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'tuned' sensor that yields an optical response at physiological values. Mutations to the S2 domain improved the speed of the optical response to less than 5 msec Tau on/off. Double mutations incorporating the S2 and S4 mutations were not necessarily additive suggesting potential interaction sites between the S2 and S4 transmembrane domains.

3754-Pos Board B482

The Effect of Interhelical Loop Length and Composition on the Electrostatic Interactions of the Voltage Sensor Domain of JShak1

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Voltage gated potassium ion (Kv) channels are trans-membrane proteins that open and close their ion selective pore in response to changes in membrane voltage. Three negatively charged residues in the S2 and S3 helices are conserved in most Kv channels and form an electrostatic interaction network with positively charged residues of the S4, voltage sensing, helix. Previous studies with mouse Kv1.2 have shown that the length and composition of the S3-S4 linker are important in shaping the voltage response of Kv channels. To analyze how this S3-S4 loop effect has been adapted in a different lineage of the channel, we have studied the Kv1-family channel jShak1 from the jellyfish Polyorchis penicillatus. In jShak1 the conserved negatively charged glutamate residue in the S2 helix (position 227) is replaced by neutral asparagine. jShak1 has one less positively charged motif in the S4 helix and a very short S3-S4 linker, consisting of only five amino acid residues. Previous studies with jShak1 indicate that these factors might be responsible for constraining the interactions between the charged residues of the voltage sensing domain by limiting the movement of the S4 helix. We have replaced the natural short loop of jShak1 with different homo-polymers, varying both in length and composition and have mutated the last positively charged residue (K) in both natural and synthetic homo-loops. N227D and N227E mutations were combined with all synthetic loops to make double mutant channels. The homo-loops without a negatively charged residue at 227 do not shift the equilibrium significantly, but that the nature of the C-terminal residue alone can affect the V50 and both the V50 and the Boltzmann slope factor, when combined with a negatively charged residue at 227.

3755-Pos Board B483

Direct Evidence of Conformational Changes Associated with Voltage-Gating in a Voltage Sensor Protein by Time-Resolved X-Ray/Neutron Interferometry

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Voltage-gated cation (e.g. Na^+, K^+) channels are central to neurological signal transmission. The mechanism of voltage-gating, namely the coupling of conformational changes in the voltage sensing domain (VSD) in response to depolarizing potentials with respect to the resting transmembrane electric potential, to opening the pore domain (PD) resulting in transmembrane ionic current, remains unresolved. We report the direct measurement of changes in the scattering-length density (SLD) profile of the VSD protein, vectorially-oriented within a reconstituted phospholipid bilayer membrane, as a function of the transmembrane electric potential by time-resolved x-ray and neutron interferometry. The changes in the experimental SLD profiles are predicted by molecular dynamics simulations, thus providing an interpretation in terms of the VSD's atomic-level 3-D structure.

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Networks of Coevolving Residues in Voltage Sensor Domains

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Voltage sensors (VSDs) are ubiquitous protein domains acting as transducers of transmembrane electric signals. Owing to the fundamental nature of this task, VSDs emerged before the division between prokaryotes and eukaryotes and are accordingly found in extant genes from long-diverged phylogenetic lineages. Unrelated members of this class of domains show a striking degree of structural similarity and, more importantly, a unique voltage-driven conformational transition. The universal operating principles of these exquisite molecular devices are encoded in a surprisingly short sequence of amino acids. Here, we investigate this intriguing sequence-function relationship using approaches from information theory and probabilistic models. We find that VSD sequences conform to a small set of rules, or design principles, constraining the chemical identity of relatively few amino acids. We then recapitulate previous experimental findings and, importantly, formulate novel hypotheses. Notably, our approach opens the fascinating perspective of an evolutionarily-informed de novo design of VSDs.

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A Structure Based Course-Graining Physical Modeling of the Voltage Activated Kv1.2 Channel - Simulating and Analyzing the Fast Gating Current

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K^+ ion (Kv) channels catalyze ion permeation across membrane in a voltage dependent way, regulating electrical activities in cells. Several functional and structural studies demonstrate that Kv has a voltage sensor domain (VSD) that is coupled to changes in the membrane potential. An increase in the membrane potential activates the VSD, which drives the gate domain to open, generating voltage dependent ionic currents. There have been intense studies of the mechanism of the activation of Kv channels, using the kinetic models built from electrophysiological measurements. Recent structural studies also offered a detailed structural mechanism (with a phenomenological free energy landscape), suggesting how the movements of the conserved ARG residues in the VSD, upon changes in the membrane potential, can be facilitated. Starting with structural models of the voltage activation of the K1.2 channel and using our coarse-graining (CG) model allowed us to simulate the response of the channel to voltage changes. The simulations allowed us to evaluate the gating charge in terms of the change in the simulated electrolytes distribution. The change in gating charge was then converted to gating current using our renormalization model and the resulting time dependent of the protein fluctuation. The CG free energy landscapes allowed us to simulate the slow component of the gating current for the activation processes. Most significantly, our unique ability to simulate the time dependence of the fast gating current reproduces the observed trend, thereby providing a detailed picture of its molecular origin.

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Role of Charged Residues in the Regulation of Voltage Sensor Movement in Herg K^+ Channels

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The human Ether-a-go-go Related Gene (hERG) K^+ channel plays a central role in cardiac action potential repolarization. Compared to other Kv channels where fast activation is due to rapid voltage sensor (S4) movement, hERG activation is slow and the mechanisms remain under debate. Previous studies have shown that charges in the voltage sensing domain contribute to determining the rate of channel activation, which includes transition of the S4 and downstream events in S4-pore coupling. The functional role of individual charges in S4 movement alone is still unclear in hERG channels. We have investigated the effect of neutralization of positive charges (K525-K538) on the voltage dependence of S4 equilibrium by examining the voltage and time-dependent accessibility of cysteine substituted I521 in the tip of the S4. At −120 mV, 521C is almost inaccessible to MTSET for R531Q, R537Q and K538Q, but is accessible for R528Q, R534Q and K525Q. This suggests that at −120 mV, position 521 in the R531Q and R537Q, K538Q mutants is mostly buried, whereas it is extruded in the other mutants. The slopes of the S4 equilibrium curves of K538Q and R531Q are greatly altered, indicating that K538Q favors the transition of S4 from the resting to activated states, while R531Q favors the reverse transitions. To extract the kinetics of S4 movement, we examined the dependence of the MTSET modification rate on pulse duration. We found that time constants of S4 equilibrium for R528:I521C, R537:I521C and R531Q:I521C are slower than control, with the largest effect seen for R531Q:I521C. Our data suggest that K525, R528, R534 normally play a role in stabilizing S4 in the resting state; while R528, R537 and R531 facilitate the transition of S4 from the resting to the activated state.

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The N-Terminus of Auxiliary β Subunit is Involved in the Modulation of Voltage Sensor BK Pore-Forming α Subunit

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BK channels and their modulatory β subunits are found in most mammalian tissues ranging from brain to liver to smooth muscle, where they could be modulated by specific β subunits (β1-β4). Recently, we showed that auxiliary β1 and β2 subunits stabilize the BK voltage sensor in the active conformation, and that β3 has no effect on voltage sensor equilibrium (Contreras et al., 2012). Despite the fact that the BK phenotype produced by each of the β subunits has been well characterized, controversies exist regarding the molecular mechanisms by means of which these auxiliary subunits can modify the gating of BK channel. To identify the molecular determinants that confer to the β1 the ability to modulate the voltage sensor of BK channels, we constructed fifteen chimeras of β1/β2 and β1/β3 subunits by exchanging the N- and C-terminus, the transmembrane (TM) segments, and the extracellular loops, between them. The RNA of chimeric constructs were injected in Xenopus laevis oocites and gating currents were recorded in permeant ions free-solutions and in the absence of

internal Ca^{2+} by patch clamp on inside-out configuration. Gating currents were elicited by steps of 1 ms to increasing voltages ranging from −90 to 350 mV in increments of 10 mV, and integrated during the onset of depolarizing pulses to generate charge versus voltage (Q/V) curves. Our results show that the effects of the β1 subunit on the resting-active equilibrium of the voltage sensor is determined by the N- and C-terminal with a major contribution of the N-terminus.

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Whole-Cell Gating-Charge Measurements for Analysis of Allosteric Domain Coupling in Hslo1 BK Channels

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Large-conductance calcium- and voltage-activated potassium (Slo1 BK) channels are important for smooth muscle contraction and neurotransmitter release. Since BK channel gating is allosteric in nature, the conformations of the domains sensing transmembrane voltage (VSD) and intracellular calcium (CSD) reciprocally influence each other, either directly or via the central pore/gate domain (PGD). Thus, to fully understand the gating of BK channels involving multiple domains, determination of voltage dependence of both conductance (GV) and gating-charge movement (QV) is required. Here we evaluated how VSD-linker segments facing the CSD influence channel gating. Whole-cell gating currents were measured upon BK expression in tsa201 cells. In the absence of intracellular Ca^{2+}, the voltage for half-maximal off-gating charge movement ($V_{0.5,Q}$) was 159 ± 2 mV (n=6) with a corresponding apparent gating charge (Q_{app}) of 0.80 ± 0.02 e_0. Coexpression of the human β1 subunit or increasing intracellular $[Ca^{2+}]$ to 10 μM left-shifted $V_{0.5,Q}$ by 42 mV and 70 mV, respectively, without significant changes in Q_{app}. To investigate allosteric domain interactions in BK, we analyzed the effect of combining mutations within S4/S5 and S6/C linkers on GV and QV in the absence of Ca^{2+}. S6/C-linker mutations G327F and K330F shifted the GV by 109 mV and −133 mV and the QV by 7 mV and −10 mV, respectively. S4/S5-linker mutations F223A+L224A and E219R shifted the GV by −22 mV and 120 mV, and the QV by −97 mV and −109 mV, respectively. In the background of G327F or K330F, GVs were further shifted by up to −200 mV only by F223A+L224A. The data suggest that residues F223 and L224 (S4/S5 linker) and G327 and K330 (S6/C linker) are involved in allosteric coupling in BK channels. Supported in part by DFG HE 2993/8 and NIH.

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Probing the Voltage Gated Proton Channel Hv1 with FRET

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The Hv1 proton channel has been shown to contribute to acid extrusion after acid loading in cells, it is involved in pH regulation in the airway epithelium and in the innate immune system during the respiratory burst and it has been proposed to play a role in the capacitation process of the sperm, aditionally the proton channel can also help set the membrane potential. The Hv1 channel is similar to the voltage sensing domain of voltage gated K^+ and Na^+ channels, it assembles into a dimer were each of the subunits forms a permeation pathway which is highly cooperative. Here, by measuring Foster Resonance Energy Transfer (FRET) between fluorescent proteins and with the non-fluorescent molecule dipicrylamine (DPA), we attempt to get some insigths about the organization and function of the Hv1 proton channel.

mCitrine or mCerulean fluorescent proteins were attached to the C-termini of the channel. HEK 293 cells were cotransfected with the constructs and FRET was measured by the spectral FRET method. DPA was used as an energy acceptor for the mCitrine fluorescence in injected Xenopus oocytes membrane sheets. DPA intercalates in the membrane, quenching the fluorescence by a FRET mechanisms, allowing distance measurements between the membrane and the C-termini.

We have been able to determine the relative separation between the C-termini of the dimer and its relation to the membrane plane. Results are consistent with the X-ray structure of the coiled-coiled C-terminal domain.

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3762-Pos Board B490

The Activation Kinetics of the Voltage-Gated Proton Channel is Drastically Accelerated by Unsaturated Fatty Acids

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The unsaturated fatty acids are important component of the biological membranes and the precursors of mediators of inter- and intra-cellular signaling. It is well-known that the unsaturated fatty acids, including arachidonic acid (AA, C20:4), modulate functions of various ion channels in a distinct manner; some ion channels are activated whereas others are inhibited. The voltage-gated proton channel (VSOP/Hv1) can control the proton conductance by membrane voltage and pH. It helps the production of reactive oxygen species by NADPH oxidase in immunocytes. The enhancement of the production of reactive oxygen species by AA has been reported to be accompanied by the increase of Hv currents in neutrophils, macrophages, and eosinophils. However, the detailed molecular mechanisms of actions of AA on the voltage-gated proton channel have remained elusive. Here we report the effects of AA on mouse Hv1 heterologously expressed in HEK293T cells by electrophysiological analysis. We measured Hv currents in the inside-out patch clamp configuration under pH 7.0 in both solutions of pipette and bath. The addition of 50 uM AA with rapid-perfusion system immediately increased the magnitude of Hv currents through mHv1 that are evoked during one second depolarizing step to 100 mV by 20 times. After washout of AA, the currents rapidly returned to the original current level. The analysis with sixty second depolarizing pulse showed that Hv currents exhibited more than 15 times faster activation kinetics and 1.5 times larger steady-state conductance upon the addition of AA. We also examined the effects of other fatty acids and constructs of mHv1. Based on these results, we would discuss the molecular mechanisms and the essential sites for the actions of unsaturated fatty acids on Hv1.

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Electrostatic Interactions in the Closed and Open States of Voltage-Gated Proton Channels

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Voltage-gated proton channels (Hv1) play important roles in the respiratory burst, in pH regulation, in spermatozoa, in apoptosis, and in cancer metastasis. Unlike other voltage-gated cation channels, the Hv1 channel lacks a centrally located pore formed by the assembly of subunits. Instead, the proton permeation pathway in the Hv1 channel is within the voltage-sensing domain of each subunit. The gating mechanism of this pathway is still unclear. Mutagenic and fluorescence studies suggest that the fourth transmembrane segment, S4, functions as the voltage sensor and that there is an outward movement of S4 during channel activation. Using thermodynamic mutant cycle analysis, we find that the conserved positively charged residues in S4 are stabilized by counter charges in the other transmembrane segments both in the closed and open states. We construct models of both the closed and open states of Hv1 that are consistent with the mutant cycle analysis. These structural models suggest that electrostatic interactions between transmembrane segments in the closed state pull hydrophobic residues together to form a hydrophobic plug in the center of the voltage-sensing domain. Outward movement of S4 removes the hydrophobic plug, as if this hydrophobic plug works as the gate that prevents protons to permeate the Hv1 channel in the closed state.

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On the Location of Binding Sites of 2-Guanidinobenzimidazole in the Voltage-Gated Proton Channel

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Voltage-gated proton channels (Hv1) play important roles in the respiratory burst, in pH regulation, in spermatozoa, in apoptosis, and in cancer metastasis. The ability to block them selectively is an important target for drug development and for biophysical studies of channel function. Recently, 2-guanidobenzimidazole (2-GBI), a selective and state-dependent blocker of Hv1 channels was identified. However, the exact location of the binding site for a blocker as well as its apparent state-dependence has yet to be established. Recently, we have investigated the structure of the closed and open states of the voltage-gated proton channel through a combination of modeling and experimental analysis. To understand the mode binding and blocking and to hopefully identify the binding pocket, an initial docking study was performed for all of the ligands reported by Hong *et al* (25) as having significant binding affinity with the open channel monomer.

We tested whether our open- and closed-state models could explain the state-dependence of the binding of the guanidine analogue 2-guanidobenzimidazole (2-GBI) from the cytosolic side of the channel. A combination of free-energy simulations and molecular docking established the architecture of the cytosolic binding site for 2-GBI. The site is centered near F198, consistent with the experimental data allowing for interactions between the positively-charged guanidine moiety of 2-GBI and the negatively-charged residues E201, D222, and E219. The aromatic ring of 2-GBI is stabilized by residues in the proximity of F198. In the closed state model, E201, D222, and E219, are occupied by the