1533-Pos Board B443
Cytoplasmic Domain Filter Function in the Mechanosensitive Channel of Small Conductance
Ramya Gamini, Marcos Sotomayor, Christophe Chipot, Klaus Schulten.
Mechanosensitive (MS) channels, inner membrane proteins of bacteria, open and close in response to mechanical stimuli such as changes in membrane tension during osmotic stress. In bacteria, these channels act as safety valves preventing cell lysis upon hypo-osmotic cell swelling: the channels open under membrane tension to release osmolytes along with water. The MS channels of small conductance, MscS, consist of a large cytoplasmic domain (CD) that features a balloon-like, water filled chamber opening to the cytoplasm through seven side pores and a very narrow distal pore. The CD is apparently a molecular sieve, which minimizes loss of osmolytes and metabolytes during osmoadaptation. Here we use diffusion theory and molecular dynamics simulations to explore the transport kinetics of Glu- and K+ as representative osmolytes. We suggest that MscS through the openings of its CD acts as a filter that balances passage of Glu- and K+, and possibly other osmolytes, to yield a largely neutral efflux and, thereby, reduce cell depolarization in its open state.

Voltage-gated K Channels - Gating I

1534-Pos Board B444
Structural Dynamics of the S4 Voltage Sensor Helix in Bilayers Lacking Lipid Phosphates
Magnus Andersson, Alfredo J. Freites, Stephen H. White, Douglas J. Tobias.
Voltage-dependent K+ (Kv) channels require lipid phosphate groups for proper functioning. The S4 helix, which carries the gating charges in the voltage-sensing domain, can be inserted into biological membranes while being stabilized by an hydrogen-bonding network in which lipid phosphates play an essential role. To identify protein-lipid dynamics and interactions in the absence of lipid phosphates, we performed molecular dynamics (MD) simulations of a variant of the KvAP S4 sensor (S4mut) in bilayers with and without lipid phosphates. We find that in bilayers lacking lipid phosphates (DOTAP), the snorkeling Arg residues are not anchored to the polar headgroup region and hence display more structural flexibility along the bilayer normal compared to phosphate-containing lipid bilayers (POPC). The increased flexibility of the Arg resi-dues and their close interaction with water molecules leads to a collapsed hydrophobic core and a water-permeable DOTAP bilayer. These results show that lipid phosphates are required for stabilizing the S4mut-containing bilayer.

1535-Pos Board B445
A potassium Channel Voltage-Sensing Domain in a Non-Phospholipid Bilayer
Harindar S. Keer, **J. Alfredo Freites**, Stephen H. White, Douglas J. Tobias.
Non-phospholipid, cationic 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) lipid based membranes fail to support the function of a voltage-dependent K+ channel due to the lack of phosphate groups (Schmidt et al., Nature 444, 775, 2006). However, the specific effects of the presence or absence of phosphate groups in the channel membrane environment on the voltage-sensing mechanism remain unknown. To characterize the conformational dynamics and protein-lipid interactions in the absence of lipid phosphates, we performed all-atom simulations of the KvAP channel voltage-sensing domain (VSD) in a DOTAP bilayer in excess water. We generated prolonged trajectories for an up-state (Krepkiy et al., Nature 462, 473, 2009) and a down-state (Schow et al., Biophys J 98, 2857, 2010) conformation of the VSD, and contrasted the results with simulations of the VSD embedded in a POPC bilayer. Pair interaction energies reveal differences in the way each VSD conformation interacts with the different components of the membrane environment in the presence and absence of phosphate groups, which result in significant changes to the system electrostatics. This work is supported by NIH grants GM74637 and GM86685 and NSF grant CHE-0750175. This research was supported in part by NSF through Teragrid resources provided by the TACC at UT Austin.

1536-Pos Board B446
Conformational Changes in Lipid Reconstituted Potassium Channel Voltage-Sensing Domains
Dmitriy Krepkiy, Sonya Hanson, Klaus Gawrisch, Kenton J. Swartz.
In the voltage-activated potassium channels, S1-S4 voltage-sensing domains control opening and closing of an associated pore domain. Electrophysiology experiments show that in the presence of lipids with positively charged headgroups, voltage sensors appear to be confined to the resting state (Schmidt, D., 2006). In order to define the structural changes in voltage sensing domains we purified the S1-S4 domain from KvAP and reconstituted it in either a POPC:POPG (1:1) lipid mixture or DOTAP, a positively charged lipid without a phosphate group. Although the α-helical secondary structure is identical in these lipids as observed by circular dichroism spectroscopy, the fluorescence properties of single Trp70 in the middle of S2 helix are different. Trp70 fluorescence is quenched when the S1-S4 domain is reconstituted into DOTAP. This observation is consistent with the changes of the chemical environment of the Trp70 due to cation-π interactions when voltage-sensing domain is in the resting state (Tao, X., 2010). We are using solid-state NMR spectroscopy on voltage-sensing domains reconstituted in different lipids to test this hypothesis. Saturation transfer difference NMR experiments on the ^{13}C uniformly labeled S1-S4 protein indicate that protein hydration and exposure to lipids are not significantly different in POPC:POPG and DOTAP. ^{13}C NMR spectra of (u)-^{13}C-Trp70 indicate that C_α chemical shifts do not change for Trp70 in different lipids, arguing that the secondary structure is unchanged in this segment of the S2 helix. On the other hand, ^{13}C and ^{15}N NMR spectra indicate changes for indole nitrogen N_ϵ and carbon C_{z2} consistent with the difference in the chemical environment of indole ring when S1-S4 domain reconstituted in different lipids. By using two- dimensional solid-state NMR spectroscopy we are evaluating possible conformational changes between resting and activated states of S1-S4 domains.

1537-Pos Board B447
Phospholipids as a Structural and Functional Determinant for Voltage Sensors in a Kv Channel
Liang Shi, Hui Zheng, Weiran Liu, Lingyan Anderson, **Qiu-Xing Jiang**.
Recent studies proposed that phosphodiester groups in a phospholipid bilayer help stabilize a voltage sensor in its activated conformation. However, the nature of such channel-lipid interaction is unclear. We are studying the structure and function of the KvAP channel in lipid bilayers. We obtain the structural information of the channel in membranes by electron crystallographic study of two-dimensional crystals, and utilize both biochemical assays and electrical recordings to determine the conformational states of the voltage sensors and the pore domain in reconstituted channels. Our electron crystallographic studies start to reveal that the KvAP voltage sensors in a phospholipid membrane can take an alternative conformation, which is different from the expected four-helix bundle structure observed in the X-ray crystallographic studies of Kv channel proteins in detergents or mixed detergent/lipid micelles. The functional implications of such a new conformation of the voltage sensor are being investigated. Our functional studies converge to the conclusion that annular phospholipids around the channel are required for its voltage sensors to switch from the deactivated to the activated state. Our data suggest that a phospholipid bilayer may be an essential factor for the structure and function of a voltage sensor.

1538-Pos Board B448
Molecular Dynamics Study of Structural Elements Relevant to Gating of Kv Channels
Greg Starek, Simon Bernèche.
Voltage-gated potassium ion (Kv) channels respond to changes in membrane voltage, enabling K^+ transport across the membrane. While several crystal structures have been presented in the open conformation, the closed structure remains unsolved, leaving the gating mechanism of Kv channels unclear. Here, we present preliminary results of molecular dynamics simulations on voltage-gated potassium ion channels, with emphasis on the gating of the channel. Particular attention is placed on potential structural rearrangements of the S1-S4 helices, including inter-helical and intra-helical interactions within the voltage sensor. Of interest in the study is the occurrence of 3_{10} helix motifs in S4, as reported in several crystal structures of potassium ion channels (Long et al., Science 2005; Long et al., Nature 2007; Clayton et al., Proc.Natl.Acad.Sci.USA, 2008).

1539-Pos Board B449
Barium Block of Kv4.2 is Enhanced by Channel Inactivation
Steven J. Kehl, Yen May Cheng.
Ba^{2+} is frequently used infunctional studies of the topology of potassium-selective pores because it is a permeant blocker. Consequently, the rate and extent of current block, or unblock, can be quantified to assess changes of the