488-Pos
Development of Bicelles Containing Anionic Lipids to Characterize Cationic Membrane Active Peptides by NMR Spectroscopy
Joshua D. Brown, Denise V. Greathouse.
University of Arkansas, Fayetteville, AR, USA.
Aqueous dispersions of bicelles (bilayered micelles) offer an alternative to mechanically aligned bilayer samples for solid-state NMR spectroscopy (Sanders and Landis, 1995, *Biochemistry* 43:4030-4040). Bicelle formation requires at least a binary mixture of zwitterionic long-chain and short-chain lipids. The long lipid (such as 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) forms a planar bilayer surface, while the short-chain lipid (such as 1,2-di-O-hexyl-*sn*-glycero-3-phosphocholine), caps the edges and isolates the bilayer hydrophobic core from water. When placed in a magnetic field, bicelles may spontaneously align. Because bacterial cell membranes are composed of 20%-25% negatively-charged lipids, we have sought to include an anionic lipid (such as 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol) in our bicelle preparations. To find optimal conditions, we have varied the content of the negatively charged lipid, the ratio of long to short-chain lipid (q), and the buffer used to prepare the bicelles. ^{31}P NMR spectroscopy was used to assess the alignment of the bicelles under the various conditions. Membrane active peptides, selectively deuterated at specific amino acids, will be incorporated into bicelles under optimal conditions and into mechanically aligned bilayers on glass plates. NMR spectra from membrane active peptides in mixed lipid bicelles will be compared with spectra from peptides in macroscopically oriented bilayers prepared on glass slides. Studies of cationic antimicrobial peptides, such as lactoferricin B6 (RRWQWR-NH_2) are significant because of the increased emergence of antibiotic resistant bacterial strains. It is therefore important to understand the mechanism by which such peptides exert effects on membranes which mimic the composition of bacterial cell membranes.

489-Pos
Acylated Lactoferrin Peptides Using Solid State NMR and All-Atom Molecular Dynamics Simulations
Tod D. Romo[1], **Alan Grossfield**[1], Laura Bradney[2], Denise V. Greathouse[2].
[1]University of Rochester Medical Center, Rochester, NY, USA, [2]University of Arkansas, Fayetteville, AR, USA.
Lactoferricin B is a cationic antimicrobial peptide with broad spectrum effectiveness. A small piece extracted from this peptide, LfB6 (RRWQWR-NH_2), has similar antimicrobial properties, which can be further enhanced by attaching a short fatty acid to the N-terminus (C6-LfB6). The exact mechanism by which antimicrobial peptides interact with bacterial cell membranes is not well understood, but it is proposed to depend on lipid composition. In contrast to mammalian membranes which are comprised primarily of neutral lipids, bacterial membranes contain a significant (~20-25%) fraction of negatively charged lipids. In the case of LfB6, the presence of two tryptophans and three arginines are thought to promote selective interaction with bacterial cell membranes. Here, we investigate the interactions of C6-LfB6 with lipid bilayers by combining solid state ^{2}H and ^{31}P NMR with an ensemble of all-atom molecular dynamics simulations running in aggregate more than 10 microseconds. In particular, we have investigated the peptides interactions with bilayers with two distinct compositions: 3:1 POPE:POPG (bacteria-like) and POPC (mammal-like). The results show that at low concentration the peptide has very little effect on the acyl chain concentration of the anionic membrane, and a more substantial effect on the zwitterionic POPC membrane. The synergy between the experimental and simulation results generates new insights into the molecular-level physics driving antimicrobial function.

Intracellular Communications & Gap Junctions

490-Pos
Functional Mapping of Connexin Pores Reveals Different Pore Topologies and the Location of a Channel Gate
Bruce J. Nicholson.
University of Texas Heath Science Center at San Antonio, San Antonio, TX, USA.
Using the substituted cysteine accessibility method (SCAM), residues in M1 in hemichannels of Cx46 and a Cx32/43 chimera, and M2 and M3 in gap junctions of Cx32 have been identified as lining the pore. We have now conducted a comprehensive comparison map of Cx50 gap junction channels and found that the orthologous residues mapped in M3 of Cx32 are also reactive in Cx50, with one exception near the extracellular end of the helix. Residues in M2 are also reactive in Cx50, but only one is in common with Cx32 (V84), while the other three sites define a different face of M2. Unlike hemichannels, no reactivity was evident in M1 of gap junction channels. We have used several strategies to minimize artifacts by reacting sites from both ends of the gap junction channel and showing that charged thiol reagents could predictably change the ion selectivity of the pore at specific sites proposed to line the pore. These studies provide the first evidence that different connexin channels have distinct topologies that can explain large and often uncorrelated differences in their channel conductance and size exclusion limits. It also suggests that the pore lining topologies of hemichannels and gap junctions may be different. We have extended these studies to map the site where the Cx32 channel closes in response to voltage. Utilizing a disease associated mutation (M34T) to induce a closed state of the channel that can be opened with voltage, we have tested accessibility of residues in M3 from both ends of the channel, and mapped the site of occlusion in the pore to one turn of the M3 helix and also detected a conformational change in M1 consistent with a rotation of this helix.

491-Pos
Gating by Voltage and Ca^{2+} in Human Connexin (cx26) Hemichannels
Jorge E. Contreras[1,2], Agenor Limon[3,2], Angelica Lopez-Rodriguez[1,2].
[1]NIH, Bethesda, MD, USA, [2]Grass laboratory, MBL, Woods Hole, MA, USA, [3]University California Irvine, Irvine, CA, USA.
Opening of connexin hemichannels permits the release of small metabolites, such as ATP and glutamate, which play an important autocrine/paracrine signaling in a variety of cell types. The recently solved crystal structure of the Cx26 gap junction channel allows us to explore in greater detail the relationship between the structure and function of both, hemichannels and gap junction channels. Here, we begin by revisiting the activation mechanisms of human Cx26 (hCx26) hemichannels by voltage and Ca^{2+}. Using the two electrode oocyte voltage-clamp technique, we found that depolarization up to +60 mV induced activation of hemichannel currents, and repolarization produced large tail-currents with slow deactivation (τ ~10 s). Interestingly, the magnitudes of the tail-currents were dependent on the lengths of the depolarizating pulses rather than the magnitudes of the currents activated during the pulses. Strikingly, cell-attached single channel recordings showed that depolarizating pulses ($\leq$40 mV) stimulated only infrequent and brief openings of hCx26 hemichannels. However, the repolarizating pulses induced opening of single hemichannel currents (likely corresponding to the tail currents) with smaller conductance and longer mean open times. In addition, we found that low Ca^{2+} (500 μM) increased macroscopic hemichannel currents at positive potentials and slowed deactivation of the tail currents. We are currently performing single channel recordings to elucidate how Ca^{2+} modulates channel activation and deactivation kinetics in response to depolarizing and repolarizing voltages. Our results indicate that while depolarization causes opening of some hCx26 hemichannels, it mostly shifts the hemichannels to a non-conductive state that will likely open after repolarization; and that Ca^{2+} ions may play a role by regulating the energetics of these transitions. Supported by The Grass Foundation.

492-Pos
Structures of Connexin26 Mutants Demonstrate a Global Flexibility of Subunits and N-Terminal Rearrangements in the Pore
Atsunori Oshima[1], Kazutoshi Tani[1], Masoud M. Toloue[2], Yoko Hiroaki[1], Amy Smock[3], Sayaka Inukai[1], Nicholson J. Bruce[2], Gina E. Sosinsky[3], Yoshinori Fujiyoshi[1].
[1]Kyoto University, Kyoto, Japan, [2]University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [3]University of California San Diego, San Diego, CA, USA.
Gap junction channels are unique in that they possess multiple mechanisms for channel closure, however, structural insights into the gating mechanism have been limited by the lack of isolation of closed versus open channels for each gating mechanism. Here, we present three dimensional maps of the mutant connexin26 (Cx26M34A) and an N-terminal deletion of this mutant (Cx26M34Adel2-7) at 6 and 10 Å resolution, respectively, determined by electron crystallography. Three of the six connexin subunits in the Cx26M34A hemichannels have non-equivalent configurations resulting in a departure from strict hexagonal symmetry. The volume of the density seen in the pore of the Cx26M34A channels is prominently decreased in the Cx26M34Adel2-7 pore, but a slim density still resides. A projection map of Cx26 wild type (Cx26WT) channel at 10Å resolution and crystallized under conditions promoting a closed state reveals a density in the pore that is weaker than the Cx26M34A plug, however the high variance peak from the crystallographic