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movements within each of the domains in the human cardiac voltage-gated $Na^+$ channel, $hNa_V1.5$, in conjunction with the cut-open oocyte technique. Our results with $hNa_V1.5$ show significant differences compared to previous VCF results from the rat skeletal muscle isoform ($rNa_V1.4$). Previously, the $rNa_V1.4$ DIII-S4 was shown to immobilize with kinetics that correlated with fast inactivation. In contrast, we show that $hNa_V1.5$ DIII-S4 rapidly returns to the down-state even when fast inactivation has not yet recovered. Based on this result, we hypothesize that the local anesthetic lidocaine, which was shown to alter the DIII-S4 voltage dependence in $rNa_V1.4$, also has a distinct interaction with $hNa_V1.5$. Supporting this hypothesis, we show no significant change in DIII-S4 voltage-dependence with the application of lidocaine to $hNa_V1.5$. Thus, previous lidocaine-$hNa_V1.5$ results that conflict with $rNa_V1.4$ VCF observations are now reconciled by our observation that the mechanism of interaction with the DIII-S4 differs between the two isoforms. In conclusion, despite high homology, significant functional differences exist between hNav1.5 and rNav1.4, in particular in the links between the DIII-S4, inactivation gating and lidocaine interaction.

**203-Plat**

**Crystal Structure of the Navβ4 Extracellular Domain**

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Voltage gated $Na^+$ channels (Nav) are present in all excitable cells and play a crucial role in the generation and propagation of action potentials. The eukaryotic channels are composed of a main α subunit together with one of four different auxiliary β subunits (β1-β4). These β subunits are largely extracellular, with a single transmembrane helix and a short cytoplasmic C-terminal tail. The extracellular domains of β subunits are thought to interact with exposed loops of the pore-forming α subunit and thusmodulate the functional propertiesof NaV channel. Here we present the crystal structure of the extracellular domain of Navβ4 at 1.7Å resolution. The protein displays an immunoglobulin-like fold, with a conserved buried cystine bridge. A reactive cysteine (C58) is located at the surface and forms a likely interaction site with the α subunit. Recent unpublished functional data show that the C58A mutation causes a loss of Navβ4-mediated modulation. We mapped the positions of several disease-causing mutations identified in different Navβ subunits. One of these (C131W) affects the conserved buried cystine bridge, but surprisingly does not cause unfolding of the protein. Rather, a crystal structure of the mutant shows that it causes rearrangements in two distinct areas of the protein, including conformational changes in the loop containing the reactive cysteine.

**204-Plat**

**Molecular Dynamics Studies of Ion Conduction in a Prokaryotic Channel**

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Recently, the crystal structures of putative bacterial, voltage-gated sodium channels have been published, giving an atomic scale view of the NaChBac family of voltage-gated sodium channels. The selectivity filters of the open pore structure of NavMs (McCusker et al (2012) Nature Comm. 3, 1102) and the closed pore structure of NavAb (Payandeh et al (2011) Nature 475, 353) differ only on the order of 1-2 Å in diameter. Previously published studies aimed at predicting the mechanism of ion selectivity through free energy surfaces have not provided a consistent free energy surface. Electrophysiology studies suggest that sodium selectivity in the NaChBac family is slight when compared to the potassium selectivity in potassium-selective ion channels (Shaya et al (2011) PNAS 108, 12313). Multi-microsecond equilibrium simulations of NavAb by Chakrabarti et al. suggest that conformational movements of the glutamate side chains are correlated to sodium movement through the selectivity filter (PNAS (2013) 110, 11331).

We present microsecond timescale molecular dynamics simulations of ion conduction through a truncated model of the NavAb pore as a function of applied voltage, in solutions containing sodium, potassium, and both, and as a function of concentration. The model NavAb is constructed from the NavAb pore in which the S5 and S6 helices are truncated, creating an open pore, and embedded in a neon support. The S5 and S6 helices and the support are restrained, but the pore helices and selectivity filter are unrestrained. We show that conformational distribution of the glutamate side chains is voltage dependent. The dependence of selectivity on voltage, concentration, and cations present is demonstrated. Additionally, it is apparent that the rate of and predominant mechanism of conduction is not only dependent upon the voltage and cation but also upon the direction of current.

**205-Plat**

**Phosphoproteomic Identification of CaMKII- and Heart Failure-Dependent Phosphorylation Sites on the Native Cardiac Nav1.5 Channel Protein**

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Voltage-gated $Na^+$ (Nav) channels are key determinants of myocardial excitability and defects in Nav channel functioning or regulation, associated with inherited and acquired cardiac disease, increase the risk of life-threatening arrhythmias. In heart failure, the inactivation gating properties of Nav1.5 channels are altered, resulting in decreased channel availability and increased late $Na^+$ current. Although previous studies have suggested roles for CaMKII and CaMKII-dependent Nav1.5 phosphorylation sites, the global native phosphorylation pattern of Nav1.5 channels associated with these pathophysiological alterations is unknown. Mass spectrometric (MS)-based phosphoproteomic analyses were undertaken to identify in situ the native phosphorylation sites on the Nav1.5 protein purified from ventricles isolated from mice (CaMKIIdc-Tg) overexpressing CaMKIIdc in the heart. Quantitative analyses of Nav1.5 phosphopeptides allowed comparing the relative abundances of Nav1.5 phosphorylation sites in CaMKIIdc-Tg, versus wild-type, ventricles. A total of eighteen Nav1.5 phosphorylation sites were identified, seven of which are novel as compared with those reported in our previous LC-MS/MS analyses. Fourteen of these sites are located in the first loop, one in the second loop, one in the N-terminus and two in the C-terminus of Nav1.5. Interestingly, out of these eighteen phosphorylation sites, the C-terminal phosphoserine pS1938 is present in the CaMKIIdc-Tg IPs (n=3/4) and absent in the WT IPs (n=0/4), and pS1989 is 9-fold more represented ($p<0.05$, n=4 in each condition) in the CaMKIIdc-Tg, compared with the WT, IPs. Relative abundances of the other phosphopeptides, identifying the other phosphosites, are similar to the mean relative abundance found for all the other unmodified Nav1.5 peptides. These analyses (1) provide seven novel native cardiac Nav1.5 phosphorylation sites, and (2) identify two novel C-terminal Nav1.5 phosphorylation sites as potential critical determinants of the pathophysiological alterations of Nav1.5 channels in heart failure.

## Platform: Molecular Dynamics I

**206-Plat**

**Conformational Dynamics During GPCR - G Protein Coupling**

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Upon activation by agonists, G-protein coupled receptors (GPCRs) in their active R* form transmit extracellular signals into the cell by catalysing GDP/GTP exchange in heterotrimeric G-proteins (Gαßγ, G). The GPCR rhodopsin activates Gt to transmit the light signal into retinal rod cells. After photon capture, the rhodopsin activity jumps by more than one billion fold implying both high fidelity and speed of active rhodopsin (RhR*)/Gt coupling. We employed all-atom molecular dynamics (MD) simulations to study the conformational diversity of membrane embedded rhodopsin and extend the static picture provided by the available crystal structures. An intrinsically unstructured cytoplasmic loop region connecting transmembrane helices 5 and 6 (ICL3) is identified. The MD data show how each protein state is split into several conformational substates. Only a single conformational substate with largely helical ICL3 is selectively stabilized by the Gtα C-terminus (GtαCT), as in the crystal structures. A mechanism for the fast and precise signal transfer from rhodopsin to Gt is proposed, which assumes a stepwise and mutual reduction of their conformational space. The analysis was extended to the interaction of GsαCT and GiαCT with activated β2-adrenoceptor (β2AR*) to address the issue of receptor promiscuity. We find that a short region of GsαCT stabilizes the open β2AR* conformation provided by the β2AR*/Gsαßγ crystal structure. In the crystal structures of RhR* with GtαCT, a close homologue of GiαCT, RhR* exhibits a more closed active conformation. With the slim GiαCT peptide we find that β2AR* adopts a similarly closed conformation as RhR* with GtαCT. This analysis elucidates how G-protein coupling specificity relies on the fold and primary structure of the Gα C-terminus.

Scheerer et al. (2008), Crystal structure of opsin in its G-protein-interacting conformation, NATURE
Elgeti et al. (2013) Precision vs Flexibility in GPCR signaling, JACS