channels. Replacing the tail of SUR2A with that of SUR1 switched the ATP sensitivity in the presence of MgADP to that of SUR1 (and v.v.). These data suggest that the 'tail' of SUR plays a critical role in coupling nucleotide binding/hydrolysis to channel activation, and that this differs between cardiac and beta-cell KATP channels.

1025-Plat Structural Details of an Allosteric Mechanism for Bimodal Anesthetic Modulation of Pentameric Ligand-Gated Ion Channels Rebecca J. Howard,1 Zaineb Fourati,1 Stephanie A. Heusser,1 Haidai Hu,1 Reinis R. Ruža,2 Ludovic Sauguet,3 Erik Lindahl,1,3 Marc Delarue,2 1Science for Life Laboratory, Department of Biochemistry & Biophysics, Stockholm University, Solna, Sweden, 2Unit of Structural Dynamics of Macromolecules, Institut Pasteur, Paris, France, 3Theoretical and Computational Biophysics, KTH Royal Institute of Technology, Stockholm, Sweden.

Ion channel modulation by general anesthetics is a vital pharmacological process with implications for receptor biophysics and drug development. Although functional studies have identified conserved sites of both positive and negative anesthetic modulation in pentameric ligand-gated ion channels, a structural understanding of these bimodal effects is lacking. The prokaryotic model GLIC recapitulates anesthetic modulation of human ion channels, and is accessible to structure determination in both open and closed states. Here, we report new mutagenesis, electrophysiology, and crystallography data on GLIC variants in the presence and absence of general anesthetics, mapping a mechanism for allosteric modulation. In particular, we document the crystallographic conversion of two GLIC variants from apparent closed to open states, solely attributable to anesthetic binding within the transmembrane domain of each receptor subunit. These and other targeted mutations alter anesthetic effects by shifting the relative stability of closed and open, apo and holo states, involving at least three binding sites in the transmembrane domain. Our results support an integrated, multi-site model for allosteric modulation, and provide atomic details of both positive and negative modulation by one of the most common general anesthetics, providing potentially fruitful templates for drug development.


Inhibition of the M2 proton channel in the influenza A virus prevents viral replication from occurring. Two of the four FDA-approved drugs for the treatment of influenza infections, amantadine and rimantadine, target the M2 channel. However, multiple M2 structures have been determined in the presence of lipidic cubic phase (LCP) crystallization techniques. We present the first crystal structures of rimantadine bound to M2 in both the Inwardclosed and Inwardopen conformations of the channel (2.0 Å, 2.5 Å), as well as amantadine bound to the Inwardclosed configuration (2.0 Å). At this resolution range the orientation of the bound drug is unambiguous, and the ammonium group of the adamantane drugs can be seen interacting with ordered water molecules present within the channel. Additionally, we report the binding of a dual-inhibiting compound to both the wild type channel (2.6 Å) and the drug-resistant V27A mutant (2.5 Å). The position of the bound inhibitor within the channel shifts in the presence of the V27A mutation. These structures further our understanding of drug binding and inhibition within the M2 proton channel and will help guide the design of compounds for the inhibition of drug-resistant mutants of the influenza M2 channel.

1027-Plat Azobenzene-Based Photoswitches for the Control of the Voltage-Gated Proton Channel Hv1 Andreas Rennhack, Elena Grahn, U. Benjamin Kaupp, Thomas K. Berger. Molecular Sensory Systems, Research Center Caesar, Bonn, Germany.

The voltage-gated proton channel Hv1 is expressed in the plasma membrane of various human cells, including macrophages, lung epithelial cells, and sperm. Hv1 opening leads to an efflux of protons and thereby alkalizes the cell. In macrophages, Hv1 counteracts cytosolic acidification caused by NADPH oxidase activity during the respiratory burst. In sperm, Hv1 might be involved in maturation. To better understand the physiological role of Hv1, we developed a light-controlled inhibitor that allows us to control Hv1 with spatiotemporal precision. We synthesized photosomerizable Hv1 inhibitors (photoGBIs) by combining a light-sensitive azobenzene group with a known Hv1 inhibitor, 2-guanidinobenzimidazole (2GBI). The azobenzene group isomerizes from cis to trans under blue light illumination and thermally relaxes back from cis to trans in the dark. Four analogs (photoGBI1-4) were characterized on Hv1 channels heterologously expressed in X. laevis oocytes using the excised patch-clamp technique. Light-induced conformational changes of the photoGBIs changed their efficacy of inhibition: in the dark, photoGBIs inhibit Hv1, and under blue light, inhibition is released. PhotoGBI4 modulates proton currents of human sperm and macrophages and holds the promise to further our understanding of Hv1-mediated pH regulation.

Platform: Systems Biophysics

1028-Plat Biophysical Techniques for the Study of Phase Transitions in Protein Droplets and Cells Raimund Schlüessler1, Shada Abuhattum1, Gheorge Cojoc1, Timon Beck2, Felix Reichel2, Kyoo Hyun Kim2, Mirjam Schürmann3, Paul Müller3, Jürgen Czarske2, Vasily Zubarev4, Titus Franzmann5, Simon Alberti5, Jochen Guck6.

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Recently, phase transitions of protein-RNA droplets in cells, and of the cytoplasm of entire cells, have been shown to play important roles in physiological and pathological processes in biology. Their molecular control is still unclear at this point. We have developed and are applying a range of biophysical techniques for the specific study and quantitative characterization of such phase transitions. We use a dual-beam laser trap, real-time deformability cytometry, and atomic force microscopy for the viscoelastic characterization of cells and protein-RNA granules in vitro. We combine opto-fluidic object rotation with quantitative phase microscopy to obtain phase images from multiple angles, which in turn are tomographically reconstructed by a back-propagation algorithm to obtain 3D distributions of refractive index and mass density inside trapped objects. Finally, we have also established Brillouin microscopy for the 3D mapping of mechanical properties inside cells with diffraction-limited resolution. I will present and discuss our findings obtained on phase-separated FUS protein droplets and on phase transitions in yeast cells under different kinds of stress with this unique toolset.

1029-Plat Analysis of Apoptotic Event Time Correlations in Single Cells Alexandra Murchhauser1, Peter Rüttgermann1, Daniel Wosche1, David Garry1, Martina Ober1, Kenneth Dawson1, Joachim O. Rädler1, 1Faculty of Physics and Center for NanoScience (CENs), Ludwig-Maximilians University (LMU) Munich, Munich, Germany, 2Centre for BioNano Interactions, School of Chemistry and Chemical Biology, University College Dublin, Dublin, Ireland.

Signaling cascades in apoptosis are known to be highly heterogeneous at the single-cell level both in effect and dynamics. Cell fate decisions are the final result of various possible pathways, each involving organelle-specific signals as key players. Lysosemes as well as mitochondria are believed to be central actors in apoptosis, but also the impact of reactive oxygen species (ROS) production is known to be involved in cell death. The timing of cell death events and their sequence, however, are not fully explored yet. Here, we establish a method to analyze fluorescence time courses of single cells during apoptosis induced by amino-functionalized polylysine nanoparticles (PS-NH2) and the protein kinase c inhibitor staurosporine with automated time-lapse microscopy on micro-structured arrays. We monitor chronological order of cell death events with high temporal resolution using time-correlation analysis by the use of two different fluorescence markers per cell. In particular, we studied: lysosomal membrane permeabilization (LMP), mitochondrial outer membrane permeabilization (MOMP), increase of ROS level and oxidative burst (Ox-Burst), caspase 3 activation (CASP-3), exposure of phosphatidylserine to the outer membrane (PhS-FLIP) and loss of plasma membrane integrity with the subsequent nucleus staining (PMP). We find distinct timing in the signal pathway and variance in event correlations indicative of cross-talk in the event cascade of apoptosis. Our results indicate that both the mitochondria and the lysosomes catalyze separately the subsequent processes. In summary, multi-dimensional time-correlation provides a dynamic fingerprint of signal progression that is capable to identify effects at the single-cell level.