

Calculating Conductance of Ion Channels - Linking Molecular Dynamics and Electrophysiology

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Abstract. Molecular dynamics computer simulations were combined with an electrodiffusion model to compute conduction of simple ion channels. The main assumptions of the model, and the consistency, efficiency and accuracy of the ion current calculations were tested and found satisfactory. The calculated current-voltage dependence for a synthetic peptide channel is in agreement with experiments and correctly captures the asymmetry of current with respect to applied field.

1. Introduction

Ion channels are pore-forming assemblies of transmembrane proteins that mediate and regulate ion transport through cell membranes [1]. They are ubiquitous to all life. In humans and other higher organisms, they are essential for conducting nerve impulses, cardiac processes, muscle contraction and epithelial transport. In lower organisms, they can act as toxins or antimicrobial agents, and are involved in infectious diseases. Because of their important and diverse biological functions they are frequent targets of drug action. Ion channels also have numerous applications in biotechnology. For these reasons, studies of ion channels are at the forefront of biophysics, structural biology and cellular biology.

The availability of X-ray structures has greatly advanced our understanding of ion channels [2]. However, their mechanism of action remains elusive: ion channels are dynamic by nature, but X-ray crystallography captures the channel in a single, sometimes non-native state. To explain how ion channels work, X-ray structures have to be supplemented with dynamic information that, in principle, can be obtained from molecular dynamics (MD) simulations[3]. However, MD simulations suffer from their own problems, such as inability to access sufficiently long time scales or limited accuracy of force fields. A direct way to assess their reliability is to compare calculated ionic conductances with electrophysiological measurements obtained under similar conditions.

In MD simulations, channel conductance, defined as the ratio of ionic current through the channel to applied voltage, can be calculated once the current, the number of ions that traverse the channel per unit time when an external electric field is applied to the system, has been determined [3]. If the conductance is small, a voltage significantly higher than the experimental one needs to be applied to collect sufficient statistics of ion crossing events. Then, the calculated conductance is extrapolated to the experimental voltage using procedures of unknown accuracy. We propose an alternative approach in which MD simulations at a single voltage are combined with an electrodiffusion (ED) model to recover the full current/voltage (I-V) dependence. Here, we first test the assumptions of the ED model and determine the reliability of the calculated conductance in a model channel built of trichotoxin (TTX), a peptide containing 18 amino acids [4]. The backbone of the TTX was restrained to limit channel fluctuations.



Next, we investigate without any restraints a hexameric channel, LS3, in which each monomer is built of two amino acids, leucine (L) and serine (S) in the sequence (LSSLLSL)₃. For this channel we compare calculated and experimentally measured [5] conductance at several applied fields.

2. Theory and Methods

We assume that ion transport through a transmembrane channel can be described as a diffusive process in the presence of an external electric field and the intrinsic potential of mean force (PMF) due to the channel, the membrane, water and other ions. This implies that the channel structure does not undergo substantial changes when an electric field is applied. The transport of ions across channels in the presence of an electric field can be obtained from the time evolution of the probability density of ions in the system, which is governed by the Smoluchowski equation, obtained from the Fokker-Planck equation with the condition that the equilibrium probability distribution is the Boltzmann distribution:

$$\frac{\partial P(z, t)}{\partial t} = -\nabla J(z), \quad J(z) = -\nabla[D(z)P(z, t)] + \frac{D(z)}{k_B T} F(z)P(z, t) \quad (1)$$

where $J(z)$ is the current, $D(z)$ is the diffusivity, $P(z, t)$ is the probability density of ions, $F(z)$ is the mean force acting on the ion at position z in the channel along the normal to the membrane, k_B is the Boltzmann constant and T is temperature. This 1-dimensional representation is justified if equilibration of ion positions along directions parallel to the membrane is markedly faster than transport across the channel. This means, for example, that there are no strong ion binding sites along the channel.

If the bulk concentrations of ions on both sides of the membrane and the applied field remain constant over time, then the transport of ions through a channel is a stationary process with absorbing boundary conditions. In other words, the system reaches a steady state and the Fokker-Planck equation reduces to the ED equation, which is a generalized form of the Nernst-Planck (NP) equation [1]. If we assume that the diffusivity in the channel is constant and known (see Results) integration of this equation yields:

$$\frac{J}{D} = \frac{\rho(z_1) \exp(E(z_1)/k_B T) - \rho(z_2) \exp(E(z_2)/k_B T)}{\int_{z_1}^{z_2} \exp(E(z)/k_B T) dz}, \quad (2)$$

Here, the number density per unit length, $\rho(z)$, is proportional to the steady-state probability distribution. The total potential of mean force, $E(z)$, is the sum of the intrinsic PMF of an ion crossing the channel in the absence of an electric field, $A(z)$, and the electrostatic potential energy of the applied field, $qV(z)$. z_1 and z_2 define two planes inside the channel. In principle, their choice is arbitrary, but generally, the accuracy improves if they are well separated. Eq. 2 is not the only way to solve the ED equation for J , but it is the only expression that does not require knowledge of the full density profile, $\rho(z)$, inside the channel. For a given applied voltage, only the boundary densities $\rho(z_1)$ and $\rho(z_2)$ need to be calculated, which should be markedly more efficient than determining $\rho(z)$. This makes calculations of the full I-V dependence facile.

The equilibrium PMF, $A(z)$, can be obtained from separate simulations of the channel in the absence of an electric field [6]. Alternatively, it can be determined through integration of Eq. 1, which yields:

$$A(z_2) - A(z_1) = k_B \left[\ln \rho(z_1) - \ln \rho(z_2) - \frac{J}{D} \int_{z_1}^{z_2} \frac{1}{\rho(z)} dz \right] - q\mathcal{E}_{el}(z_2 - z_1), \quad (3)$$

where $\mathcal{E}_{el}(z_2 - z_1)$ is the change in electric field between z_1 and z_2 . This means that $A(z)$ can be determined from a non-equilibrium simulation that is sufficiently long to calculate J and $\rho(z)$ with satisfactory accuracy. Then, only the boundary terms are needed to calculate currents at other applied fields, as described above.

To initiate our MD simulations, both the TTX and LS3 channels were placed in a phospholipid bilayer that was in contact with water lamellae containing 1 M KCl on both sides. A schematic of the system is shown in Fig. 1. Simulations were carried out at several different applied fields for times of 0.5–2 μ sec. The simulation protocol resembled that described in our earlier study on ion channels[7]. From these simulations the ion currents, density profiles and/or the density boundary terms were obtained.

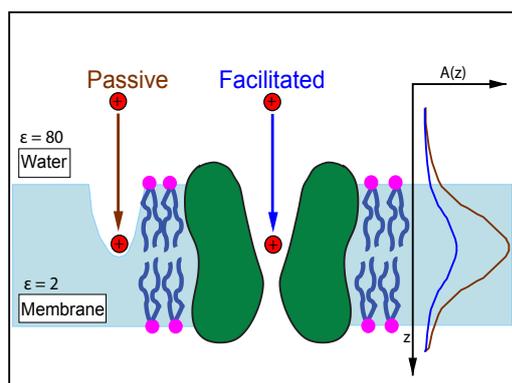


Figure 1. Schematic of an ion channel in a membrane. The water-filled pore significantly lowers the free energy barrier to ion transport, as shown on the right side of the figure.

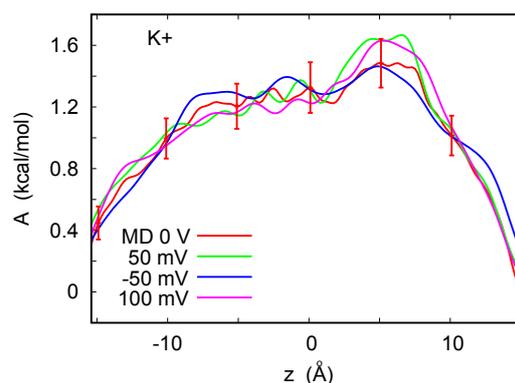


Figure 2. Free energy profile of K^+ in TTX at 0 V (red) and reconstructed from MD at 50 mV (green), -50 mV (blue), and 100 mV (magenta). $z = 0$ is the center of the membrane.

3. Results

In the ED equation, it is assumed that the probability of ion crossing is stationary and the numbers of crossing events in disjointed intervals are independent of each other, which implies that ion crossing is a Poisson process. However, if motions of ions in a channel are correlated or a channel undergoes slow conformational changes these assumptions would not be satisfied. Thus, we constructed semi-log plots of the cumulative probability distribution of waiting times between consecutive ion crossing events vs. time. For a Poisson process, they should yield straight lines with the negative slope equal to the inverse of the average waiting time, which is precisely what we found from our simulations.

The diffusivity was calculated by way of the Einstein relation from short simulations in which an ion was placed at different positions along z , as described previously [7]. Its values were found to be constant to within the statistical errors, so the dependence of $D(z)$ on z was suppressed in the ED equation.

The free energy profiles for K^+ in TTX reconstructed from Eq. 3 are shown in Fig. 2. We see that $A(z)$ obtained from simulations at ± 50 mV and 100 mV are in good agreement with $A(z)$ calculated from the equilibrium simulation. Similarly, the reconstructions for the LS3 channel (not shown) are very good for voltages of ± 100 mV, but not for -200 mV. This indicates that the ED model begins to break down at larger voltages.

For a stationary process, J is independent of the limits of integration in Eq. 2. Thus, calculating J for different ranges of z provides a valuable consistency test. If J is not approximately constant something is amiss; either $A(z)$ or the densities at the endpoints, $\rho(z_1)$ and $\rho(z_2)$, are inaccurate, or the assumption that the drift term in the Fokker-Planck equation is only due to the equilibrium free energy profile and applied field is incorrect. As shown in the example of K^+ in the TTX channel (see Fig. 3), the ED currents integrated in the range $[z_1 (= -z_2), z_2]$ are quite stable for z_2 between 10 and 15 Å. For smaller integration ranges, the errors increase, most likely due to the larger relative contributions from the density profiles deep inside the channel, which are known less accurately.

For the LS3 channel, in addition to long MD trajectories at -200, -100 and 100 mV, we also generated shorter trajectories at -50, 50 and 200 mV, from which we extracted $\rho(z_1)$ and $\rho(z_2)$ and computed the current by way of the procedure described in the previous section. In Fig. 4, the results are compared with the experimentally determined I-V curve. The K^+ current calculated directly from MD simulations is in excellent agreement with the total experimental current. The corresponding ED currents are very similar with the exception of that at -200 mV, indicating that the ED equation might become less accurate at large applied fields. Our calculations correctly capture the non-Ohmic character of the currents, which are smaller for the positive voltages than for negative voltages. There is, however, one discrepancy between

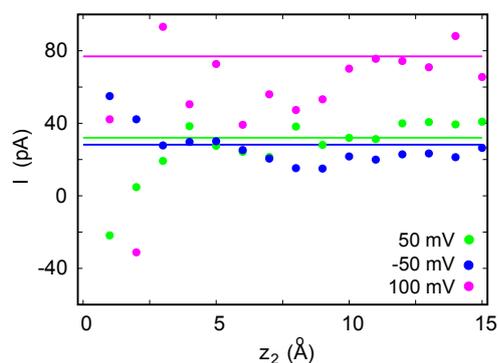


Figure 3. K^+ ED model currents for TTX at 50 mV (green), -50 mV (blue) and 100 mV (magenta). Solid lines are the currents observed in the simulations.

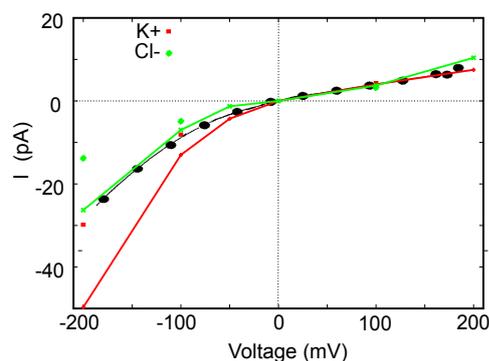


Figure 4. The I-V curves for K^+ (red) and Cl^- (green) in the LS3 channel. Experimental data extracted from [5] are shown as black circles.

the calculated and experimental currents. The channel is selective for K^+ over Cl^- by a factor of 10, which means that Cl^- currents should be quite small. This is not the case in the simulations, as currents of K^+ and Cl^- are comparable. The most likely reason for this outcome is the lack of balance between force fields describing interactions of Cl^- with bulk water and the environment inside the channel.

4. Conclusions

Ion currents were calculated directly from MD simulations and computed from a 1-dimensional ED model, and were found to be in good agreement for a model system based on a heptameric TTX channel structure. The main assumptions underlying the ED model have been shown to be valid: the distribution of ion-crossing events follows Poisson statistics and the thermodynamic force on an ion can be obtained from the intrinsic (zero-voltage) PMF and the applied electric field. The intrinsic PMF can be obtained equally accurately from equilibrium simulations at 0 V and from non-equilibrium density profiles. Once this PMF is known, the current at a desired voltage can be calculated efficiently only from the knowledge of ion densities near the ends of the channel. The extension of this approach to the LS3 channel yields an I-V curve that reproduces the experimentally observed non-Ohmic behavior of the channel. Capturing this feature, called rectification, is a valuable and sensitive test of reliability of computer simulations. The simulations also revealed that standard force fields for Cl^- are, most likely, improperly balanced.

Acknowledgments

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