closely related potassium channels display differences of up to two orders of magnitude in their single channel conductance. The substitution of Proline 475 by Aspartate increases Shaker K^+ transport rate by 7-8 fold. Previous work of our lab suggests that such a dramatic increase in K^+ transport rate could arise from increased pore occupancy (Moscoso et al 2012). We decided to test the occupancy hypothesis by introducing charged residues along the pore of Shaker in order to fill the permeation pathway and compare their maximal transport rate to that of BK channels (600pS). The occupancy was tested with Molecular Dynamic simulations while the channel conductance was tested by single channel recordings at several K^+ concentrations. Fully occupied Shaker variants were still far below of BK single channel conductance values. A possible explanation for the latter is that inner entrance dimensions could limit the maximal ion transport rate to 1/3 of BK channel. To test the latter we determined the radius capture of our Shaker channels by measuring the diffusion limited currents in 2M of sucrose. Our result shows that Kv channels have a smaller inner entrance than large conductance K-channels which lead us to propose that increased occupancy raises single channel conductance but pore dimensions imposes an upper limit for the maximal transport rate of K-channels.

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2728-Pos Board B420

Amino Acid Substitutions for T75 in KCSA Alter Ion Selectivity

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The K^+/Na^+ ion selectivity of the bacterial ion channel KcsA is $\approx$400. We have shown from non-equilibrium molecular dynamics (MD) simulations that entry of the ions into the selectivity filter of KcsA is associated with a free energy barrier that is approximately 3.7 kcal/mol higher for Na^+ than for K^+, and that this free energy barrier effectively excludes Na^+ from the selectivity filter of KcsA. Na^+ is stabilized just outside the selectivity filter of KcsA in the water-filled central cavity (vestibule) by interactions with the side chain of the T75 residues of each KcsA subunit. In silico amino acid substitutions were made for T75 in KcsA to serine, valine, and cysteine in order to examine the consequences of replacing the side chain groups in threonine on Na^+ and K^+ permeation. Threonine has side chain methyl and hydroxyl groups, and serine has a proton and a hydroxyl group, valine has two methyl groups, and cysteine has a proton and a thiol group in their side chains. Single Na^+ or K^+ ions were pulled through the wild type and mutant channels using a step-wise pulling protocol and Jarzynski's Equality to obtain work distributions and free energy values for each ion moving through the channel. The simulations showed that valine and serine excluded both Na^+ and K^+ from the selectivity filter under conditions where the wild type channel excluded only Na^+; however, cysteine allowed both ions to enter the selectivity filter. These results suggest that mutant KcsA channels having serine, valine, or cysteine in the position of T75 will have reduced K^+/Na^+ selectivity. The simulations also indicate that differential dehydration of the ions is not correlated with changes in ion selectivity in the mutants.

2729-Pos Board B421

Ion Permeation Efficiency through Potassium Channels

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Potassium channels underlie important physiological functions such as cellular ionic homeostasis and nerve signal transduction. Alongside the discrimination of potassium over sodium, the channels' capability of finely tuning their permeability for potassium is crucial for their function. In most potassium channels, these two features are combined in the selectivity filter, a narrow region at the outer mouth of the channel that is permeated by potassium ions in a single file. Here we present our findings from simulations of the wild-type and mutants of the bacterial KcsA potassium channel pore-region under near-physiological trans-membrane voltages. The simulations accurately reproduce important electrophysiological parameters of these KcsA variants, such as peak conductance and rectification. Based on the recording of thousands of permeation events, we were able to statistically investigate the relationship between filter flexibility, conformation and permeation efficiency. The results show a clear correlation between filter flexibility and ion permeation efficiency, indicating that the channel provides more than just a static scaffold to facilitate permeation. In addition, a heterogeneous distribution of ions in the selectivity filter was found during permeation events, indicating that multiple permeation mechanisms concurrently underlie efficient ion permeation.

2730-Pos Board B422

Strategies to Achieve Selective Conductance in K- and Na- Selective Ion Channels

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Ion channels are proteins spanning the membrane that conduct the ions across the cell membranes. They play a vital role in generating and regulating the electrical singling in living organisms such as pace-making, neuronal signaling and smooth muscle function. In this work, we used multi-dimensional free energy simulations to unravel key principles governing ion-selective conductance across four ion channels with known crystal structures e.g NaK2K K^+-selective channel, NaK2CNG non-selective channel, NavAb Na^+-selective channel and NavAb-E177D non-selective channel. Free energy simulations allow for resolution of binding sites, barriers, transport stoichiometry and actual binding thermodynamics. The results indicate that a single permeant ion binds far too tightly to most of the channels and rapid permeation relies on the multi ionic effects. However, as the filter of Na^+ channel is much wider and shorter than that of K^+ channel, they employ their unique selective strategies comparing to K-selective channels. In Na^+ selective channel, the ions are highly hydrated and the binding sites are flexible allowing for a transit of partially hydrated ions. Presence of high-field ligands allows for a better binding and smaller barriers for Na^+ as compared to K^+. We found that barriers and wells on the potential surface controlling permeation are highly dependent on the type of co-permeant ion.

2731-Pos Board B423

Insights into the Ion Permeation Process of High and Low Conductance K-Channels using Non-Equilibrium Molecular Dynamics

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Potassium channels are membrane proteins that allow fast and selective flow of K^+ ions across membranes, participating on the regulation of the electrical properties of the cell, generation and propagation of electrical impulses in nervous systems, gene expression regulation, neurotransmitters release. K^+ channels have a selectivity filter (SF) composed by a highly conserved sequence TVGYGD, which forms the narrowest part of the channel. Despite the fact that the structure of the SF is conserved among K^+ channels, they show different conductance rates e.g. 250 pS for BK channel and 20 pS for Shaker channel. Moreover, a single mutation in Shaker (P475D) can increase its conductance from 20 pS to 180 pS.

Molecular dynamics simulations of the aforementioned K^+ channels were performed in order to describe the molecular properties that modulate the conductance process. To analyze these properties an external electric fields were applied, allowing a faster permeation of K^+ ions and therefore to have a further approximation of the patch clamp experimental conditions.

Using this non-equilibrium approach a number of outward K^+ transport events were observed in a high and low conductance K^+ channels. The properties involved in the K^+ ion conductance process were characterized at molecular level through computing the electrostatic potentials profile, PMF (ABF method) profiles, permeation events number, K^+ desolvation process and K^+ ion density inside the pore. This study provides new perspectives to understand the ion conductance observed in high and low conductance K^+ channels, allowing to propose new hypotheses which were validated through site directed mutagenesis and electrophysiological assays.

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2732-Pos Board B424

2D Ir as an Experimental Probe of Ion-Induced Structural Changes in KCSA

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