compound exposure. The inhibition profile of PT-0509977 suggests that a
conformational change in the Domain 4 VSD couples to multiple downstream
inactivated states and immobilizing the voltage-sensor via a small molecule
interaction with this site may lock the channel into long term inactivation
from which recovery is slow.

2903-Pos Board B333
Structural Modeling of Local Anesthetic Binding to the Pore-Domain of
Human Nav1.5 in Open and Closed States using Rosetta
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Mutations in voltage-gated sodium (Nav) channel isoforms are correlated with
a wide range of cardiovascular and neurological diseases in humans, and are therefore important targets for the rational design of novel drugs. The
cardiac isoform of the Nav channel, Nav1.5, presents a unique target for the
development of antiarrhythmic drugs. In this work, we identify key structural
motifs for local anesthetic binding in the pore-domain of the open and closed
states of the human Nav1.5 channel. The Rosetta structural modeling method
was used to construct models of human Nav1.5 isoform based on the 3D crystal
structures of bacterial Nav channels: NavRh (closed state) and NavMs (open state).
The resulting lowest free-energy models were selected for local anesthetic
docking simulations. Rosetta loop modeling and global relaxation of 10,000 models yielded a convergent motif in the selectivity filter region of a
stabilizing hydrogen bond network between Tryptophan and Threonine pairs.
A membrane-facings fenestration near the S6 helix of domain IV and the S5 he-
lix of domain III was also structurally conserved, and is a proposed site
of neutral drug entry. Docking simulations of the channel blocker, lidocaine,
reveal key protein-ligand binding configurations within the pore. Our prelimi-
nary models of the human Nav1.5 channel in the open and closed states reveal
highly conserved structural motifs important for both stabilization of the pore
domain, as well as for drug entry and binding. Future work will use structural
models of drug interaction with human Nav1.5 as a dynamic testing platform
for the calculation of the kinetics of drug binding and unbinding.

2904-Pos Board B334
Understanding the State Dependence of Voltage Sensor Toxin Action on
Voltage Gated Sodium Channels
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Voltage gated sodium (Nav) channels are responsible for initiation and propa-
gation of action potentials in nerve and muscle. Due to their physiological roles,
Nav channels are prime targets of natural toxins from a variety of organisms
such as spiders, scorpions, snakes and cone snails. ProTx-II, from the tarantula
Thrixopelma pruriens, is a 30-residue peptide toxin that is a potent inhibitor of
Nav channels. It binds to voltage sensor domains (VSDs) II and IV of human
Nav1.7 channels. It is more than 100-fold selective for Nav1.7 versus all other
human Nav channel isoforms. Magi-5, from Macrothele gigas, is a 29-residue
peptide toxin that stabilizes an activated state of the domain II VSD of Nav
channels. Both spider toxins share a structural fold stabilized by the same disul-
fide bridge network, yet they have opposite effects on Nav function: ProTx-II
stabilizes a resting state of VSDII, while Magi-5 stabilizes an activated state.
We use solid phase peptide synthesis to generate ProTx-II - Magi-5 chimeras
by inserting loop regions between conserved cystines of Magi-5 into ProTx-
II. Molecular modeling, protein-protein docking and electrophysiology tech-
niques are used to identify critical residues responsible for opposite effects of
ProTx-II and Magi-5 on Nav channel function. Our findings may be useful in
the design of novel modulators of human Nav1.7 channel and may elucidate
important structural determinants of VSD toxin activity.

2905-Pos Board B335
Targeting Protein:Protein Interaction Sites for Drug Development against
Voltage-Gated Sodium Channels
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Fibroblast growth factor 14 (FGF14) is a functionally relevant accessory protein of
the neuronal Nav channel. Through a monomeric interaction with the intra-
cellular C-termius of neuronal Nav channels, FGF14 modulates Na+ currents
in a Nav isoform-specific manner serving as a fine-tuning regulator of excit-
ability. In previous studies we have reconstructed the PPI interaction of
FGF14 and Nav1.6 in live cells using the split-luciferase complementation assay
(LCA) and through site-direct mutagenesis identified “hot-spots” at the FGF14
surface critical for binding to Nav1.6. Based on the FGF14 monomer structure
generated in silico, we have designed short peptide fragments that align with
pockets defined by the FGF14 β12-strand and β8-9 loop and validated their
in-cell activity as inhibitors of the FGF14:Nav1.6 complex. We then applied
patch-clamp electrophysiology and show exciting preliminary data indicating
that two peptides, Fpep1 and Epep1, exhibit either a negative allosteric modu-
lators (NAM)-like or a positive allosteric modulators (PAM)-like activity
against Nav1.6-encoded currents. For one peptide, Fpep1, we have begun me-
dicating discovery efforts to generate NAM- and PAM-based small molecules
that are currently being evaluated. These breakthrough results identify the FGF14 (β8-9 and
β12 as part of potential druggable pockets against the FGF14:Nav1.6 complex
and indicate that small molecule inhibitors (SMI) and/or peptidomimetics
targeting these pockets might give rise to a new class of unconventional PPI-
based allosteric modulators of Nav channels that could restore dysfunction
of neuronal excitability and plasticity in brain disorders. These results provide
fundamental new knowledge for the design of new leads targeting the Nav chan-
nel macromolecular complex. We expect our studies to have a broad impact in
the drug design against a wide range of still untreatable brain disorders

2906-Pos Board B336
Sodium Selective Conduction, Inactivation and Inhibition Mechanisms
using the Bacterial NavAb Channel
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Voltage-gated sodium channels play essential roles in electrical signaling in the
nervous system and are key pharmacological targets. We have carried out
tomographic and Anton simulations of the bacterial NavAb channel to reveal
ion conduction intimately connected with conformational fluctuations of the
protein pore (C Boiteux, I Vorobyov & TW Allen, 2014 PNAS 111:3454),
and a multiple-ion mechanism underlying Na+ versus K+ selectivity. We
observe collapse of the pore domain, involving residues identified in pore-
based slow inactivation and drug binding in prokaryotic and eukaryotic chan-
nels. We demonstrate high affinity binding of anti-epileptic and local anesthetic
drugs to F203, that is a surrogate for the highly conserved F68 in mammalian
sodium channels, as well as low affinity sites with potential roles in channel
inhibition. We observe two drug access pathways, including a previously
suggested lipophilic route via membrane-bound fenestrations, and an aqueous
pathway through the channel pore, despite being closed (C Boiteux, et al. 2014
PNAS 111:13057). These studies provide new insight into Nav function and
modulation, and predictions to assist future drug development.

2907-Pos Board B337
Molecular Dynamics Simulations Describe the Mechanism of K Block in
Bacterial Nav Channels
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Although extensive electrophysiological characterization is available for eu-
karyotic voltage-gated Na+ channels (Nav), no high-resolution structures of
these channels are available. Crystal structures of several bacterial Nav chan-
nels have been published and molecular dynamics simulations of ion perme-
ation through these channels are consistent with many electrophysiological
properties of the eukaryotic channels. Unlike eukaryotic Nav channels, howev-
er, the bacterial Nav channels are strongly outwardly rectifying, and the mech-
anism of this rectification has not previously been described. We used step-wise
pulling protocols to implement Jarzynski’s Equality in non-equilibrium molec-
ular dynamics simulations of ion permeation through the bacterial NavAb
channel to obtain a mechanistic description of this outward rectification.
Results of the simulations indicate that two or three extracellular K+ ions
bind tightly at the same z-coordinate along the selectivity filter of NavAb
and can effectively block the channel in the presence of modest voltages or con-
centrations driving forces. The conformational state of the pore, located at
the same z-coordinate is also found in the two-dimensional potential of mean
forces generated from umbrella sampling and weighted histogram analysis.
In contrast to K+, three Na+ ions move through the selectivity filter together
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 Na 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 provide 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 “dumping” 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, modulate both 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 purifica-
tion of isolated VSDs of the isolated VSDs of human Nav1.5 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\(_{\text{v}}\)) is responsible for the rapid upstroke of action potentials. A key player during myocardial excitation is the cardiac channel isoform Na\(_{\text{v},1.5}\). The general architecture of mammalian Na\(_{\text{v},s}\) 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 domain 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 with Analgesia
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The contribution of the peripheral nerve voltage-gated sodium (Na\(_{\text{v}}\)) channel Na\(_{\text{v},1,9}\) to nociception has been demonstrated in Na\(_{\text{v},1,9}\) knockout mice that...