

# Crowded Charge in Biological Ion Channels

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## ABSTRACT

Biological ion channels are proteins that conduct ions across lipid membranes that are otherwise impermeable to them. Modern theories of electrolyte solutions that include the finite size of the ions has been shown to be a very successful method for modeling ion channels and we continue that work here. We consider a model of ion flux through a calcium-selective ion channel and calculate the components of the chemical potential using density functional theory of electrolyte solutions. For different bath ion concentrations of  $K^+$  and  $Ca^{2+}$  current/voltage curves are computed and the three components of the chemical potentials of the ions are examined.

**Keywords:** ion channels, density functional theory

## 1 INTRODUCTION

Biological ion channels are proteins that conduct ions (mainly  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ , and  $Cl^-$ ) across lipid membranes that are otherwise impermeable to them [1]. There are hundreds of different channel types, each distinguished by a different amino acid sequence that determines the channel's conductance, selectivity, and gating (opening and closing) properties. Here we will focus on modeling the conductance and selectivity properties of a calcium-selective channel we have considered previously [2].

Of the different kinds of calcium-selective channels, the L-type is the most selective; adding 1  $\mu M$  of  $CaCl_2$  to a solution of 0.1 M  $NaCl$  reduces the sodium current by half. Studies have shown that four glutamate (abbreviated E) amino acid residues (the so-called EEEE locus) are necessary and sufficient to give this channel its selectivity properties. The model calcium channel we consider here is built on this motif: the channel is modeled as four glutamates confined to the selectivity filter of the channel.

In this paper we will consider the  $K^+$  selectivity properties of this channel with respect to  $Ca^{2+}$ . Previously,  $Na^+$  selectivity was considered and it was shown that the channel (whose model is specifically described in the next section) is calcium-selective, but to a lesser extent than the physiological channel.

## 2 THEORY

We use the same model of calculating ion flux that we have used previously [2]. It is summarized briefly in this section.

### 2.1 Flux

The starting point for computing flux is the following constitutive relation for the flux  $\mathbf{J}$  of each ion species  $i$ :

$$-\mathbf{J}_i = \frac{1}{kT} D_i(\mathbf{x}) \rho_i(\mathbf{x}) \nabla \mu_i(\mathbf{x}) \quad (1)$$

where  $D$  is the local diffusion coefficient,  $\rho$  is the local density, and  $\mu$  is the local chemical potential. In our model, the ions are represented as charged, hard spheres and therefore the chemical potential has three components:

$$\mu_i(\mathbf{x}) = \mu_i^{\text{id}}(\mathbf{x}) + \mu_i^{\text{HS}}(\mathbf{x}) + \mu_i^{\text{ES}}(\mathbf{x}) \quad (2)$$

where the ideal (id) component is the chemical potential of point charges in the electrostatic mean field (see below); the hard-sphere (HS) component is due to the finite size of the ions; the electrostatic (ES) component is due to charging up the ions of finite size.

For point charges, the chemical potential is

$$\mu_i^{\text{id}}(\mathbf{x}) = \ln \left( \frac{\rho_i(\mathbf{x})}{\rho_{\text{scale}}} \right) + z_i e \phi(\mathbf{x}) \quad (3)$$

where  $\rho_{\text{scale}}$  is a typical concentration (for example, 1 M),  $z_i$  is the valence of species  $i$ ,  $e$  is the elementary charge, and  $\phi$  is the electrostatic potential given by the Poisson equation

$$-\nabla \cdot (\epsilon(\mathbf{x}) \nabla \phi(\mathbf{x})) = e \sum_j z_j \rho_j(\mathbf{x}) \quad (4)$$

where  $\epsilon$  is the local dielectric coefficient. In this paper we take the dielectric coefficient to be constant throughout the system.

The formulation of the HS and ES terms are those given in Ref. [2]. Due to their complexity we do not give them here. It is these terms, however, that are essential to modeling ion flux in general, but especially through ion channels; in channels, the density of ions is much greater than in bulk electrolyte solutions and therefore the highly non-linear effects of two ions not being able to occupy the same space (they are hard spheres which may not overlap)

– excluded-volume effects – become magnified. In this paper we will also discuss the size of the various terms and how they change as the occupancy of the channel changes.

In this paper the ions are assigned the following diameters (in nm):  $K^+$ : 0.276,  $Ca^{2+}$ : 0.2,  $Cl^-$ : 0.362. These are crystal diameters and are not adjusted. Water was treated as an uncharged, hard sphere with 0.28 nm diameter.

The diffusion coefficients were given bulk values in the baths and chosen to be small in the channel (in  $10^{-12} m^2/sec$ :  $H_2O$ : 21.3,  $K^+$ : 3.25,  $Ca^{2+}$ : 0.1,  $Cl^-$ : 3.25) and the piecewise constant profile was Gauss-filtered with a 0.2 nm filter width. The same values were used in the previous study (with  $Na^+$  given the same value as  $K^+$  here) [2].

## 2.2 Channel

The model of the channel is minimal, including only the features essential for selectivity. Specifically, we model the four glutamate residues (each of which has a charged  $COO^-$  at the end) as eight half-charged oxygen atoms ( $O^{1/2-}$ ) that are confined to the selectivity filter of the channel that is 0.1 nm in length. Each oxygen ion is given a size (0.28 nm diameter) and in every respect is treated like the mobile ions (except that they cannot leave the selectivity filter); they interact through their charge and must compete for space with the other ions inside the filter.

## 3 RESULTS

### 3.1 Current/Voltage Curves

The experiments we simulate are similar to the classical experiments that have been used to quantify the selectivity of the L-type calcium channel to  $Ca^{2+}$  with respect to  $Na^+$ . Specifically, the baths on the two sides of the membrane containing the channel contain an electrolyte solution of 0.1 M KCl and 1  $\mu$ M  $CaCl_2$ . Next more  $CaCl_2$  is added to the right bath. In this paper we consider having  $10^{-6}$  M,  $10^{-5}$  M,  $10^{-4}$  M, and  $10^{-3}$  M  $CaCl_2$  in the right bath. For each case a current/voltage curve is calculated. These are shown in Fig. 1.

At low  $Ca^{2+}$  concentrations the current/voltage curves are linear, but the currents becomes smaller as the concentration increases; this is the “calcium block” of potassium currents. At a saturating  $Ca^{2+}$  concentration the current/voltage curve is linear for the negative currents, but becomes super-linear as more positive voltage is applied; this is characteristic of calcium channels.

### 3.2 Magnitude of Chemical Potential Components

Next we examine the size of the three components of the chemical potential [Eq. (2)] for  $Ca^{2+}$  and  $K^+$  as the contents of the channel change.

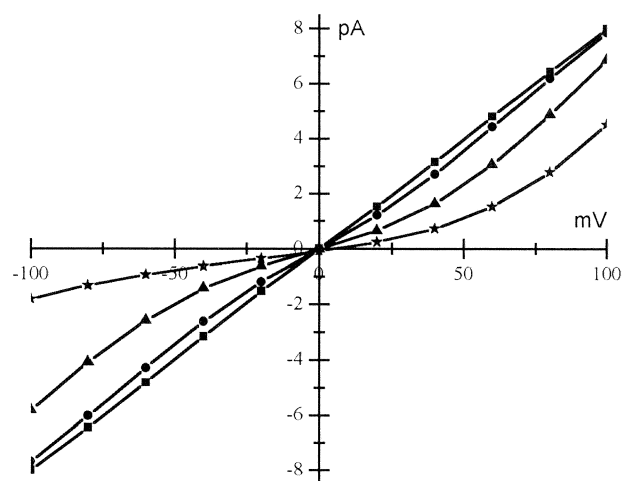


Figure 1: Current/voltage curves. The left bath contains 0.1 M KCl and 1  $\mu$ M  $CaCl_2$  and the right bath contains 0.1 M KCl and  $10^{-6}$  M (squares),  $10^{-5}$  M (circles),  $10^{-4}$  M (triangles),  $10^{-3}$  M (stars)  $CaCl_2$ .

In Fig. 2 are shown the concentrations of  $Ca^{2+}$  and  $K^+$  for the four concentration sets when no voltage is applied. In order to compare the components for the two species, for each component and species the potential is shown relative to its left-bath value; that is, left-bath value of the component is subtracted from the profile. For example, for the HS component for  $K^+$ , the  $\mu_K^{HS}$  profile is shown with the left-bath value of  $\mu_K^{HS}$  subtracted. This then shows the difference in these potentials between the left-bath and the channel and brings both species' potentials to the same baseline so they may be easily compared. If the two baths were identical, then this representation is the energy barrier or well experienced by the ions for each component.

**Ideal (id) Component.** The ideal component of the chemical potential for the four right bath mixtures is shown in Fig. 3. For  $Ca^{2+}$  this term is always positive and becomes large as  $Ca^{2+}$  saturates the channel contents. For  $K^+$ , on the other hand, this term is always negative, relatively small in magnitude, and does not change as the contents of the channel changes under the different conditions.

**Hard-Sphere (HS) Component.** The hard-sphere (HS) component of the chemical potential for the four right bath mixtures is shown in Fig. 4. Because of its larger size,  $K^+$  has a larger HS component compared to that of  $Ca^{2+}$ . It is interesting to note that this component does not change greatly for either ion species although the contents of the channel changes dramatically (cf. Fig. 2). Because of the high density of oxygens in the selectivity filter (eight atoms in a cylinder of length 0.1 nm and radius 0.4 nm), an approximately equal amount of counter charge is attracted to the filter under all bath conditions shown here. This constancy of the total channel contents also makes the HS component relatively constant.

Another feature to note about the HS component is the range over which this potential acts; because the ideal

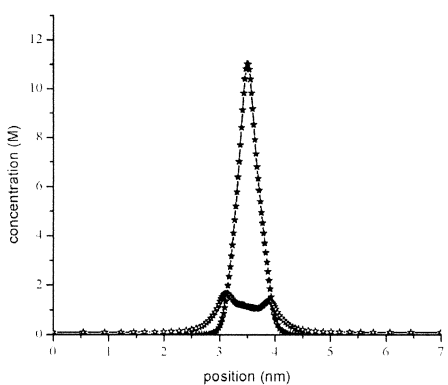
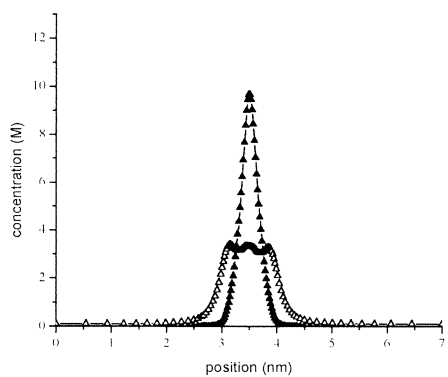
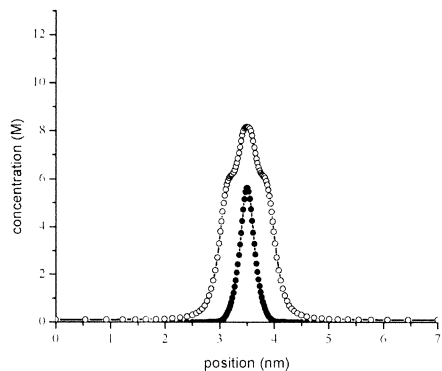
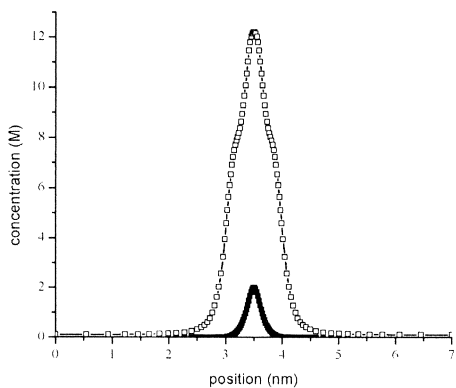


Figure 2: Concentrations of  $\text{Ca}^{2+}$  and  $\text{K}^+$  vs. position along the axis of the channel. The selectivity filter extends from 3 to 4 nm on this scale. From top to bottom:  $10^{-6}$  M  $\text{CaCl}_2$ ,  $10^{-5}$  M  $\text{CaCl}_2$ ,  $10^{-4}$  M  $\text{CaCl}_2$ , and  $10^{-3}$  M  $\text{CaCl}_2$  in the right bath. Solid symbols:  $\text{Ca}^{2+}$ ; hollow symbols:  $\text{K}^+$ .

component includes the long-ranging electrostatic potential, the ideal component acts over the entire system, while the HS component acts over approximately 1 nm.

**Electrostatic (ES) Component.** The excess electrostatic (ES) component of the chemical potential for the four right bath mixtures is shown in Fig. 5. This component has a different magnitude for the two species considered here ( $\text{K}^+$  and  $\text{Ca}^{2+}$ ). In bulk electrolyte solutions this term is approximately proportional to  $z_j^2$  which is also true in this case. Because of this, although the term is not large for  $\text{K}^+$ , it is for  $\text{Ca}^{2+}$ . Furthermore, the potential well for  $\text{Ca}^{2+}$  becomes deeper as more  $\text{Ca}^{2+}$  enters the selectivity filter.

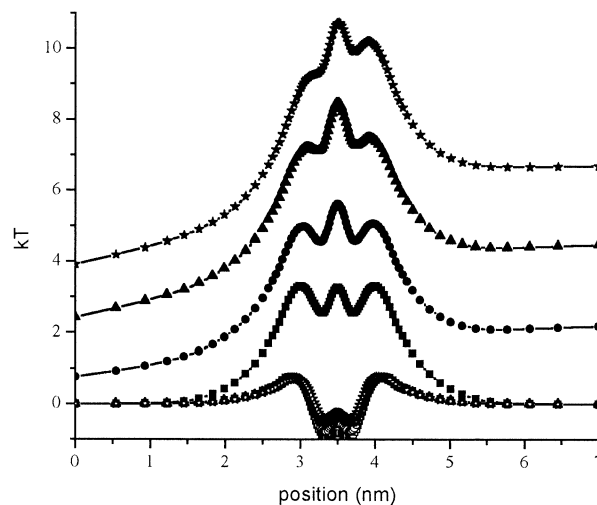


Figure 3: The ideal component for the four right bath mixtures (see Fig. 2). The curves decay to 0 at negative positions. The symbols are the same as in Fig. 1. Solid symbols:  $\text{Ca}^{2+}$ ; hollow symbols:  $\text{K}^+$ .

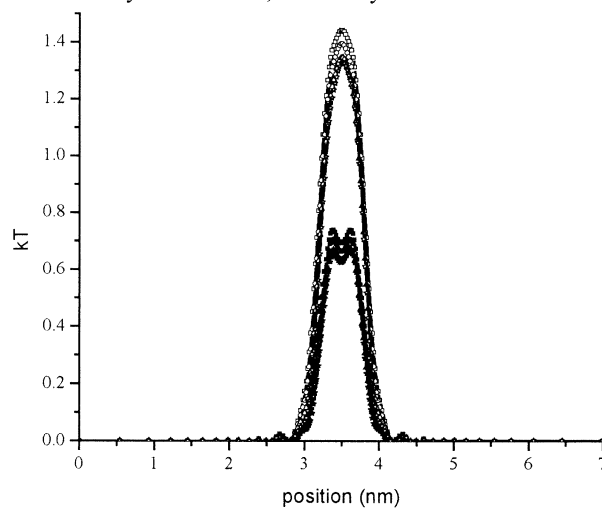


Figure 4: The hard-sphere (HS) component for the four right bath mixtures (see Fig. 2). The symbols are the same as in Fig. 1. Solid symbols:  $\text{Ca}^{2+}$ ; hollow symbols:  $\text{K}^+$ .

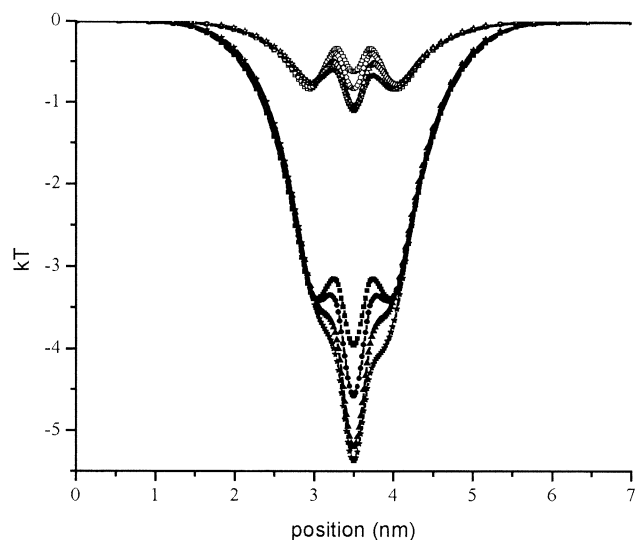


Figure 5: The electrostatic (ES) component for the four right bath mixtures (see Fig. 2). The symbols are the same as in Fig. 1. Solid symbols:  $\text{Ca}^{2+}$ ; hollow symbols:  $\text{K}^+$ .

Similarly to the ideal term, the ES term is an electrostatic term and therefore is long-ranging; in this case, the ES component acts over approximately 3 nm and is therefore intermediate in range to the ideal and the HS components.

#### 4 CONCLUSION

In this study of a calcium selective ion channel we considered the selectivity of the channel for  $\text{Ca}^{2+}$  versus  $\text{K}^+$ . For different bath ion concentrations current/voltage curves were calculated. In comparison with previous work on this model channel studying  $\text{Ca}^{2+}$  and  $\text{Na}^+$  [2], this channel rejects the larger  $\text{K}^+$  more easily; it takes less  $\text{Ca}^{2+}$  in the right bath to remove  $\text{K}^+$  than it did to remove  $\text{Na}^+$  in the previous work ( $10^{-4}$  for  $\text{K}^+$  versus  $10^{-3}$  for  $\text{Na}^+$ ).

#### REFERENCES

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