the noisy signals and estimate rotations to within a few photons. These improvements enabled new insights into the walking mechanism of myosin V: we directly observe 10-20 ms periods of high mobility while myosin V detached heads search for actin. The gradual left-handed path of myosin V is produced by intermittent large side-ways motions among long stretches of straight walking where the lever arms mostly tilt in a plane very close to that containing the actin filament. For in vivo studies, detecting forces and motions of intracellular cargos have been compromised, until recently, by uncertainties about calibrating optical traps in cells. In the last year, four groups published novel methods for improving trap calibration in living cells. In our method, the thermal fluctuations of a phagocytosed bead are analyzed together with its motions upon sinusoidal excitation over a series of frequencies, giving trap sensitivity and local sub-diffusive viscoelastic parameters of the cytoplasm. This method is insensitive to active biological processes in the cell, as the forced response is used to resolve the elastic responses of the trap and the environment. Forces on these cargos indicate several kinesin and dynein motors operating near force balance. Supported by NIH grants P01GM087253 and R01GM086352.

Platform: Mechanisms of Voltage Sensing and Gating

1178-Plat
Gating Currents of Monomeric Hv Channel Reveals a Permeation Pathway Coupled to the Voltage Activation
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Recently, a family of voltage-gated proton channels, called (Hv) was cloned from different organisms. Hv channel shares a common structure with the Voltage Sensor Domain (VSD) of voltage-gated K channels. Despite off the high homology between Hv channel and the VSD, they have distant quaternary structures. Hv channel lacks a pore domain, and is fully functional either as a monomer or dimer. The S4 was proposed as the possible permeation pathway, resembling the omega current present in some mutants of Shaker K^+ channel. However the permeation pathway is still elusive. Fluorescence experiments suggests that the voltage activation in Hv is in two steps, where the second step is a cooperative conformational change, involving interactions between subunits that opens both permeation pathways in a concerted way in the dimer. However, the relationship between the H^+ pore, and the voltage sensor activation is not fully understood for the monomer. Here we presented the first Hv gating currents recordings from the monomeric Hv form (Hv-ΔNΔC). Interestingly, the voltage sensor activation precedes the H^+ conduction, suggesting that voltage sensor and H^+ pore, are two different structures allosterically coupled. The Q-V present a zδ of 1.6 e0, in agreement with previous experiments of voltage-clamp fluorometry. The data presented support the hypothesis that the channel opening is associated to a second conformational state of the voltage sensor, associated to an extremely slow component in the gating current. Supported by Fondecyt grants ACT 1104 and Fondecyt 1120802 to CG and 1110430 to R.L.

1179-Plat
The Specialized Role of the S1 Transmembrane Segment in the Gating of the Hv1 Proton Channel
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Voltage-gated proton channels mediate proton extrusion in a large variety of cells. Hv1, the first and so far only cloned voltage-gated proton channel, is composed of the first four transmembrane segments (S1-S4) that compose voltage-sensing domains (VSD), but lacks the pore domain of "classic" voltage-gated channels. Hv1 channels associate as dimers, but each subunit contains its own pore, suggesting that the pore and the gate are contained within each VSD. Earlier work showed that, as in "classic" voltage-gated channels, the arginine-rich S4 transmembrane segment is the voltage sensor in Hv1. However, the molecular mechanism of opening is still ill-defined. In this study, we identify the S1 segment as the gate of Hv1 channels. Observed changes in the accessibility of the internal and external ends of S1 indicate a voltage-driven rearrangement of this segment. Moreover, the conformational change of S1 precisely tracks channel opening in voltage dependence and kinetics. Finally, we show that gating of Hv1 involves a close interaction between the S1 and S4 segments that depends on S4's third arginine (R3) and an Hv1 specific aspartate on S1 (D1), two residues that have been previously proposed to interact in the open state to form the proton selectivity filter. Overall, our findings suggest that the S1 segment has specialized as the determinant for gating and proton conduction in Hv1.

1180-Plat
Optically Mapping the Movement of Discrete Gating Charges in Shaker
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The nature of the movement of the voltage sensor in voltage-gated ion channels has been a subject of intense study. In the canonical voltage-gated potassium channel Shaker, one major branch of experimentation has been to examine discrete gating charges in the voltage sensor, such as R362 (R1) or R365 (R2), to determine, via site-directed mutagenesis or cross-linking, the residues that they interact with, such as F290 or F416. However, site-directed mutagenesis may uncover indirect interactions rather than direct ones, while cross-linking may uncover rare states. We have aimed to track the movement of the charged residues directly, using a positively charged bimane derivative (qBBr) conjugated to a cysteine replacing either R362 or R365. Although bulkier than arginine, the distance of the positive charge from the protein backbone is similar, and the introduction of the dye is sufficient to restore typical gating current properties to these cysteine mutant constructs, strongly suggesting that qBBr is mimicking the natural function of the endogenous arginine. We have taken advantage of the properties of the bimane class of fluorescent dyes which are highly quenched by proximity to tyrosine and tryptophan but not other amino acids. Using qBBr attached to a chosen gating charge of Shaker proteins expressed in Xenopus laevis oocytes under voltage-clamp, we can optically measure the motion of a specific gating charge in relationship to a specific quenching site in response to changes in membrane potential. The quenching site can be either naturally occurring or artificially substituted into the protein. This method for optically mapping the movement of discrete gating charges in Shaker should be transferable to a wide variety of voltage-gated membrane proteins. Support: NIH GM030376.

1181-Plat
Multi-Dimensional Free Energy Landscape of Voltage Sensor Domain Activation
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Many modulators, such as toxins, anesthetics or drugs, act on voltage-gated channels by altering the activation and/or deactivation mechanism of their voltage-sensing domains (VSD). So far, we have proposed a "static" model of the Kv1.2 VSD activation using brute force and modified molecular dynamics simulations and that agrees with a large body of experimental data. This model involves 5 states: α (activated), β, γ, δ (three intermediate) and ε (resting) [Delemotte et al. 2011, Proc. Natl. Acad. Sci. USA, 108:6109-6114], and has enabled to gain access to the contribution of transmembrane voltage to the free energy of activation via calculation of the corresponding gating charge. Crucial details, however, are still missing, among which an estimation of the thermodynamic stability of these states or of the minimum energy transition pathway linking them. In order to complete our understanding of VSD function, we produce the multi-dimensional free energy landscape (FES) of the four transitions linking the Kv1.2 VSD conformations, enabling to follow for the first time the pathway of activation of a VSD. We then investigate how the free energy landscape of VSD activation is modified by a change in the lipid environment and by the mutation of key residues.

1182-Plat
Protein Backbone Mutagenesis Reveals a Novel Link between Ion Occupancy and C-type Inactivation in K^+ Channels
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The selectivity filter of K^+ channels contains four sequential ion binding sites built primarily from backbone carbonyl oxygen atoms. In addition to ion discrimination, the selectivity filter participates in C-type inactivation, which is a conformation change at the selectivity filter that converts it from a conductive to a non-conductive state. C-type inactivation is modulated by permeant ions but the mechanism underlying this effect is not known. To investigate the link between ion occupancy and C-type inactivation, we used amide to ester substitutions in the protein backbone to alter ion binding at specific sites in the selectivity filter and determined the effect on C-type inactivation. We found that introducing an ester linkage at the 1' site in the selectivity filter of the KcsA channel did not affect inactivation but introducing ester linkages at the 2' and the 3' sites severely reduced inactivation. We determined the structure of the 2' ester mutant of the KcsA channel and found that the 2' ester substitution