transferred into PcTx1. These channel chimeras retained robust voltage-dependent activation, however they remained insensitive to PcTx1, and the toxin chimera did not inhibit Kv2.1. In addition, we used Rosetta structural modeling methods to construct docking configurations homologous to the PcTx1-ASIC structure where GxTx-1E is bound to the S3b helix of the Kv1.2/2.1 paddle chimera. Although the model has an energetically favorable peptide-protein interface, it requires that GxTx-1E span the lipid bilayer, exposing many polar residues on GxTX-1E to a hydrophobic environment. These results suggest that tarantula toxins use common surfaces to bind to structurally unrelated ion channels, yet the precise mechanisms of interaction are distinct.

3731-Pos Board B459

Binding Structure & Dynamics for Toxins Modifying the Gating Mechanism of Kv Channels

Anders Gabrielsson[1], Sara Liin[2], Fredrik Elinder[2], Erik Lindahl[3].
[1]Theoretical Physics, Royal Institute of Technology, Stockholm, Sweden, [2]Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden, [3]Science for Life Laboratory, Stockholm University, Stockholm, Sweden.

Voltage gated ion channels allow the flux of specific ions through the cell membrane to be controlled by the membrane potential, which makes them a critical component for electro-chemical signaling in the nervous system. The gating is regulated by the voltage sensor domains (VSD), in which the charged S4 helix moves to induce the conformational transition between open/closed states. This serves as an ideal model system for membrane proteins with multiple conformations, and if the gating can be modulated it would potentially enable a whole range of new drugs that could fine-tune the gating response. This is strikingly manifested in the case of tarantula spiders, which produce a potent venom that specifically target the VSD to causing dysfunctions in the neuronal and cardiac systems.

Here, we report on molecular simulations of interactions between toxins and different Kv channels, in particular related to their influence of the gating transition. We have studied the binding to multiple intermediate states and effect of Stromatoxin (ScTx1) on gating transitions in both Kv2.1 and a mutant Shaker channel with enhanced toxin affinity. Results from toxin-protein docking show the toxin binding to S2 and S3 helices. An interaction pair is formed between E277 (Kv2.1 numbering) in the top-most part of S3 and either R4 or K22 on opposite sides of the toxin, resulting in two distinct poses in the binding site. In addition to docking, we have also explored multi-microsecond molecular dynamics simulations of toxins bound in both open and closed states.

We propose a specific binding site for the toxin in the VSD of both channels, and predict that differences in residue interactions are responsible for selectively stabilizing S4 in either the open or closed state.

3732-Pos Board B460

Expression of Different Subunits of the Calcium-Regulated BK Channel in Rat Brain and Its Putative Cytoprotective Properties

Bartlomiej S. Augustynek[1], Anna Kajma-Olszewska[1], Wolfram S. Kunz[2], Adam Szewczyk[1].
[1]Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland, [2]Department of Epileptology, University of Bonn Medical Center, Bonn, Germany.

BK_{Ca} is ubiquitously expressed in the plasma membrane of both excitable and non-excitable cells. Although it was extensively studied mainly in plasma membrane, its counterpart exists also in mitochondria (mitoBK_{Ca}). It was identified in mitochondria of several cell types including astrocytes and neurons. Potassium channels are notorious for their role in cytoprotection but the mechanisms of these properties remain to be elucidated. However, it is suspected that attenuation of ROS generation and mild uncoupling in mitochondria occurring upon activation of the mitoBK_{Ca} may play some role in this phenomenon.

In the current study we have evaluated the expression pattern of different subunits of the BK_{Ca} channel within certain areas of rat brain. We could show that there is a correlation between the mtDNA copy number and the expression level of the BK_{Ca} β4 auxiliary subunit. The expression of the β1, β2 and β3 subunits in different brain areas does not seem to correlate with the mtDNA copy number.

This strongly suggest that β4 auxiliary subunit might be a constituent of mitoBK_{Ca} in rat brain. This finding supports the recent report about the expression of this subunit in the astrocytoma U-87 MG cell line[1].

The presence of the mitoBK_{Ca} in brain can explain why NS-1619 (a BK_{Ca} opener) inhibits ROS generation rate and causes mild dissipation of the membrane potential in succinate energized rat brain mitochondria.

1. Bednarczyk, P. *et al.* Putative Structural and Functional Coupling of the Mitochondrial BKCa Channel to the Respiratory Chain. *PloS one***8,** e68125 (2013).

This study is supported by the International PhD Program in Neurobiology coordinated by The Foundation for Polish Science and financed from the European Funds.

3733-Pos Board B461

Calcium Dependent Stoichiometries of an SK2 Intracellular Domain/Calmodulin Complex

David B. Halling[1], Sophia A. Kenrick[2], Richard W. Aldrich[1].
[1]Neurobiology, University of Texas at Austin, Austin, TX, USA, [2]Wyatt Technology Corporation, Santa Barbara, CA, USA.

Ca^{2+} activates SK channels through the ubiquitous Ca^{2+} sensor, calmodulin (CaM). The process of SK gating by Ca^{2+} is a dynamic process that involves Ca^{2+} binding to CaM and CaM affecting channel opening. A commonly held view is that an SK channel activates upon a change in conformation of the SK/CaM complex upon binding Ca^{2+}. We determined how Ca^{2+} affects CaM binding to a recombinant SK peptide, SKp, which is identical to a fragment of SK2 that binds CaM. Composition gradient multi-angle light scattering (CG-MALS) was used to measure the molar masses and affinities of different stoichiometries that form between SKp and CaM. Based on earlier crystallographic studies, we expected to find a 2SKp/2CaM complex. We found multiple stoichiometries can form both at <5 nM Ca^{2+} (zero Ca^{2+}) and at saturating Ca^{2+} (>2 mM). At zero Ca^{2+} we observe a 1SKp/1CaM and a 2SKp/1CaM complex. At saturating Ca^{2+} we observe the same stoichiometries plus a 1SKp/2CaM complex. In solution, we do not see a 2SKp/2CaM complex. Our data suggest that the current, simple view of SK channel gating will need to be reevaluated in the context of Ca^{2+} dependent stoichiometry.

3734-Pos Board B462

Examining Protein, Lipid and Water Distribution in Lipid Membranes with Potassium Channel KcsA

Ella Mihailescu[1], Joseph Blasic[1], David Worcester[2].
[1]Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, USA, [2]Physiology and Biophysics, University of California, Irvine, CA, USA.

The potassium channel from the soil bacteria Streptomyces lividans named KcsA, is the first of the potassium-selective channel proteins whose structure has been solved crystallographically (Doyle, D.A. et al., Science 280, 1998). KcsA has become the principal model for the pore domain in voltage-gated K^+, Na^+, and Ca^{2+} channels due to highly conserved structural motifs in the pore domain characteristic for these channels. Using deuterium labeling and neutron diffraction we have examined the distribution of water, lipid acyl chains and protein across a lipid membrane containing KcsA channels. The cavity dimensions for KcsA inferred from crystallographic structures allow less than 20 water molecules to be accommodated. We find that much more water is present in the core of the channel within a bilayer environment. We present density profiles of the protein and the lipid acyl chains. Our results reveal how the lipid adapts to the protein morphology such that to fill in density gaps in the hydrocarbon region.

3735-Pos Board B463

Influence of Lipid Bilayer Thickness on Ion Channels Using Single-Channel Voltage-Clamp Fluorescence Imaging

Hugo McGuire, Rikard Blunck.
GEPROM and department of Physics, University of Montreal, Montreal, QC, Canada.

The lipid molecules forming the cell membrane can strongly influence ion channel function dependent on several physical properties such as the electrostatic charge of the headgroup or the hydrophobic thickness. However, in contrast to other channel modulators such as voltage or ligands, the lipid membrane cannot be readily controlled. In order to modulate the surrounding lipid environment, the channels have to be purified and reconstituted into the desired lipid bilayer matrix. As these steps can prove challenging, the direct effect of lipids on ion channel function remains elusive and the underlying mechanisms poorly understood. Here, we inserted labeled KcsA (E71A) potassium channels into lipid bilayers of different thickness (length of carbon