Biophysics Austria Conference 2024

8th – 10th July 2024

Salzburg

Abstract Book

www.biophysics-austria.com

Monday, July 8th

10:00	*Conference registration desk open*
14:00	*Hotel Check-in, Bildungshaus St. Virgil*

12:00	*Arrival / snacks*	
Advanced Microscopy	Chair: Plochberger/Hinterdorfer	
13:00 - 13:45	Jonas Ries	*U Wien*
13:45 - 14:15	Alexander Jesacher	*MU Innsbruck*
14:15 - 14:35	Peter Van Oostrum	*BOKU Wien*
14:35 - 14:55	Yusuf Erdogan	*MU Graz*
14:55 - 15:25	Gerhard Schütz	*TU Wien*
	Coffee Break	
Channels & Transporters I		Chair: Derler/Horner
16:00 - 16:30	Gerald Obermair	*KLU Krems*
16:30 - 16:50	Anna Madlmayr	*U Linz*
16:50 - 17:10	Matthias Sallinger	*U Linz*
17:10 - 17:30	Simone Pelizzari	*MU Innsbruck*
17:30 - 18:00	Marta Campiglio	*MU Innsbruck*
18:00 – 19:30	*Dinner*	
Evening Talk		Chair: B. Flucher
19:30 - 20:15	Filip Van Petegem	*UBC Vancouver*
20:15 - 20:45	Poster Flash Talks	
20:45 - open end	*Posters - Beer & Wine*	

Tuesday, July 9th

07:00 - 08:20	*Breakfast*	
Protein Structure		Chair: Keller/Zangerl-Plessl
08:30 - 09:00	Anna Stary-Weinzinger	*U Wien*
09:00 - 09:20	Karin Kornmüller	*MU Graz*
09:20 - 09:40	Thomas Stockner	*MU Wien*
09:40- 10:10	Georg Krainer	*U Graz*
	Coffee Break	
Membrane/Lipids		Chair: Pohl E./Schindl
10:40 - 10:50	Sponsor presentation	*Izon*
10:50 - 11:20	Jürgen Pfeffermann	*U Linz*
11:20 - 11:40	Janice Bergen	*U Wien*
11:40 - 12:00	Zohar Klein	*Israel IT, Haifa*
12:00 - 12:30	Olga Jovanovic	*Vet Med, Wien*
12:30 - 14:00	*Lunch*	
	Afternoon Activities	
14:30	Guided city tours:	
Options:	*1. Highlights of Salzburg*	
	2. Salzburg Cloisters	
	3. University of Salzburg	
17:00 - 18:00	Biophysics Austria - General Assembly *(all BA members)*	
18:00 – 19:30	*Dinner*	
Evening Talk		Chair: G. Pabst
19:30 - 20:15	Robert Vacha	*U Brno, CZ*
20:15 - 20:45	Poster Flash Talks	
20:45 - open end	*Posters - Beer & Wine*	

Wednesday, July 10th

07:00 - 08:20	*Breakfast*	
Channels & Transporters II		Chair: Groschner/Koschak
08:30 - 09:00	Petronel Tuluc	*U Innsbruck*
09:00 - 09:20	Valentina Hopl	*U Linz*
09:20 - 09:40	Anna Stoib	*U Linz*
09:40 - 10:00	Oleksandra Tiapko	*MU Graz*
	Coffee Break	
Single Molecule Biophysics		Chair: Oh/Pohl P.
10:40 - 11:10	Frans Mulder	*U Linz*
11:10 - 11:30	Rong Zhu	*U Linz*
11:30 - 11:50	Sebastian Fürthauer	*TU Wien*
11:50 - 12:20	Kerstin Blank	*U Linz*
12:20 - 12:30	*Farewell*	
12:30 – 14:00	*Lunch*	
	Departure	

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Sponsor:

Exhibition:

AppliedPhotophysics

Organizer´s welcome

Dear Biophysics Austria Members,

Dear Participants,

Welcome to our biennial conference, July 8-10, 2024 in Salzburg. This is the second time we are meeting in this format and we are happy that so many members of the Austrian biophysical community responded to our invitation. Compared to the Strobl meeting two years ago we experienced growth in every way. We will have more participants, more talks, more poster presentations and more exhibitors. Nevertheless, the conference will continue to be our biennial “family reunion”: A welcome occasion to see the wide spectrum of biophysical research in Austria, an opportunity to meet colleagues and friends, to strengthen existing and initiate new collaborations. Our presentations will be a good mix of talks by young and experienced scientists. The topics cover a wide range of research areas and technical approaches.

I urge all of you to pay particular attention to the two poster sessions in the evenings. There you will have the first-hand encounter with ongoing research and a chance for getting to know our next generation biophysicists. The young scientists I encourage to fully dive in and get involved in the conference. Don´t be shy to ask questions at the talks and approach the experienced colleagues during the coffee breaks and meals. If need be, drag them to your poster! If you are planning to fill a postdoctoral position in your lab or if you are looking for the right place for your own next career step, in either case, this conference is a great place to do so. As mentioned before, this is our “family reunion”. Being housed together at the attractive conference venue, joining for all meals and enjoying recreational activities together will foster scientific exchange as well as personal relationships.

Owing to the broad scope of the scientific program, you may think that certain research topics or products offered by a particular exhibitor are of no relevance for your own research. Please wait with your verdict until you have heard the talk or had the products explained to you. Perhaps you will be surprised by what you have learned and how this might contribute. But apart from a concrete usefulness to your own operation, we all know to enjoy good science when explained well. In that spirit, let us look forward to three days of intensive scientific exchange between colleagues and friends of the Austrian biophysical community.

For the Biophysics Austria board and the organizing committee,

Bernhard Flucher, Vice-President

Elena Pohl, President
Rainer Schindl, General Secretary
Thomas Stockner, Secretary
Andreas Horner, Treasurer
Birgit Plochberger, past President

Oral Presentations

Abstracts

Monday, July 8th

Superresolution Microscopy for Dynamic Structural Cell Biology

Jonas Ries[1]

[1] Max Perutz Labs, University of Vienna, Department of Structural and Computational Biology, Dr.-Bohr-Gasse 9, 1030 Vienna, Austria

Optical superresolution microscopy ideally complements electron microscopy for studying protein structure and dynamics in cells. The high contrast of the fluorescence label allows studying individual structures in various conformational states without averaging, and live cell superresolution microscopy can directly probe dynamics [1].

I will first introduce single-molecule localization microscopy (SMLM) and show how we used it to study the complex protein machinery that drives endocytosis [2].

I will then discuss MINFLUX, a new SMLM variant with unprecedented precision for imaging and tracking [3]. I will introduce the principle of MINFLUX and its opportunities and limitations for dynamic cellular imaging. I will share our recent result in which we used MINFLUX to track the stepping motion of the motor protein kinesin-1 as it walks on microtubules in living cells [4].

[1] Liu, S., P. Hoess, and J. Ries. Super-Resolution Microscopy for Structural Cell Biology. Annu. Rev. Biophys 2022.

[2] Mund, M., A. Tschanz, Y.-L. Wu, F. Frey, J.L. Mehl, M. Kaksonen, O. Avinoam, U.S. Schwarz, and J. Ries. Clathrin coats partially preassemble and subsequently bend during endocytosis. Journal of Cell Biology. 2023.

[3] Gwosch, K.C., J.K. Pape, F. Balzarotti, P. Hoess, J. Ellenberg, J. Ries, and S.W. Hell. MINFLUX nanoscopy delivers 3D multicolor nanometer resolution in cells. Nature Methods 2020.

[4] Deguchi, T., M.K. Iwanski,…, K.-M. Noh, L.C. Kapitein, and J. Ries. Direct observation of motor protein stepping in living cells using MINFLUX. Science 2023.

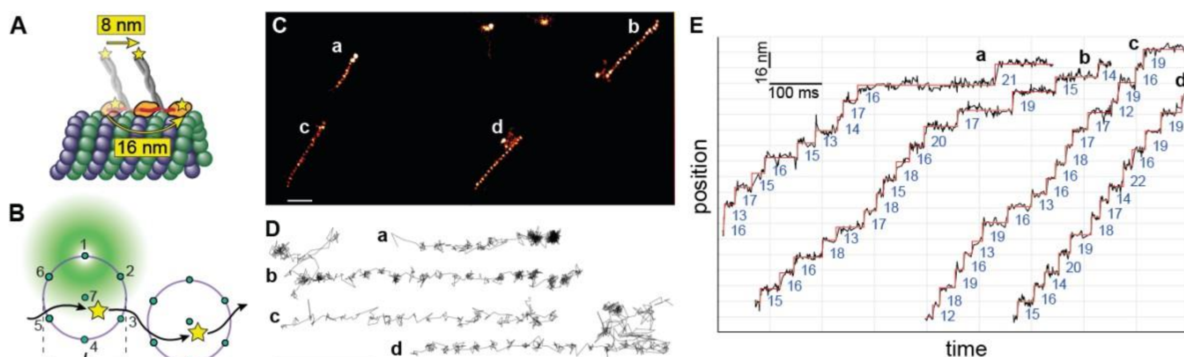

Figure 1: (A) Kinesin walks in hand-over-hand manner with the step sizes of either 8 nm for C-terminal or 16 nm for N-terminal labelling. (B) 2D MINFLUX tracking of a single molecule. A donut beam probes seven positions around a fluorophore to determine its location with nanometer precision. The scan pattern is iteratively centered on the fluorophore during tracking. (C) Tracks of truncated kinesin-1 (HaloTag-K560) labeled with JF646 HaloTag ligand where the localizations are rendered as a super-resolution image, and (D) line plots connecting each localization showing clear walking steps. (E) Time versus position plots of kinesin-1 tracks as indicated in (C), showing clear 16 nm stepwise movements.

Prospects and Challenges of using Adaptive Optics in Biomedical Imaging

Alexander Jesacher[1]

[1] Institute of Biomedical Physics, Medical University of Innsbruck, Austria

Clear, high-resolution images are an essential source of information in the life sciences. Light microscopy is widely used as the workhorse for this purpose.

While optical microscopes provide excellent image quality at the surface of a glass coverslip, their performance inevitably degrades as one attempts to image deeper into heterogeneous cellular networks and tissues. Yet many important biophysical questions require the observation and behavior of cells in their natural 3D environment.

Adaptive optics (AO) has been developed to counteract these wavefront distortions (aberrations) to restore diffraction-limited imaging performance at deep image points. Originally developed for astronomical imaging, it combines wavefront sensing with physical correction of aberrations using a spatial light modulator.

In my talk, I will give an overview of the current state of the art in the use of AO in biomedical optical microscopy, ranging from single molecule localization to deep tissue multiphoton microscopy. I will also address the challenges that lie ahead in this field.

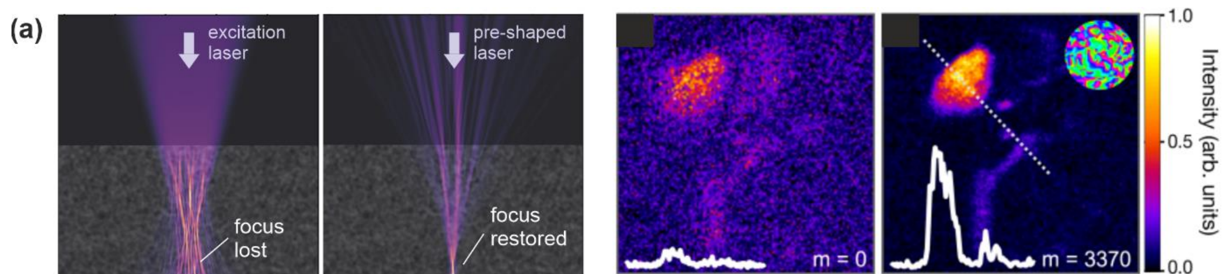

Figure 1(a) Concept of AO in multi-photon imaging: The excitation laser is "pre-shaped" such that a tight focus is formed in the depth of the tissue. (b) GFP expressing microglial cell in 250µm mouse hippocampus tissue, before (left) and after applying AO.

May, M.A., Barré, N., Kummer, K.K. *et al.* Fast holographic scattering compensation for deep tissue biological imaging. *Nat Commun* **12**, 4340 (2021). https://doi.org/10.1038/s41467-021-24666-9]

New contrasts for holographic microscopy might enable an industrial revolution

Peter van Oostrum[1], Erik Reimhult[1]

[1] Institute of Colloid and Biointerface Science (ICBS), Department of Bionanosciences (DBNS), BOKU University, Vienna, Austria.

In holographic microscopy, monochromatic light shines through a medium in which particles or microorganisms scatter some of it. Mie theory describes how particles in the size range of the wavelength scatter light. Even details that are significantly smaller than the wavelength matter. The scattered light can interfere with a coherent reference beam, forming a hologram that encrypts a lot of that information. This hologram can be recorded with intensity sensitive cameras, after magnification using a microscope objective, or also by geometry in a lens-less design. In in-line holography, the un-scattered part of the illumination serves as reference beam. This fixes the phase relation between the object and reference beams and guarantees perfect alignment, making in-line holographic microscopy inherently robust.

Background corrected holograms can be analysed either by fitting a scattering theory, expanded with interference, to the data, or by back-propagation to approximate the scattered field throughout the sample volume. The fitting approach relies on models with simplifications that are not general. The back-propagation route on the other hand, makes no a-priori assumptions. However, the approximation of the scattered field contains many interference artefacts, including the influence of the so-called twin-image of objects 'mirrored' in the recording plane. Its existence is inherent to interference, and the in-line geometry does not allow to separate the twins [1].

We propose a simple yet elegant criterion based on the phase of the scattered field that allows to suppress the twin image and effectively enhances the resolution by a factor of two [2]. In addition, we propose a family of interference contrasts that boost the signal to noise ratio further [3]. Combined, these improvements allow to swiftly locate and characterize thousands of individual microorganisms, including bacteria, in large volumes of liquid. This opens up both the possibility of monitoring the microbiological cleanliness of water and to monitor processes in bioreactors in terms of phenotype distributions and infections. We present a few possible applications in industry that might enable radical changes to food safety and the use of resources.

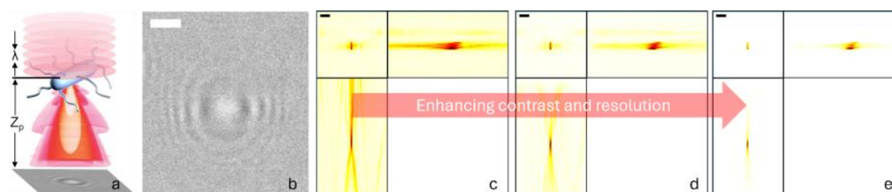

Figure 1: In-line holographic microscopy applied to E. coli. a: Schematic. b. A background corrected hologram of an E coli bacterium 25 µm above the recording plane illuminated with light from a 405 nm laser diode, magnified with a 64X water immersion objective. c. Maximum intensity projections of the intensity of the backpropagated light. d. The same, of the holographic phase contrast from [4]. e. The same of an enhanced contrast [3] combined with a phase criterion [2]. The scalebars are 4 µm.

[1] Cheong FC, Krishnatreya BJ, Grier DG. Optics Express. 2010;18(13):13563
[2] van OOSTRUM, REIMHULT; EP3575773A1.
[3] van OOSTRUM, REIMHULT; EP3922977A1.
[4] Cheong FC, Wong CC, Gao YF, et al. Biophys. J. 2015;106, 1248-1256

Development of a New Dual Reporter System to Simultaneously Monitor Ca^{2+} Signals and AMPK activity

Yusuf Ceyhun Erdoğan[1,2], Johannes Pilic[1], Benjamin Gottschalk[1], Esra N. Yiğit[3], Asal G. Zaki[3], Gürkan Öztürk[3], Emrah Eroğlu[3], Begüm Okutan[4], Nicole G. Sommer[4], Annelie M. Weinberg[4], Rainer Schindl[5], Wolfgang F. Graier[1,2], and Roland Malli[1,2]

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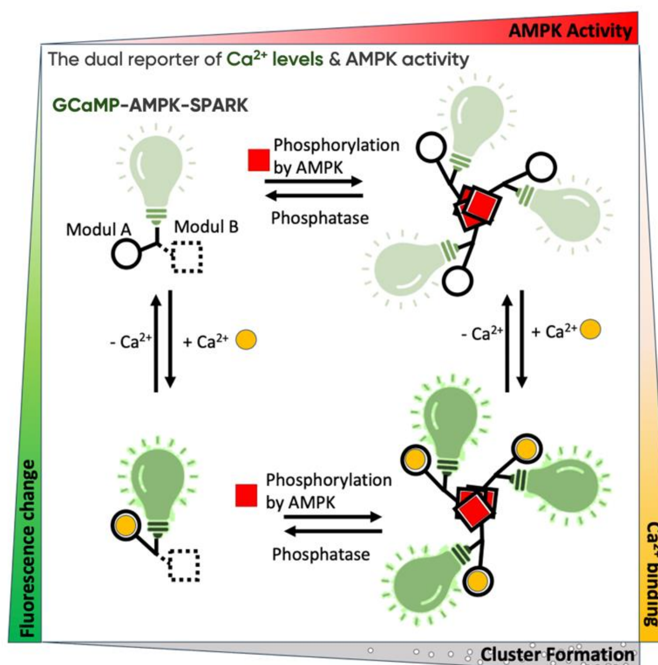
AMP-activated kinase (AMPK) works as a principal regulator of cellular metabolism, phosphorylating a wide range of targets in response to cellular stresses such as energy deficiency or elevation of cytosolic Ca^{2+} levels. Illuminating signaling crosstalks around AMPK activity is challenging, as co-imaging with existing fluorescent biosensors requires elaborate filter layouts to avert spectral restrictions. Here, we introduce novel reporters that provide the fundamental quality of information in a simpler experimental setup, circumventing the limitations posed by available strategies. First, we developed a novel separation of phases-based activity reporter of kinase (SPARK)-based AMPK reporter, named AMPK-SPARK, that forms bright fluorescent clusters upon the kinase activation. Using AMPK-SPARK, we recorded endogenous AMPK activity in various cell types and imaged the dynamics of AMPK activity in response to cellular stresses. Next, we integrated a genetic Ca^{2+} biosensor, GCaMP6f, into our initial design, allowing simultaneous measurement of Ca^{2+} levels and AMPK activity by single-channel fluorescence microscopy. Using the new dual reporter, GCaMP-AMPK-SPARK, we identified Ca^{2+} signals that trigger immediate, delayed, or no AMPK activation in single HeLa cells. We anticipate that our next-generation dual reporter strategy to study Ca^{2+}-AMPK interplay can be extended and applied to investigate other intricate signaling networks around kinase activities.



How T-cells probe antigen - a single molecule perspective

Gerhard J. Schütz[1]

[1] TU Wien, Austria

T-cells readily detect the presence of even a single antigenic peptide/MHC complex (pMHC) among thousands of endogenous pMHCs via T-cell receptors (TCRs) on the surface of antigen-presenting cells. The mechanisms underlying this phenomenal sensitivity have remained elusive. Recent studies suggest that the topography of the immunological synapse formed between the T cell and the antigen-presenting cell is of pivotal importance for these processes. We hence were first interested, how the TCR is distributed within the immunological synapse. For this, we used single molecule localization microscopy in combination with supercritical angle detection to localize single TCR molecules at an isotropic precision below 15nm [1, 2]. Second, researchers speculated that mechanical forces could be instrumental for the high specificity and sensitivity of the T cell response. Force magnitude, spread, and temporal behavior, however, are still poorly defined. We developed a calibrated FRET-based sensor equipped either with a TCR-reactive single chain antibody fragment or peptide-loaded MHC. The sensor was tethered to planar glass-supported lipid bilayers and informed most directly on the magnitude and kinetics of TCR-imposed forces at the single molecule level. From the single molecule FRET signals we quantified the magnitude of tensile forces exerted by T cells via single TCR molecules. In addition, the data allowed us to draw conclusions on the directionality of the observed forces, as well as on the pulling speed.

References

[1] Rossboth, B., A.M. Arnold, H. Ta, R. Platzer, F. Kellner, J.B. Huppa, M. Brameshuber, F. Baumgart, and G.J. Schütz, TCRs are randomly distributed on the plasma membrane of resting antigen-experienced T cells. Nature Immunology **19**, 821-827 (2018).

[2] Velas, L., M. Brameshuber, J.B. Huppa, E. Kurz, M.L. Dustin, P. Zelger, A. Jesacher, and G.J. Schütz, Three-Dimensional Single Molecule Localization Microscopy Reveals the Topography of the Immunological Synapse at Isotropic Precision below 15 nm. Nano Letters **21**, 9247-9255 (2021)

[3] Göhring, J., F. Kellner, L. Schrangl, R. Platzer, E. Klotzsch, H. Stockinger, J.B. Huppa, and G.J. Schütz. Temporal analysis of T-cell receptor-imposed forces via quantitative single molecule FRET measurements. Nature Communications. **12**, 2502 (2021).

Disease-associated mutations of $\alpha_2\delta$ proteins affect calcium channels and channel-independent synaptic functions

Gerald J. Obermair[1]

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$\alpha_2\delta$ proteins serve as auxiliary subunits of voltage-gated calcium channels and regulate channel membrane expression and biophysical current properties. Besides their channel-function, $\alpha_2\delta$ proteins regulate synapse formation, differentiation, and synaptic wiring. Considering these important functions, it is not surprising that CACNA2D1-4, the genes encoding for $\alpha_2\delta$-1 to -4 isoforms, have been implicated in neurological, neurodevelopmental, and neuropsychiatric disorders. However, how mutations in $\alpha_2\delta$ proteins can lead to brain disorders is insufficiently understood. By analyzing mutations linked to autism spectrum disorder ($\alpha_2\delta$-1 and $\alpha_2\delta$-3) and epileptic encephalopathy and cerebellar atrophy ($\alpha_2\delta$-2) we show that, besides altering calcium channel functions, dysfunctional $\alpha_2\delta$ proteins can affect synapse differentiation, trans-synaptic signaling, as well as pre- and postsynaptic functions. Moreover, a recently identified mutation in $\alpha_2\delta$-2 dissociates trans-synaptic signaling from calcium channels, proving that this function does not involve the calcium channel complex. Taken together, our data strongly link the human mutations to the underlying brain disorders and highlight the importance of studying $\alpha_2\delta$ mutations not only in the context of channelopathies but also synaptopathies.

Deciphering the role of TRPM7 in Ca^{2+} signaling

Anna Madlmayr[1], Kilian Hoelting[2], Birgit Hoeger[1], Johanna Tauber[1], Tanja Haider[1], Marius Weng[1], Susanna Zierler[1,2]

[1]Institute of Pharmacology, Faculty of Medicine, Johannes Kepler University Linz, Austria
[2]Walther Straub Institute of Pharmacology and Toxicology, Faculty of Medicine, Ludwig-Maximilians-Universität Munich, Munich, Germany

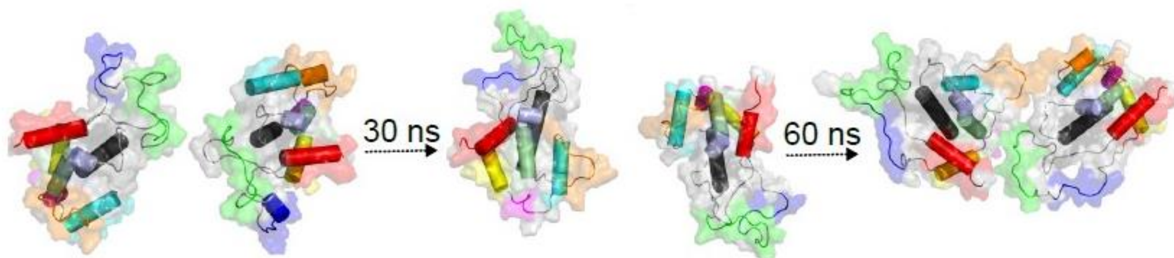
Ions serve as the smallest signaling molecules within a biological system. Importantly, systemic and cellular ion homeostasis is maintained via strictly controlled ion channels. The most prominent and potent signaling ion is Ca^{2+}, essential for many cellular processes including immune responses. In T cells, Ca^{2+} signaling occurs via store-operated Ca^{2+} channels and Ca^{2+} influx triggers T-cell activation, differentiation, proliferation and cytokine expression. Besides these store-operated Ca^{2+} channels, TRPM7, a member of the TRP channel family, a highly conserved, ubiquitously expressed cation channel, plays a crucial role in T-cell signaling. TRPM7 is the primary regulator of cellular Mg^{2+} homeostasis and thus contributes to cellular survival and proliferation. TRPM7 channel domain is covalently connected to an intracellular kinase domain, making TRPM7 an intriguing signaling molecule acting via distinct signaling hubs. Our group has identified TRPM7 as an important modulator of immune homeostasis, driving proinflammatory functions also affecting Th17 differentiation and shaping gut immunity. In the past, TRPM7 has been heavily discussed to be involved in modulating Ca^{2+} signaling in T cells. We aim to address this question in more detail utilizing a CRISPR-Cas9 engineered Jurkat TRPM7 knock-out (KO) T cell line. Functional characterization of TRPM7 KO cells revealed reduced viability and proliferation, impaired cellular ion homeostasis and suggested an involvement of TRPM7 in T-cell receptor mediated Ca^{2+} influx. In KO cells, Ca^{2+} signaling as well as downstream Ca^{2+} dependent signals were hampered. Here, we aim to elucidate the molecular interplay of TRPM7 with this Ca^{2+} signaling pathway by applying a combination of state-of-the-art methods such as imaging and electrophysiological techniques. Ultimately, we aim for a better understanding of the role of TRPM7 in Ca^{2+} signaling, immune cell homeostasis and ultimately in pro-inflammatory diseases such as autoimmunity.

Essential role of N-terminal SAM regions in STIM1 multimerization and function

Matthias Sallinger[1], Christina Humer[1], Hwei Ling Ong[2], Sasirekha Narayanasamy[2], Qi Tong Lin[3], Marc Fahrner[1], Herwig Grabmayr[1], Sascha Berlansky[1], Sean Choi[2], Tony Schmidt[4], Lena Maltan[1], Lara Atzgerstorfer[1], Martin Niederwieser[4], Irene Frischauf[1], Christoph Romanin[1], Peter B. Stathopulos[3], Indu Ambudkar[2], Romana Leitner[1], Daniel Bonhenry[5] and Rainer Schindl[4]

1) Institute of Biophysics, Johannes Kepler University Linz, Austria
2) Secretory Physiology Section, National Institute of Dental and Craniofacial Research, NIH, Bethesda, USA
3) Department of Physiology and Pharmacology, Western University, London, Canada
4) Department of Medical Physics and Biophysics, Medical University of Graz, Austria
5) Department of Physics and Materials Science, Faculty of Science, Technology and Medicine, University of Luxembourg

STIM1, a single-pass transmembrane protein in the ER membrane, serves two key roles: sensing ER-Ca^{2+} levels and activating Orai1 Ca^{2+} channels located in the plasma membrane when Ca^{2+} is low. Upon ER Ca^{2+} depletion, STIM1 undergoes multimerization, essential for activating Orai1. Using molecular dynamics simulations, we identified two crucial segments, α7 and an adjacent region near the α9-helix in the STIM1 SAM domain, responsible for multimerization. Point mutations in these segments disrupted multimerization, reducing Ca^{2+} entry via Orai1 and changed oscillation frequency. Co-expressing of Orai1 as well as constitutively active STIM1 mutants partly restored function, suggesting SAM domain interactions are vital for proper STIM1 activation. Our findings underscore the hydrophobicity-driven interactions in STIM1 multimerization, pivotal for transitioning between monomeric inactive and multimeric activated states of the protein, revealing the importance of SAM domain interactions in STIM1 function.

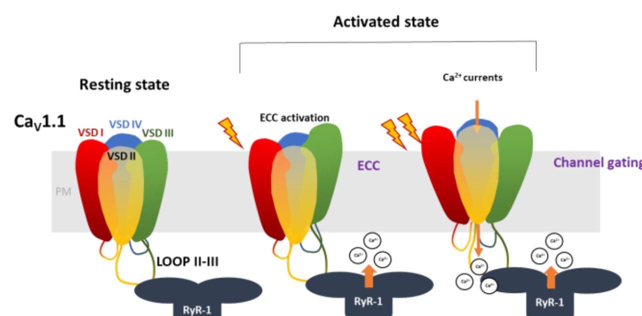


A single voltage sensor of $Ca_V1.1$ activates skeletal muscle excitation-contraction coupling

Simone Pelizzari[1], Martin C. Heiss[1], Monica L. Fernández-Quintero[2], Yousra El Ghaleb[1], Petronel Tuluc[3], Marta Campiglio[1], and Bernhard E. Flucher[1,*]

[1] Institute of Physiology, Department of Physiology and Medical Biophysics, Medical University Innsbruck, 6020 Innsbruck, Austria
[2] Integrative Structural and Computational Biology, SCRIPPS research institute, San Diego, USA
[3] Department of Pharmacology and Toxicology, Center for Molecular Biosciences Innsbruck, University of Innsbruck, 6020 Innsbruck, Austria

The skeletal muscle $Ca_V1.1$ is a voltage gated calcium channel, but it primarily functions as voltage sensor of excitation-contraction coupling (ECC). In response to membrane depolarization $Ca_V1.1$ triggers the opening of RyR1, which releases calcium into the myoplasm and thus initiates contraction. Upon strong stimulation, $Ca_V1.1$ also elicits calcium currents (ICa) with kinetics and voltage-dependence distinct from those of ECC. $Ca_V1.1$ contains four structurally analogous voltage-sensing domains (VSDs), each consisting of four transmembrane helices (S1-S4), which independently activate these channel gating and ECC. Previous results indicate the importance of VSDs I and IV in controlling ICa. In particular, charge neutralization in VSD I speeds up the ICa activation kinetics, while alternative splicing of exon 29 in the IVS3-S4 loop alters the voltage-dependence of ICa, in both cases without affecting ECC. Here we apply two different mutation strategies to alter the functions of VSDs II and III of $Ca_V1.1$. We examined the effects on current gating and activation of ECC using combined patch-clamp and fluorescence calcium recordings. Neutralizing the innermost gating charge of VSD I caused an ablation of ICa, while ECC remained unchanged. Analogous mutants in VSDs II (VSDII_K4A) and III (VSDIII_R4A) showed comparable effects on ICa. However, only VSDIII_R4A shifted the voltage-dependence of ECC by 45 mV to more depolarizing potentials. In a second approach, we transferred the extracellular IVS3-S4 loop of $Ca_V1.1$ from its native domain, to the analogous positions of VSDs II and III. While this had no effect on ICa, insertion of the IVS3-S4 loop in VSD III (VSDIII-L_{IV}) shifted the activation of ECC by 60 mV to less depolarized potentials. Together, these results clearly demonstrate that VSD III of $Ca_V1.1$ is the exclusive activator of ECC. Structure modeling revealed non-canonical state transitions of VSD III, which might initiate the conformational coupling of $Ca_V1.1$ to RyR1 opening. Notably, the insertion of the IVS3-S4 loop including exon 29 into different VSDs perturbs their respective functions in the regulation of kinetics (VSD I), voltage-dependence (VSD IV), and ECC (VSD III). This regulatory activity of this loop can be ascribed either to new interaction created between the loop and the recipient VSD, or to altered structural characteristics. We addressed this question by mutating potential interaction partners or by changing the length and flexibility of the loop. Surprisingly, the translocation of a six amino acid sequence from the IVS3-S4 loop into VSD III was sufficient to recapitulate the striking left-shift of ECC. Structure modeling of this mutant is expected to reveal the structural alterations underlying the facilitated activation of ECC. Supported by FWF grant P35618 to B.E.F.



Function of STAC3 in skeletal muscle: $Ca_V1.1$ modulator or linker of EC coupling?

Marta Campiglio[1], Wietske Tuinte[1], Enikö Török[1], Petronel Tuluc[2], Filip Van Petegem[3]

[1] Institute of Physiology, Medical University Innsbruck, Austria; [2] Institute of Pharmacology and Toxicology, University of Innsbruck, Austria; [3] Department of Biochemistry and Molecular Biology, University of British Columbia, Canada

Skeletal muscle excitation-contraction (EC) coupling is the fundamental mechanism by which an electrical signal, the action potential, is transduced into a mechanical response, muscle contraction. Recently the hitherto unnoticed scaffold protein STAC3 has been shown to be essential for this process, as it is crucial for both the stability and function of the voltage-sensor of EC coupling $Ca_V1.1$ and for voltage-induced calcium release from the sarcoplasmic reticulum (SR). Furthermore, STAC3 was shown to establish two distinct interactions with $Ca_V1.1$. However, whether a single interaction or both contribute to each function of STAC3 remains elusive. In this study, we used reconstitution of different STAC3 fragments and mutants in $Ca_V1.1$/Stac3-null myotubes to investigate the role of the two $Ca_V1.1$/STAC3 interactions in controlling $Ca_V1.1$ functional expression, as well as the voltage-induced calcium release from the SR. Our results indicate that the STAC3-NT interaction with the proximal C-terminus of $Ca_V1.1$ governs the functional expression of $Ca_V1.1$ and it is sufficient for nominal voltage-induced calcium release from the RyR1. On the other hand, the interaction of STAC3-CT with the II-III loop of $Ca_V1.1$, which has long ago been identified as the critical domain specific for skeletal muscle EC coupling, merely enhances the conformational coupling with the RyR1.

Moreover, the STAC3-CT interaction with the II-III loop, the affinity of which was measured in the low micro molar range, does not take place in the absence of STAC3-NT, which interacts with the proximal C-terminus of $Ca_V1.1$ and drives STAC3 to the channel complex. Here, we demonstrate that targeting the SH3 domains of STAC3 to the channel complex via fusion to the $Ca_V\beta_{1a}$ subunit rescues EC coupling both in cells expressing STAC3-NT or the STAC3 disorder causing mutant STAC3-W280S, providing a potential therapeutic strategy for reversing the pathophysiology associated to STAC3 disorder.

Finally, as STAC3 was suggested to be the linker between the voltage sensor of EC coupling $Ca_V1.1$ and the calcium release channel RyR1, but only interactions with $Ca_V1.1$ have been conclusively demonstrated, we investigated the importance of the SH3-2 domain, the only protein interaction domain of STAC3 for which no binding partner has yet been assigned. Deletion of the SH3-2 domain significantly reduces EC coupling, but further mutagenesis of key residues demonstrated that the reduction is due to the destabilization of the SH3-1 interaction with the II-III loop, rather than to the loss of a protein-protein interaction. These results suggest that STAC3 functions as an allosteric modulator of $Ca_V1.1$, rather than a linker to the calcium release channel.

Ryanodine Receptors: regulation, disease mechanisms and pharmacology

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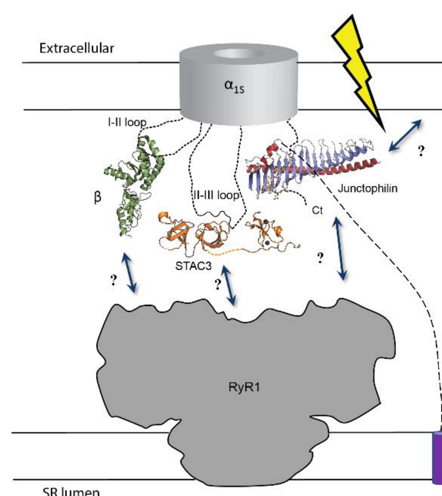
Ryanodine Receptors (RyR)s are giant ion channels found in the membranes of the endoplasmic (ER) and sarcoplasmic reticulum (SR). Mostly known for their role in muscle excitation-contraction coupling, RyRs control the release of Ca^{2+} from the SR into the cytosol. The timing of this event is absolutely key: too little, too much, too early, or too late can have devastating consequences. For example, mutations in the skeletal muscle isoform (RyR1) can cause malignant hyperthermia, a potentially lethal condition arising from volatile anaesthetics. Mutations in the cardiac RyR2 often cause stress-induced arrhythmias that may result in sudden cardiac death. Faulty regulation of wild-type RyRs has also been associated with atrial fibrillation and Alzheimer's disease.

Despite their importance, few molecules are available to correct misbehaving RyR channels in clinical settings. We set out to solve cryo-EM structures of wild-type and disease mutant RyRs in different conditions, revealing detailed disease mechanisms. Single point mutations drastically change the conformational landscape, resulting in facilitated channel opening. Investigating RyRs in the presence of channel openers and inhibitors has revealed various allosteric pathways through which the large cytosolic cap of the channel can influence the pore. RyRs have been found to be 'off-targets' for several drugs used to treat other disorders, resulting in muscle weakness and arrhythmias. Identifying these sites provides critical clues for generating derivatives with reduced side effects.

RyRs receive a plethora of regulatory inputs from auxiliary proteins and post-translational modifications. Most prominent among these is an ion channel located in the plasma membrane of skeletal muscle: the voltage-gated calcium channel CaV1.1. Upon depolarization of the plasma membrane, this channel triggers opening of RyR1 through mechanical coupling. Despite decades of research, the precise mechanism has remained a mystery, but is known to involve several auxiliary proteins. We solved structures of these proteins in complex with their binding sites in CaV1.1 and explored how these interfaces influence the mechanical coupling. Current efforts are focused on how they engage RyR1.



Oral Presentations

Abstracts

Tuesday, July 9th

Allosteric mechanisms of inward rectifier K^+ channel gating

Anna Stary-Weinzinger[1], Boris Shalomov[2], Theres Friesacher[1], Daniel Yakubovich[2], J Carlo Combista[2], Haritha P Reddy[2], Shoham Dabbah[2], Harald Bernsteiner[1], Eva-Maria Zangerl-Plessl[1], Nathan Dascal[2]

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G-protein-regulated inward rectifier K^+ channels (GIRKs) underlie the inhibitory effects of major $G_{i/o}$ coupled neurotransmitters in the heart, and brain and regulate heartbeat, and neuronal excitability. GIRKs are involved in drug and alcohol addiction, pain, depression, seizures, and cognitive impairment of Down syndrome. Phosphatidylinositol 4,5-bisphosphate (PIP_2), Gβγ and Na^+ ions are essential key regulators, required to open the pore. Despite the availability of x-ray structures revealing the atomistic interaction with all three key activators, gating related conformational changes are still poorly understood. Here we report on disease-linked mutations and an allosteric blocker that serve as tools for probing gating-related conformational changes. Molecular dynamics (MD) simulations on the G154S disease mutant provide insights into changes in the selectivity filter of the channel, which lead to loss of optimal K^+ coordination and subsequent water-mediated Na^+ flux. Further, aberrant filter dynamics are correlated with changes at the binding site of the Gβγ dimer, providing information on the constitutive activity of the mutant [1]. The antiepileptic drug ethosuximide (ETX) suppresses epileptiform activity in a mouse model of *GNB1* syndrome, through inhibition of GIRK channels [2]. Combining electrophysiology and µs-long MD simulations, we identified an allosteric binding site of the drug, close to the physiological activator PIP_2. Remarkably, Gβγ, the physiological activator of GIRKs, increases the potency of ETX block. Our research suggests that ETX is a potent allosteric GIRK blocker and can serve as a tool for probing gating-related conformational changes in GIRK.

Acknowledgments: Support from Austrian Science Fund grant nr. W1232 is acknowledged. Calculations were performed at the Vienna Scientific Cluster (VSC).

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[2] Colombo S, Reddy HP, Petri S, Williams DJ, et al. *Front Cell Neurosci*. **2023** 17,1175895.

Low Density Lipoprotein Structure and Dynamics

Tamás Földes[1], Sarah Stainer[2], Aline Cisse[1,3], Ambroise Desfosses[4], Peter Hinterdorfer[2], Ruth Prassl[5,6], Judith Peters[1,3,7], Karin Kornmueller[5,6]

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Given its prominent role in the development of atherosclerosis, we believe that a deep understanding of low density lipoprotein's (LDL) structure and dynamics is the key to develop novel treatment strategies. We aim to achieve a high-resolution structure of LDL, explicitly taking into account its massive range of conformational dynamics. In this study we combined several powerful techniques to investigate LDL in its near-native state: high-speed atomic force microscopy (HS-AFM) and cryo-electron microscopy (cryo-EM) were complemented with coarse-grained simulation studies. Based on our recent cryo-EM map of LDL [1] we applied novel algorithms to approach LDL's conformational heterogeneity from different perspectives. We describe several highly dynamic protrusions, which we attribute to specific domains of apolipoprotein B-100 (apo B-100) via mapping of an AlphaFold 2 model of the protein. With HS-AFM the protrusion's dynamics and flexibility were directly visualized: large domain fluctuations of apo B-100 were tracked over several milliseconds with nanometer spatial resolution. For the first time we get a highly dynamic direct description of LDL, with apo B-100's flexibility being a crucial property, well in accordance with its role in metabolism. In addition, cryo-EM studies revealed novel features of the cholesterol-ester (CE) core layer organization – fine substructures that are in evidence of a domain-like organization within one CE plane. This finding was confirmed by simulation studies: we achieved to construct a coarse-grained representation of the whole LDL particle. An in-depth knowledge of structural and dynamical features of LDL will open new avenues for rational drug-design, combating the development or progression of cardiovascular diseases.

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Transport by SLC6 transporters is energized by the co-transported sodium ions, but the substrate provides the energy for transporter occlusion and for opening of the inner gate

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The fold of the Solute Carrier (SLC) family 6 was initially revealed by the structure of LeuT from the thermophile Aquifex aeolicus and was confirmed to be conserved to the human SLC6 family members. Substrates of human SLC6 family members are amino acids, creatine, osmolytes, gamma-aminobutyric acid (GABA) and monoamines. The transporters play essential roles in neurotransmission through removing neurotransmitters from the synaptic cleft, thereby regulating neurotransmitter homeostasis. The SLC6 fold consists of 12 transmembrane helices that are assembled as structural inverted repeats and arranged into two domains, the scaffold domain that anchors the transporter in the membrane and the bundle domain that moves to allow for substrate translocation. The substrate binding site S1 is located in the centre of the transporter, half-way through the membrane. The alternating access model is a generalized model that describes substrate translocation by membrane transporters, which in SLC6 transporters is achieved by motions of the bundle domain and the imperfect complementarity of the bundle and the scaffold domains, establishing a structural seesaw model.

Macroscopically it was shown that the sodium gradient energizes the substrate transport, the static structures revealed the two sodium binding sites and the three main conformations, but at the single transporter level, key elements of transporter function including dynamics, driving forces, free energy profiles and the mechanism of structural and energy coupling remains ill-defined. Here we show at the all atom level how sodium contribute to transport and show that substrate binding is the essential energetic trigger for transport.

By combining MD simulations with in vitro experiments, we identified functionally important differences in sodium binding to NA1 between the monoamine transporters (SERT, DAT) and the GABA transporters and quantify the energetic coupling between substrate and the NA1-bound sodium. While sodium binding stabilizes the substrate-binding competent outward-open state, only substrate binding can provide the energy and the forces required for transporter occlusion. Here we show that the substrate induced occlusion has a second function. It provides the energy for priming the occluded transporter for opening towards the cytosol by a weakening of the inner gate, an essential step of the transport mechanism, with which several disease causing mutants interfere. This dataset allows us to shed light on the mechanism of structural and energetic coupling in SLC6 transporters.

Next-generation microfluidic approaches for protein biophysics

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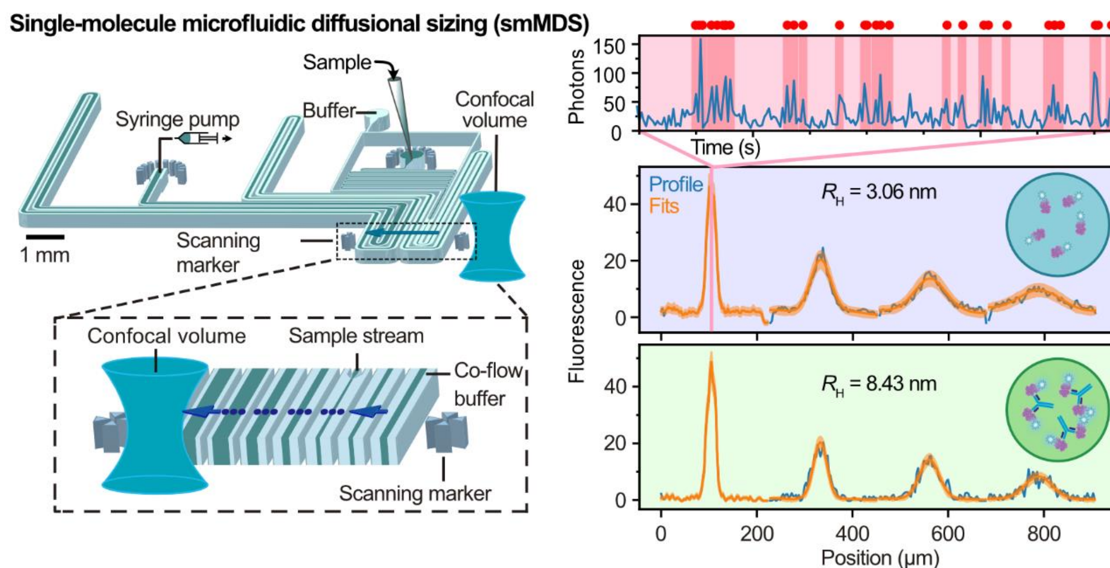
Proteins constitute the molecular machinery of life, and their biophysical characterization has become a pivotal aspect in many areas of modern biosciences. Innovative biophysical approaches are continually expanding the capabilities of protein analysis, enabling a deeper understanding of the function, malfunction, and pharmaceutical design of proteins. Particularly, technologies based on the control of fluids at the micron scale, along with orthogonal methods such as single-molecule techniques, are transforming our understanding of protein behavior. In my talk, I will share insights into our work in this area and introduce a recently developed single-molecule microfluidic approach for protein analysis, known as single-molecule microfluidic diffusional sizing (smMDS) (Krainer et al. 2024, *Nat. Commun.*, accepted). This technique allows for calibration-free and absolute measurement of protein hydrodynamic radii at the single-molecule level. I will explain the working principle of smMDS and demonstrate its capabilities for protein analysis. These include sizing of proteins and protein assemblies down to femtomolar concentrations, affinity profiling of protein interactions in solution, and resolving different assembly states of high- and low-molecular-weight protein oligomers. Additionally, I will show that smMDS is effective in sizing multiple protein species within complex aggregation mixtures and characterizing nanoscale condensate clusters. Overall, smMDS constitutes a versatile, in-solution approach for characterizing the sizes, interactions, and assembly states of proteins. It promises wide-ranging applications in the biological and biomedical sciences including the mechanistic and functional analysis of proteins, the molecular design of protein therapeutics, and the characterization of new nanomedicines and biomaterials.



Optomechanical and optocapacitive modulation of membrane proteins using photolipids

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Institute of Biophysics, Johannes Kepler University Linz, Linz, Austria

Azobenzene-containing photolipids respond to light by changing their molecular structure – light-driven nanoscale actuation. Embedded in lipid bilayers, these individual structural changes lead to alterations in the material properties of the embedding matrix. To that effect, our group has explored photolipids as regulators of in-plane lateral phase separation, i.e., membrane domains[1]. Based on the known sensitivity of mechanosensitive and mechanically-modulated membrane proteins to membrane mechanical properties, we reasoned that photolipids can be used as their regulators. Indeed, we demonstrated the sensitivity of a model molecular force probe, the peptide ion channel gramicidin A, embedded in a photolipid-containing lipid bilayer to light exposure[2]. A key structural change incurred by photoisomerization was a reduction in membrane thickness with UV light which led to improved hydrophobic matching. Likewise, this reduction was reflected in an increase in bilayer electrical capacitance. This observation eventually led us to explore photolipids as putative optocapacitive regulators. Optocapacitive approaches achieve regulation of neuronal activity by light without requiring genetic modifications. Instead, they rely on rapid changes in membrane capacitance which were previously achieved by heating, necessitating intense infrared light or exogenous membrane-associated nanomaterials for photothermal conversion. Putting our hypothesis to the test, we drastically amplified the rate of photolipid photoisomerization and observed light-triggered de- and hyperpolarizing optocapacitive currents under voltage-clamp conditions[3]. Using the reductionist planar lipid bilayer model system and a combination of high-frequency current and capacitance recordings, we demonstrated that these currents emerge from millisecond-timescale changes in bilayer capacitance. In whole-cell patch-clamp experiments, intense UV light exposure led to photolipid-induced depolarization triggering action potentials. Further, we found that endogenous mechanosensitive ion channels are sensitive to blue light exposure. We attribute the latter to a transient photolipid-induced increase in membrane tension. In conclusion, membrane self-insertion of administered photolipids in combination with focused illumination may allow for optocapacitive and optomechanical cell stimulation with high spatiotemporal control.

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[2]Pfeffermann *et al.*, Photoswitching of model ion channels in lipid bilayers. *J. Photochem. Photobiol. B*. **224,** 112320 (2021).
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Food constituents and contaminants modulate lipid metabolism and affect intestinal cells' morphology and biomechanical compliance

Janice Bergen[1,2,3], Francesco Crudo[1], Doris Marko[1], Franz Berthiller[4], Giorgia Del Favero[1,2]

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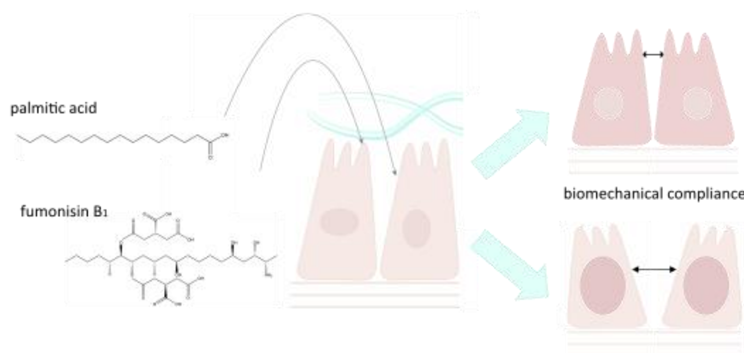
Developing models *via* cell-based assays are of utmost importance to bridge the gap between *in vivo* and *in vitro* methods and to increase the predictivity of the latter. Especially for food constituents and contaminants where exposure can vary largely on individual basis, *in vitro* models promise to ease and speed up risk assessment. Here we present a novel workflow based on the multiparametric morphological profiling of intestinal cells for the evaluation of toxicity of food contaminants. Particularly, several mycotoxins were described to change membranes biophysical properties with potential repercussions on cell biomechanical compliance [1-3]. Pathological potential of mycotoxins raises increasing concern especially in light of global crop contamination now exceeding former estimates, with affected commodities reaching up to 80% [4]. Therefore, we chose mycotoxin fumonisin B_1 (FB1) due to its persistent occurrence and described activity at intestinal level - including hampering lipid metabolism - and palmitic acid (PA), one of the most ubiquitous fatty acids in the human diet as test compounds [5-7]. Basic cell compliance in response to FB1 (10-100µM) and PA (25-100µM) was initially elucidated in HCT116 and HCEC-1CT cells (2D-models), determining the sphinganine to sphingosine (Sa/So) ratio, significant changes in membrane fluidity and force response to fluid flow by implementing a pump system. Combinatory effect of the two compounds were furthermore explored. Building on this and increasing model complexity, a Caco2/HT29-MTX-E12 co-culture was differentiated to form a functional 3D layer with villi-like structures and covered by mucus. Intercellular distances (ICD) and cell permeability were assessed, and a significant change of the 3D morphology of the cell layer was observed for FB1. Notably, these data were obtained in a non-cytotoxic concentration range, offering novel insights into the understanding of probable detrimental effects stemming from chronic toxins' exposures.



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Excited State Proton Transfer of Super Photoacid Tethered to Lipid Membranes Reveals the Presence of Hydrogen-Bonded Ion Pair

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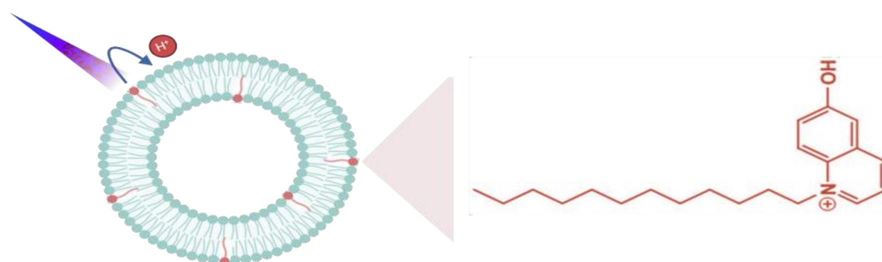
Photoacid molecules experience a significant decrease in pK_a upon electronic excitation, facilitating excited state proton transfer (ESPT) to nearby proton acceptors. Super photoacids are characterized by $pK_a^* < 0$, hence, they are 'super' proton donors to even acidic acceptors. Here, we synthesized a new super photoacid molecule – C_{12}-Hydroxyquinoline (C_{12}HQ). According to the Eigen–Weller model, a hydrogen-bonded ion pair forms during the dissociation process, where the proton transfers to the acceptor while still hydrogen-bonded to the photoacid. However, to date, the experimental evidence of this ion pair formation was elusive. In our study, we use C_{12}HQ tethered to a lipid membrane composed of either phosphatidylcholine (PC) or phosphatidic acid (PA). Using ultrafast spectroscopy, we were able to observe the transient formation on a hydrogen-bonded ion pair between C_{12}HQ and the lipid membrane. By comparing PC membranes to PA membranes, we concluded that it is the phosphate group that acts as the proton acceptor and forms the ion pair with the dissociated proton. This capability of the phosphate group to form an ion pair with the proton is only possible while using a super photoacid, while a regular photoacid is too 'weak' to serve as the proton donor to the acidic phosphate group.



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Diversity of interactions of 2,4-dinitrophenol with membrane proteins and lipids

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2,4-dinitrophenol (DNP) is an artificial uncoupler of oxidative phosphorylation in mitochondria. Due to its high toxicity and the lack of an antidote, DNP has been banned from therapeutic use to combat obesity. The renewed interest in DNP arises from its potential to treat neuronal, metabolic and inflammatory diseases at low doses. Initially, the effect of DNP on mitochondria was attributed to its protonophoric activity in the lipid phase of the inner mitochondrial membrane (IMM). Recent studies have also implicated mitochondrial proteins in DNP-mediated uncoupling. However, certain aspects of the mechanism remain under debate [1-3].

In our research, we reconstituted recombinant ANT1, UCP1-UCP3 and OGC proteins in a planar bilayer lipid membrane. Remarkably, all proteins significantly increased the total membrane conductance (G_m) at low DNP concentrations. Carboxyatractyloside and adenosine-5-triphosphate inhibited DNP-mediated protein activation only when added prior to DNP [1, 3]. Site-directed mutagenesis and molecular dynamics (MD) simulations revealed that arginine 79 of ANT1 plays a crucial role in DNP-mediated uncoupling [1]. Considering the specific lipid composition of organelle membranes, we investigated DNP anion interactions using two models: (i) the IMM and (ii) the plasma membrane. Our results showed that in model (i), the combination of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and cardiolipin significantly reduced DNP-mediated G_m. In contrast, cholesterol substantially enhanced DNP-mediated G_m in model (ii). MD simulations revealed that the concentration of DNP within the lipid membrane strongly depends on the lipid composition. However, in both models DNP anions are localized in the lipid headgroup region, while protonated DNPs are shifted towards the membrane center. Calculations of the average distance between molecules indicated that DNP anions and DOPE headgroups form hydrogen bonds that compete with DNP protonation.

Specific binding sites in lipid and protein phases, as well as membrane capacity for DNP adsorption, could serve as potential targets for regulating the uncoupling efficiency of DNP and its analogues in the inner mitochondrial membrane.

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Design Guidelines for Antimicrobial Peptides that Kill Antibiotic-Resistant Bacteria via Transmembrane Pores

Rahul Deb[1,2], Ivo Kabelka[1], Marcelo D. T. Torres[3,4,5,6], Markéta Kobĕrská[7], Floriana Cappiello[8], Martina Drabinová[1], Jan Přibyl[1], Edo Vreeker[9], Giovanni Maglia[9], Katy Jeannot[10,11], Maria Luisa Mangoni[8], Gabriela Balíková Novotná[7], Cesar de la Fuente-Nunez[3,4,5,6], and Robert Vácha[1,2]

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Pore-forming peptides can be utilized in various medical and biotechnological applications, including antimicrobial peptides that can kill antibiotic-resistant bacteria. However, designing peptides that self-assemble into transmembrane barrel-like nanopore structures is challenging due to the complexity of several competing interactions involving peptides, lipids, water, and ions. We develop a computational approach for the de novo design of α-helical peptides that self-assemble into stable and large transmembrane barrel pores with a central nano-sized functional channel. We address the lack of existing design guidelines for the de novo pore-forming peptides and propose 52 sequence patterns, each of which can be tailored for different applications using the identified role of its residues. Atomic force microscopy, channel electrical recording, leakage of small fluorescent molecule and transport of macromolecule experiments confirm that the designed peptides form stable, large, and functional barrel-shaped nanopores in model membranes. The custom-designed peptides act as potent antimicrobial agents able to kill even antibiotic-resistant ESKAPE bacteria at nanomolar concentrations, while exhibiting low toxicity to human cells. Peptides and their assembled nanopore structures can be similarly fine-tuned for other medical and biotechnological application.

Oral Presentations

Abstracts

Wednesday, July 10th

Cav1.3 calcium channel regulates pancreatic β-cell gene transcription, electrical activity, and insulin release.

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High voltage-gated calcium channels (HVCC) are critical for pancreatic β-cell electrical activity, insulin release, cell differentiation, and survival. Both human and mouse β-cells express several HVCCs isoforms with Cav1.3 L-type calcium channel showing the highest mRNA expression level. In humans, genetic polymorphisms leading to Cav1.3 loss-of-function associate with increased susceptibility for diabetes while Cav1.3 gain-of-function mutations can cause hyperinsulinaemic hypoglycemia. Nevertheless, the physiological role of Cav1.3 L-type calcium channel in pancreatic β-cell functions is unknown. Single cell RNAseq analysis showed that Cav1.3 deletion alters β-cell gene transcription suppressing cell-differentiation and activating protein degradation pathways. Consequently, Cav1.3 genetic ablation leads to a ~6-fold increase in DNA damage, and a 3-fold decrease in β-cell proliferation markers causing a ~20% reduction in β-cell count. Functionally, Cav1.3 deletion reduces the calcium influx by ~25 % and significantly alters the β-cells calcium current activation and inactivation kinetics. AP-clamp experiments indicated that Cav1.3 contributes with calcium influx required for the initiation of the glucose-induced electrical activity as well as during the repetitive action-potential firing. Consequently, $Cav1.3^{-/-}$ β-cells exhibited reduced glucose-induced electrical activity characterized by delayed AP-onset, reduced AP-firing and AP-train frequency over a wide range of glucose concentrations. This led to a significantly reduced 15 mM glucose-induced second phase insulin release. Overall, our data demonstrate that Cav1.3 calcium channel exerts a very complex multifactorial modulation on glucose homeostasis by controlling gene transcription, β-cell mass as well as glucose-stimulated electrical activity and insulin release.

Resolution of Orai1 dynamics at the channel periphery

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In the human body Calcium (Ca^{2+}) is not only essential for the bone structure but also for cellular processes such as gene transcription, proliferation, and apoptosis. A dysregulation of the Ca^{2+} homeostasis therefore might result in severe conditions such as developmental issues, cancer, and immune deficiencies. Here, the ubiquitously expressed Ca^{2+} release-activated Ca^{2+} (CRAC) channel has been identified as one of the most crucial Ca^{2+} entry pathways. This specific ion channel is fully composed of two proteins, the Ca^{2+} sensor located in the endoplasmic reticulum (ER), Stromal Interaction Molecule (STIM1), and the ion conducting pore Orai1 situated in the plasma membrane (PM). Upon depletion of the intracellular Ca^{2+} stores STIM1 activates, oligomerizes, translocates to ER-PM junctions and activates Orai1. The coupling of STIM1 to mainly the C-terminus of Orai1 and the consequent signal propagation from the channel periphery all the way to the pore finally results in Ca^{2+} influx. Our previous findings stress the significance of specific Orai1 gating checkpoints inducing a local conformational change resulting in an overall global transmembrane (TM) domain movement essential for pore opening. The precise underlying motions of the individual TM domains within Orai1 still remain elusive. Therefore, via a combined approach of Ca^{2+} imaging, electrophysiology, and molecular dynamic (MD) simulations together with conventional site directed mutagenesis and genetic code expansion (GCE), we offer an overview of critical sites and dynamics in the channel periphery essential for pore opening. Especially the application of a combination of GCE and the photocrosslinking unnatural amino acids (UAAs) azido-phenylalanine (Azi) and benzo-phenylalanine (Bpa) helped us to gain a better resolution of the structure/function relationship of the ion channel on an amino acid level. Here, we identified the positions V181 in TM3 and A254 in TM4 as critical sites for the mechanism of pore opening.

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Investigating the pH gating mechanism of *Helicobacter pyloris* UreI

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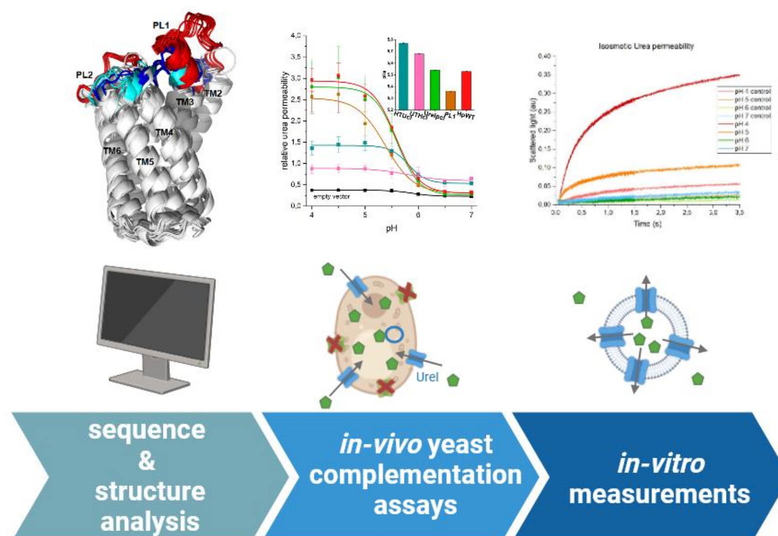
Chronic gastric infections with *Helicobacter pylori* are present in over 50% of the world population and are linked to diseases like peptic ulcer and stomach cancer. The commonly used antibiotic therapy is getting less efficient due to increasing antibiotic resistance. An alternative drug target could be the pH-gated inner membrane urea channel UreI which is crucial for the survival of the bacteria in the acidic environment of the human stomach. However, despite *in vivo* studies and high-resolution structures in the open and closed state, *Hp*UreIs gating mechanism is still elusive. To gain molecular insight into its gating mechanism we also tested the homologous urea channel from *Streptococcus salivarius* using yeast complementation assays. Relative urea and ammonia permeabilities are compared to the wild-type protein in the physiologically relevant pH range of 4.0 - 7.0. The results question the hypothesis of PL1 and PL2 constituting the main pH-sensor of *Hp*UreI as the urea channel from *S. salivarius*, lacking such extensive periplasmic loops, shows similar pH-dependence as *Hp*UreI. These results could be verified for overexpressed, purified and reconstituted *Ss*UreI in *in vitro* measurements. Our experimental studies are guided and accompanied by an extensive sequence- and structure-based analysis of homologous bacterial urea channels, which spot interesting sub-group variants and conserved protein features as well as positions. Yeast complementation assays constitute a cost-effective tool for testing such qualitative differences in protein variant activity for a broad range of solutes.



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Schematic figures created with Biorender.com

Calcium influx through TRPC3 induced SKCa-dependent hyperpolarisation

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Background: Ca^{2+}-permeable transient receptor potential canonical 3 (TRPC3) channel is involved in memory modulation by regulating the excitability of hippocampal neurons, contributing to enhanced afterhyperpolarization (AHP).

Calcium-activated potassium channels (KCa) are fundamental modulators of neuronal excitability and contribute to the generation of AHPs following action potential bursts.

Hypothesis: TRPC3 channels enhance the activity of Ca^{2+}-dependent K^{+} channels by local, microdomain communication.

Methods: To uncover downstream targets of TRPC3 signaling in hippocampal neurons, we performed electrophysiological recordings, fluorescent imaging, and co-immunoprecipitation (co-IP).

Results: TRPC3 channel stimulation with a selective TRPC3/6/7 agonist, GSK1703924A (GSK) led to a reversible decrease in the firing frequency of cultured neurons. On contrary, GSK did not exhibit any effects on the excitability of hippocampal neurons derived from TRPC1-7 knockout mice. Ca^{2+} levels in Fluo-4 loaded dissociated hippocampal neurons revealed an increase upon application of GSK. Subsequently, we investigated the relationship between these channels using transiently transfected HEK293 cells. Calcium imaging conducted on HEK293 cells overexpressing TRPC3, unveiled that particularly small-conductance (SKCa) potassium channels resulted in a notable impairment in cytoplasmic calcium loading while co-expression large-conductance (BKCa) potassium channels has no effect on calcium levels. Furthermore, co-expression of TRPC3 and SKCa induced significant membrane hyperpolarization compared to TRPC3 alone in the presence of extracellular calcium. Conversely, exclusion of calcium from the buffer or employing a Ca^{2+}-impermeable mutant TRPC3 (E630Q) depolarized plasma membrane in transfected HEK293 cells even in presence of SKCa channel. Moreover, co-IP demonstrated a physical TRPC3 and SKCa channel interactions. Application of selective SKCa channel inhibitor eliminated effect of GSK in dissociated hippocampal neurons.

Conclusion: Both constitutive and transient TRPC3 activity serve as a calcium source that specifically enhances SKCa currents, rather than BKCa currents, to regulate excitability of hippocampal neurons.

Studying protein electrostatics by NMR experiments

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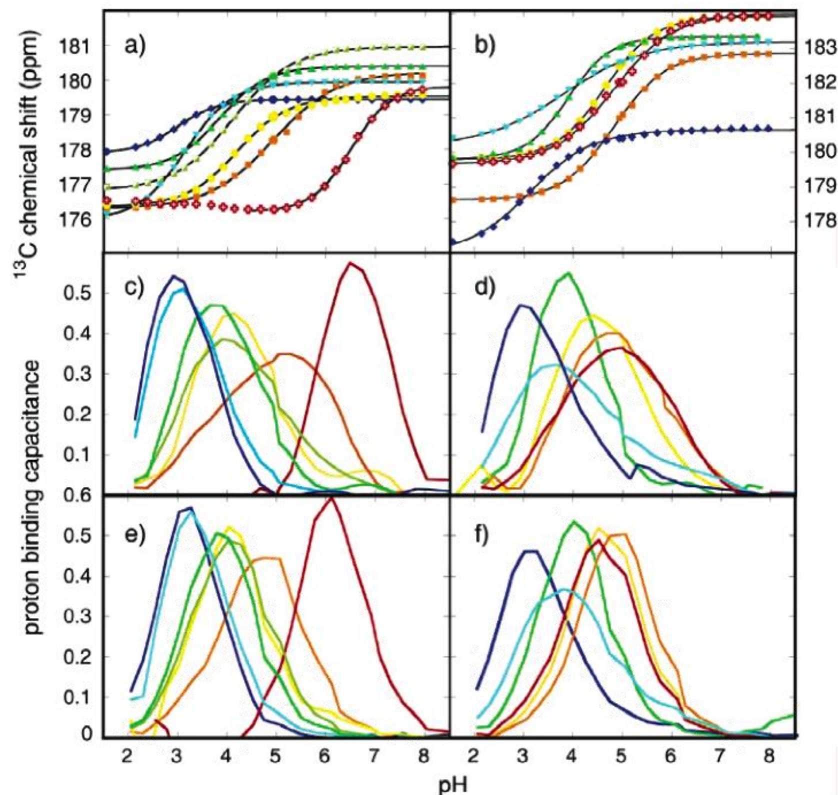
Nuclear Magnetic Resonance (NMR) spectroscopy has the unrivalled potential to quantify electrostatic interactions in proteins experimentally, and allows unambiguous access to all amino acids in proteins, using ^{13}C and ^{15}N chemical shifts as strategic reporters [1]. This improved methodology has allowed accurate insights into the microscopic charge states and nature of electrostatic coupling in small proteins. We have recently developed a novel way to study electrostatics in intrinsically disordered proteins (IDPs) and demonstrated that the electrochemical potential significantly influences hydrogen exchange (HX) kinetics of IDPs [3]. In addition, studies with charged paramagnetic co-solutes allows this potential to be determined in an independent experimental way [3,4].



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Nanopharmacological force sensing on monoamine transporters

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The monoamine transporters (MATs) consist of serotonin transporter (SERT), dopamine transporter (DAT), and norepinephrine transporter (NET), which are neurotransmitter sodium symporters. MATs reuptake the released monoamines from the synaptic cleft into the presynaptic neurons, thus modulating the monoaminergic neurotransmission. MATs are targeted not only by their physiological substrates, but also by therapeutic and addictive drugs. Cocaine binds to all MATs and inhibit the uptake of monoamines. Several other psychoactive drugs, e.g. D-amphetamine and its congeners including 3,4-methylenedioxymeth-amphetamine (MDMA), enter the presynaptic neuron through MATs and induce efflux of monoamines, thereby enhancing monoaminergic neurotransmission. By contrast, the antidepressant citalopram exclusively binds to SERT and methylphenidate, the drug for treatment of attention deficit hyperactivity disorder (ADHD) specifically binds to DAT. Controversy regarding the number and function of ligand binding sites in neurotransmitter-sodium symporters arose from conflicting data in crystal structures and molecular pharmacology. We designed novel molecular tools for atomic force microscopy that directly measure the interaction forces between the serotonin transporter (SERT) and the S- and R-enantiomers of citalopram on the single molecule level. Our approach is based on force spectroscopy, which allows for the extraction of dynamic information under physiological conditions inaccessible via X-ray crystallography. Two distinct populations of characteristic binding strengths of citalopram to SERT were revealed in Na^+-containing buffer. In contrast, in Li^+-containing buffer, SERT showed only low force interactions. Conversely, the vestibular mutant SERT-G402H merely displayed the high force population. These observations provide physical evidence for the existence of two binding sites in SERT, i.e. a central (S1) site and a vestibular (S2) site, when accessed in a physiological context. Competition experiments revealed that these two sites are allosterically coupled and exert reciprocal modulation. Interaction forces between the cocaine analogue, MFZ2-12, and the dopamine transporter (DAT) revealed that two populations of binding strength were pronounced in the presence of Zn^{2+} (10 µM), accompanied by an elevated binding activity. These findings are in accordance with a Zn^{2+} induced outward facing conformational change. Absence of Na^+, Zn^{2+}, or mutation at S422A dramatically reduced the population of strong interaction forces, indicating that it originated from the central S1 binding site. Our nanopharmacological approach paves a new avenue to explore transient binding sites in clinically relevant membrane transporters and opens the door to quantitatively address the modulation of interaction forces between ligands and allosterically coupled binding sites.

The physics of highly crosslinked cytoskeletal networks

Sebastian Fürthauer

TU Wien, Austria

Living cells move, deform and divide. The engine of these behaviors is the cytoskeleton, a highly crosslinked network of polymer filaments and molecular scale motors that use chemical energy to do work. We develop a theory that predicts how the micro-scale properties of molecular motors and crosslinks tune the networks emergent material properties and generate predictable, and possibly controllable, behaviors. I will present how this theory is constructed, and discuss its implications for cytoskeletal networks in vitro and in vivo.

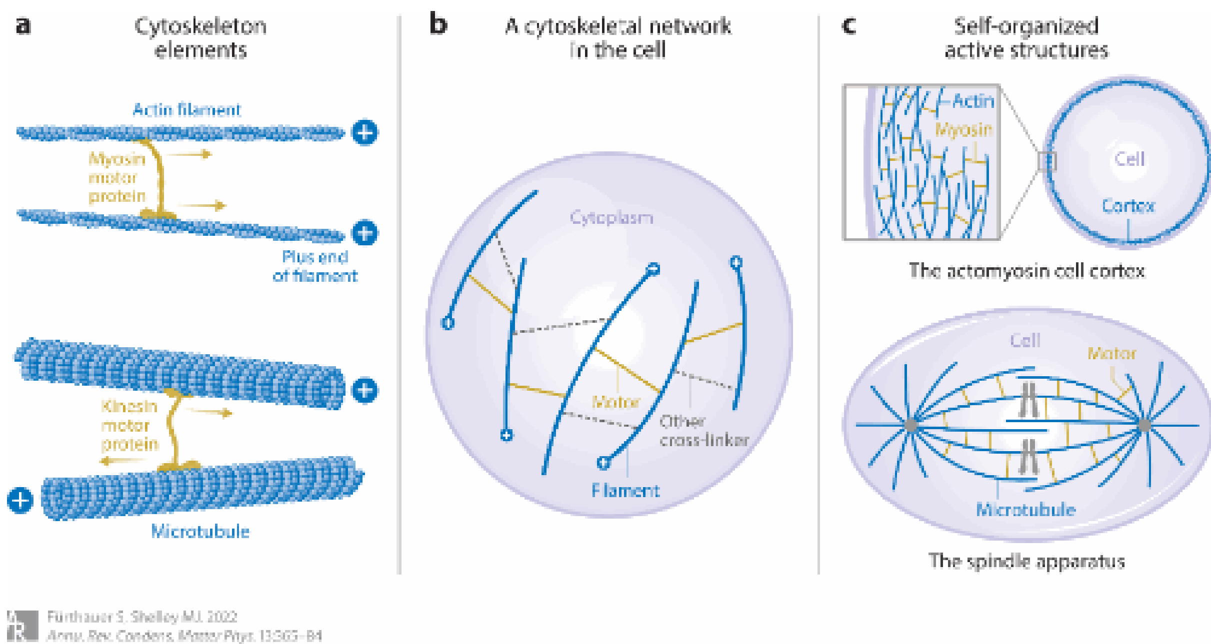

Figure from [3].

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Coiled Coils as Molecular Force Sensors: from Molecular Mechanisms to Applications in Cell Biology

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Coiled coils (CCs) are highly abundant motifs in structural proteins. Consisting of two (or more) α-helices wound around each other in a superhelical fashion, they represent essential structural elements of the cytoskeleton and the extracellular matrix. Considering their function as mechanical building blocks, surprisingly little is known about the structural determinants that define their molecular mechanical properties. Using atomic force microscope-based single-molecule force spectroscopy we have established the sequence-structure-MECHANICS relationship of a series of *de novo* designed, synthetic CCs (Figure 1A). We show that CC mechanical stability depends on CC length [1,2], helix stability and hydrophobic core packing [3-5], as well as on the pulling geometry [1,5].

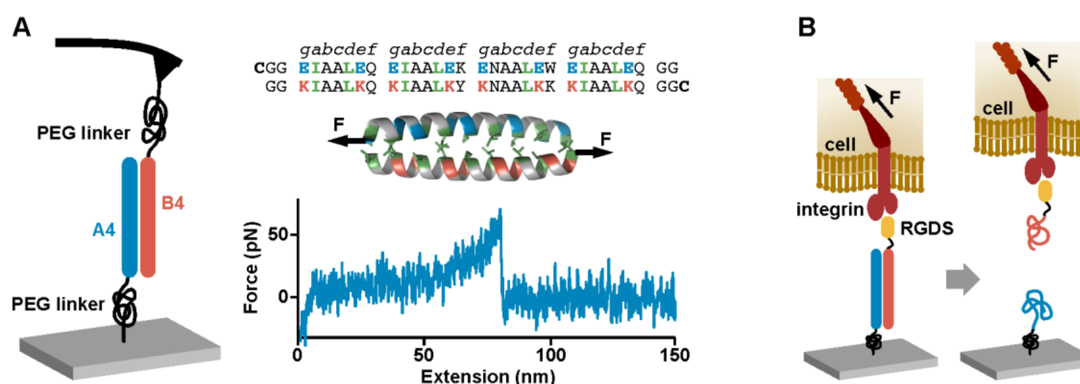

Figure 1. Coiled coil (CC)-based molecular force sensors. **A**. Characterization of CC strength with AFM-based dynamic single-molecule force spectroscopy (SMFS). SMFS probes the loading-rate dependent interaction strength of heterodimeric CCs. One chain is immobilized to a glass surface and the second to the AFM cantilever. The coupling sites are chosen such that a shear loading geometry is obtained. **B**. Application of mechanically calibrated CCs as molecular force sensors in cell culture experiments. Instead of the AFM cantilever, cells now pull on pre-calibrated CCs. For this purpose, the CCs carry a cell-adhesive RGDS sequence.

Based on this knowledge, we have developed a library of CCs with tuneable thermodynamic, kinetic and mechanical properties. This library now serves as the basis for the development of CC-based molecular force sensors for 2D cell culture applications and allows for determining the threshold force for cell attachment (Figure 1B). Towards 3D cell culture, we are developing hydrogels where the CCs act as mechanosensitive and self-reporting crosslinks [4,6,7]. Equipped with a fluorescence reporter system, these CC building blocks will allow for observing mechanical processes at the cell-material interface at the molecular level in real-time.

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Poster Session

Monday Flash-Talks

Posters by presenters in alphabetical order:

Asfia, Shima	Univ. Saarbrücken, DT
Einschütz López, Alexander	BOKU, Wien
Ezsias, Bence	JKU, Linz
Friesacher, Theres	Univ. Wien
Ganglberger, Matthias	Univ. Innsbruck
Geisler, Stefanie	Univ. Innsbruck
Hermenean, Horia-Constantin	Univ. Innsbruck
Kaltenegger, Michael	Univ. Graz
Kopittke, Caroline	TU, Wien
Pabst, Georg	Univ. Graz
Ramanthrikkovil Variyam, Ambili	Technion, Haifa, Israel
Roticiani, Giorgia	Vetmed, Wien
Shojaei, Sahar	JKU, Linz
Steiner, Philip	JKU, Linz
Sumarokova, Maria	ISBM, Moscow
Tuinte, Wietske	MU, Innsbruck
Yadav, Rohit	JKU, Linz

The Role of Perilipin 5 for the Contact Sites between Lipid Droplets and Bilayer: Protein Tether, or Lipid Bridge?

Shima Asfia[1], Mahsa Mohammadian[1], Ralf Seemann[1] and Jean-Baptiste Fleury[1]

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Lipid droplets (LDs) play a pivotal role in cellular energy storage and supplying components for the structure of organelle membranes. As the biology of lipid droplets relies on close coordination and communication with other cellular organelles, it is important to understand this interaction. In particular, the role of the protein Perilipin 5 (PLIN5), which is known as a mediator in regulating LDs dynamics and metabolism in cells is of interest. Thus, we investigate LDs (triolein oil droplets) surrounded by a phospholipid monolayer composed of DOPC and DOPE in different ratios with and without PLIN5 are brought in contact with small unilamellar vesicles (SUV)s and large unilamellar vesicles (LUV)s, which are also composed of DOPC and DOPE with ratios close to that of an ER membrane. To detect different contact interactions of these vesicles with the monolayer coating the LDs, the vesicles were double fluorescent labeled with a phospholipid Rhodamine dye in the bilayer and Cy5 dye in the core. Protein tethers can be assumed when the vesicles stay in contact with LDs via protein attachment; in this case, spots with both fluorescent dyes are observed on the surface of the LDs. Lipid bridges can be assumed when vesicles fuse to the LD monolayer, and a uniform colored “Rhodamine ring” appears on the surface of LDs revealing that only the phospholipid dye of the vesicles merged with LDs monolayer.

Substrate influence on cell mechanics: an AFM frequency dependent study

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Atomic Force Microscopy (AFM) has emerged as a versatile technique for probing the mechanical properties of biological samples with unprecedented resolution. In the context of cancer research, understanding the biomechanical properties of tumor cells is of important, as these properties are closely linked to cancer progression, metastasis, and response to therapy. Among the mechanical properties of interest, cell elasticity (i.e., elastic modulus), plays a pivotal role in various cellular processes, including migration, invasion, and interaction with the extracellular matrix. [1,2]

Recent studies have highlighted the dynamic nature of tumor cell viscoelasticity, which can undergo rapid changes in response to various stimuli, such as mechanical forces, biochemical cues, and changes in the cellular microenvironment. In particular, the frequency at which mechanical measurements are performed using AFM has been shown to influence the observed viscoelastic behavior of cells. In this phenomenon, the response to an applied force depends not only on the magnitude but also on the frequency of the force. [3,4]

In this study, we compare three cell lines: MCF-7, MCF-10A, and MDA-MB-231 cultured on hydrogels of polyacrylamide with varying stiffness (low, medium, and high). We investigate how changes in substrate stiffness and Atomic Force Microscopy (AFM) measurement frequencies (initially 1, 10, and 50 Hz) affect the viscoelastic behavior of tumor cells.

Our goal is to gain insights into the dynamic mechanical properties of MCF-7 cancer cells and to understand their implications for disease progression and treatment response. We plan to explore the cells' mechanical response under different conditions, such as exposure to drugs and variations in frequency range.

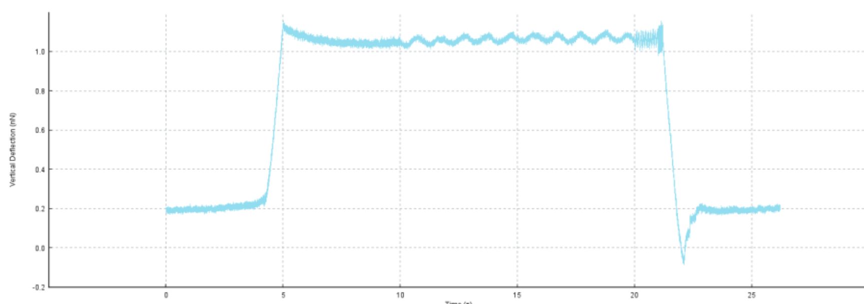

Figure1: Results of a frequency dependent measurement on MCF-7 cells seeded on a polyacrylamide hydrogel (hard). The first segment (0 - 5) is the approach of the cantilever, the second segment (5 - 10) is a relaxation step, the third segment (10 – 21) represent the three frequencies and finally the fourth segment (21 – 26) is the retract of the cantilever from the surface of the cell.

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High resolution clear native gel electrophoresis of native nanodiscs assisted by fluorescence correlation spectroscopy

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Native gel electrophoresis techniques, such as blue or clear native PAGE, are used to separate and characterize membrane proteins at the microscale. Since clear native electrophoresis (CNE) does not use charged compounds that could bind to proteins and cause a charge shift, proteins migrate according to their intrinsic isoelectric point [1]. Consequently, alternative tools to the weight markers commonly used for SDS pages must be used for identification. Here we propose to use fluorescence correlation spectroscopy (FCS) for this purpose. First, we extracted membrane proteins using glyco-DIBMA, an amphilic copolymer [2]. The resulting nanodiscs contained the protein in its native lipid environment. Second, we subjected the discs to CNE. The discs remained intact during the procedure. This greatly reduced the risk of protein aggregation. Third, we excised different bands from the gels and extracted the intact nanodiscs from the gel matrix. Fourth, we determined the molecular brightness of our fluorescently labelled membrane proteins and the diffusion time of the nanodiscs using FCS. These parameters allowed us to (i) confirm that the nanodisc size had not changed during CNE and (ii) select the protein in the desired oligomeric state. Importantly, the whole process does not denature the protein as it remains in its native environment throughout. The CNE-purified proteins can therefore be used for functional studies, for example by reconstitution into vesicles

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Allosteric inhibition of GIRK channels by ETX

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G-protein coupled inwardly rectifying potassium (GIRK) channels are key players in inhibitory neurotransmission in the heart and the brain. Misregulation of GIRK is associated with a variety of neurological disorders, such as epilepsy, Alzheimer's disease, and the rare Keppen-Lubinsky syndrome. Ethosuximide (ETX) is an antiseizure drug that inhibits GIRK channels and therefore has therapeutic potential for GIRK-related disorders. Using electrophysiology experiments and µs-long molecular dynamics (MD) simulations, we identify an allosteric ETX binding site close to the binding site of the endogenous GIRK activator phosphatidylinositol 4,5-bisphosphat (PIP_2). We propose an inhibition mechanism where drug binding leads to the displacement of PIP_2 and the closing of the channel. Furthermore, the inhibition is drastically potentiated when another GIRK activator, the Gβγ subunit of the G-protein, is bound. We use this dependence to explore the complex allosteric network underlying GIRK regulation.

A novel calcium channel Cavβ2 splice variant with unique properties predominates in the retina

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Cavβ subunits play a crucial role in modulating the properties of voltage-gated calcium channel complexes, including regulating their membrane expression and biophysical properties. Our research team has recently discovered and characterized a novel splice variant of Cavβ2, which we have named β2i.

We identified β2i by analyzing RNA-Seq data and subsequent quantitative real-time PCR. Our data showed that β2i is predominantly expressed in the retina, particularly in photoreceptors and bipolar cells. We therefore cloned the splice variant from the mouse retina and investigated the biophysical properties of calcium currents co-expressed with this variant in a heterologous expression system as well as its intrinsic membrane interaction, when expressed alone. The biophysical properties resembled those of known membrane-associated β2 variants. Indeed, β2i exhibited both strong membrane association, but also a tendency to cluster at the Golgi apparatus, possibly due to the unusually high density of hydrophobic amino acids in the N-terminus of β2i, a characteristic not found in other variants.

We also examined available Cavβ structural data to gain insights into the mechanisms underlying these observed characteristics. Our AlphaFold2 model suggested an alpha helix at the N-terminal end of β2i composed of the first 19 amino acids (M1 to K19) and a long, disordered loop connecting to another alpha helix. The SH3 domain (residues 72-131) is most likely properly folded in solution. Regarding the size and structural properties, the protein 1-152 Cavβ2i is an ideal candidate for nuclear magnetic resonance (NMR) studies of intrinsically disordered proteins within the context of a folded protein. We are currently setting up the process to express and purify the first 152 amino acids of the β2i protein for NMR studies. Given the poor solubility of the β2i N-terminus, we have fused the protein via a TEV cleavage site to an MBP tag to enhance solubility. Followed by protein enrichment using an MBP trap on the FPLC system, we then eliminate the MBP tag using TEV protease achieving an efficiency of approximately 7%. The suboptimal cleavage efficiency may be attributed to the size of the MBP tag and steric hindrance. Our comprehensive approach will broaden our understanding of the role of the novel N-terminally spliced β2 subunit and provide insights into the molecular mechanisms underlying the features of β2i.

Genetic deletion of Stac2 affects electrical activity of chromaffin cells & mouse behavior

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Voltage-gated Cav1.2 and Cav1.3 L-type calcium channels (LTCC) are important regulators of neuronal morphology, hormone release and action potential (AP) firing. Src homology 3 and cysteine-rich domain adaptor proteins (Stac) have recently been identified as new regulators of LTCC expression and current properties. Upon overexpression in cultured hippocampal neurons Stac2 isoform abolished LTCC Ca^{2+} dependent inactivation via an allosteric inhibition of calmodulin binding. Additionally, in Drosophila deletion of the homologous *DStac* gene results in deficient neuronal calcium transients and locomotion; and human *STAC2* mutations have been identified in patients with schizophrenia. However, the importance of Stac2 in endogenous Cav channel modulation, cellular functions and neurological disorders is still elusive.

Therefore, we here investigate the first mouse model bearing a constitutive Stac2 deletion achieved by inserting a LacZ targeting construct. Litters from heterozygous mice showed typical Mendelian inheritance. Moreover, male and female heterozygous and knockout mice displayed normal viability and body weight, indicating that Stac2 is not essential for survival and general development. To investigate where neuronal Stac2 might be functionally relevant we further analyzed LacZ expression on brain sections. β-galactosidase staining revealed high expression of Stac2 in regions important for motor function and anxiety-related behaviors. Therefore, behavioral phenotypes were investigated in male and female mice by using a series of tests. Currently ongoing behavioral analysis suggests that female knockout mice display reduced motor function obvious by impaired performance in the accelerating rotarod and challenging beam test. Interestingly, male knockout mice show normal motor coordination but display enhanced anxiety-like behavior in the elevated plus and light/dark test. Nissl-stained sections revealed no gross morphological changes in brain structure which might explain the phenotypes. Therefore, we next tested if genetic deletion of Stac2 affects cellular functions using patch-clamp electrophysiology. Current-clamp experiments in isolated mouse chromaffin cells (MCCs) revealed normal resting membrane potential and spontaneous firing frequency in male knockout mice. However, step current injection showed that knockout MCCs responded with a reduced rheobase and an increased action potential firing frequency at the onset of the pulse, suggesting that they are easier to depolarize. This increase in excitability might be caused by a shift in the voltage dependency of Cav activation toward more negative membrane potentials, while whole-cell Ca^{2+} current density and kinetics were not different compared to wildtypes.

Altogether, our data show for the first time that Stac2 regulates neuron-like AP firing behaviour and calcium currents in its native environment. Performing slice electrophysiology and c-Fos staining we will next investigate if abnormal neuronal or synaptic signalling might underly the behavioural phenotypes observed in male and female mice.

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Complex channel gating changes induced by germline *CACNA1D* (Cav1.3) missense variants lead to a broad symptomatic spectrum

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Background: Ca^{2+}-influx through voltage-gated Cav1.3 Ca^{2+} channels (encoded by the *CACNA1D* gene) is essential for several physiological processes, such as sinoatrial node pacemaking, glutamate release at auditory ribbon synapses, excitation-transcription coupling and cellular excitability. In humans, homozygous *CACNA1D* loss of function (LOF) causes bradycardia and deafness (SANDD syndrome) whereas *de novo* (heterozygous) variants causing complex activity-enhancing gating changes are associated with a neurodevelopmental disorder (NDD). Herein, we aim to functionally characterize three missense mutations causing SANDD (inherited homozygous variant A376V, reported in 10 patients) or NDD (*de novo* variant V1447L and the premature STOP codon C1436X variant) to test if distinct gating changes could explain the different clinical phenotypes.

Methods: Whole-cell voltage-clamp recordings were performed in tsA201 cells transfected with full-length wild-type (WT), A376V, V1447L or C1436X human Cav1.3 α_1 subunit co- transfected with β_3 and $\alpha_2\delta_1$ subunits and EGFP. 15 mM Ca^{2+} was used as charge carrier.

Results: Cells expressing the V1447L variant alone or co-expressed with WT revealed a significant hyperpolarising shift in the half-maximal activation/inactivation voltage by ~-11 mV together with other complex gating changes as previously observed with several NDD-associated *CACNA1D* variants, such as increased window currents and slower deactivation kinetics. A376V, associated with clinical LOF, unexpectedly showed complex gating changes, such as increased current density but reduced plasma membrane expression (Q_{ON} area reduced by approximately 2-fold), hyperpolarising voltage-dependent activation/inactivation shifts of ~-6.5 mV and ~-8 mV, respectively, and faster inactivation kinetics. Lastly, C1436X expressed alone resulted in a non-conducting channel. However, when co-expressed with WT, C1436X also induced complex gating changes (hyperpolarising shift of the voltage-dependence of activation by ~-7 mV and faster inactivation kinetics).

Conclusion: Our data demonstrate the pathogenicity of the V1447L variant, allowing us to reclassify it as 'pathogenic' and revealed a dominant effect on WT channels when expressed together. Surprisingly, A376V is the first complex gating-modifier leading to a clinical LOF phenotype (SANDD syndrome). Further studies using more physiological model systems and *in silico* modelling are required to elucidate how these complex gating changes can mimic the SANDD phenotype so far only described in individuals with non-functional Cav1.3 channels. Finally, although C1436X alone is non-functional, when co-expressed with WT channels it induces typical NDD-associated gating changes, which could explain the ASD phenotype of the patient. Further experiments will be required to show if this interaction renders truncated C1436X channels functional, or if C1436X causes gating changes of co-expressed WT channels. Overall, our results show that *CACNA1D* variants induce distinct functional changes associated with diverse clinical phenotypes. Increasing the number of well-characterised variants and patients will be crucial to uncover genotype-phenotype correlations, with implications for diagnosis and treatment.

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Shape-based design of bitopic proteins as probes for membrane elastic stress

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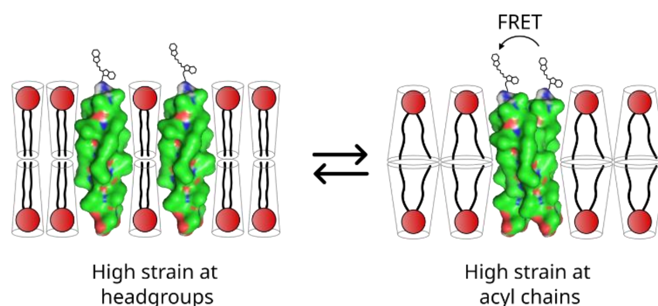
The function of integral membrane proteins is influenced by their hosting lipid membrane environment. Apart from specific lipid- protein interactions, also the properties of the bulk membrane affect the structure and function of transmembrane proteins (TMPs) [1].

We hypothesize that TMPs respond to the elastic stress distribution of the lipid bilayer. To this end we designed bitopic proteins considering their geometrical shape along the lipid bilayer normal while matching the overall membrane thickness. The transmembrane sequence was chosen in a way to rule out specific interactions between amino acids by exclusively using hydrophobic residues. The self-association of these "probes" is then to be measured by single molecular Förster resonance energy transfer microscopy in large unilamellar vesicles of varying lipid composition to simulate different membrane stress environments.

The artificial proteins were expressed in E. coli using a maltose binding protein (MBP) solubility tag and purified the proteins in the presence of detergent [2].

The current iteration of our TMP sequence is of asymmetric shape and was designed to improve purification with detergent as well as the interaction with lipid bilayers.

Currently, we are experimenting on the reconstitution into lipid vesicles. In particular, we are exploring the possibility of the MBP-tag as hydrophilic "anchor" on the extravesicular side of the liposomes by side-specific phosphine-quenching of cyanine dyes [3]. This would enable the unidirectional insertion of the TMP.



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Febrile temperatures enhance early T cell activation

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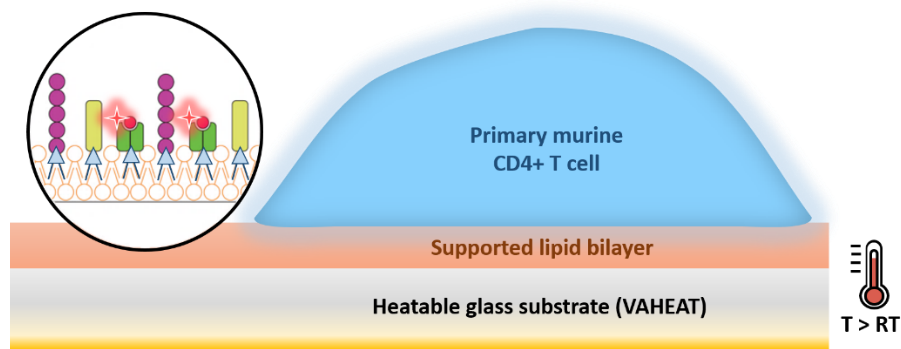
The immune system is highly sensitive to changes in body temperature. While hyperthermia enhances the immune response (e.g. during fever) [1-3], hypothermia has immuno-suppressive properties that are used in anti-inflammatory treatments [4-5]. Interestingly, the cellular or molecular mechanisms of temperature-induced immuno-modulation are barely understood.

We investigated the influence of temperature on the early signalling of primary CD4+ T cells from 5c.c7 TCR-transgenic mice. Functionalized supported lipid bilayers (SLB) mimic the surface of antigen presenting cells and allow for specific activation by supplying the cognate antigen as well as costimulatory and adhesion proteins. Glass substrates with microscopy-compatible heating elements (VAHEAT, Interherence) allow for precise temperature control at the interface between bilayer and T cell. Via ratiometric calcium imaging, we detect activated T cells and show that hyperthermia not only increases T cell sensitivity but also accelerates the activation process. Additionally, we characterize how increased temperatures damage functionalized SLBs and present strategies to maintain bilayer integrity.



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Ion-mediated changes of spontaneous monolayer curvature activate the integral enzyme OmpLA

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Transbilayer asymmetry of lipids and proteins is a poorly understood distinct feature across all plasma membranes. In our pursuit to elucidate the biophysics underlying plasma membrane asymmetry, we have devised protocols for generating synthetic asymmetric lipid membranes, incorporating reconstituted integral membrane proteins. Specifically, we interrogated the behavior of outer membrane phospholipase A (OmpLA), an integral enzyme found in Gram-negative bacteria, in diverse asymmetric lipid membranes composed of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin. Our experiments reveal a notable reduction in the enzyme's hydrolytic activity in compositionally asymmetric charge-neutral lipid bilayers. In charged asymmetric membranes, we observed an overall enhancement of protein activity, further influenced by mono and divalent ions. All observations can be primarily explained by an allosteric coupling between protein activity and lateral asymmetry stress, which can be modulated by compositional asymmetry and electrostatic interactions between ions and lipid headgroups. The generic nature of our findings suggests that analogous mechanisms likely govern the behavior of many other plasma membrane.

Proton diffusion across mixed lipid membranes highlights the role of membrane composition

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Abstract

Proton circuits within biological membranes are at the heart of natural bioenergetic systems, whereas different biological membranes are characterized by different lipid compositions. In this study, we investigate how the composition of mixed lipid membranes influences the proton transfer (PT) properties of the membrane by following the excited-state PT (ESPT) process from a tethered probe to the membrane with time-scales and length-scales of PT that are relevant to bioenergetic systems. Two processes can happen during ESPT: the initial PT from the probe to the membrane at short timescales, followed by diffusion of dissociated protons around the probe on the membrane, and the possible geminate recombination with the probe at longer timescales. Here, we use membranes that are composed of mixtures of phosphatidylcholine (PC) and phosphatidic acid (PA). We show that the changes in the ESPT properties are not monotonous with the concentration of the lipid mixture; at low concentration of PA in PC, we find that the membrane is a poor proton acceptor. Molecular dynamics simulations indicate that at this certain lipid mixture, the membrane is more structured with the least defects. Accordingly, we suggest that the structure of the membrane is an important factor in facilitating PT. We further show that the composition of the membrane affects the geminate proton diffusion around the probe, whereas, on a time-scale of tens of nanoseconds, the dissociated proton is mostly lateral restricted to the membrane plane in PA membranes, while in PC, the diffusion is less restricted by the membrane.

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Role of the amino acid residues in the transport of free fatty acid anions in Uncoupling Protein 1

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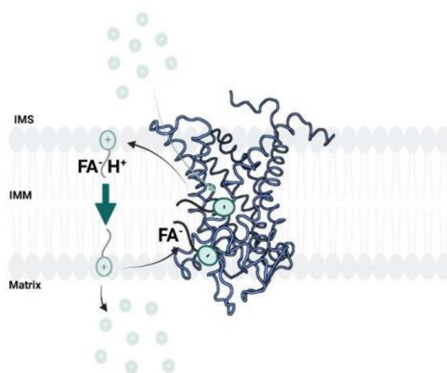
Uncoupling protein 1 (UCP1) drives heat production during the non-shivering thermogenesis by dissipating proton gradient without the formation of ATP. This process is critical in newborns, in hibernation and is also involved in obesity and insulin sensitivity. Over the past three decades, several mechanisms have been proposed to explain the protonophoric

function of UCP1 in the presence of free long-chain fatty acids (FAs). One such model is the fatty acid cycling model, which claims that FAs flip-flop through the membrane delivering a proton to the matrix side (Skulachev, 1991). UCP1 catalyzes the return of FA anions back to the cytosolic side of the membrane and the molecular mechanism, which has already been proposed for the mitochondrial adenine nucleotide translocase (ANT) (Kreiter et al., 2023). This mechanism elucidates the movement of fatty acid (FA) anions, assisted by UCP1, to the cytosolic side of the inner mitochondrial membrane (IMM), thus completing the cycle of proton transport in the FA cycling model.The well-defined model of planar bilayer lipid membranes reconstituted with UCP1, combined with site-directed mutagenesis and molecular dynamics simulations, allows us to show how UCP1 can drive the passage of FA

anions across the IMM. FA anions are first attracted to positively charged residues facing the matrix side of the IMM. They can then slide along the positive protein-lipid interface and bind to one of the three positive charged residues (R84, R183, R277) in the middle of the protein cavity. At this position, the anions can be protonated. Negatively charged residues near this arginine triplet, such as E135, assist in protonation. FA in the neutral form can spontaneously be released from the protein. The results reveal the precise mechanism by which UCP1 facilitates the passage of fatty acid anions across the inner mitochondrial membrane and provide valuable insights into its protonophoric function.



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Novel insights into the pH-gating mechanism of bacterial urea channels

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Bacterial urea channels, known as UreI channels, are present in both gram-positive and gram-negative bacteria. UreI's work together with cytoplasmic urease to form an acid acclimation strategy in bacteria. One important protein in this family of bacterial urea channels is *Hp*UreI, which is crucial for the survival of the pathogen *Helicobacter pylori* in the acidic environment of the human stomach. *Hp*UreI channels urea in a pH-dependent manner from the periplasm into the cytoplasm, where urea is hydrolyzed into ammonia and carbon dioxide, which in turn increases internal pH. Despite in vivo studies and high-resolution structures in the open and closed states, the gating mechanism of this protein remains elusive. We systematically tested point mutations of protonatable residues located at the periplasmic and cytoplasmic sides of *Hp*UreI, as well as homologous channels from *Helicobacter hepaticus* and *Streptococcus salivarius*. We used yeast complementation assays to compare their functionality and pH-gating behavior to the wild-type proteins within the physiologically relevant pH range between

4.0 to 7.0. Therefore, we expressed our constructs in urea or ammonia-uptake-deficient *Saccharomyces cerevisiae* and grew them in different pH environments with urea or ammonia as the sole nitrogen source, respectively. By doing so, we were able to correlate increased cell survival with urea or ammonia permeability of the channels. Our results show that UreI gating involves an intricate network of residues, which challenges the notion of periplasmic histidine residues as the sole pH sensor. Overall, yeast complementation assays can be a powerful tool for assessing exogenous protein functionality across diverse solutes and pH conditions.

Keywords: bacterial urea channel, UreI, pH-gating mechanism, Helicobacter pylori, Helicobacter hepaticus, Streptococcus salivarius, yeast complementation assays, ammonia permeability

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Thapsigargin induces non-apoptotic, caspase-independent programmed cell death in RBL-1 cells

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Thapsigargin (TG) is a potent inhibitor of the sarco/endoplasmic reticulum (ER) Ca^{2+}-ATPase (SERCA), leading to an increase in intracellular Ca^{2+} levels. Recent studies demonstrate the ability of TG to induce programmed cell death (PCD) in numerous cancer cell types, highlighting TG as a potential anticancer drug [1-3]. Although it is commonly believed that TG induces apoptosis-mediated, caspase-dependent PCD, our cytological and physiological investigations on rat basophilic leukemia cells (RBL-1) suggest an alternative, non-apoptotic, caspase-independent mechanism of PCD. Since the ultrastructural hallmarks of TG-induced cell death are only sparsely described, we studied the morphological consequences of TG exposure in RBL-1 cells using 2D transmission electron microscopy (TEM) and 3D TEM tomography in correlation with laser scanning microscopy (LSM). To visualize and quantify the effects on different compartments, organelle specific live-cell fluorescence markers were used. In addition, caspase-dependent cell death assays were carried out. TG-exposed RBL-1 cells showed prominent ballooning of the perinuclear space, vacuolization, increased vesicle formation, mitochondrial enlargement and degradation as well as initial ER-swelling. In particular, the TEM data did not show apoptotic hallmarks such as nuclear fragmentation or the formation of apoptotic bodies in TG-exposed RBL-1 cells. In addition to the morphological analysis, physiological cell death assays revealed a caspase independent mode of PCD after TG treatment. Contrary to the prevailing theory that TG triggers caspase-dependent apoptosis, our results suggest a non-apoptotic, caspase independent modality of PCD. Moreover, numerous non-apoptotic morphological hallmarks were found, which are reminiscent of non-apoptotic PCD modalities such as autosis and paraptosis. TG might be indeed a potential tumor therapeutic agent, but the underlying mechanisms of the associated PCD are still unclear and could differ in various cell types.

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Membrane reshaping by amphipathic helices: the role of conical lipids

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Membrane reshaping is a critical aspect of various cellular processes, including intracellular trafficking, signal transduction, protein binding, and enzyme activity regulation. The transition of membrane shape is controlled by specialized proteins, whose activities are coordinated in space and time. Proteins involved in generating membrane curvature typically contain an amphipathic helix on a site that interacts with the membrane. This helix is believed to act as a "wedge" that induces or assists in outward membrane bending when it is shallowly inserted into the lipid bilayer. However, recent research has challenged the idea that this amphipathic insertion is responsible for curvature generation. Instead, protein steric interactions (crowding) have been shown to be primarily responsible for membrane bending, while the role of the amphipathic helix has been reduced to anchoring in the membrane. In this study, we examine the ability of the H0 helix of the epsin N-terminal homology domain (ENTH), along with designed peptides with a similar length and hydrophobic profile, to cause membrane shape transition. Our findings indicate that while H0 and its analogs exhibit a significant positive spontaneous curvature when embedded in the lipid monolayer, they have little effect on the flat shape of the membrane. Consequently, a high surface density of peptides and low lateral tension are necessary to induce spontaneous tubulation of membranes. However, the inclusion of conical lipid PE in the membrane significantly facilitates the destabilization of its flat shape, reducing the critical density of peptides and increasing lateral tension that can resist membrane shape transition. Furthermore, the radii of the forming tubes showed a strong dependence on the amount of PE, decreasing as it increased. A theoretical model is proposed that considers the curvature-composition coupling [3] for both amphipathic peptides and conical lipids to explain their synergy in membrane shape transition. This work was supported by the Russian Science Foundation (grant # № 22-15-00265).

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Dissecting the functions of multiple interactions of STAC3 in skeletal muscle excitation-contraction coupling

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The adaptor protein STAC3 was reported to be essential for skeletal muscle EC coupling and exerts three distinct functions. It facilitates membrane expression of CaV1.1. It is crucial for CaV1.1 function as the voltage sensor of EC coupling. Lastly, it is essential for the conformational coupling between CaV1.1 and RyR1. This raises the question whether STAC3 could be the missing link between the two calcium channels. Previously, two distinct STAC3/CaV1.1 interactions were identified: the one between the SH3-1 domain of STAC3 and the II-III intracellular loop of CaV1.1, and the one between the C1-linker region of STAC3 and the proximal C-terminus of CaV1.1. To determine which interaction is important for each function, two STAC3 fragments, each containing the domain responsible for one interaction, were reconstituted in a double CaV1.1/STAC3 KO skeletal muscle cell line. Electrophysiological recordings revealed that the STAC3 C1-linker fragment expression rescued CaV1.1 charge movement and calcium currents. However, the calcium release from the SR was severely reduced. Conversely, reconstitution of only the STAC3-SH3s domains did not rescue any function. Simultaneous reconstitution of both fragments also did not fully rescue EC coupling, suggesting that the isolated SH3 domains interact with low affinity. To increase the local concentration, we linked the SH3 domains to the CaVß1a subunit. This fragment alone rescued minimal EC coupling, but no calcium currents. However, when co-expressed with the other STAC3 fragment, full currents and EC coupling were reconstituted. While the SH3-1 domain interacts with the II-III loop of CaV1.1, the role of the SH3-2 domain remains unclear. Therefore, we made a STAC3 construct lacking the SH3-2 domain, confirming its importance for EC coupling. To study its importance in more detail, two mutations were introduced. The first one resembled the STAC3 disorder mutation of the SH3-1 domain, which resulted in no decrease in EC coupling. However, when three residues, involved in forming water-mediated hydrogen bonds with the II-III loop of CaV1.1, were neutralized, it resulted in a significant decrease in EC coupling.

Altogether, these results demonstrate that the C1-linker/C-terminus interaction is responsible for STAC3 targeting to the EC coupling machinery and CaV1.1 functional expression, while the low-affinity SH3s/II-III loop interaction merely enhances EC coupling. The role of the SH3-2 domain is suggested to be in stabilizing the interaction with the CaV1.1 II-III loop.

Photoactivation of Voltage-Gated Potassium Channels by a photolipid-based approach

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Voltage-gated ion channels play a pivotal role in facilitating rapid cellular excitability, particularly within neurons. Among these channels, voltage-gated potassium (Kv) channels are instrumental in modulating the shape and duration of action potentials. Kv channels exhibit a notable sensitivity to mechanical stimuli. However, it remains unclear whether membrane tension alone or changes in membrane thickness and curvature govern the response. To answer this question, we used azobenzene-containing diacylglycerols that allow upon excitation by light to alter membrane shape[1], bilayer mechanical properties[2], and to induce mechanical stress. The generation of tension requires membrane thickness changes on a millisecond timescale. The associated rates of capacity change induce depolarizing or hyperpolarizing currents depending on whether UV or blue light has been used[3]. Here, we first clarified that the increase in membrane tension generated by photoswitchable lipids in a solvent-depleted planar lipid bilayer is transient. Further, we reconstituted KvAP, the voltage-gated potassium channel from *Aeropyrum pernix* into solvent-depleted planar lipid bilayers containing the photoswitchable lipid OptoDArG, and observed alterations of its open probability upon light exposure. Blue light rapidly decreased the capacitance. This triggered hyperpolarizing currents within milliseconds, mediated by the opening of more KvAP channels. Most of these channels remained open even after the mechanical tension was released, suggesting that tension is not required to stabilize their open conformation. Instead, the increment in membrane thickness must have stabilized the "up" conformation as indicated by channel closure after membrane thinning due to UV light irradiation. Repeating the ensemble measurements at the single channel level confirmed the conclusion. Our study shows how photolipids can be used to exert light-triggered changes in the activity of membrane proteins that are not inherently sensitive to membrane tension.

1. Saitov *et al.*, Ordered Lipid Domains Assemble via Concerted Recruitment of Constituents from Both Membrane Leaflets. *Phys Rev Lett.* **124,** 108102 (2020).

2. Pfeffermann *et al.,* Photoswitching of model ion channels in lipid bilayers. *J. Photochem. Photobiol. B.* **224,** 112320 (2021).

3. Bassetto†, Pfeffermann†, Yadav†, Strassgschwandtner *et al.,* Photolipid excitation triggers depolarizing optocapacitive currents and action potentials. *Nature Communications* **15,** 1139 (2024).

Poster Session

Tuesday Flash-Talks

Posters by presenters in alphabetical order:

Clarke, Amy	MU, Graz
El Ghaleb, Yousra	MU, Innsbruck
Fedirko, Tetiana	Natl. Univ Kiev
Ganesh, Adithya	JKU, Linz
Gaugutz, Anna	TU, Wien
Heiss, Martin	MU, Innsbruck
Horner, Andreas	JKU, Linz
Keller, Sandro	Univ. Graz
Oh, Yoojin	JKU, Linz
Posch, Sandra	JKU, Linz
Roth, Elisa	Univ. Innsbruck
Schrangl, Lukas	BOKU, Wien
Skerjanz, Julia	MU, Graz
Sudjarwo, Wisnu	BOKU, Wien
Török, Enikő	MU, Innsbruck
Vargas, Carolyn	Univ. Graz
Plessl, Eva-Maria	Univ. Wien

PIP_2 modulates TRPC3 activity via binding to the L3 lipid binding site

Amy Clarke[1], Julia Skerjanz[2], Mathias AF Gsell[2], Patrick Wiedner[2], Hazel Erkan-Candag[2], Klaus Groschner[2], Thomas Stockner[1], Oleksandra Tiapko[23]

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The transient receptor potential canonical type 3 (TRPC3) channel is a Ca^{2+}-selective ion channel expressed in the plasma membrane of smooth and cardiac muscle cells, as well as the Purkinje cells of the human adult brain. In addition to activation by diacylglycerol (DAG), TRPC3 is positively modulated by phosphatidylinositol-4,5-bisphosphate (PIP_2), however studies aimed at localising the regulatory PIP_2 binding site have reached inconsistent conclusions. Here, using molecular dynamics simulations and patch clamp techniques, we reveal that PIP_2 predominantly interacts with TRPC3 at the L3 lipid binding site, located at the intersection of pre-S1 and S1 helices. We propose that PIP_2 sensing involves a multistep mechanism propagating from the L3 to the pore domain via the TRP helix and S4-S5 linker. These structural insights into the function of TRPC3 are invaluable for understanding the mechanism by which members of the TRPC subfamily of channels are regulated by their lipid environment.

Structural variants of the gate, selectivity filter and voltage sensors determining function and pathogenicity of T-type calcium channels

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[1] Medicinal University of Innsbruck, Austria; [2] Scripps Research Institute, USA; [3] University of Innsbruck, Austria

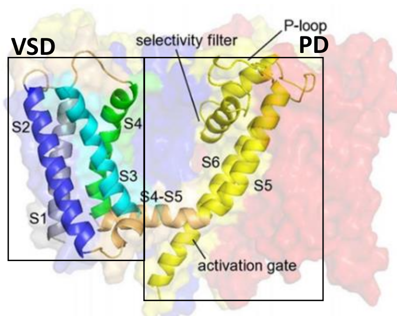
The voltage-gated calcium channels (VGCC) family consists of ten isoforms that can be divided in high voltage activated (HVA) and low-voltage-activated (LVA) channels. $Ca_V3.1$-$Ca_V3.3$ are the LVA channels, also known as T-type channels. These channels activate and inactivate at potentials close to the resting membrane potential of the neurons in which they are expressed, making them perfectly equipped to regulate cellular excitability.

We identified the gene for $Ca_V3.3$ (CACNA1I) for the first time as a disease gene for neurodevelopmental disorder and epilepsy [1]. Using a combination of structure modelling, site-directed mutagenesis, patch-clamp recording, and NEURON computer modelling, we identified four gain-of-function disease variants located in the channel activation-gate. These variants show left-shifted voltage-dependence of activation and inactivation, and slower inactivation and deactivation kinetics, resulting in increased calcium influx during rest and activity. Our results indicated that these changes lead to hyper-excitability and calcium toxicity, underlying the epilepsy and neurodevelopmental disorder in the patients. We are currently studying three new CACNA1I activation gate variants linked to epilepsy and/or developmental delay. Because even small alterations of gating properties result in disease, we are characterizing further sites critical for the tuning of activation and inactivation properties.

While voltage-sensitivity is typically orchestrated by interactions of the gating charges with ion-pair partners within the voltage-sensor domains (VSDs), we previously reported non-canonical inter-domain interactions between the VSD and a pore-domain (PD), essential for setting the voltage-dependence of channel gate opening in Ca_V1 channels [2]. Here we report homologous inter-domain interactions in all T-type channels, identifying these inter-domain interactions as an important general mechanism used by VGCCs to regulate their voltage sensitivity. Furthermore, preliminary results reveal specific structural determinants in the selectivity filter, involved in regulating various biophysical properties of $Ca_V3.3$. Surprisingly, not only do these selectivity filter residues regulate voltage sensitivity, they also seem to be involved in a mechanism that regulates the kinetics of channel inactivation.

Our results link structure to function and show how changes of a single amino acid can have tremendous effects on channel gating. The exact gating property that is altered (e.g. voltage-dependence and kinetics of activation vs. inactivation), depends on the precise location and the specific substitution of the amino acid. To understand the function and pathogenicity of T-type channels, it is important to study the complete channel structure, how different functional units are connected and ultimately all cooperate in opening the channel gate.

Side-view of VGCC structure model: One repeat depicted in structure density of the whole channel. VSD consists of S1-S4, PD of S5 and S6 connected by the P-loop including the selectivity filter. The activation gate consists of the intracellular sides of S5 and S6.



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2. El Ghaleb, Y. *et al*. Ion-pair interactions between voltage-sensing domain IV and pore domain I regulate Ca V 1 . 1 gating. *Biophys. J.* **120**, 1–13 (2021).

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Study of cholinomimetic properties of compound 146539 on multicellular preparations of smooth muscles of large intestine of rats

[1] T. O. Fedirko, O. V. [1] Tsymbalyuk, [1] I. S. Voiteshenko, [2] S. A. Starosyla, [3] V.G. Bdzhola, [3] A. O. Prykhod'ko, [1] A. Yu. Nyporko

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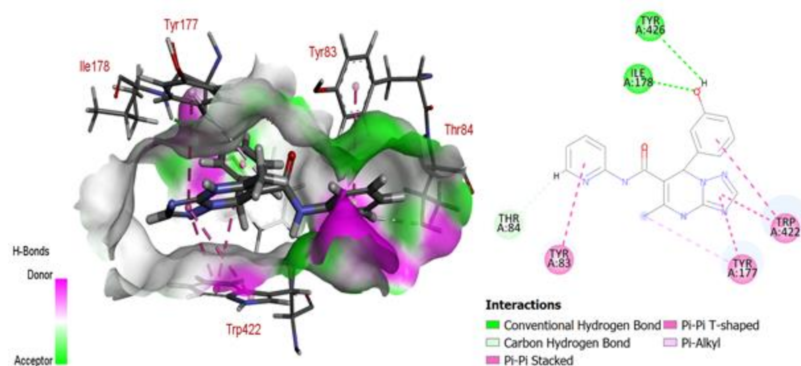
In visceral smooth muscles, acetylcholine is the main excitatory neurotransmitter, which activates contractile activity through the activation of muscarinic acetylcholine receptors (mAChRs) of M_2 and M_3 subtypes [1], and dysfunction of mAChRs is the basis many pathologies [2]. There is still an urgent need for the development of pharmacological agents - agonists of mAChRs of individual subtypes, in particular M_2-cholinergic receptors. The work aimed to study the effect of the compound with code 146539 on spontaneous and acetylcholine-induced contractions of the smooth muscles of the large intestine of rats. The ability to activate mAChRs was predicted by *in silico* methods.

Spontaneous and acetylcholine-induced contractions were studied tensometrically in isometric mode on preparations of circular smooth muscles of the *caecum* [3]. It has been established that compound 146539 (10^{-9} – 10^{-6} M) induces the activation of acetylcholine-induced (10^{-5} M) contractions. The maximum effective concentration of 146539 was found to be 10^{-8} M, wherein the phasic component of contractions increased threefold compared to the control. Furthermore, the absolute values of the strength of the tonic components of acetylcholine contractions, primarily associated with the activation of the M_2 subtype of mAChRs, increased in a dose-independent manner (on average two and a half times compared to the control) across all tested concentrations of compound 146539. It was also observed that at concentrations ranging from 10^{-8} to 10^{-6} M compound 146539 dose-dependently suppressed the spontaneous contractile activity. Notably, at concentrations of 10^{-8} and 10^{-7} M only the amplitude of spontaneous contractions decreased, while at a concentration of 10^{-6} M, both the amplitude and frequency decreased significantly. Analyzing the effects of, it can be predicted that compound 146539 does show properties of M_2-cholinomimetic.



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2 Nyporko, A., Tsymbalyuk, O., Voiteshenko, I., Starosyla, S., Protopopov, M., & Bdzhola, V. (2023). New promising agents against COPD and asthma among the amides of 1-oxo-3-phenyl-isochroman-6-carboxylic acid. *Biophysical Bulletin*, (50), 36–47.

Functional test of membrane proteins reconstituted into GUVs from native nanodiscs

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The purification and reconstitution of membrane proteins is a challenge as success depends on the empirical choice of detergents [1], and the choice of the lipid environment. Nanodiscs formed by amphiphilic polymer GlycoDIBMA can circumnavigate these problems as the polymer extracts a lipid patch from the cell membrane, keeping the protein in its native environment [2]. Apart from being time-efficient, this versatile technique allows extracting proteins from diverse cells. However, assessing membrane channel functionality within these nanodiscs poses a challenge. To address this, we developed an assay to transfer the proteins from nanodiscs to giant unilamellar vesicles (GUVs). It employs fluorescence correlation spectroscopy (FCS) to first measure nanodisc abundance and size. Transfer success is also assessed by FCS, which provides membrane protein density and diffusion coefficient in the GUV bilayer. Elucidation of protein functionality is based on a water flux assay using the micropipette aspiration technique [3]. Rapid buffer exchange minimises the unstirred layer around the membrane and improves reproducibility. The approach is useful not only for determining the functionality of water channel proteins, but also for ion channels, most of which are capable of facilitating water transport.

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3D localisation microscopy and single molecule tracking of membrane-bound DNA nanostructures using defocused imaging

Anna Gaugutz[1], Lukáš Veľas[1], Elena Georgiou[2], Yongzheng Xing[2], Stefan Howorka[2], Gerhard J. Schütz[1]

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The topography of the immune synapse plays a key role in the activation of T cells. Diffusion of membrane proteins is often hindered due to steric effects in close cell-cell contacts. A prime example is the exclusion of the phosphatase CD45 from the close-contact zone due to its large extracellular domain. To study exclusion effects, tracking of proteins in all three dimensions is required. Standard optical microscopy provides high localisation precision in lateral dimensions but is limited in the axial direction. 3D single molecule localisation microscopy (3D SMLM) based on defocused imaging achieves a resolution of 10 nm along the axial direction [1]. The method is validated by tracking the diffusion of two nanoprobes (DNA-NP) on lipid membranes. The nanostructures are custom-designed with DNA nanotechnology and are fluorescently labelled at two z-positions differing by 11 nm. With 3D SMLM, we determine a lateral diffusion coefficient of $D \approx 0.3\ \mu m^2/s$ and a relative height difference of $\Delta z = 11.57 \pm 1.01$ nm, which is in line with the rational design. 3D tracking of nanostructures provides a powerful tool to map out exclusion zones in the immunological synapse with high resolution.

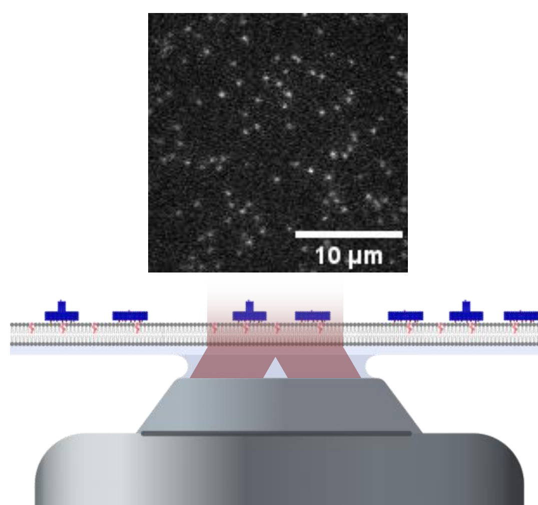

Figure 1: 3D SMLM of DNA nanoprobes on lipid membranes using defocused imaging.

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Role of the charge transfer center as a bimodal regulator of voltage-dependence and kinetics of Ca_V voltage sensors action

Martin C. Heiss[1], Monica L. Fernández-Quintero[2], Yousra El Ghaleb[1], Simone Pelizzari[1], Marta Campiglio[1], Petronel Tuluc[3], Klaus R. Liedl[4], Bernhard E. Flucher[1]

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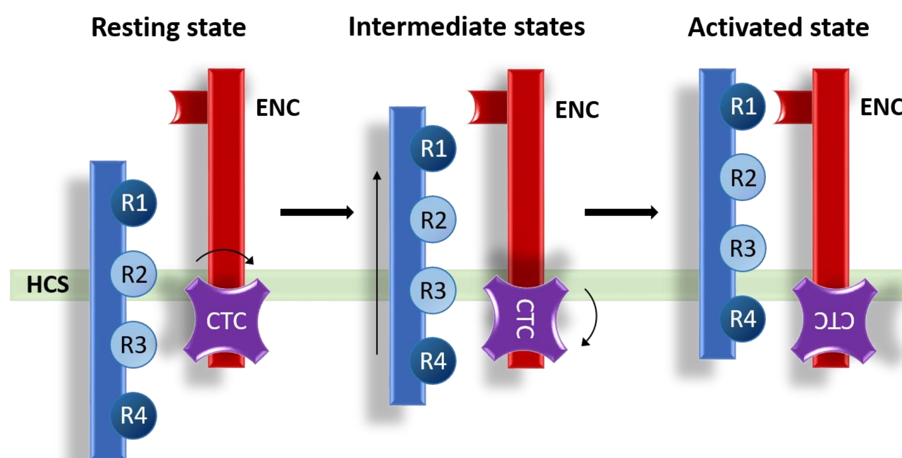
The response of voltage-gated calcium channels to changes in the membrane potential is mediated by four distinct voltage-sensing domains (VSD I-IV) coupled to a common pore. Each of these VSDs consist of four transmembrane helices (S1-S4) with S4 containing four to five gating charges. Upon depolarization, these gating charges sense changes in membrane potential and initiate an upward movement of the S4 segment, resulting in the opening of the channel pore. This sliding S4 helix movement is enabled by the formation of consecutive ionic interactions between the gating charges and negative countercharges in the surrounding helices. The charge transfer center (CTC), a highly conserved structure of the voltage sensor, is crucial for catalyzing the movement of the positive gating charges across the membrane electric field. However, the exact mechanism how the VSD state transitions are regulated and the role therein of the interactions between gating- and countercharges is unclear. To investigate the functions of these transient ionic interactions in the CTC and their role in regulating calcium channel gating, we combined structure-guided site-directed mutagenesis with patch-clamp analysis in dysgenic (Ca_V1.1-null) myotubes reconstituted with mutated Ca_V1.1 constructs. Our findings indicate that interactions between the S4 gating charges and countercharges of the CTC serve two roles in the voltage-sensing process of Ca_V channels. The delicate balance between interactions stabilizing the resting or activated states fine-tunes the voltage-dependence of activation. Their action in catalyzing the sequential transitions of the gating charges across the hydrophobic constriction site determines the activation kinetics of calcium currents.



Factors influencing the thermal stability of the bacterial water channel AQPZ and their functional consequences

Wieland Lackinger[1], Sascha Gratzl[1], Julian Beck[2], Johannes Wachlmayr[1], Christine Siligan[1], Sandra Posch[1], Nikolaus Gössweiner-Mohr[1], Gustav Oberdorfer[2], Andreas Horner[1]

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Lipid-protein interactions are crucial in determining the stability and function of transmembrane proteins. The interactions that occur at the lipid-protein interface or bilayer properties can affect protein conformation. Protein oligomerization can lead to structural and proteolytic stability, functional diversity, regulatory mechanisms, and the formation of binding cavities. However, the contributions of lipid-protein interactions and oligomerization to membrane protein stability remain unclear. We aimed to examine the contributions of the oligomeric assembly and specific lipid interactions on the thermal stability of AQPZ, a tetrameric bacterial water channel. To investigate this, we compared the wild-type protein to a de novo-designed protein variant with decreased oligomeric stability and increased monomeric stability, both reconstituted into three different lipid compositions. To examine the interplay between protein stability and function we also utilized purified and reconstituted AQPZ to estimate quantitative water permeability values via a combination of stopped-flow assays, fluorescence correlation spectroscopy and dynamic light scattering. Our results show that the tetrameric assembly is the primary determinant of thermal stability. Furthermore, both variants were more stable in the nature-like lipid environment (PLE) as compared to simplified lipid mixtures, indicating the importance of lipid tail complexity in protein stability. The effect of cardiolipin on protein stability was more pronounced in the destabilized AQPZ variant. Interestingly, in contrast to the importance of lipid chain complexity for protein stability, cardiolipin seems to be essential for protein function, as indicated by an increased single-channel water permeability similar to PLE. These findings improve our understanding of the structure-function relationship of oligomeric transmembrane channels and aid the development of optimized AQPZ variants for use in synthetic biology.

New Lipid-Bilayer Nanodiscs for Membrane-Protein Biophysics

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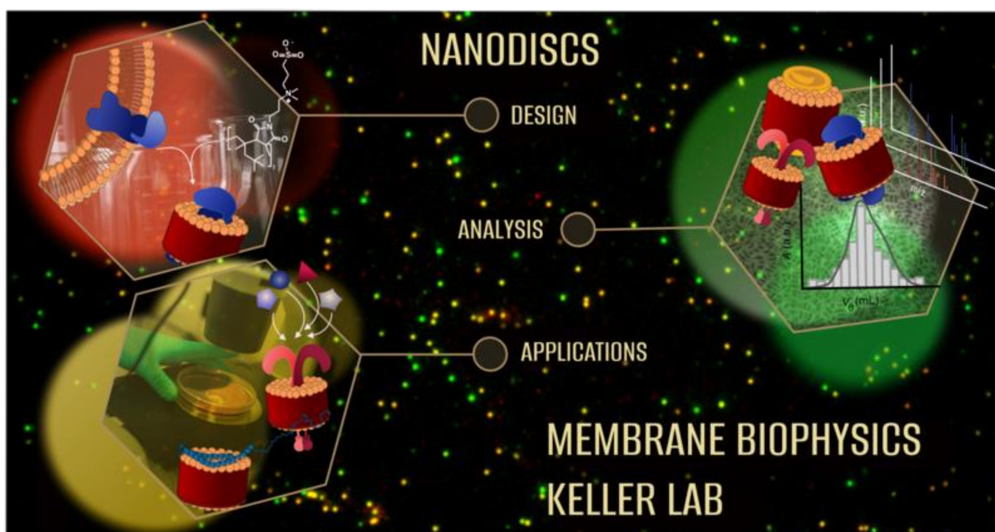
Nanodiscs that harbour individual membrane proteins or membrane-protein complexes in a nanoscale lipid-bilayer environment hold great promise for biophysical investigations under well-controlled yet native-like conditions. Our laboratory focusses on new methods for the direct, detergent-free extraction of membrane proteins from cellular membranes into native nanodiscs, which preserve both the overall bilayer architecture and the local lipid composition of the original cellular membrane. Thus, these native nanodiscs render membrane proteins amenable to *in vitro* biophysical investigations without ever removing the proteins from a lipid-bilayer environment. Recently, we have developed and used novel amphiphilic polymers and small-molecule amphiphiles with improved properties for forming native nanodiscs that are compatible with a broad range of ensemble and single-molecule biophysical techniques.[1–3] In this contribution, we will present selected examples including (i) clinical antibody–receptor interactions (Trastuzumab/HER2) and lipid-specific membrane partitioning of proteins (α-synuclein) studied by microfluidic diffusional sizing (MDS); (ii) native membrane-protein libraries investigated by mass spectrometry (MS); and (iii) cell-free translation of functional membrane proteins (G protein-coupled receptors, GPCRs) with co-translational protein insertion into native nanodiscs.



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Single molecule characterization of lectin binding to SARS-CoV-2 spike by atomic force microscopy

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New SARS-CoV-2 variants are continuously emerging with critical implications for therapies or vaccinations. The 22 N-glycan sites of the Spike protein remain highly conserved among SARS-CoV-2 variants, opening an avenue for robust therapeutic intervention. By using a nanomechanical force-sensing approach, we obtained real-time information about the molecular bonds involved in the binding of various carbohydrate-binding proteins, so-called lectins, to viral spike proteins. Out of a lectin library, two lectins, Clec4g and CD209c, were identified to strongly bind to the spike protein of SARS-CoV-2. To study spike binding of lectins at the single molecule level, we applied atomic force microscopy based single molecular force spectroscopy. We quantified unbinding forces and analysed the number of bond ruptures between Clec4g/CD209c and the trimeric spike protein. Multiple bond formations lead to stable complex formation, in which the number of formed bonds enhanced the overall interaction strength and dynamic stability of the lectin/spike complexes. Equipped with suchlike molecular modalities, Clec4g/CD209c are multivalent efficient competitors in SARS-CoV-2 spike binding to cellular ACE2.[1]

We also determined the binding capacity of a molecularly engineered lectin cloned from banana, BanLec H84T, which was shown to display broad-spectrum antiviral activity against several RNA viruses.[2] Our studies revealed that H84T-BanLec interacts with the Spike protein of the original viral strain, Wuhan-1 and several variants of concern (Delta, Omicron). Based on our force probing technique, dynamic molecular interaction patterns with accurate rupture force and length distributions were depicted. The complex multiple binding features between the dimeric H84T and trimeric spike protein were analysed with respect to the distribution of the glycosylation sites on the spike. Using high speed AFM, we additionally imaged spike proteins complexed with isolated lectin molecules to visualize oligomeric states and complex formation. Our data obtained by AFM techniques elucidate lectin-spike interactions at the single molecule level and uncover candidate receptors involved in spike binding and SARS-CoV-2 infections. The capacity of lectins to block SARS-CoV-2 viral entry holds promise for pan-variant therapeutic interventions.

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[2] A molecularly engineered, broad-spectrum anti-coronavirus Lectin Inhibits SARS-CoV-2 and MERS-CoV Infection In Vivo, Cell Reports Medicine, 3 (2022) 100774.

Fluorescence correlation spectroscopy-based optimization of membrane protein reconstitution into liposomes

Sandra Posch[1], Xenia Fischer[1], Maryam Marefat[2], Martina Hofmann[2], Andreas Karner[2], Johannes Preiner[2], Andreas Horner[1]

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Reconstitution of membrane proteins into liposomes is an indispensable technique in the field of membrane protein research. It enables the structural and functional characterization of membrane channels, transporters and receptors in a simplified experimental system. However, the development of reconstitution protocols is often based on a biased trial-and-error approach, even though it is vital in membrane protein biophysics, nanobiotechnology, and medical research. Additionally, existing methods to produce proteoliposomes are facing protein loss, protein aggregation, and heterogeneity in the number of proteins per vesicle. To overcome these issues, we introduce a method based on fluorescence correlation spectroscopy (FCS). FCS is a powerful technique that quantifies molecular dynamics to deduce the average number of fluorescent molecules and their diffusion time through the detection volume. Our procedure involves the detergent-based extraction and purification of membrane proteins, followed by their reconstitution into lipid mimetic systems. Thereby, we measure the number, brightness and the mobility of fluorescently labeled membrane proteins and lipids over time for each step of the reconstitution protocol using FCS. In a feedback loop, we use the insights gained by systematically varying all relevant experimental parameters, such as temperature, type, concentration of detergent and lipid, buffer and the method and speed of detergent extraction to adjust the reconstitution protocol. We applied our method to the reconstitution of the pH-dependent bacterial urea channel *Ss*UreI, which plays a crucial role in the acid acclimation of Streptococcus salivarius. This bacterium is involved in maintaining oral pH balance and has a significant impact on dental health, including dental caries, calculus formation, gingivitis, and periodontitis. Additionally, we applied our method to an immunoglobulin G (IgG) Fc receptor (FcγR), which is expressed on most innate and adaptive immune cells and plays crucial roles in antibody-mediated immune responses such as phagocytosis, release of inflammatory mediators, and antibody-dependent cellular cytotoxicity. Overall, our method is suitable for a wide variety of fluorescently membrane proteins and has the potential to streamline reconstitution into proteoliposomes.

Mouse retinal rod bipolar cells express different L-type Ca channel transcripts

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Retinal ribbon synapses provide sustained glutamatergic transmitter release that, in photoreceptors, is controlled by Cav1.4 L-type calcium channels. Because bipolar cell reports are less conclusive, we addressed the voltage-gated calcium channels (VGCC) in mouse rod bipolar cells (RBC). Among the L-type calcium channel family we found transcripts of Cav1.3 in short and long forms, Cav1.4 and a novel splice variant of Cav1.1, as well as the accessory subunits β2 and α2δ-4 in FACS-sorted RBCs. Our RNAseq data screening revealed all annotated Cav1.4 exons and splice variants of Cav1.3 with exons 8a, Δ11, 31b and 32. The retinal Cav1.1 transcript lacks the first initial 15 exons, but includes two novel exons at the N-terminus that get alternatively spliced, which we confirmed by RT-PCR. Though there was substantial Cav1.4 transcript, our whole-cell patch-clamp recordings in RBCs showed that VGCC current persisted when knocking out Cav1.4 (I_{max} WT: -34,6 ± 7,5 pA (n=8), Cav1.4-KO: -19,7 ± 4,8 pA (n=8); p = 0,0003; unpaired students t-test). While it is unclear, if the retinal Cav1.1 transcript can even form functional channels, Cav1.3 could compensate for Cav1.4. However, preliminary data from Cav1.3 deficient mice suggested that Cav1.3 has no compensatory role, because calcium currents were not significantly decreased compared to WT (I_{max} WT -34,6 ± 7,5 pA (n=8), Cav1.3-KO -44,5 ± 5,7 pA (n=4); p=0,19; Mann-Whitney test). Our patch-clamp recordings further indicated the presence of at least two different types of VGCC's in RBCs. Recording from different holding potentials (HP), we observed a sustained high-voltage activated L-type (HP -50 mV) and a transient, low-voltage activated T-type calcium current (HP -100 mV). The analysis revealed distinct activation thresholds in WT RBCs: -36,4 ± 2,2 mV at -50mV and -48,7 ± 4,9 mV at -100mV; p<0,0001; unpaired students t-test. In conclusion, our investigation identified multiple Cav-transcripts in RBC's. We were also able to distinguish between L-type and T-type calcium currents using patch-clamp electrophysiology recordings. However, the source of the remaining VGCC component still needs to be explored through additional functional and pharmacological experiments.

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Mechanical forces in T cell receptor–ligand interactions

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Using their T cell receptors (TCRs), T cells scan the surfaces of antigen-presenting cells (APCs) for antigenic peptides mounted on major histocompatibility complexes (pMHCs). This interaction is of fundamental importance for the adaptive immune system as it constitutes the basis for the specific identification of harmful substances. Despite its importance, critical facets of this recognition process have remained elusive, including the impact of biophysical parameters such as mechanical forces. We developed a FRET-based molecular force sensor to be inserted into the immunological synapse in order to quantify the exact amount of mechanical force exerted by TCRs. The sensor contains a peptide derived from spider silk acting as a molecular spring. A donor and an acceptor fluorophore attached near the opposing termini allow for monitoring force-induced changes in the end-to-end distance via the single-molecule FRET efficiency.

Experiments performed with primary murine CD4+ T cells carrying a specific TCR and sensors functionalized with stimulating pMHC revealed only minute forces (~2.5 % of events exceeding 4 pN) when seeded on gel-phase lipid bilayers serving as surrogate APCs, and negligible forces (< 0.5 % exceeding 4 pN) on fluid-phase bilayers. Higher overall forces were observed when choosing receptor–ligand pairs establishing longer-lived bonds. In all cases the forces on gel-phase bilayers exceeded those on liquid-phase bilayers, indicating that their origin is mainly the lateral motion of TCRs within the T cell membrane.

It has been postulated that TCR–pMHC binding lifetime—presumably a critical parameter in T cell antigen recognition—is increased by mechanical forces for stimulating peptides, i.e., dynamic catch–slip bonds are formed. To investigate, we directly measured lifetimes via single-molecule FRET between donor fluorophores on TCRs and acceptor fluorophores coupled to pMHCs. Considering the aforementioned influence of lipid bilayer fluidity on the forces exerted on the TCR–pMHC bonds, pMHCs anchored to gel-phase bilayers were contrasted with pMHCs anchored to fluid-phase bilayers. These experiments revealed no evidence of dynamic bonds.

Exploring the role of TRPC1-IP3R cross-talk in the ER homeostasis

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Background: Transient receptor potential canonical proteins (TRPC1-7) are a group of calcium (Ca^{2+}) permeable cation channels that exhibit a significant presence in the brain. Among the seven isoforms, TRPC1 stands out for its role in hippocampal physiology and pathophysiology via the regulation of cellular Ca^{2+} homeostasis. A peculiar feature of TRPC1 is its targeting to the ER membrane, where it can potentially physically associate with other proteins involved in Ca^{2+} release mechanisms such as inositol 1,4,5-trisphosphate receptors (IP3R) or function as a Ca^{2+} leak channel on its own. The engagement of TRPC1 in the ER Ca^{2+} release could potentially affect the extent of Ca^{2+} signaling within the cell, influencing various cellular processes.

Aims: Study the role of TRPC1 in ER Ca^{2+} dynamics and its potential interaction with IP3R

Methods: In this study we conducted confocal microscopy, fluorescence protease protection (FPP) assay, FRET microscopy (with ER-targeted D1ER) and Ca^{2+} imaging using FURA 2-AM dye in transiently transfected HEK293 and HEK IP3R KO cells.

Results: We confirmed TRPC1 channels cellular targeting to the ER membrane via confocal microscopy. Furthermore, FPP assay allowed us to determine the topology of TRPC1 within the ER membrane. FRET experiments showed a significantly lower Ca^{2+} efflux via IP3R after application of carbachol (CCh) when TRPC1 was present, as opposed to control cells without TRPC1. When introducing various mutations in TRPC1, we observed alterations in the channel's activity with the most prominent changes observed in the D582K mutant. The presence of the D582K mutation restored Ca^{2+} release levels to match those observed in control cells. Moreover, Ca^{2+} imaging experiments using FURA 2-AM dye revealed alterations in the Ca^{2+} release behavior of IP3Rs in the presence of TRPC1.

Conclusions: TRPC1 is targeted to the ER membrane and affects ER Ca^{2+} release mechanisms. This indicates that TRPC1 potentially acts as a Ca^{2+} leak channel in the ER. Furthermore, TRPC1 alters the IP3R activity through mechanisms that are yet to be determined.

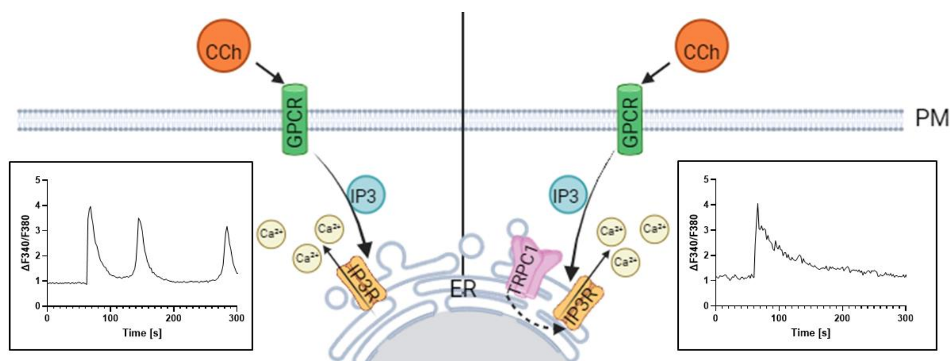

Figure 2: TRPC1 alters the IP3R calcium release behavior. Binding of carbachol (CCh) to G protein-coupled receptors (GPCR) leads to the activation of the Phospholipase C pathway and the subsequent binding of inositol 1,4,5-triphosphate (IP3) to IP3 receptors (IP3Rs) in the ER which triggers ER calcium depletion via IP3Rs. TRPC1 potentially interacts with IP3Rs and alters their calcium release pattern.

Surface Symphony: Orchestrating DPPC Monolayer Behavior

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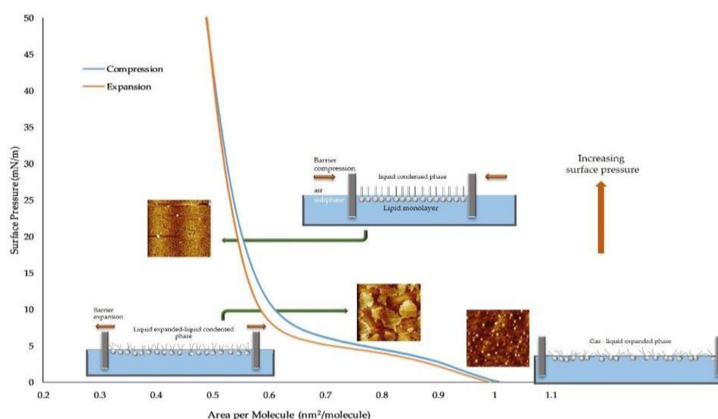
Lipid monolayers, which mimic key features of biological membrane lipid bilayers, provide an ideal experimental platform for the study of membrane-related phenomena. Their simplicity allows systematic manipulation of lipid composition, packing density and surface pressure, facilitating the elucidation of membrane behavior under different conditions. This study uses atomic force microscopy (AFM) to investigate the behavior of DPPC monolayers, focusing on subphase variations, changes in lipid fluidity and protein interactions. Systematic experiments reveal significant differences in DPPC monolayer morphology between subphases, highlighting their sensitivity to environmental changes and the importance of subphase composition in modulating lipid behavior. Furthermore, manipulation of lipid fluidity by the introduction of additional lipid (POPC) leads to a change in lipid domain organization. This suggests a critical role for lipid fluidity in shaping DPPC monolayer architecture and influencing structural properties. The study also examines the effects of protein penetration, focusing on lysozyme and human serum albumin (HSA). This demonstrates protein-induced disruption of lipid domains within the monolayer, suggesting potential protein-lipid interactions that could affect membrane integrity and functionality. Overall, this research provides valuable insights into the behavior of DPPC monolayers, enhancing the understanding of lipid membrane dynamics and having implications for biomedical and drug delivery systems.

Keywords: DPPC-POPC monolayers, AFM, lipid domains, subphase variations, protein interaction.



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Modulation of the membrane and functional expression of the voltage sensor of EC coupling $Ca_V1.1$ by ERC1

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ERC1, a member of the family of CAST/ELKS scaffold protein, is responsible for supporting the structure of presynaptic active zones. ERC1 directly interacts with the $Ca_V\beta$ subunit of voltage-gated Ca^{2+} channels (VGCC) through the GK domain [1]. Importantly, this interaction affects VGCC activity, as ERC1 deletion leads to reduced calcium influx at inhibitory synapses in the hippocampus, the calyx of Held, rod photoreceptors, and pancreatic β-cells [2; 3; 4]. Here, we hypothesized that ERC1, which is endogenously expressed in skeletal muscle, might influence the membrane and functional expression of $Ca_V1.1$, as well as voltage-induced Ca^{2+} release from the sarcoplasmic reticulum.

First, to analyse the effect of ERC1 on $Ca_V1.1$ functional expression, we performed patch-clamp experiments in HEK cells and found that ERC1 increases $Ca_V1.1$ current density by 63%. The suggested $Ca_V\beta$ binding fragment ERC1 1-404 [1], only partially modulates $Ca_V1.1$ currents in HEK cells (29.4%). Furthermore, we generated skeletal muscle ERC1 knockout cell lines with CRISPR/Cas9 and examined the impact of ERC1 deletion on $Ca_V1.1$ and RyR1 levels using immunocytochemistry. Whereas $Ca_V1.1$ cluster intensity is reduced (by 15%), RyR1 expression remains unchanged. Additionally, we implemented electrophysiology coupled with calcium transients recordings on muscle cells, upon ERC1 deletion or reconstitution. ERC1 deletion does not significantly affect $Ca_V1.1$ current amplitude, but it reduces EC coupling by 54%.

Our results demonstrate that ERC1 increases the number of $Ca_V1.1$ channels in the membrane of skeletal muscle cells and $Ca_V1.1$ current density in HEK cells. In muscle cells, ERC1 plays a role in the conformational coupling with RyR1.

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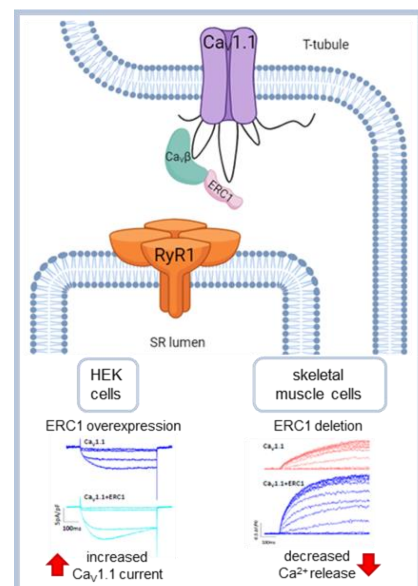

Measuring Antibody–Oncoprotein Interactions in Native Nanodiscs

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We present a method for determining absolute concentrations and affinities of target receptors in native membrane-protein libraries extracted directly from cell cultures. To demonstrate our approach, we focus on HER2, a membrane protein that is overexpressed in 20–25% of all breast cancers and serves both as a diagnostic marker and as a therapeutic target.

HER2-positive patients are commonly treated with the monoclonal antibody trastuzumab (Trz, trade name Herceptin), which we also used in our experiments. However, not all patients with HER2 overexpression respond to Trz treatment. One reason for this could be that the concentration of full-length (i.e., binding-competent) HER2 varies substantially from patient to patient. Therefore, it would be desirable to measure cellular copy numbers of binding-competent HER2, as therapies could then be personalized more accurately for each patient.

To address this need, we introduce a fast and simple method for measuring membrane-protein affinity and copy number (MAffCoN).[1] Key to our method is the use of polymer-encapsulated nanodiscs, which retain the protein in a native-like lipid-bilayer environment.[2,3] Using orthogonal microfluidic techniques—microfluidic diffusional sizing (MDS) and flow-induced dispersion analysis (FIDA)—we then measured the binding affinity of HER2 to fluorescently labeled trastuzumab, yielding sub-nanomolar dissociation constants (K_D) and absolute numbers of ~10^6 binding-competent HER2 protein copies per AU565 breast cancer cell.[4,5]

MAffCoN is straightforward to implement in standard research laboratories and has the potential to be integrated into or improve existing drug-discovery workflows. As we demonstrate, MAffCoN is easily adaptable to different microfluidic techniques, highlighting its potential to be used as a diagnostic tool for tissue analysis.

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Investigation of the neurokinin receptor 1 structures and its regulation by its endogenous activator substance P and the antagonist aprepitant

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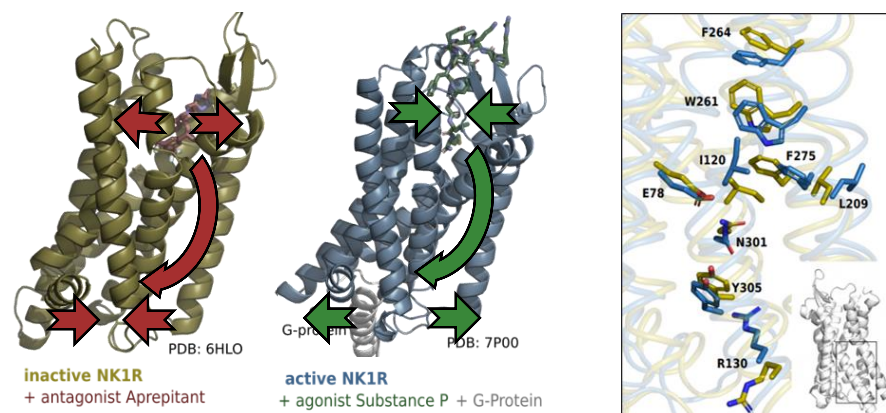
The membrane-spanning neurokinin 1 (NK1) receptor is a G-protein coupled receptor (GPCR) involved in sensory transmission and pain. The human NK1 receptor is found in endothelial cells, the nervous system, smooth muscle, and on T-cells in the immune synapse. It is implicated in an array of pathological processes, such as nausea, analgesia, depression, and inflammation. NK1 has highly selective endogenous agonist, a peptide called substance P (SP), and hemokinin/endokinin peptides. Upon binding, conformational changes of the receptor lead to the binding of heterotrimeric G protein subtypes Gq and Gs, and therefore intracellular signal transduction. Antagonists of the NK1 receptor used for the treatment of for example chemotherapy-induced nausea are aprepitant and netupitant. However, the dynamic molecular changes of the activation pathway associated with the binding of SP, Gq (or Gs), and the antagonist are hardly understood.

Several structures of the NK1 receptors have been published so far. In this study, we concentrate on the structures with SP (pdb-code: 7P00[1]) and aprepitant (pdb-code: 6HLO[2]) bound. Interestingly, the structures show that SP and aprepitant compete for the same binding site. We perform long-scale molecular dynamics simulations to probe how binding at the orthosteric pocket propagates to the intracellular site where the C-terminus of the G-protein is inserted. Therefore, we perform simulations of both structures without any ligand bound, with SP bound, and with aprepitant bound. Our first findings highlight that upon placing of aprepitant in the activated (SP bound) structure, we see conformational changes associated with receptor inactivation.



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