to identify possible candidates for drug repurposing in pain therapy, even though Na_v subtype selectivity still needs to be investigated.

Riccardo Rizzetto

Axxam SpA
Principal scientist



10:00 h - Beyond the Limits: There is a Channel in every challenge

After more than 40 years of drug discovery, ion channels still remain pivotal and promising targets for the development of new bioactive molecules. However, as our knowledge in the mechanisms involved in the physiopathology of biological processes and diseases

increases, the need of innovative, state-of-the-art quality assays is becoming prevalent in the drug discovery field.

AXXAM is an Innovative Partner Research Organization whose aim is to facilitate our customers' drug discovery programs, designing the most meaningful experimental setting while using the full power of the latest and cutting-edge technologies. In this talk we'll show two case studies as examples of standard assays that have been tailored to become new, physiologically relevant, and highly informative platforms to reach a higher level of in-vitro drug development. Furthermore, we'll present the two highly innovative assays involving organellar electrophysiology and optical stimulation at SyncroPatch 384.

Coffee & nibbles: (10:20 - 10:55 h)

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Session 4: (10:55 - 12:15 h)

Membrane Transporters

Chairperson: Cecilia George

Edmund Kunji

University of Cambridge
Professor of Biophysics



10:55 h - Dissecting the charge movements during adenine nucleotide exchange by the mitochondrial ADP/ATP carrier

The mitochondrial ADP/ATP carrier, also called adenine nucleotide translocase or translocator, imports ADP into the mitochondrial matrix, where it is converted to ATP by the ATP synthase, and exports the newly synthesized ATP to the cytosol, where it fuels the energy-requiring processes that are vital for cell survival. They belong to the largest solute carrier family in humans, called the SLC25 Mitochondrial Carrier Family. During transport, the ADP/ATP carriers cycle between a cytoplasmic-open state and matrix-open state, both of which have been structurally resolved. Comparison of the two states shows that the carrier has six dynamic elements for state interconversions, which support an alternating access mechanism, consisting of three core elements and three gate elements. When the carrier changes from the cytoplasmic-open to the matrix-open state, the three gate elements move inwards, closing the cytoplasmic side of the carrier, whereas the three core elements move outwards, opening the matrix side. The converse occurs when the carrier changes from the matrix-open to the cytoplasmic-open state. The carrier functions as a mono-

mer and has a single substrate binding site and two networks on either side of central cavity that open and close the carrier during the transport cycle in an alternating way. We have demonstrated recently that the carrier has a ping-pong kinetic mechanism in agreement with the structural mechanism, meaning that ADP is imported first before ATP is exported. However, this leaves a major bioenergetic challenge. How is ADP, which carries three negative charges, imported into mitochondria against the inside negative membrane potential? In this talk, we will dissect the molecular features of the carrier that contribute to the charge translocations during adenine nucleotide exchange.

Rocco Zerlotti

Nanion Technologies
PhD candidate



11:15 h - Functional characterization of human GAT1 through solid supported membrane-based electrophysiology

The γ -aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the brain, and its homeostasis is mainly regulated by the GABA transporter hGAT1 in the central nervous system (CNS). GAT1 is a secondary-active transport protein that exploits the Na^+ gradient to energize the uphill re-uptake of GABA from the synaptic cleft into the presynaptic neuron. Since dysregulations in GABA transport are linked to many neurological disorders, hGAT1 is an important target for their medical treatment.

The solid supported membrane-based electrophysiology (SSME) is a technique

that allows the measurement of electrogenic events in transporters, pumps, and channels. The sample (i.e., native membrane vesicles or proteoliposomes) is adsorbed on an artificial bilayer on top of a gold-coated sensor and a capacitive-coupled system is generated. The transport is triggered by substrates concentration gradients as driving force, while membrane voltage is zero. Through SSME we detected GABA-induced currents on CHO membrane vesicles overexpressing hGAT1, with a maximum amplitude of 3-5 nA, that showed a triphasic behavior leading us to identify three different electrogenic events. The transport component has been identified, showing a K_m of 15-20 μ M. We could also assess the stoichiometry of GABA versus Na^+ , which was found to be in agreement with the currently most accepted one of $\text{GABA}:\text{Na}^+=1:2$.

Adam Hyman

GSK

Senior scientist



11:35 h - High-Throughput Screening strategies for electrogenic SLC transporters

Membrane transporters are tractable but under-explored targets for drug intervention. Various SLC family members are genetically linked to diseases with unmet therapeutic need. Discovery of new small molecules by high-throughput screening is key for discovering potential new medicines. This presentation will focus on high-throughput screening strategies for electrogenic SLC transporters, including assay development for single-point screening and choosing

appropriate orthogonal assays to qualify hits.

Felix Baerenz

Sanofi

Principal scientist



11:55 h - The development and application of high-content SSM-based electrophysiology assays in drug discovery using the Nanion SURFE²R technology for the measurement of electrogenic transporters

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 EC_{50} , IC_{50} , K_m , K_D , and rate constants of electrogenic reactions. We present the development and application of Nanion SURFE²R assays which can be used as secondary assays in a drug discovery screening cascade.

Lunch (12:15 - 13:30 h)

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Session 5: (13:30 - 14:40 h)

Small yet powerful: mini devices, big results

Chairperson: Conrad Weichbrodt

Marcus Schewe

University of Kiel

Postdoctoral Researcher



13:30 h - Molecular basis of TREK K2P channel gating: new insights from an integrated approach

Two-pore domain K⁺ (K2P) channels play a crucial role in regulating electrical cell excitability and thereby are important mediators of biological processes like neurotransmission, hormone secretion, K⁺ homeostasis or temperature and pain perception. Particularly, TREK K2P channel gating is complex as a wide range of stimuli and small chemical compounds, partner proteins and second messenger molecules modulate channel activity. TREK channel regulation involves the C-terminus (Ct) as polymodal stimulus sensor and the selectivity filter (SF) as the channels gate. Moreover, two crystallographic conformations of TREK-2 K2P channels (i.e., the up- and down-state) have been resolved and their main difference lies in the position and orientation of the transmembrane helix TM4. In the down-state (but not in the up-state) large side fenestrations -branching off from the central cavity- exist that are also targeted by drugs such as the norfluoxetine (NFX). NFX is thought to inhibit TREK-2 K2P activity by preventing the transition from the down- into the up-state. The down-state is thought to represent a low activity state while the up-state is

thought to have a high open probability. Direct structural evidence showing allosteric coupling between the proximal Ct (pCt) and the SF gate is currently lacking, as both up- and down-state TREK-2 structures revealed the same SF conformation but several experimental and computational studies have suggested a possible coupling between different conformational states of the M4 helix and the SF gate. Furthermore, functional data linking the two conformational states to any physiological regulation such as pH and PIP2 regulation or phosphorylation is still largely missing.

Here, we used an integrated approach, combining functional and computational electrophysiology, including systematic cysteine scanning mutagenesis, lipid tethering and blocker experiments, molecular dynamics simulations and interaction network analysis, to establish the essential role of the pCt in TREK channel gating. Using (de-)phosphorylation mimics and by chemically attaching lipid tethers to the pCt we show that movement of the pCt towards the membrane induces the up-state and uncover two gating pathways by which movement of the pCt controls the stability (i.e., conductivity) of the filter gate. Moreover, using the crystallographic up- and down-state structures of TREK-2 as starting template for full atomistic MD simulations we show that the SF in down state undergoes inactivation via conformational changes while the up-state structure exhibited a stable and conductive SF. This provides an atomistic understanding for the low channel activity previously assigned to the down-state but not evident from the crystal structure.

Collectively, the results draw a consistent structural mechanism of TREK K2P channel gating based on crystallographic as well as now also on comprehensive functional data.

Juan Francisco Bada
EPFL
Postdoctoral Researcher



13:50 h - Single-molecule detection of protein post-translational modifications with a biological nanopore

Protein post-translational modifications (PTMs) play a crucial role in several biological processes, by profoundly modulating protein properties on both spatial and temporal scales. Protein PTMs have also emerged as reliable biomarkers for several diseases. However, only few techniques are available to accurately capture their complexity at a single molecule level and, therefore, to characterize their roles in health and disease. Nanopore sensing has emerged as a novel technique, which provides high sensitivity for the detection of low-abundance proteins, holding the potential to impact single-molecule proteomics and PTMs detection in particular. Here, we use a biological nanopore, the pore-forming toxin aerolysin, to detect and distinguish different alpha-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 alpha-synuclein peptide and its PTM variants could be confidently identified using a deep learning model for signal processing. Our work highlights the

unique advantage of using nanopore as a tool for simultaneous detection of multiple PTMs and paves the way for their use in biomarker discovery and diagnostics

Alessandra Griffo
MPI for Medical Research
Postdoctoral Researcher



14:10 h - RNA Origami Nanopores spanning lipid membranes

Artificial membrane nanopores have significance either in the field of synthetic biology and of single molecule detection. Recent advancements in DNA nanotechnology have yielded functional synthetic pores as noteworthy examples. Nevertheless, their full potential is constrained due to the requirement for chemical modification, which results in high production costs and incompatibility with cellular expression. In this study, we introduce an RNA origami nanopore that spans into lipid membranes through aptamer-target interactions, eliminating the need for chemical modification. To surmount the energy barrier for membrane insertion, we opted for aptamers that bind to lipid membranes, specifically employing a biotin aptamer to bind to biotinylated DOPE lipids. The RNA nanopore is synthesized through in vitro transcription from a DNA template, yielding a high quantity of nanopores. We validate its correct assembly and successful membrane insertion using respectively atomic force microscopy (AFM) and recordings of ionic currents. The latter demonstrates distinct insertion steps with a conductance of 0.8nS (in 150 mM NaCl)

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