1899-Plat
Probing Protein Motions and Function in Ci-VSP
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With the discovery of the Ciona intestinalis voltage sensor-containing phosphatase (Ci-VSP), voltage sensing domains (VSDs) have moved beyond the exclusive realm of ion channels. By combining a VSD with a lipid phosphatase domain, Ci-VSP is the first enzyme known to be directly regulated by voltage. Interestingly, Ci-VSP is also the first protein with a monomeric VSD. Yet, a monomeric VSD still exhibits complex protein motions indicating that other factors beyond the oligomerization state of the domain play a part. We have applied two electrode voltage clamp electrophysiology to probe phosphatase function as well as voltage clamp fluorometry to probe the conformational changes involved in voltage sensing and the propagation of that signal to the phosphatase. The observed changes in fluorescence correlate with protein motions allowing any factors affecting the protein to be teased apart. These motions are influenced by the catalytic states of the phosphatase consistent with the expected coupling between the two domains. The linker between the voltage sensing domain and the phosphatase domain also influences these motions suggesting the linker may play an active role in protein regulation. By investigating Ci-VSP, we hope to gain a greater understanding of how the VSD functions.

1900-Plat
Uncoupling Of The Phosphatase Produces A Deeper Relaxation Of Ci-VSP
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The coupling of the Voltage sensing domain (VSD) and the phosphatase domain (PD) of the Ciona intestinalis voltage sensing phosphatase (Ci-VSP) is mediated by a putative Phospholipid Biding Motif (PBM), located between the two domains. During depolarization, the movement of the S4 favors the binding of the PBM to the membrane, placing the PD in position to carry out its function. We have shown that upon prolonged depolarization, the VSD of Ci-VSP evolves to a relaxed state, as shown by a shift of the Q-V curve (Villalba-Galea et al., 2008). As a consequence of the relaxation of the VSD, fluorescence changes of a probe attached to the extracellular end of the Ci-VSP S4 were observed. However, we have now found that the relaxation produces changes along the entire S4 segment. We report here that mutations in the PBM, R253A-R254A, uncouple the sensing currents from the phosphatase activity, suggesting that the mutations abolish the coupling by disrupting the binding of the Arginine of the PBM to the phospholipid of the membrane. Interestingly, these mutations also increase the rate of the sensing currents during repolarization. A simple interpretation is that the S4 movement is restricted by the binding of the PBM to the membrane. If this is the case, it may also restrict the relaxation such that during a long depolarization the S4 could undergo a larger movement and reach a deeper relaxed state. Consistent with this interpretation we found that in the PBM mutant, the shift of the Q-V curve during prolonged depolarization is enhanced while the recovery time from the relaxed state is increased. Supported by NIHGM30376.

1901-Plat
Hv1: How A Voltage-sensor May Form A Channel
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Unlike canonical voltage sensor domains, which are associated with other functional domains such as channel pore domains, or more rarely enzymes, Hv1 is a voltage sensor that acts as a proton channel per se. We used modelling and simulation tools in order to investigate the molecular mechanisms of function for this recently discovered voltage-gated proton channel.
As no three-dimensional structure has yet been determined for this protein, we built homology models based on the available structures of voltage-gated potassium channels. We performed coarse grain (CG) simulations of the interactions of these models with a phospholipid bilayer, enabling analysis of protein-lipid interactions. The equilibrium orientation from the CG simulations was used to aid setup of full-atom (AT) simulations with the protein embedded in a POPC bilayer. Analysis of the central pore radius and hydrogen bond interactions with water throughout the trajectories suggest that the models may capture different open conformations of Hv1. The models and simulations provide insights into a potential novel mechanism of H+ permeation involving hydronium coordination by Hv1 residues. Site-directed mutagenesis is being used to test the role of specific residues in H+ permeation through Hv1.

1902-Plat
Crystal Structure Of Full-length Kcsa Trapped In Open Conformation Reveals That C-terminal Domain Fine Tunes Activation And Coupled Inactivation
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Using chaperone-assisted crystallography, we have recently determined the crystal structure of full-length (FL) KcsA in its closed conformation. The FL structure reveals that the C-terminal domain (CTD) extends ~70 Å towards the cytoplasm as a canonical four helix bundle stabilizing the closed state. Electrophysiological analysis of KcsA/Fab4 complex demonstrates that CTD remains as a bundle during activation gating. This motivated us to crystallize open conformation (OC) of FL KcsA using the same antibody fragments and a recently developed constitutively open mutant, which was crystallized in its truncated form. We solved the FL KcsA/Fab2 complex structure in the open state at 3.9 Å resolution. FL open-structure bends at G104 and exhibits ~10 Å displacement at the V115 creating four side windows large enough to accommodate hydrated K+ ions right below the gate. The CTD remains as four helix bundle, exerting strain on the bulge helices which connects the bundle and the transmembrane domain.
Based on the current structure and an earlier set of truncated KcsA structures displaying different degree of openings, we suggest that the cytoplasmic domain not only stabilizes the closed state but also fine tunes the level of opening at the activation gate and thus determine the level of inactivation occurring at the selectivity filter. Brownian Dynamics, simulation, electrophysiological studies and electrostatic calculation are ongoing efforts to dissect the ion permeation pathway.

1903-Plat
High Resolution AFM of KcsA Structure and Clustering in a Lipid Bilayer
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KcsA, a potassium channel found in *Streptomyces lividans*, is an important prototype for all other K^+ channels which share the highly conserved signature sequence TVGYG. It oligomerizes to form a tetrameric channel which is intracellular pH-activated in the range pH $<$ 5.5. The crystal structure of the transmembrane section in the closed state has been resolved at 2.0 Å which has provided an insight into the mechanisms of ion selectivity and gating. Its biological function remains poorly understood, but protein clustering observed *in-vivo* and *in-vitro* may be significant.
Amplitude Modulation Atomic Force Microscopy (AM-AFM) has been used to achieve sub nm single molecule structural information of the topology of membrane protruding segments of KcsA reconstituted in lipid bilayers under conditions approaching physiological ideal and to monitor changes induced by different pH conditions. The spatial resolution of AFM also allowed investigation of the supramolecular organization of KcsA within the membrane.
We have imaged KcsA reconstituted in 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] supported bilayers under neutral and acidic conditions (pH 3-7). We obtained high resolution images showing well-defined subunit structure with marked heterogeneity at low pH. Under neutral conditions, a change in channel height occurs, which is likely to be due to a conformation change and is accompanied by a loss of subunit detail. We also found the distribution of tetrameric KcsA in the lipid bilayer changed with pH. We infer that protein-protein interactions are responsible for lowering the 4-fold symmetry of the tetramer, and that pH-mediated clustering of KcsA and conformational change are governed by protein-protein and lipid-protein interactions. The observed dependence of KcsA spatial organization on pH has possible implications for channel activation (cooperative gating) and biological function.

1904-Plat
Using Electron Spin Echo Envelope Modulation (ESEEM) to Probe the Local Environment of Residues in the KcsA Potassium Channel
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Abstract: Recent studies on the voltage-dependent potassium channel KvAP have demonstrated the importance of the lipid bilayer in maintaining the correct orientation and packing of the voltage-sensor domain with respect to the pore domain [1, 2]. It has also been suggested that lipid may play an integral role in voltage-dependent gating [3, 4]. To date, the precise environment surrounding