T449A Shaker-IR background with different hydrophobicities according to the Wimley-White hydrophobicity scale (Ala, Cys, or Trp) and charges (Glu, Asp, Arg, Lys) and measured the inactivation kinetics. Mutant channels were transiently expressed in tsA_201 cells. All ionic current experiments were performed with excised inside-out patches. The introduction of an ionizable cysteine and the small but neutral alanine dramatically slowed entry into the inactivated state (the inactivation time constants for Shaker mutant T449A/I470C, T449A/I470A and T449A were 1.4 s, 250 ms and 50 ms, respectively). We could not detect any current on mutant T449A/I470X (X = Asp, Glu, Trp) but the transfection with a mixture of mutant and wild-type expression plasmids expressed currents. These heterotetramers had slower inactivation kinetics with respect to the T449A channels. Almost all of the channels had a midpoint voltage for activation in the range −50 mV to −20 mV. The only exception was the I470C mutant which had a large shift in voltage dependence (midpoint ~5 mV). Mutant channels containing Arg or Lys (I470R, I470K) did not give functional expression either as homotetramers or as heterotetramers. Our results show that not the size but the physicochemical properties of the side chains (hydrophobicity and charge) at position 470 determine the inactivation kinetics of voltage-gated $K^+$ channels, which may reflect interaction of the side chains with permeant ions.

**2723-Pos Board B415**

**Voltage Sensor Domain Mutations Involved in the Kv1.2 Channel Activation via MD Simulations**

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Voltage-gated cation channels (VGC) are membrane-bound proteins responsible for the generation and propagation of the action potential that regulates a number of critical biological events such as skeletal muscle contraction, neuron activity and hormone secretion. These channels are equipped with voltage sensor domains (VS) that act as electrical devices highly sensitive to transmembrane (TM) voltage variations () that modulate the gating state of VGC. Despite major structural conservation within VS from the VGC family, there are marked sequence variations among them related to their diverse kinetic rates for *up-down* turnover. For instance, the VS kinetics is markedly distinct between Kv and Nav channels, a feature that complies with their respective role in the fast and slow phases of the action potential. Currently, original results concerning single point mutations have identified key amino acids directly affecting the kinetic rates of Shaker-like channels activation, such as I237A (Lacroix J.J. *et al*, 2012), F290A (Schwaiger *et al*, 2013), and more recently I287T/V363T (Lacroix J.J. *et al*, 2013).
Here, by benefiting from the well understood activated and resting states of Kv1.2 we investigate the molecular nature of such mutations affecting the *up-down* VS turnover. Specifically, we use all atom MD simulations to investigate the structural effects of the referred mutations to the sensing domain. Anticipating our results, such amino acid changes provoke minor structural rearrangements in the *up* and *down* states of the VS, thus indicating that these mutations could actually be affecting the intermediate states withing the activation pathway of the channel.

**2724-Pos Board B416**

**Atomistic Modeling of Ion Conduction through Voltage-Sensing Domains**

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Voltage-sensing domains (VSDs) are modular membrane protein units that sense changes in the membrane electrostatic potential, and through conformational changes, regulate a specific function. The VSDs of voltage-dependent $K^+$, $Na^+$ and $Ca^{2+}$ channels do not conduct ions under physiological conditions, but they can become ion-permeable under pathological conditions through mutations in the voltage sensor, particularly of S4 basic side chains [1,2]. Relatively little is known about the underlying mechanisms of conduction through VSDs. The most detailed studies have been performed on Shaker $K^+$ channel variants that include the mutation of the outermost Arg residue in S4 to a smaller, uncharged side chain[1,2]. Ion conduction through the Shaker VSD is manifested in electrophysiology experiments as a separate voltage-dependent inward current that appears when the VSDs are in their resting state conformation[1]. Only monovalent cations permeate the Shaker VSD through a narrow and twisted pathway after reaching a vestibular region on the extracellular side of the VSD[1,2]. This permeation pathway is, at least in part, the same as that one followed by the S4 basic side chains during voltage-dependent activation. We sampled VSD ion conduction events on the microsecond timescale under a membrane potential using experimentally validated models of the Shaker VSD[3] in a resting state in order to elucidate the molecular mechanisms of ion conduction, gating, and selectivity. This work was supported by NIH Grants F30CA171717 to M.L.W. R01GM098973 to FT, P01GM86685 to DJT SHW, and P41GM103712-S1 to the National Resource for Biomedical Supercomputing.

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**2725-Pos Board B417**

**Cys Mutation + MTS Caution is Needed in Interpretation of Arg Reaction in the S4 Transmembrane Segment of a Voltage Sensing Domain (VSD) of a Voltage Gated Channel: Results of Quantum Calculations**

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In all previous work on voltage gated channels, mutation of the arginines on the S4 segment of the VSD, then reaction with an MTS reagent, followed by channel shutdown, has been taken to mean that the arginine was exposed on the surface from which the reagent was applied. This may require more care in interpretation. Cysteine is smaller than the arginine by about the size of the reactive sulfonate on the MTS; The mutation leaves a large cavity where the arginine side chain had been, so the MTS can reach the cysteine, possibly via the omega pore. The backbone atoms need not move. The distance between S4 and S2 or S3 remains largely unchanged. Salt bridges, (e.g., R297-E183) however, are disturbed; when cysteine is in the reactive (negative) form, it constitutes a charge reversal mutation, as the arginine was (presumably) positive. Quantum calculations on configurations of this region for R300C of the VSD of Kv1.2 show that the cys anion can fold away from the cavity where it could react, in a manner dependent on the water and protons present. See the preprint posted at http://arxiv.org/abs/1309.1373. Optimizations have been done at BLYP/6-31G** level. Acknowledgement: Computations were done at the Brookhaven National Laboratory CFN cluster, and the CUNY hpc facility.

**2726-Pos Board B418**

**Quantum Calculations Show How the Water at the Gate of the Voltage Gated Kv1.2 Channel Plays a Major Role in Determining Conduction through the Gate**

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We have carried out quantum calculations to optimize the structure of the gate region of a Kv1.2 (with hydrogens added as in the pdb 3Lut structure) channel as well as the structure below the gate as far as the T1 segment, starting from the X-ray structure. A key result: A PVPV–>PVVV in silico mutant disrupts the water structure. The normal gate opens just enough to allow a hydrated $K^+$ to enter the cavity that separates the gate from the selectivity filter. The mutation also alters the electrostatic potential. In addition, we have calculated the result of protonation of the H418 below the gate, which shows approximately a 3 Å rotation toward the pore on protonation, enough to alter the potential at the gate; deletion of H418 is reported to produce a non-functional channel(1). With Cl- ion at the gate, but not $K^+$, a water structure forms in the optimized channel structure, and appears to be fairly stable; this may already select against anions. Calculations in cases that involve $K^+$ include bond order and charges on all atoms, enabling calculation of electrical potentials. Optimizations were done initially at HF/6-31G* level; most have been confirmed at BLYP/6-31G** level, with little change in any case tested. Acknowledgement: Computations were done at the Brookhaven National Lab CFN cluster and the CUNY hpc facility.

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**2727-Pos Board B419**

**Turning a Small into Large Conductance K-Channel - How Far Can We Go?**

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Potassium channels are membrane proteins that allow the passage of $K^+$ ions across the hydrophobic core of the membrane. They display an extremely conserved signature sequence that elicits high ion transport rates and exquisite discrimination between ions with similar radii. Despite of this conservation,

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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