study we used a selection of peptide neurotoxins against the cardiac Nav1.5 channel to demonstrate the feasibility of screening slow-binding molecules by applying sophisticated voltage protocols, and acquiring stable, high quality recording for more than 30 mins. The toxins used were: 1) Jing/Zhaotoxin-II which is a potentiator or positive modulator of Nav1.5 channels, 2) ProTx-II, which is an inhibitor of Nav1.5 channels, and 3) u-Conotoxin PIIIA a Nav1.4 inhibitor was used as a negative control. The IonWorks Barracuda Plus platform was used for these studies. Results are presented which are in good agreement with peer-reviewed publications. Taken together, these results demonstrate the high-throughput capabilities of this platform for measuring peptide toxins targeted against ion channels.

682-Pos  Board B437
Searching for the Interaction Sites of the Betal Subunit with the Voltage-Sensing Domains of Sodium Channels Using LRET
Tomoya Kubota 1, Bobo Dang 1, Rocío K. Finol-Urdaneta 2, Jérôme J. Lacroix 1, Ludvine Frezza 1, Robert J. French 2, Stephen B.H. Kent 1, Ana M. Correa 1, Francisco Bezanilla 1.
1The University of Chicago, Chicago, IL, USA, 2University of Calgary, Calgary, AB, Canada.
Mammalian voltage-gated sodium channels (Nav) are composed of two subunits: a monomeric pore-forming subunit (α-subunit), that contains four domains (DI-DIV), each composed of a voltage-sensing domain (VSD, S1-S4) and a pore domain (S5-S6) and auxiliary subunits (β-subunits) that are transmembrane proteins with type I topology: containing an extracellular amino-terminus, a single transmembrane segment and an intracellular carboxyl terminus. A large body of literature has shown that interaction between α- and β-subunits results in Nav with altered gating kinetics suggesting that β-subunits may directly interact with VSD. However, aspects of the stoichiometry, arrangement and molecular interaction between α and β subunits remain unclear. In this study, we explored the location of β1 in relation to the rat skeletal muscle sodium channel (Nav1.4) using lanthanide-based resonance energy transfer (LRET) via two strategies. 1) Four Nav1.4 constructs were designed to encode a Tb3+ binding-tag (Nav1.4-LBT) on top of the S4 of each domain (DI-LBT, DII-LBT, DIII-LBT and DIV-LBT) as energy donor. A hexa-histidine-tag was inserted in β1 (6His-β1), which binds a Cu2+ ion to act as acceptor. 2) β1 constructs were designed to encode at an extracellular site an LBT that binds Tb3+ as energy donor while the acceptor was Alexa488 conjugated to Ts1, a β scorpion toxin which binds to DII-VSD in Nav1.4. For both experiments, we used Xenopus laevis oocytes co-expressing Nav1.4 and β1 constructs injected with the cRNA at 1:1 molar ratio. In preliminary results, DIV-LBT + 6His-β1 oocytes displayed robust energy transfer between Tb3+ and Cu2+, indicating that at least one β1-subunit is located at ~28 Å of S4-VSD. Support: 13POST14800031 (AHA), MOP-10053 (CIHR), GM68044-07, U54GM087519 and GM030376.

683-Pos  Board B438
Tracking Voltage-Dependent Conformational Changes of the VSD in Nav with LRET
Tomoya Kubota 1, Pedro Brugarolas 1, Bobo Dang 1, Rocío K. Finol-Urdaneta 2, Ludivine Frezza 1, Robert J. French 2, Stephen B.H. Kent 1, Francisco Bezanilla 1, Ana M. Correa 1.
1The University of Chicago, Chicago, IL, USA, 2University of Calgary, Calgary, AB, Canada.
Voltage-gated sodium channels (Nav) are fundamental for the generation and the propagation of action potentials. Mammalian Nav alpha subunits are single macromolecules organized in four different domains (DI-DIV). Each is composed of 6 transmembrane segments (S1-S6) from which S1-S4 constitute the voltage sensing domain (VSD) and with S5 and S6 constituting the pore. While Nav function has been studied extensively, the exact structural mechanisms of gating are not fully understood. Recently, the crystal structure of the prokaryotic sodium channel, NavAb, has been solved, but the pore and confirm their role in channel gating. Overall, our findings further elaborate on the principal residues that are predicted to alter water structures in the pore. According to molecular dynamics calculations, water polarizability appears to be important for the filling of channel. We observe that water molecules form stable structures (an enthalpy stabilized ice-like phase) inside the channel. We mutated the principal residues that are predicted to alter water structures in the pore and confirm their role in channel gating. Overall, our findings further elaborate on the principal residues that are predicted to alter water structures in the pore and confirm their role in channel gating. We report observation of a novel "remote knock-on" mechanism for enhancement of permeation in Brownian dynamics simulations of a simple monomol channel. Unlike conventional knock-on, which requires a second ion of the same species to enter the channel in order to knock forward and replace an ion already in the channel, the new mechanism does not require the instigating ion to enter the channel, nor that it be of the same species. The figure plots the conditional probability distribution as a function of the position x of the instigating ion at the instant of permeation when the ion initially trapped at x=0 escapes to the right. The curves are plotted for different solute concentrations, and CL. The most probable position of the instigating ion is clearly at the left mouth of the channel (x=-15Å). A 2nd small peak corresponding to conventional knock-on appears at x=0 for high enough CL.

684-Pos  Board B439
Observation of "Remote Knock-On", a New Permeation-Enhancement Mechanism in Ion Channels
Dmitry G. Luchinsky 1,2, Rodrigue Tindjong 2, Igor Kaufman 2, Peter V.E. McClintock 2, Igor A. Khovanov 2, Bob S. Eisenberg 2.
1Mission Critical Technologies Inc, El Segundo, CA, USA, 2Lancaster University, Lancaster, United Kingdom, 3University of Warwick, Coventry, United Kingdom, 4Rush University, Chicago, IL, USA.
We report observation of a novel "remote knock-on" mechanism for enhancement of permeation in Brownian dynamics simulations of a simple monomol channel. Unlike conventional knock-on, which requires a second ion of the same species to enter the channel in order to knock forward and replace an ion already in the channel, the new mechanism does not require the instigating ion to enter the channel, nor that it be of the same species. The figure plots the conditional probability distribution as a function of the position x of the instigating ion at the instant of permeation when the ion initially trapped at x=0 escapes to the right. The curves are plotted for different solute concentrations, and CL. The most probable position of the instigating ion is clearly at the left mouth of the channel (x=-15Å). A 2nd small peak corresponding to conventional knock-on appears at x=0 for high enough CL.

685-Pos  Board B440
A Mutualational and Computational Study of Water and Ion Movement through the S6 Bundle-Crossing of CaV1.2 Channel
Roman Shirokov.
Pharmacology and Physiology, NJMS Rutgers University, Newark, NJ, USA. How exactly voltage-gated calcium and sodium channels open their physical gate formed by the S6 TM segments is not clear yet. To study this, we modeled the pore-forming region of CaV1.2 channel using a combination of homology-based modeling and ab initio Rosetta algorithm. The obtained fold was stable during 100-200 ns long molecular dynamics runs with implicit membrane and solvent. In explicit membrane/water systems, it remained stable during multiple 40-50 ns runs. When tested with the ZDOCK protein-docking algorithm, the fold binds toxin Calichicudine at a site that incorporates residues, which previously were shown to be involved in binding of the toxin. Molecular dynamics with SW4M-NDP water and polarizable ions reveals the presence of novel water-filled cavities on the intracellular side of the channel. We mutated the principal residues that form them and show their critical role in channel gating.

According to molecular dynamics calculations, water polarizability appears to be important for the filling of channel. We observe that water molecules form stable structures (an enthalpy stabilized ice-like phase) inside the channel. We mutated the principal residues that are predicted to alter water structures in the pore and confirm their role in channel gating. Overall, our findings further elaborate on the principal residues that are predicted to alter water structures in the pore and confirm their role in channel gating. We report observation of a novel "remote knock-on" mechanism for enhancement of permeation in Brownian dynamics simulations of a simple monomol channel. Unlike conventional knock-on, which requires a second ion of the same species to enter the channel in order to knock forward and replace an ion already in the channel, the new mechanism does not require the instigating ion to enter the channel, nor that it be of the same species. The figure plots the conditional probability distribution as a function of the position x of the instigating ion at the instant of permeation when the ion initially trapped at x=0 escapes to the right. The curves are plotted for different solute concentrations, and CL. The most probable position of the instigating ion is clearly at the left mouth of the channel (x=-15Å). A 2nd small peak corresponding to conventional knock-on appears at x=0 for high enough CL.

686-Pos  Board B441
Poison-Fermi Model of a Calcium Channel: Correlations and Dielectric Coefficient are Complementary
Bob Eisenberg 1, Jim-Liang Liu 2.
1Rush University, Chicago, IL, USA, 2National Hsinchu University of Education, Hsinchu, Taiwan.
We derive a continuum model of biological calcium channels, called the Poison-Fermi equation, designed to deal with crowded systems in which ionic species and side chains nearly fill space. The model is evaluated in three dimensions. It includes steric and correlation effects and is derived from conjugated with a HiLyte fluor488 (TTX-F), and the peptide β scorpion toxin Ts1, from the Brazilian scorpion Tityus serrulatus, conjugated with Alexa488 (Ts1-Alexa488). Having several donor positions (Tb3+) ions in LBT’s and two different acceptor positions (TTX-F and Ts1-Alexa488), we calculated multiple distances in voltage-clamped Xenopus laevis oocytes expressing our Nav1.4 constructs that remained functionally active. The results provide new insight to structure-function information in mammalian Nav channels. Support: 13P01ST14800031 (AHA), MOP-10053 (CIHR), GM68044-07, U54GM087519 and GM030376.

Voltage-gated Ca Channels