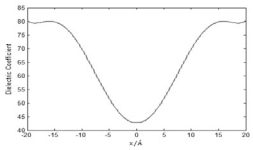
classical hard-sphere lattice models of the configurational entropy of spherical ions and solvent molecules. Formulae are given for all ionic species with different sizes and valences. Unphysical overcrowding does not occur with Fermi distributions, unlike with Boltzmann distributions. We provide an analytical description of the implicit dielectric ('primitive') model of electrolytes that yields global and local formulae for chemical potential. Poisson-Fermi equations are local, with different correlations at different places. Correlations produce spatial variations of dielectric permittivity as an output of analysis. Computations of binding are consistent with Monte Carlo binding curves. They have anomalous mole fraction effects, an effective blockage of sodium binding by a tiny concentration of calcium ions. Symbols and details in J.Computational Physics **2013**, *15*, 88; J Phys Chem B **2013** doi:10.1021/jp408330f

$\varepsilon_w(l_c^2\nabla^2-1)\nabla^2\varphi=\sum_{i=1}^{\mathcal{K}}q_iC_i=\rho.$ OR $\varepsilon_w(l_c^2\nabla^2-1)\Psi=\rho;\ \nabla^2\varphi=\Psi.$



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The Functional Heterogeneity of the Human $Ca_V1.2$ Voltage Sensors

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Voltage-dependent Ca^{2+} channels (Ca_V) are the principal pathway of excitation-evoked Ca^{2+} entry. Their pore-forming α_{1C} subunit encodes four homologous, but non-identical, concatenated repeats, each including a voltage-sensing domain (VSD). The four VSDs confer voltage dependence to the ion-conductive pore. A potential consequence of the independent evolution of each VSD is that the strength and timing of Ca^{2+} influx are governed by up to four VSDs with distinct voltage-sensing properties and contributions to channel opening. To test this hypothesis, and resolve the voltage dependent conformational rearrangements that initiate excitation-coupled Ca^{2+} signaling, we used voltage-clamp fluorometry, tracking the activation of each VSD in human $Ca_V1.2$ (L-type) channels coexpressed with β_3 and $\alpha_2\delta$ subunits. We found that each VSD possesses distinct voltage-sensitivity, as estimated by fitting normalized fluorescence deflections from each VSD to Boltzmann distributions: VSD-I: $V_{0.5}$= 8.3 ± 2.7mV; z=2.0 ± 0.090e^0; VSD-II: $V_{0.5}$=-27 ± 1.8mV; z=2.4 ± 0.17e^0; VSD-III: $V_{0.5}$=-18 ± 2.1mV; z=1.4 ± 0.085e^0; VSD-IV: $V_{0.5}$=-61 ± 2.3mV; z=1.3 ± 0.18e^0. Upon depolarization to 0mV, VSDs I-III activate with a similar time course to channel opening ($\tau_{ON}\approx$2-4ms), while VSD-IV is significantly slower (τ_{ON}=17 ± 2.3ms). Upon repolarization, VSD-II deactivates with a kinetic component practically synchronous to the tail current (τ_{OFF}=0.57 ± 0.22ms), while other VSDs were several fold slower: VSD-I τ_{OFF}=3.7 ± 0.69ms; VSD-III τ_{OFF}=6.6 ± 1.0ms; VSD-IV τ_{OFF}=28 ± 5.3ms. How do the disparate voltage dependences and kinetics of the four VSDs associate with open probability and global charge displacement? All experimental data were fit by an allosteric model comprising five ''gating particles'' representing the four VSDs and the pore. This model revealed that the pore Closed/Open transition in the α_{1C}-β_3-$\alpha_2\delta$ channel is primarily regulated by VSD-II and -III, which stabilize the Open state by ~-37meV and ~-68meV, respectively. This finding sets $Ca_V1.2$ channels apart from K_V and Na_V channels, where activation is governed by four and three VSDs, respectively. Funded by: NIH,AHA,FONDECYT,ACT.

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Functional Interaction between the N-Termini of Murine L-Type Calcium Channel $Ca_V1.2$- and β-Subunit Splice Variants

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L-type calcium channels composed of a pore-forming $Ca_v1.2$ (α_{1C}) subunit and auxiliary $\alpha_2\delta$ and β-subunits are important for cardiovascular and neuronal function. $Ca_v1.2$ and β-subunits are subject to alternative splicing, which affects their biophysical and pharmacological properties. Here we describe an example of reciprocal modulation of such splice variants.
Kanevsky and Dascal (J Gen Physiol. 2006;128:15-36) demonstrated that the modulation of $Ca_v1.2$ by a β_{2b}-subunit depends on the $Ca_v1.2$ N-terminus (NT) and its natural and artificial variants. On the other hand, studies on the structure-activity relationship of β_2-subunit NT splice variants yielded conflicting results (Takahashi et al., Biophys J 2003; 84:3007-21, Herzig et al., FASEB J 2007;21:1527-38, Link et al., J Biol Chem 2009;284:30129-37), possibly due to the different Cav1.2 subunits chosen.
Here we examine the putative interaction between the two NT by coexpressing two variants of $Ca_v1.2$ (short NT and long NT) with three splice variants of β_2-subunit β_{2a}, β_{2b} and β_{2d}), which differ in size and composition of their NT. All possible combinations of murine constructs (including $\alpha_2\delta$-1) were transiently expressed in HEK293 cells. Whole-cell and single-channel currents were recorded.
Whole-cell currents of β_{2a}-containing channel complexes display slower time-dependent inactivation compared with β_{2b} and β_{2d}, irrespective of the $Ca_v1.2$ NT. In contrast, whole-cell current density is higher with β_{2a} (compared to β_{2b} and β_{2d}), but only when co-expressed with long NT $Ca_v1.2$. At the single-channel level, this is reflected by an elevated open probability and availability for the β_{2a}-containing complex when co-expressed with long NT $Ca_v1.2$, but not with short NT $Ca_v1.2$. The respective NT of $Ca_v1.2$ and its β_2-subunit can interact in a biophysically important manner. This mechanism may contribute to functional fine-tuning of channel complexes by alternative splicing.

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Gating Properties of Cav1.3 Calcium Channels: Insight from Alternative Splicing and Human Mutations

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Cav1.3 voltage-gated L-type calcium channels can activate at more negative membrane potentials than other high-voltage activated calcium channels. This allows them to contribute to specific physiological functions, such as cardiac pacemaking and hearing. Cav1.3 α1-subunits are regulated by a C-terminal modulatory domain (CTM) serving an auto-inhibitory function. Alternative splicing removes the CTM in C-terminally short variants (e.g. Cav1.342A) thereby stabilizing an even more negative activation voltage-range than in the long variant (Cav1.3L). It is unknown if the CTM affects gating of the voltage-sensor, its coupling to pore opening or both. We therefore investigated CTM effects on Cav1.3 voltage-sensor function (ON-gating charge). We compared Cav1.3 gating properties with low voltage-gated Cav3.1 T-type channels and with somatic Cav1.3 mutants recently discovered in human adrenal aldosterone-producing adenomas (APA).
We expressed Cav3.1, Cav1.3 and Cav1.2 α1 subunits (the latter with α2-δ1 and β3 subunits) in tsA-201 cells. ON-gating charge (QON) and inward calcium currents (ICa) were measured using whole cell patch-clamp.
Cav3.1 ICa activated about 25mV more negative than Cav1.3L despite a much lower overall voltage-sensitivity of Cav3.1 voltage-sensor movements (QON-V). Half-maximal QON-V of Cav1.3L was also more negative than of Cav1.2. Although a proportionally higher fraction of QON had to be moved to activate Cav1.3L ICa, Cav1.3L channel activated at lower voltages than Cav1.2. Removal of the Cav1.3L CTM lowered Cav1.342A ICa half-maximal activation-voltage without affecting QON-V. The CTM therefore must enhance ICa at lower potentials by facilitating pore-opening upon voltage-sensor movements. APA mutations V259D (IS4-S5), I750M (IIS6) and P1336R (IVS4-S5) induced about 15 mV negative shifts in activation-voltage and/or slowed inactivation. Gain of Cav1.3 channel function can therefore explain enhanced calcium-dependent aldosterone synthesis in these tumors.
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Hill Analysis of Ion Channel Activation: Theory and Practice

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The opening of an ion channel pore involves the cooperative activation of environmentally sensitive gating particles. The logarithmic sensitivity of particle-specific activity A reaches asymptotic expression with saturating force (e.g. membrane potential). The asymptotes consist of the ''particle potential'' (Greek letter eta) augmented by allosteric interactions W with neighboring activated particles. In ''weak'' allosterism, where within the range of measurable A one observes the maximum effect exerted by the W,