in the gating properties of Hv1 when coexpressed with NVS, and NVS was not capable of interacting with Hv1 in pull down assays. Having no apparent interaction with Hv1, we set out to determine whether NVS forms oligomers. Using chemical crosslinking and pull-down assays, we see formation of oligomers that are consistent with dimers and dependent on the C-terminus. Taken together, our results support the hypothesis that NVS is a voltage sensing protein capable of dimerization, with a function independent of Hv1. We are currently using these findings and approaches to investigate the structural assembly of NVS and to identify interaction partners.

2141-Pos Board B278
Putative Voltage Sensitive Enzymes in Prokaryotes
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A voltage sensor domain (VSD) is a protein module that rearranges its conformation based on the electric potential of the cell membrane. VSDs have classically been described as N-terminal modules that confer voltage sensitivity to C-terminal pore domains in ion channels. More recently, N-terminal VSDs have been shown to confer voltage response to C-terminal enzyme modules in voltage sensitive enzymes (VSE), while the isolated VSDs of voltage gated proton channels (Hv1) perform both voltage sensing and proton channel functions. So far, VSEs and Hv1s have been found only in eukaryotes. We have identified a set of prokaryotic sequences that contain a VSD homolog; however, the C-terminal domains of these sequences, which we refer to as putative prokaryotic voltage sensitive enzymes (ppVSE), are dramatically different from ion pores. As expected, predicted secondary structures of the N-terminal domain are similar to those for bona fide VSDs; however, unlike the pore domains of ion channels, which contain two transmembrane helices, predicted structures of the C-terminal domains of the ppVSEs do not contain transmembrane helices. Alignment of individual domains to the HMM of the ion channel pfam pf00520 indicates significant similarity of ppVSE N-terminal domains but no detectable similarity of ppVSE C-terminal domains to the pf00520 HMM. A phylogenetic analysis of VSDs from prokaryotic sequences indicates a distinct lineage of the ppVSE VSD. This is the first documented evidence of a prokaryotic VSD-containing protein that does not have a pore domain.

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Sequence Signature of Voltage Sensing Detected via Dimensionality Reduction Techniques
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The family of six-transmembrane-helices channels shows recognizable sequence homology and a strictly conserved structural architecture; yet these channels are involved in a significantly heterogeneous set of physiological functions, ranging from reporting noxious environmental conditions, to shaping the neuronal action potential, to syncing the beating of the heart. A striking example of this heterogeneity is provided by the comparison between the polymodal transient receptor channels (TRPs), whose activation can be regulated by several stimuli, and the voltage-gated ion channels (VGCs), which are activated by the variations of the transmembrane potential through. By analyzing multiple sequence alignments spanning the first four transmembrane segments (the voltage sensor domain in VGCs) and comparing TRPs and VGCs, we highlight the sequence determinants of voltage-driven activation. To this end we exploit the concept of fractal dimension to characterize the complexity of the two datasets. Moreover, we use a novel feature-selection approach, to identify the so-called maximally informative samples, i.e. set of residues whose distribution is maximally informative about the selection pressure that generated and differentiate the sequence ensembles.

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Lipid-Dependent Conformational Transitions in KvAP are Driven by Voltage Sensing Domain
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Recent electrophysiological studies have shown that the mechanism of voltage-driven gating in Kv channels is exquisitely sensitive to the composition of the lipid membrane. For instance, non-phosphate lipids can dramatically right-shift the voltage dependence (G-V curve) of KvAP (1,2) in a way that promotes the stabilization of the ‘‘down’’ conformation of the voltage sensor. We evaluated the conformations of KvAP’s isolated VSD by means of site directed spin labeling CW EPR and DEER spectroscopy through reconstitution in lipids with (POPC:POPG) or without (DOTAP) phosphate groups. Our data suggested a novel Tilt-Shift model for the mechanism of voltage sensing with a ~3 Å upward tilt and simultaneous ~2 Å axial shift of S4 (3). We have extended these measurements to evaluate the effects on the KvAP pore domain and quantify the DOTAP molar fraction dependence on the conformation of the inner gate. While in the full-length channel DOTAP reconstitution triggers conformational rearrangements in both the S4 segment and the S6 inner bundle gate, in the absence of voltage sensing domain, DOTAP is unable to generate significant rearrangements in S6. This result is consistent with the idea that S4 movements are allosterically transmitted to the inner bundle gate. Although the mechanistic correlation between voltage-dependent and lipid-dependent gating in voltage sensing domains remains to be fully characterized, it is clear that just as with electric fields, the interacting lipids play a determinant role in defining the equilibrium between the activated and resting states of voltage sensing domain.
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2144-Pos Board B281
Molecular Determinants of Temperature Dependent Gating of Ion Channels
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Physiological sensation of heat or cold in higher organisms is mediated by specialized ion channels whose opening and closing is exquisitely regulated by ambient temperatures. Members of TRP channel family, a branch of the much larger voltage-gated ion channel superfamily, serve as the primary physiological thermo-sensors. However, the physicochemical underpinnings of high temperature-sensitivity of channel gating remain poorly understood. Here, using a heuristic protein design approach, we have transmuted a temperature-insensitive potassium channel into a heat or a cold-sensitive channel. By varying amino acid polarities at sites undergoing state-dependent changes in solvation, we were able to systematically confer temperature-sensing phenotype to a prototypical voltage-dependent potassium channel. We also demonstrate that magnitude of voltage-sensing charges inversely modulate temperature-sensitivity consistent with predictions of thermodynamic coupling. These emerging molecular principles provide a template to understand varied temperature-dependent gating phenotype in channels with conserved transmembrane architecture.

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The Gating Charge of Kv1.2 is Less than Expected from its Similarity to Shaker
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Much has been learned about the voltage sensors of ion channels since the X-ray structure of the mammalian voltage-gated potassium channel Kv1.2 was published in 2005. The availability of high-resolution structural data of a Kv channel paved the way for the structural interpretation of numerous electrophysiological findings collected over many years in a variety of ion channels, most notably Shaker, and allowed the development of meticulous computational simulations of the activation mechanism of Kv1.2. The fundamental premise of the validity of the interpretation of functional measurements from Shaker using the structure of Kv1.2 is that both channels are related closely enough such that correlation of their data is a trivial task. We set out to confirm these assumptions by measuring the voltage sensitivity of the channel using the limiting slope method, followed by the determination of the gating charge through gating current recordings. We found that the gating charge, as measured by both techniques, is 10 e0, ~25% less than the well-established 13-14 e0 in Shaker. Next, we neutralized each of the six positive residues in S4 of Kv1.2 to probe the cause of the reduction of the gating charge, and found that while replacing R1 with glutamine decreased voltage sensitivity to just about 50% of the wild-type channel value, mutation of the subsequent arginines did not have an effect nearly as large. These data stand out as different to Shaker’s, where removal of the first four positive residues reduces the gating charge by roughly the same amount of elementary charges. We propose that the septum that separates the aqueous crevices in the VSD of Kv1.2 is thicker than Shaker’s, and that this accounts for a smaller gating charge in Kv1.2.