Na channel can both be described (over a wide range of solutions and concentrations) by the same reduced model with the same unchanging two parameters (dielectric coefficient and diameter) in which side chains are spheres (Ca channel = EEEE or EEEA; Na channel = DEKA). In the EEEE channel, Ca^{2+} selectivity is driven by charge/space competition in which selectivity arises from a balance of electrostatics and the excluded volume of ions in the crowded selectivity filter. Electrostatics selects Ca^{2+} over monovalent cations. Excluded volume selects Ca^{2+} over larger divalent cations. All these combine to create depletion zones in the ionic density profiles that are crucial determinants of the current carried by each ionic species.

2666-Pos

Energetic Variational Analysis *EnVarA* of Ions in Calcium and Sodium Channels

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Selective binding in both calcium and sodium channels can be described (in many solutions and concentrations: Biophysical Journal (2007) 93:p.1960) by the same reduced model and unchanging two parameters (dielectric coefficient and diameter) despite the very different primary structure of the two proteins (Ca channel EEEA/EEEE; Na channel DEKA) and properties, even though amino-acid side-chains (E, D, etc.) are represented only as charged spheres. Monte Carlo *MC* simulations, reported in ~30 publications, work well (we think) because they do not specify structure as an input, independent of conditions. Rather, *MC*calculates the structure as an output, as a self-organized, induced fit of side-chains to ions (and vice-versa). **Structure is different in different solutions** in self-organized systems. Self-organized systems can be powerfully analyzed using the calculus of variations, specifically, energetic variational analysis (*EnVarA*). We optimize *both* action and dissipation integrals (Least Action and Maximum Dissipation Principles), motivated by Rayleigh, then Onsager who optimized just one, or the other. The resulting systems of coupled partial differential equations automatically satisfy the First and Second Laws of Thermodynamics and electrostatic Poisson equations, with physical boundary conditions that can produce flow. *EnVarA* extends Navier-Stokes equations to complex fluids containing deformable droplets (Journal of Fluid Mechanics (2004) 515:p.293). ***EnVarA* provides a seamless extension of conservative Hamiltonian systems** (perhaps at thermodynamic equilibrium) **to dissipative systems.***EnVarA* **is a** field theory of ions in channels and solutions with entropy, friction, and flow. *EnVarA* computes current where *MC* computes only binding. *EnVarA* applied to EEEE/DEKA channels gives binding like real calcium/sodium channels. Time dependent currents computed with *EnVarA* resemble time dependent currents in either voltage activated sodium or potassium (squid axon) channels (depending on parameters), **although the *EnVarA* model has only one unchanging conformation.**

2667-Pos

3D Structure of a Recombinant DHPR Expressed in Mouse

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The dihydropyridine receptor (DHPR) is an L-type Ca^{2+} channel that acts as the voltage sensor for excitation-contraction coupling in skeletal muscle by tightly controlling the intracellular Ca^{2+} channel RyR1. Because previous 3D electron microscopic studies have largely not resolved the spatial organization of the DHPR subunits (α1s, α2-δ, β1a, and γ), we constructed mice expressing a β1a subunit with YFP and a biotin acceptor domain attached to its N- and C- termini respectively. This engineered β1a sustains a functional DHPR-RyR1 interaction and viable animals in a β1a null background. DHPRs were purified from mice by means of the (biotinylated) biotin acceptor domain, negatively stained and imaged with electron microscopy. 8,662 individual DHPRs were analyzed using single-particle image processing algorithms. Multivariate statistical analysis, classification, and multi-reference alignment yielded distinct 2D class averages corresponding to different orientations of the macromolecule. The 3D reconstruction, with 25 Å resolution, shows two distinct parts: a main body shaped like an irregular pentagon (~150x125x75 Å) with distinct corners, and a hook-shaped feature that extends ~60 Å from the main body. Consistent with the considerable conservation of membrane topology among voltage-gated channels, a good part of the main body can be closely fitted with an atomic structure of a full-length potassium channel, suggesting that the main body contains the α1s subunit. Besides the fitted potassium channel the main body has extra volume that can accommodate the YFP atomic coordinates and other subunits.

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2668-Pos

$Ca_v2.3$ Calcium Channels Inactivate from the Open State with Partial Charge Immobilization and Altered Deactivation Kinetic

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Voltage-dependent ion channels undergo inactivation following activation. In the sodium and potassium channels, the molecular determinants that govern the mechanism of inactivation involve pore blocking by a cytoplasmic particle. In calcium channels the consensus model is that the intracellular loop joining the first two homology domains of the pore forming subunit contributes to the inactivation gate or hinged-lid. To investigate key features of this model, we expressed $Ca_V2.3$ channel in *Xenopus oocytes* without auxiliary subunits and recorded ionic and gating currents using the cut-open voltage-clamp technique. Ionic current were recorded in either Ba^{2+} or Ca^{2+}. Here we report that consistent with a hinge-lead mechanisms, charge movement at the end of a depolarizing pulse decreases up to a 50% with channel inactivation. In contrast with a previous report by Patil et al (1998, Neuron 20:1020) that co-expresses auxiliary subunit with $Ca_V2.3$, trains of pulse elicited minimal inactivation. It appears then that when expressed alone, $Ca_V2.3$ channels inactivate mostly from the open state. We also found that as channels inactivate, a slow component emerges in tail-current recordings. This component contributes to about 20% of the tail currents in 80% inactivated currents and can be accounted for with a classic allosterically-coupled model provided that channels can re-open multiple times from the last closed state and that some closed-inactive channels reopen during membrane repolarization.

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2669-Pos

Action Potential Hyperpolarization Kinetics Abets the Modulation of Alpha1h T-Type Calcium Channels by KLHL1 Mutants Lacking the Actin-Binding Domain (ΔKelch)

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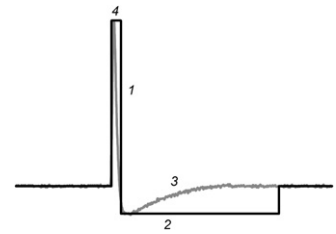
The Kelch-like 1 protein (KLHL1) is a neuronal actin-binding protein that increases the current density and channel number of $Ca_v3.2$ calcium channels *via* actin-F mediated increases in recycling endosomal activity. Removal of the actin-binding kelch motif (ΔKelch) prevents the increase in α_{1H} current density seen with wild-type KLHL1 when tested with square pulse protocols but not the increase in calcium influx seen during action potentials (AP).

Here we set out to dissect the kinetic properties of AP that confer the mutant kelch the ability to interact with α_{1H} and induce an increase in calcium influx. Square waveforms (black trace) following the AP did not significantly increase calcium influx (25%, $p>0.05$) compared to the AP (red). We investigated the effects of altering the slope of the repolarization (1), the length of hyperpolarization (2), the slope of repolarization from hyperpolarization (3) and the duration of depolarization (4) on the modulation of α_{1H} by ΔKelch. Our results show that the slope of repolarization from hyperpolarization induces the conformational changes that allow the channel to properly interact with ΔKelch, leading to increased Ca influx.



2670-Pos

C-terminal Alternative Splicing Modulates Single-Channel Gating of $Ca_v1.3$ L-Type Calcium Channel

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We have recently discovered a novel mechanism of channel modulation in $Ca_V1.3$ channels enabling cells to tightly control gating by C-terminal alternative splicing. The absence of a C-terminal modulatory motif (CTM) within a short splice form facilitates $Ca_V1.3$ channel activation at lower voltages and induces pronounced Ca^{2+} dependent-inactivation (CDI) (Singh *et al*., JBC 2008). Intriguingly, whole-cell measurements revealed a significant