as a unit in a “knock-on” mechanism of permeation. Differences in the amount of work required to move three Na$^+$ ions through the selectivity filter of NavAb compared to three K$^+$ ions predict the large negative reversal potentials observed for bacterial Nav channels in instantaneous current-voltage plots. The results of the simulations suggest that the block of bacterial voltage-gated Na$^+$ channels by extracellular K$^+$ does not occur in eukaryotic voltage-gated Na$^+$ channels because of differences in the amino acids present in the selectivity filters of the different channels.

2908-Pos Board B338
Molecular Dynamics Study of Ion Conduction and Selectivity in a Prokaryotic Ion Channel
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Since the publication of crystal structure of NavAb, the first of a (prokaryotic) voltage gated sodium channel, several computational studies have been aimed at determining the mechanisms of ion selectivity and ion conduction in voltage gated sodium channels. We provide a two-part study, well-converged free energy surfaces involving one, two and three ions predict results consistent with microsecond timescale simulations of ion conduction over concentration and voltages. The position of ions in the pore and cavity correlate to coordination number and side chain orientation, showing, as suggested by Chakrabarti et al. (PNAS (2013) 110, 11331). More surprising, presence of an ion in the aqueous cavity beneath the selectivity filter was sufficient to influence glutamate conformation.

Because the crystal structure of NavAb was in a closed pore conformation, we truncated the S5 and S6 helices and restrained these outer helices harmonically in a membrane represented by supporting lattice of neon. The turrets, pore helices and selectivity filter were free to move. Conductances were in the range of the experimental values; however, our studies show no preference for sodium conduction over potassium conduction at physiological conditions (∼200mV, 0.15 M salt). While sodium conductance varied little with respect to concentration and presence of potassium in solution, stronger potassium selectivity is observed at 1 M and in mixed solutions. Our results are consistent with the observation of anomalous mole fraction effect in NaChBac by Finol-Urdaneta et al. (JGP (2014) 143, 157-171), though not sufficient to confirm such an effect. Additionally, we provide mechanisms for ion conduction showing a greater variety of states and transitions occupied during potassium conduction, and note that at higher concentrations of salt the mechanism of conduction is not as clearly cut.

2909-Pos Board B339
Coupling of Channel Fluctuations in Ion Permeation and Selectivity in Bacterial Sodium Channel NavAb
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Even though crystallographic structures of several cation channels are known at atomistic resolution, the molecular basis for selective ion permeation, and in particular, the role of structural fluctuations of the channel in that process, remains unclear. The determination of structures of voltage-gated sodium channels opens the way to elucidating the mechanism of sodium permeation and selectivity. Recent molecular simulation studies of bacterial sodium channel NavAb (Chakrabarti et al., PNAS 110, 11331-11336, 2013) suggest that Na$^+$ binding and permeation through the selectivity filter are coupled to the conformational isomerization of Glu177 side chains from an out-facing conformation to a lumen-facing conformation, resulting in a high rate of Na$^+$ diffusion through the selectivity filter.

To clarify the role of channel dynamics on ion permeation and selectivity, we examine the effect of structural constraints systematically. Specifically, we characterize the mechanism of cation permeation in the absence of conformational “dunking” of Glu177 side chains. In addition, we investigate the effect of structural restraints imposed on the pore helices to prevent channel closure, as well as of applied voltage, on channel fluctuations and transport properties. Results of simulations totaling over 100 microseconds indicate that restricting Glu177 conformations, either directly or through global structural restraints on the helices of the pore domain, modulates Na$^+$ binding and permeation. Further, applying strong external voltage gradients significantly displaces the conformational equilibrium of the Glu177 side chains, thereby also modulating the mechanism of ion permeation in NavAb.

2910-Pos Board B340
Expression, Purification, and Preliminary Characterization of a Human Cardiac Sodium Channel Voltage Sensing Domain
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Voltage-sensing domains (VSDs) of voltage-gated ion channels sense changes in the membrane potential and as a result alter the conduction state of the channel. The human voltage-gated sodium channel NaV1.5 is primarily expressed in cardiac muscle and is responsible for the rising phase of the cardiac action potential. Mutations within NaV1.5 can lead to fatal cardiac arrhythmias. Such mutations have been found throughout the gene, including missense mutations within the VSD of repeat IV that have been shown to lead to Brugada Syndrome and LQT3. The VSDs also play an important role as binding sites for gating modifier peptide toxins from tarantula spider venoms. Such toxins could serve as good lead compounds for drug development due to their high specificity and more subtle mode of action compared to pore blockers. Therefore, it would be important to know the structures of the human sodium channels VSDs. While no structures of whole eukaryotic sodium channel proteins exist, isolated VSDs of other ion channels have been shown to fold into their native conformation in the absence of the pore forming domain. Therefore, we are pursuing the expression and purification of the isolated VSDs of the isolated VSDs of NavAb and NavNav1.5 in order to investigate the structural changes within the VSD caused by pathogenic mutations and by the binding of gating-modifier toxins. Here, we present the expression and purification of the human NaV1.5 VSD of repeat IV in a bacterial expression system in isotopically labeled form and preliminary characterization of the truncated protein. NaV1.5 VSD IV is expressed in Escherichia coli in minimal media, and extracted from membranes by solubilization into n-decylphosphocholine micelles. Purified NaV1.5 VSD IV was characterized by mass spectrometry and gel filtration chromatography and used for preliminary NMR structural studies.

2911-Pos Board B341
A Thermodynamic Analysis of Disease-Causing Mutations in the Nav1.5 C-Terminus
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The opening of voltage-gated sodium channels (Na$^+$) is responsible for the rapid upstroke of action potentials. A key player during myocardial excitation is the cardiac channel isoform Na$_{1.5}$. The general architecture of mammalian Na$_s$s is comprised of four homologous domains, containing six transmembrane segments each, and a C-terminal intracellular domain (CTD) carrying an IQ-motif. The individual domains are connected by large intracellular linkers. The linker connecting domains three and four as well as the CTD seem to play a role in channel inactivation which is different from regular channel closing but poses an important function to modulate ion conductance. The rapid inactivation of channels limits the influx of ions and therefore depolarization of the cell per opening signal. In this context the CTD is of particular interest as an interaction partner for regulatory proteins as calmodulin (CaM) as well as hotspot for disease-causing mutations that have a profound influence on channel inactivation. To elucidate the functional effects of disease-causing mutations we expressed mutant channels in Xenopus laevis oocytes and studied them by two-electrode voltage clamp. To complement the data we analyzed the thermostability of isolated mutant CTDs and performed isothermal titration calorimetry experiments. Isothermal titration calorimetry experiments in the absence and presence of Ca$^{2+}$ were used to determine binding profiles of individual CaM lobes to WT and mutant CTDs. Our data shows that mutations have distinct effects on the folding stability and ability to bind CaM. Whereas some mutations cause misfolding of the CTD, others selectively affect binding of apoCaM or both apoCaM and Ca$^{2+}$/CaM, and these changes correlate with the disease phenotype.

2912-Pos Board B342
Functional Consequences of a Novel Nav1.9 Mutation (L1302F) causing Congenital Insensitivity to Pain
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The contribution of the peripheral nerve voltage-gated sodium (Na$^+$) channel Na$_{1.9}$ to nociception has been demonstrated in Na$_{1.9}$ knockout mice that