Competition of Steric Repulsion and Electrostatic Attraction in Model Calcium Channels

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Abstract

Calcium channels conduct Na ions in the absence of Ca^{2+}, but they selectively conduct Ca^{2+} ions when Ca^{2+} ions are present at physiological concentrations. In the anomalous mole fraction effect (AMFE), even a micromolar amount of Ca^{2+} ions effectively blocks Na current. Many attempts have been made to explain the mechanism behind these phenomena. In our model of the selectivity filter of Ca channels, the end-groups of the side chains of amino acids - four glutamates - in the selectivity filter are represented as mobile ions that are restricted so they move inside the filter. These structural ions form a liquid-like self-adjusting environment for the passing ions so that the system assumes minimum free energy. They also fill part of the pore so the counterions have to compete for space in the crowded selectivity filter. In this picture electrostatic attraction and repulsive entropic excluded volume effects compete with each other to determine which ions can enter the selectivity filter. We argue that this competition is crucial in explaining the selectivity mechanism of Ca channels. We show Monte Carlo simulation results for competition between ions of different valence and diameter. We predict that Ca^{2+}-selectivity depends on the background concentration of NaCl. We show that our model can explain the micromolar Ca^{2+}-selectivity observed in the L-type Ca channel. We also show results for an alternative model of Ca^{2+} channels developed by Corry et al. In this model, the structural ions are placed in fixed positions behind the protein wall. We show that this rigid model cannot reproduce the micromolar selectivity of the L-type Ca channel.

The CSC model

The Charge Space Competition (CSC) model states that cations are attracted into the selectivity filter by charged amino acids making for a very crowded filter where it is difficult for ions to find space. This competition of energy and entropy favors small and/or divalent ions because they provide more charge in less space to balance the charge of the structural ions of the selectivity filter.
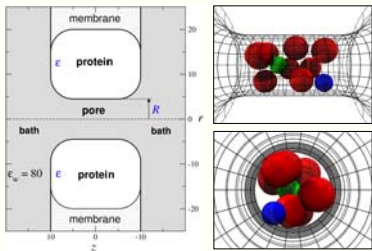
- **Channel**: a doughnut shaped object with a pore in the middle with **protein dielectric coefficient ε=10** and **pore radius R=3.5Å**.
- It is embedded in a membrane that separates two baths.
- **Ions**: charged hard spheres, **solvent**: continuum dielectric ($\varepsilon_w = 80$).
- **Selectivity filter**: central pore containing characteristic amino acid **side chains**: hard sphere ions model the terminal groups of the side chains: **E**: two half charged oxygens ($O^{1/2-}$, **red** balls in figures to the left).

### The Ca channel model of Corry et al. (*Biophys. J.* 2001, 80: 195)
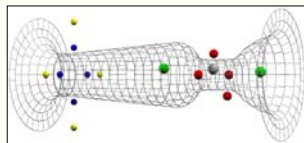
The Corry et al. model contains
- a narrow **selectivity filter** surrounded by structural charges in **fixed positions** behind the wall (**red** balls in the figure),
- a large **cavity** that imposes a dielectric barrier,
- **dipoles** at the intracellular entrance (**yellow** and **blue** balls in the figure)

Methods

- **Equilibrium profiles** are computed with **Grand Canonical Monte Carlo** simulations (**micromolar** Ca^{2+} concentrations can be simulated **directly**).
- **Normalized currents** are computed by substituting these profiles into the integrated **Nernst-Planck** equation with the assumptions that the electrolytes are *symmetric* on the two sides of the membrane, *voltage is small*, and that *flux is limited* in the selectivity filter.
- The adjustable parameter of this calculation is the ratio of the diffusion coefficients of the two ions (for example, $D_{Ca}/D_{Na}=0.1$ was used here).
- For details, see neighboring poster **2221-POS/B336.**

CSC model has micromolar Ca^{2+} selectivity
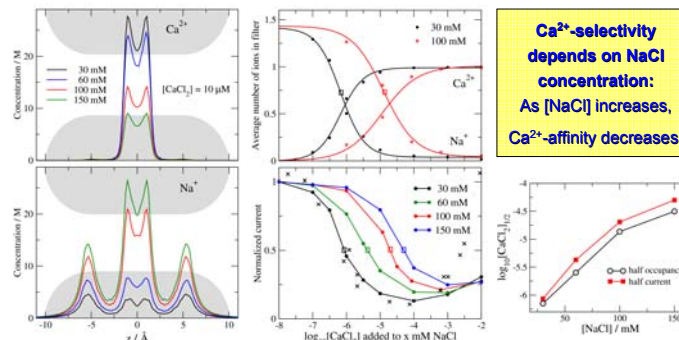
Ca^{2+}-selectivity depends on NaCl concentration:
As [NaCl] increases, Ca^{2+}-affinity decreases.

Concentration **profiles** of Ca^{2+} and Na^+ along the pore for various [NaCl]. In the CSC model there is lots of Ca^{2+} in the selectivity filter at micromolar $[CaCl_2]$.

Occupancy curves of Ca^{2+} and Na^+ and **normalized currents** for various [NaCl]. X symbols are the **experimental** data of Almers et al. (*J. Physiol.* 353: 565) for 32 mM NaCl.

Occupancy of Na^+ in the selectivity filter is a measure of the Ca^{2+}-block of Na^+ current because they are strongly **correlated**.

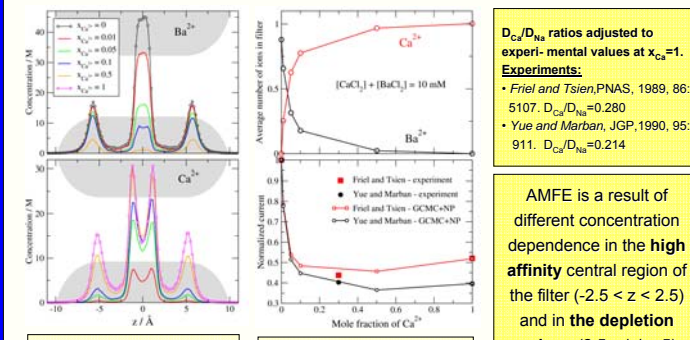## The Ca channel model of Corry et al. is only weakly Ca^{2+}-selective
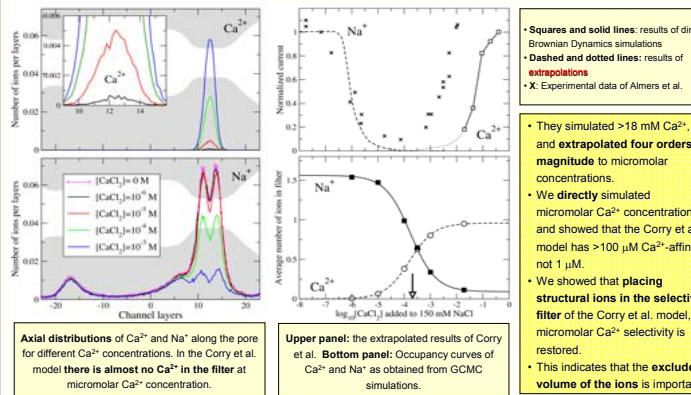
- **Squares and solid lines**: results of direct Brownian Dynamics simulations
- **Dashed and dotted lines**: results of **extrapolations**
- **X**: Experimental data of Almers et al.

- They simulated >18 mM Ca^{2+}, and **extrapolated four orders of magnitude** to micromolar concentrations.
- We **directly** simulated micromolar Ca^{2+} concentrations and showed that the Corry et al. model has >100 µM Ca^{2+}-affinity, not 1 µM.
- We showed that **placing structural ions in the selectivity filter** of the Corry et al. model, micromolar Ca^{2+} selectivity is restored.
- This indicates that the **excluded volume of the ions** is important.

Axial distributions of Ca^{2+} and Na^+ along the pore for different Ca^{2+} concentrations. In the Corry et al. model **there is almost no Ca^{2+} in the filter** at micromolar Ca^{2+} concentration.

Upper panel: the extrapolated results of Corry et al. **Bottom panel:** Occupancy curves of Ca^{2+} and Na^+ as obtained from GCMC simulations.

Size selectivity: Ca^{2+} vs. Ba^{2+} AMFE

D_{Ca}/D_{Na} ratios adjusted to experimental values at $x_{Ca}=1$.
Experiments:
- *Friel and Tsien*, PNAS, 1989, 86: 5107. $D_{Ca}/D_{Na}=0.280$
- *Yue and Marban*, JGP, 1990, 95: 911. $D_{Ca}/D_{Na}=0.214$

AMFE is a result of different concentration dependence in the **high affinity** central region of the filter ($-2.5 < z < 2.5$) and in **the depletion regions** ($2.5 < |z| < 5$).

For details, see neighboring poster 2221-POS/B336.

Concentration **profiles** of Ca^{2+} and Ba^{2+} along the pore for various Ca^{2+} mole fractions $x_{Ca}=[Ca^{2+}]/c_{total}$, where the total concentration is kept fixed at $c_{total}=10$ mM.

Occupancy curves of Ca^{2+} and Ba^{2+} and **normalized currents** for two experiments. One group found an AMFE, one group did not. We reproduce both results.

Loss of Ca^{2+} selectivity in E → A mutations
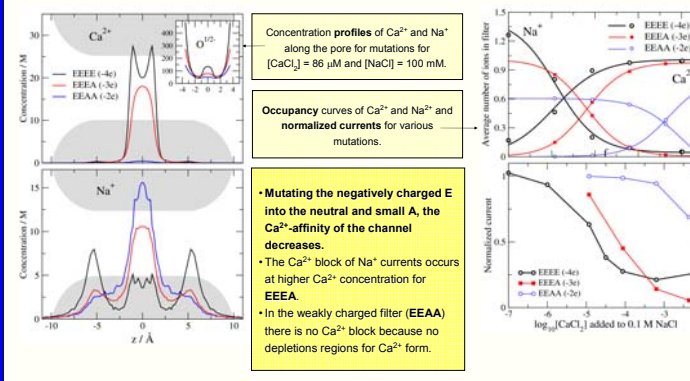
Concentration **profiles** of Ca^{2+} and Na^+ along the pore for mutations for $[CaCl_2] = 86$ µM and [NaCl] = 100 mM.

Occupancy curves of Ca^{2+} and Na^+ and **normalized currents** for various mutations.

- **Mutating the negatively charged E into the neutral and small A, the Ca^{2+}-affinity of the channel decreases.**
- The Ca^{2+} block of Na^+ currents occurs at higher Ca^{2+} concentration for EEEA.
- In the weakly charged filter (**EEAA**) there is no Ca^{2+} block because no depletions regions for Ca^{2+} form.

Conclusions

- Selectivity is a result of the **balance** of electrostatic forces (energy) and excluded volume of ions (entropy).
- The mobile terminal groups of protein side chains have a close interaction with ions: they form a **liquid-like flexible environment** that is more similar to an ionic liquid than to a dilute electrolyte solution.
- **The CSC model reproduces experimental results** and makes predictions (experiments are needed).
- A large range of phenomena is explained using only **basic physical forces** and robust, reproducible methods.

References

- **CSC model:** Nonner, W., Catacuzzeno, L., and B. Eisenberg. 2000. *Biophys. J.* 79:1976.
- **Effect of protein dielectric coefficient and pore radius:** Boda et al., 2006, *J. Chem. Phys.* 125: 034901; Boda et al., 2007. *Phys. Rev. Lett.* 98:168102.
- **Application to Na channels:** Boda et al. 2007, *Biophys. J.* 93: 1960.
- **Application to the model of Corry et al.:** Boda et al. 2008, *Biophys. J.* in press, doi:10.1529/biophysj.107.122796