guanidine moieties of R255 and R258 from S4, thereby preventing 2-GBI access to its binding site. Additional binding modes were investigated by a combination of MD and Free Energy simulations.

3765-Pos Board B493
Inhibition of Voltage-Gated Hv1 Channel by Guanidine Derivatives
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The voltage-gated proton channel Hv1 plays a key role in the regulation of ROS production by NOX enzymes in phagocytic cells. Excessive channel activity enhances proliferation and invasiveness in cancer cells, and worsens brain damage after ischemic stroke. The Hv1 channel is composed of two subunits, each containing a proton-permeable voltage-sensing domain (VSD) and lacking the pore domain typical of other voltage-gated ion channels. We have previously shown that the compound 2-guanidinobenzimidazole (2GBI) inhibits the Hv1 channel and that its binding site in the VSD is accessible from the intracellular side of the membrane only when the channel is open. Here, we examine the apparent binding affinities of a series of 2GBI derivatives on Hv1 channels mutated at positions located in the core of the VSD and apply mutant cycle analysis to determine the most likely interactions between channel and inhibitor. We identify four residues involved in 2GBI binding and determine the orientation of the blocker inside the VSD. Our data provide a simple explanation for the very large difference in binding affinity between 2GBI and the related compound 2-guanidinobenzoxazole (2GBOZ), and suggest ways to design more effective Hv1 inhibitors. We also investigate the molecular characteristics that allow guanidine derivatives to reach the binding site when added from the extracellular side of the membrane so that they can be used to block endogenous proton channels under physiological conditions. This work is supported by NIH -National Institute of General Medical Sciences, grant R01GM098973.

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Exploring Conformational Rearrangements in a Novel Voltage-Sensing Protein
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Through bioinformatic searches, we have identified a protein coded by the C15orf27 gene that we named NVS (Novel Voltage Sensor). NVS contains 531 residues and consists of 3 parts: an S1-S4 domain, a 90 residue N-terminus and a 307 residue C-terminus, both of which are predicted to be intracellular. The most critical residues found in S1-S4 domains of other voltage sensors are conserved in NVS, including 3 Arg and a Lys in the S4 helix, 4 conserved acidic residues in S1-S3 and the charge-transfer Phe in S2. In addition, the C-terminus is predicted to contain a coiled-coil domain, similar to Hv1. Our working hypothesis is that NVS functions as a voltage sensor that couples to intracellular signaling pathways (as yet undefined) or interacts with Hv1 to form hetero-oligomers through the C-terminal coiled-coil domain. In the present study we used site-specific voltage-clamp fluorometry to look for evidence that NVS may undergo conformational rearrangements in response to changes in membrane potential. We identified several positions on the S3-S4 loop where introduced and labeled Cys residues produced changes in fluorescence as a function of membrane potential. Several positions give complex fluorescence responses, starting with a rapid increase in fluorescence followed by slower decrease in fluorescence. Additionally we applied fluorescence quenchers extracellularly and examined the voltage dependence of quenching. Our results show that depolarization increases fluorescence quenching, suggesting the quencher has increased access to the fluorophore at positive membrane potentials. Taken together, our results support the hypothesis that NVS undergoes a conformational change in responses to membrane depolarization, and we are currently investigating the oligomeric state of NVS testing whether it can form heteromeric complexes with Hv1.

3767-Pos Board B495
The Resting State of Human Proton Channel from Functional and Structural Determinations
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The human voltage gated proton channel (Hv1) plays an important role in a wide variety of physiological functions, including male fertility, immune response and metastatic proliferation. Upon transmembrane voltage stimulus, the rearrangement of positively charged S4 leads to highly selective and directional conduction of proton. Hv1 carries out this function on the structural blueprint of the S4-containing voltage sensing domains (VSD). As a way to understand Hv1 gating, we have developed a simple biochemical preparation of functional Hv1 as shown by established fluorescence based proton flux assay. We have probed Hv1 in the functional proteoliposome by site-directed spin labeling EPR spectroscopic (SDSL-EPR) methods. Mobility and accessibility information show that the secondary structure of transmembrane segments is generally consistent with the expected VSD topology. Solvent accessibility measurements revealed that the gating Arginines (205, 208 and 211) are located on the lower part of S4, the internal leaflet of the bilayer. The presence of a narrow solvent occluded region strongly suggests a short proton conduction pathway, and does not seem to be compatible with the idea of a long water wire across membrane. Furthermore, the Hv1 represents a resting state VSD at 0 mV according to its G-V curve . The structural details from Hv1 provide unique insight to not only the proton conduction, but also the mechanism of voltage sensing.

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Electromechanical Coupling in Gating of the Hv1 Voltage Sensor is Revealed by Resting-State Currents in an S4 Arg to His Mutation (R205H)
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In the tetrameric Shaker voltage-gated K^+ channel, the gating of a resting-state proton currents in the S4 mutant R362H reports a conformational change associated with voltage sensor (VS) activation (Starace, et al., 2004). Here we show that the analogous R205H mutation in the Hv1 proton channel mediates a novel H^+ 'shuttle' conductance (G_{SH}) that is gated off by membrane depolarization. The similarity of G_{SH} in Hv1 R205H to the channel-like Shaker R362H suggests that G_{SH} gating can be used to measure VS activation in Hv1. The intrinsic or 'aqueous' H^+ conductance (G_{AQ}) in Hv1 R205H occurs mainly at more positive voltages than G_{SH}, producing a distinctive U-shaped G-V relation. The separation of G_{SH} from G_{AQ} gating along the voltage axis implies that initial voltage sensor activation precedes the opening of G_{AQ} in Hv1. Changes in the transmembrane pH gradient that are known to shift the apparent voltage dependence of G_{AQ} gating similarly affect G_{SH}, indicating that pH-dependent gating occurs early in the Hv1 activation pathway. Second-site mutations of an S3 acidic residue (D185) that is selectively conserved in Hv1 orthologues shift the voltage dependence of G_{AQ} gating, but do not alter G_{SH} gating. D185 mutations are thus interpreted to perturb coupling between VS activation and the opening of G_{AQ}. Another second-site mutation that results in voltage-dependent block of G_{AQ} (R205H-N214R) does not affect the voltage dependence of either G_{SH} or G_{AQ} gating. The existence of VS-pore coupling in Hv1, which lacks a canonical pore domain, demonstrates that electromechanical coupling is not exclusive to tetrameric voltage-gated cation channels.

3769-Pos Board B497
Long Alpha-Helices Projecting from the Membrane as the Dimer Interface in the Voltage-Gated H^+ Channel
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The voltage-gated H^+ channel (Hv) is a H^+ permeable voltage-sensor domain (VSD) protein that consists of four transmembrane segments (S1-S4). Hv assembles as a dimeric channel and two transmembrane channel domains function cooperatively; which is mediated by the coiled-coil assembly domain in the cytoplasmic C-terminus. However, the structural basis of the inter-domain interactions remains unknown. Here, we provide a picture of the dimer configuration based on the analyses of interactions among two VSDs and a coiled-coil domain. Systematic mutations of the linker region between S4 of VSD and the coiled-coil showed that the channel gating was altered in the helical periodicity with the linker length, demonstrating that two domains are linked by helices. Cross-linking analyses revealed that the two S4 helices were situated closely in the dimeric channel. Thus, continuous helices stretching from the transmembrane to the cytoplasmic region in the dimeric interface regulate the channel activation in the Hv dimer.

3770-Pos Board B498
Ph Sensitivity of Voltage Sensing Domain Relaxation
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Voltage sensing domain (VSD) relaxation is a process involving a voltage-independent transition from the activated state into the relaxed state. This process has been shown to shift the voltage dependence for sensing charge movement (sensing current) in the voltage sensitive phosphatase (VSP) known as Ci-VSP and to slow down the deactivation of potassium currents in *Shaker* and Kv1.2. Given the effect of VSD relaxation on the dynamics of these voltage sensitive proteins, it is thought that the relaxed and the active states are comprised of different set of conformations. Thus, it is possible that these states are sensitive to different physiological parameters. Here, it is shown that VSD activation and relaxation in Ci-VSP display different sensitivity to external pH