# Monte Carlo Simulations of Free Energy Components
# Energetics of Selective Binding in A Reduced Model of L-type Ca Channel


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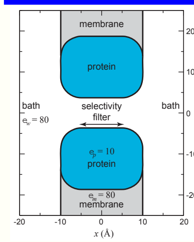
## Abstract

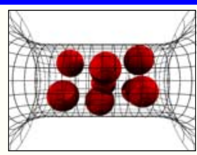
A reduced model of voltage-gated L-type Ca channels is used to study the energetics of selective binding of $Ca^{2+}$ versus monovalent and divalent cations. Widom's particle insertion method is combined with Grand Canonical Monte Carlo simulations to compute the electrostatic and excluded volume components of the free energy difference between channel and bath. We have shown (in ~ 30 papers) that selectivity of the L-type Ca channel and voltage activated 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.


## Why is the channel $Ca^{2+}$ selective?

**$Ca^{2+}$ selectivity arises from a balance of *electrostatics* and the *excluded volume of ions* in the crowded selectivity filter (*charge/space competition*).**

## Reduced Model of L-type Ca Channel



The selectivity filter of the L-type $Ca^{2+}$ channel is modeled as having **radius 3.5 Å, length 10 Å and protein dielectric 10.**

We have previously shown that this model captures the micromolar $Ca^{2+}$ selectivity [2].

## Excess Chemical Potential: The Widom Method

Chemical potential (μ) measures the change in energy when a particle is inserted.

The configurational chemical potential of an ionic species $i$ can be given as :

$$\mu_i(\mathbf{r}) = kT \log c_i(\mathbf{r}) + \mu_i^{ex}(\mathbf{r}), \qquad (1)$$

where $k$ is Boltzmann's constant, $T$ is temperature, $c_i(\mathbf{r})$ s the density profile, and $\mu_i^{ex}(\mathbf{r})$ is the excess chemical potential profile which describes the deviation of the system from the ideal solution.

Widom's particle insertion method : A ghost ion is inserted in position $\mathbf{r}$ and the total interaction energy of the inserted ghost ion with the system $U_i(\mathbf{r})$ is computed to obtain $\mu_i^{ex}(\mathbf{r})$ [1] :

$$\mu_i^{ex}(\mathbf{r}) = W(U_i(\mathbf{r})) = -kT \log \left\langle e^{-U_i(\mathbf{r})/kT} \right\rangle, \qquad (2)$$

where $\langle \ \rangle$ denotes the Grand Canonical ensemble average.

## Components of Excess Chemical Potential

The excess chemical potential of ion $i$, $\mu_i^{ex}(\mathbf{r})$ can be broken down into following components :

$$\mu_i^{ex}(\mathbf{r}) = \mu_i^{HS}(\mathbf{r}) + \mu_i^{MF}(\mathbf{r}) + \mu_i^{self}(\mathbf{r}) + \mu_i^{SC}(\mathbf{r}), \qquad (3)$$

where $\mu_i^{HS}(\mathbf{r})$ is the hard sphere component implying that ions cannot overlap (computed by inserting hard spheres from Eq. (2)), $\mu_i^{MF}(\mathbf{r})$ is the mean field component (obtained by averaging the ion's mean electrostatic potential), $\mu_i^{self}(\mathbf{r})$ is the self component due to the interaction of an ion with charges induced by ion itself (obtained by inserting charged hard spheres from Eq. (2)) and $\mu_i^{SC}(\mathbf{r})$ is the screening term which describes the efficiency of an ion to screen other ions.

The SC term cannot be computed by Widom's method and so we define the screening from Eq. (3) as :

$$\mu_i^{SC}(\mathbf{r}) = \mu_i^{ex}(\mathbf{r}) - [\mu_i^{HS}(\mathbf{r}) + \mu_i^{MF}(\mathbf{r}) + \mu_i^{self}(\mathbf{r})]. \qquad (4)$$

## Energetics of Equilibrium Binding Selectivity

At equilibrium, $\mu_i^{Bath} = \mu_i^{Channel} \Rightarrow$ From Eq. (1) we obtain : $\Delta\mu_i^{ex}(\mathbf{r}) = \mu_i^{ex}(\mathbf{r}) - \mu_i^{ex}(B) = kT \log\left(\frac{c_i(B)}{c_i(r)}\right)$ (5)

From Eq. (3) : $\Delta\mu_i^{ex}(\mathbf{r}) = \Delta\mu_i^{HS}(\mathbf{r}) + \Delta\mu_i^{MF}(\mathbf{r}) + \Delta\mu_i^{self}(\mathbf{r}) + \Delta\mu_i^{SC}(\mathbf{r})$ (6)

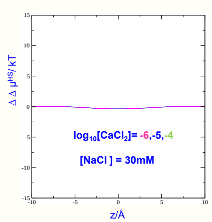
Binding Selectivity of $Ca^{2+}$ over $Na^+$ : Gives the underlying energetics of $Ca^{2+}$ favorability in the channel. From Eq. (5) and (6) for $Ca^{2+}$ and $Na^+$ respectively, we obtain the binding selectivity of $Ca^{2+}$ over $Na^+$ as follows [3]:

$$\underbrace{\log\left(\frac{c_{Ca^{2+}}(\mathbf{r})}{c_{Na^+}(\mathbf{r})}\right)}_{\text{binding selectivity}} = \underbrace{\log\left(\frac{c_{Ca^{2+}}(B)}{c_{Na^+}(B)}\right)}_{\text{number advantage}} + \underbrace{\frac{1}{kT}\Delta\Delta\mu^{HS}(\mathbf{r})}_{\text{excluded volume advantage}} + \underbrace{\frac{1}{kT}\Delta\Delta\mu^{MF}(\mathbf{r})}_{\text{mean field advantage}} + \underbrace{\frac{1}{kT}\Delta\Delta\mu^{self}(\mathbf{r})}_{\text{self advantage}} + \underbrace{\frac{1}{kT}\Delta\Delta\mu^{SC}(\mathbf{r})}_{\text{screening advantage}}.$$

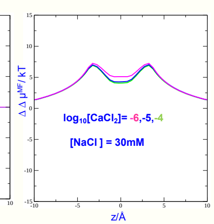
where $\Delta\Delta\mu = \Delta\mu_{Na^+} - \Delta\mu_{Ca^{2+}}$.

## Advantages for $Ca^{2+}$ Selectivity in Channel

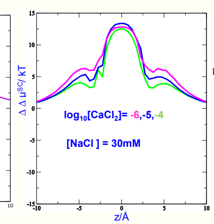
Excluded Volume Advantage



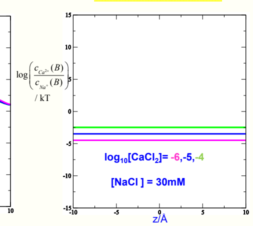

Mean Field Advantage




Screening Advantage



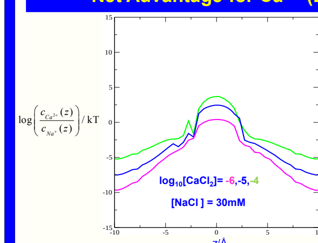

Number Advantage




- Excluded Volume Advantage – zero because $Ca^{2+}$ and $Na^+$ are the same size. NO size selectivity.
- Mean Field Advantage – positive and favors $Ca^{2+}$ over $Na^+$.
- Screening Advantage – large, positive and favors $Ca^{2+}$ over $Na^+$.
- Number Advantage – favors $Na^+$ because of its higher concentration in bath.
- Self Advantage – negative, favors $Na^+$ and does not change (not shown).

**positive = favors $Ca^{2+}$ binding**
**negative = favors $Na^+$ binding**

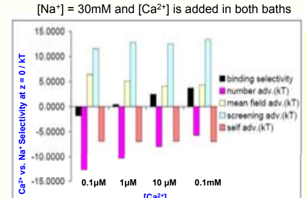
## Net Advantage for $Ca^{2+}$ (Binding Selectivity)




**Selectivity : Competition of electrostatics and excluded volume forces. In L-type Ca channels, electrostatics (mean field + screening) dominates the selective binding of $Ca^{2+}$ over $Na^+$.**

## Energetics of $Ca^{2+}$ vs. $Na^+$ Selectivity

$[Na^+]$ = 30mM and $[Ca^{2+}]$ is added in both baths




**positive = favors $Ca^{2+}$ negative = favors $Na^+$**

**Screening term dominates $Ca^{2+}$ selectivity**

binding selectivity : increases with increasing $[Ca^{2+}]$ as more and more $Ca^{2+}$ accumulates

number advantage: of $Na^+$ decreases with increasing $[Ca^{2+}]$

mean field advantage: positive, always favors $Ca^{2+}$ and decreases as pore becomes more and more electroneutral

screening advantage: positive, large and always favors $Ca^{2+}$

self advantage : negative, favors $Na^+$ and remains unchanged

excluded volume advantage : zero, equal size ions


## References

1. Widom Method For Computing Excess Chemical Potential: Widom B, Journal of Chemical Physics,39,1963.
2. Reduced Model and Selectivity of L-type Ca channel: Boda et al, Journal of General Physiology,133, 497, 2009.
3. Energetics of Divalent Selectivity in RyR: Gillespie, Biophysical Journal, 94,1169,2008.


## Conclusions

$Ca^{2+}$ selectivity is determined by electrostatics, in particular screening. Similar results were found in RyR [3].

## Future Work

DEKA Na Channel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