membrane. Multiple Kv channels appear to have affinities for lipid rafts, including the Iks channel complex. Here, we describe the overexpression and purification of the isolated voltage sensor domain of KCNQ1, as well as reconstitution and NMR characterization of this protein in model membranes with varying levels of cholesterol and sphingomyelin. The results suggest that these special lipids induce unique effects on the structure and dynamics of this voltage sensor domain. This work is supported by US NIH grant RO1 DC007416.

3473-Pos Board B334
Protein- Lipid Interactions in Lipid Reconstituted Potassium Channel Voltage-Sensing Domains
Dmitriy Krepkiy, Klaus Gawrisch, Kenton J. Swartz.
NIH, Bethesda, MD, USA.
S1-S4 voltage-sensing domains in voltage-activated ion channels control opening and closing of an associated pore domain. Electrophysiology experiments suggest that the transition of S1-S4 domains from resting to activated conformations is dependent on annular lipids. For example, experiments on the archaebacterial KvAP channel show that the voltage-activation relation for the channel shifts to depolarized voltages in the presence of lipids with positively charged head-groups (Schmidt, D., 2006). To investigate protein-lipid interactions in voltage-sensing domains, we purified the full-length KvAP channel and the isolated S1-S4 domain, and reconstituted them in either a POPC:POPG (1:1) lipid mixture or DOTAP, a positively charged lipid without a phosphate group, and used solid-state NMR spectroscopy to study protein-lipid interactions. Saturation transfer difference solid-state NMR experiments indicate that the lipid exposure is equivalent for the isolated S1-S4 domain and S1-S4 domain within KvAP channel, suggesting that S1-S4 domains provide the primary contact with the lipid matrix. ^{13}C detected INEPT based saturation transfer difference experiments indicate that protein-lipid interactions are transient and relatively weak, but reveal a preference for lipids with negatively charged head-groups. Comparisons between the S1-S4 domain reconstituted in POPC:POPG and DOTAP suggest that the positively-charged lipid does not cause significant changes in the structure of S1-S4 domains, or inter-helical ionic bonds, as reported by protein chemical shifts and spin correlations. ^{15}N and ^{13}C detected ^{1}H spin diffusion experiments indicated that the guanidinium groups of arginine residues in the S1-S4 domain are heavily exposed to water, while Trp70 in the middle of S2 helix is not, demonstrating the existing of a hydrophobic core in the middle of the voltage sensing domain.

3474-Pos Board B335
Characterization of a Novel Voltage-Sensing Protein
Jaime J. Smith.
National Institutes of Health/ NINDS, Bethesda, MD, USA.
The discovery of Ci-VSP, a voltage-sensing phosphatase, revealed that S1-S4 domains can exist in proteins independent of an ion-conducting pore (Nature, 2005). The voltage-activated proton channel, Hv1, was subsequently discovered and shown to consist of a voltage-sensing domain that conducts protons in response to membrane depolarization (Nature, 2006). Through bioinformatic searches, we identified a protein 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 other S1-S4 domains are conserved in NVS, including 3 Arg and a Lys in the S4 helix, and 4 conserved acidic residues in S1-S3. Other than an S1-S4 domain, NVS contains no conserved domains that offer clues about its function. However, the C-terminus does contain a coiled-coil domain, several SH3 binding motifs and a region that has 30% identity with a Ras-GAP binding protein thought to regulate Ras signaling pathways. Here, we show that NVS traffics to the membrane as determined by surface biotinylation. Additionally, tissue distribution studies show expression of NVS in brain, heart, kidney, liver and testes. Within the brain, NVS is enriched in cerebellum and immunofluorescence studies on frozen tissue sections indicate that NVS localizes to pre-synaptic terminals of granule cells. Furthermore, the S4 helix of NVS is capable of sensing changes in membrane potential as revealed by transferring this region into Hv1. Our guiding hypothesis is that NVS functions as a voltage sensor that interacts with signaling proteins to provide intracellular pathways with information about voltage changes across the membrane. To this end, experiments are underway to identify interacting proteins for clues about the function of NVS.

3475-Pos Board B336
Structural Dynamics in the Resting and Activated States of the Voltage Sensor of Ci-VSP from Dipolar Distance Measurements
H. Raghuraman[1], Qufei Li[1], Sherry Wanderling[1], Carlos A. Villalba-Galea[2], Eduardo Perozo[1].
[1]Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL, USA, [2]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA, USA.
The mechanism of electromechanical transduction in voltage sensing domains remains controversial. Here, we have probed the conformation of the voltage sensor of Ci-VSP in different functional states by means of EPR-based distance measurements. Ci-VSP is a voltage-sensing phosphatase from *Ciona intestinalis*. Although it is coupled to a cytoplasmic phosphatase, its voltage-sensing domain (VSD) is homologous to voltage sensors found in voltage-gated ion channels. It therefore serves as an excellent model to study voltage sensor movement independent of the interaction with pore domain. On the basis of voltage dependence of Ci-VSP sensing currents (Q-V curves), it is agreed that, at 0 mV, the S4 of wild-type Ci-VSP is in the resting conformation (down state). The arginine at position 217, located in the extracellular end of S4, has a strong effect on the voltage dependence of Ci-VSP sensing currents. Mutations at arginine 217 with a neutral or negative residue (R217Q or R217E), lead to a large leftward shifts in the Q-V curve so that, at 0 mV, the sensor is in the activated conformation (up state). This provides a unique opportunity to monitor the conformational differences in the VSD between resting and activated states in the absence of membrane potential. We expressed and purified a series of double cysteine mutants in the isolated voltage sensor (S1 to S4) of Ci-VSP in wild-type and R217E backgrounds, and measured distances using CW-based dipolar broadenings (for short distances, 8 to 20 Å) and double electron-electron resonance (DEER) spectroscopy (for longer distances, 20 to 50 Å). Our preliminary analysis of the distance measurements suggest defined conformational differences between resting and activated states of the VSD.

3476-Pos Board B337
Gating of Trimerized or Tetramerized Voltage-Gated H+ Channels
Yuichiro Fujiwara[1], Marta Perez[2], Tatsuki Kurokawa[1], Peter H. Larsson[2], Yasushi Okamura[1].
[1]Department of Physiology, Graduate School and Faculty of Medicine, Osaka University, Suita, Osaka, Japan, [2]Department of Physiology and Biophysics, Univ. of Miami Miller School of Medicine, Miami, FL, USA.
VSOP/Hv1 is a dimeric voltage-gated H+ channel unlike other tetrameric voltage-gated channels. Each subunit has its own permeation pathway, and the gating of one subunit is coupled to that of the other subunit within the dimer. We previously reported that the cytoplasmic dimer coiled-coil mediated the dimeric assembly and the gating coupling, based on the crystal structure analysis of the coiled-coil domain. The crystal structure of the VSOP coiled-coil shows an I/L core packing pattern, in which well-packed Ile/Leu residues are situated at positions 'a'/'d' in the heptad repeat and are periodically observed along the entire length of the coiled-coil. However, the functional significance of the patterned sequence for the channel function remains unknown. To address this issue, we changed the packing pattern to I/I, L/L and L/I-types, and analyzed the stoichiometry of the mutant coiled-coils' assembly. Sedimentation and crystal structure analyses showed that coiled-coils with I/I and L/L-type cores formed trimers, and the version harboring the L/I core formed a tetramer. These were consistent with the results of cross-linking analysis followed by Western blotting of the full-length proteins, indicating that the assembly stoichiometry was determined by the core type. Electrophysiological analysis revealed that only the dimer types showed slow and sigmoidal activation kinetics suggestive of cooperative gating. Thus, the I/L sequence pattern of the coiled-coil core in the natural channel is optimally designed to form a dimeric channel with slow and cooperative gating.

3477-Pos Board B338
Hv1 Inhibitors Reveal Gating Properties Typical of Pore Domains in a Voltage-Sensing Domain
Liang Hong, Iris Kim, Medha M. Pathak, Dennis Ta, Francesco Tombola.
UC Irvine, Irvine, CA, USA.
In voltage-gated sodium, potassium, and calcium channels the ion permeation pathway is located in the pore domain, which is surrounded by four voltage-sensing domains (VSDs). The pore domain also contains an intracellular gate-called the activation gate- which is opened and closed by the VSDs in response to changes in membrane potential. The Hv1 voltage-gated proton