1339-Pos Board B109
Structural Insights for the Full Length Influenza A M2 Proton Channel in Native E. Coli Membranes
Huajun Qin[1], Yimin Miao[2], Riqiang Fu[3], Mukesh Sharma[4], Thach Can[5], David D. Busath[6], Huan-Xiang Zhou[7], Timothy A. Cross[8].
[1]Department of Chemistry and Biochemistry, National High Magnetic Field Lab, Florida State University, Tallahassee, FL, USA, [2]Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL, USA, [3]National High Magnetic Field Lab, Florida State University, Tallahassee, FL, USA, [4]National High Magnetic Field Lab, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, USA, [5]Department of Physics, Florida State University, Tallahassee, FL, USA, [6]Department of Physiology and Developmental Biology, Brigham Young University, Prova, UT, USA, [7]Department of Physics, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, USA, [8]Department of Chemistry and Biochemistry, Institute of Molecular Biophysics, National High Magnetic Field Lab, Florida State University, Tallahassee, FL, USA.
Membrane proteins are structurally characterized in membrane mimetic environments, environments that may or may not reflect the properties of native membrane proteins. This is critically important since the structure of membrane proteins is influenced by the protein's environment. Here we will show structural data from magic angle spinning solid state NMR of the full length M2 protein from Influenza A observed in *E. coli* membranes. This protein has never been exposed to the denaturing influence of detergents nor to the length isolation, purification and reconstitution protocols that typify membrane protein sample preparation for structural studies. The data will be compared to the purified full length protein in synthetic liposomes and to spectra of smaller constructs for which there is a high resolution structure in lipid bilayers.

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Structure and Topology of the 114kDa Serca-Sarcolipin Complex by Solid State NMR Spectroscopy
Kaustubh R. Mote, Tata Gopinath, Gianluigi Veglia.
University of Minnesota, Minneapolis, MN, USA.
Solid State NMR Spectroscopy is rapidly emerging as a powerful technique to determine structures of membrane proteins and complexes in fully-functional, native-like, lipid-bilayer environments. Developments leading to increased sensitivity and resolution of oriented as well as magic-angle spinning NMR spectra have made it possible to study increasingly bigger proteins by these methods. These techniques allow for the determination of tilt and rotation angles of transmembrane segments, topological parameters that are believed to be dictated by essential changes in membrane protein structure and hence, provide insights into functional aspects. A further advantage of solid state NMR is that being a size-independent technique, it can be applied to large proteins and complexes. A combination of oriented (OSS-NMR) and magic-angle spinning (MAS-NMR) solid state NMR spectroscopy is used here to determine the structure of the 114kDa - Sarcoplasmic Reticulum Calcium ATPase (SERCA) - Sarcolipin (SLN) complex in fully functional lipid bilayer environment.
SLN is small transmembrane protein that regulates SERCA, the calcium ion pump of cardiac and skeletal muscle, by inhibiting its Ca2+ uptake in atria and slow/fast-twitch skeletal muscle cells. Evidence shows a direct association of SLN with the transmembrane helices of SERCA as the driving force behind this functional interaction. OSS-NMR was used to map changes in backbone topology of SLN upon binding with SERCA, while MAS-NMR was used to determine the binding interface and relevant changes in side chains. The determination of this structure will help in understanding the mechanism with which SLN inhibits SERCA. Improvements in solid state NMR techniques reported here will improve the rapid determination of membrane protein and complex structures using solid state NMR spectroscopy.

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Unchanged Transmembrane Segment Kink and Topology Despite Variation in Membrane-Mimetic Environment
David N. Langleaan, Jan K. Rainey.
Dalhousie University, Halifax, NS, Canada.
Membrane mimetics such as micelles, bicelles or bilayers provide an essential tool for studying membrane proteins and their fragments. Following the ''divide and conquer'' strategy, we have determined the structure of the N-terminal tail and first transmembrane segment of the human apelin receptor (residues 1-55, AR55). This was solved using solution-state nuclear magnetic resonance (NMR) spectroscopy in sodium dodecylsuphate (SDS), dodecylphosphocholine (DPC) and 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (LPPG) micelles as well as in a solution of 50% H_2O and 50% hexafluoroisopropanol (HFIP). This high-resolution structure of AR55 consists of a helix-kink-helix motif in the micelle-spanning transmembrane region in all of the membrane mimetics studied. The N-terminal tail of AR55 has a similar structure in all three micelle conditions and has a much higher helical content in HFIP. Dynamics along the polypeptide backbone from ^{15}N nuclear spin relaxation measurements of AR55 are correlated to solvent accessibility determined by paramagnetic relaxation enhancement, allowing definitive comparison of the manner of embedding of the protein. We show that AR55 adopts a highly similar conformation and topology in SDS, DPC and LPPG micelles despite the dramatic differences between these micelles in both headgroup and tailgroup. The transmembrane domain conformation of AR55 in HFIP is largely similar, including at the kink, despite the induction of helical character in the solution exposed N-terminal tail.

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Structure of the Pore Module of a Voltage-Gated Channel in a Lipid Environment at 3.1 Å Resolution
Jose S. Santos[1], Guillermo Asmar-Rovira[2], GyeWon Han[2], Wei Liu[2], Ruhma Syeda[1], Raymond C. Stevens[2], Mauricoo Montal[1].
[1]University of California at San Diego, La Jolla, CA, USA, [2]The Scripps Research Institute, La Jolla, CA, USA.
KvLm is a voltage-gated K^+-selective channel encoded in the genome of *Listeria monocytogenes*. The expression and reconstitution of the isolated pore module (KvLm-PM) has shown that, in the absence of the sensor, the pore module gates with low but measurable voltage-dependence and remains highly selective for K^+. Here we present the first structure of an ion conducting channel crystallized in the lipid cubic phase (LCP) at 3.1 Å resolution. The structure of the tetramer depicts a filter gate in a conductive conformation but with decreased ion occupancy and an activation gate closed both sterically and electrostatically. Comparison with other K^+ channel structures in which the activation gate is closed highlights for KvLm-PM a permeation path between the filter and the activation gate that is significantly narrower and shorter. The lipid environment results in the immobilization of four lipid molecules per monomer all clustered in a crevice between subunits at the cytoplasmic face of the pore. The position of these lipid molecules overlaps with those found in other K^+-selective channel structures consistent with the presence of a conserved lipid immobilization site. Reconstitution of KvLm-PM in lipid bilayers that mimic the crystallization condition shows that the channel exhibits the full complement of functional properties.
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Probing S4 Length Changes during Gating with LRET
Tomoya Kubota, Jérôme J. Lacroix, Francisco Bezanilla, Ana M. Correa.
The University of Chicago, Chicago, IL, USA.
Voltage-activated proteins containing a Voltage Sensor Domain (VSD) respond to changes in the membrane potential by transferring across the electric field several positively charged residues (gating charges) located in the fourth transmembrane segment (S4). Even though the mechanistic details of gating charge translocation and S4 re-arrangement are presently unclear, a prevailing hypothesis suggests that the S4 segment adopts a 3_{10} helix conformation during gating, thus aligning the gating charges and facilitating their transport across the membrane electric field. If a whole typical S4 segment were to change its conformation from an α-helix to a 3_{10} helix, its length would stretch by about 8 Å. Here we tested the existence of such transition by measuring the length of the S4 segment during gating using the LRET technique. We used the VSD domain of the *Ciona intestinalis* Voltage-Sensitive Phosphatase (C_iVSP), truncated from its phosphatase and phospholipid-binding domains, to genetically encode a lanthanide (Tb^{3+})-binding-tag at the extracellular end of S4 and the red fluorescent protein mCherry at its intracellular end. We expressed the protein in *Xenopus* oocytes from which we recorded gating currents using the cut-open and two-electrode voltage-clamp techniques. The distance-dependent efficiency of energy transfer between the two probes was measured at steady-state voltages and also during voltage pulse protocols of varying amplitudes and durations. Our technique could only detect distance changes larger than 2.5 Å. We found that in our C_iVSP construct, the results are not consistent with the expected length change if the whole S4 were converted (and remained converted) from a 3_{10} helix to an α-helix (or vice-versa) when the membrane potential is changed from −100 to +80 mV.
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In Vitro Folding of KvAP, a Voltage Gated K+ Channel
Prasanna K. Devaraneni, Francis I. Valiyaveetil.
OHSU, Portland, OR, USA.
In this contribution, we report *in vitro* folding of the archaebacterial voltage gated K+ channel, KvAP. We show that *in vitro* folding of the KvAP channel from the extensively unfolded state requires lipid vesicles and that the refolded channel is biochemically and functionally similar to the native channel. The *in vitro* folding process is slow at room temperature and the folding yield depends on the composition of the lipid bilayer. The major factor influencing refolding