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Nonequilibrium molecular dynamics calculation of the conductance of the KcsA potassium ion channel

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We have developed an efficient method for the atomistic determination of the conductance of a biological ion channel model by applying an external field to the conducting ions only. The underlying theory is discussed and demonstrated in a simple test system consisting of two ions in a box of water. Finally, the theory is applied to the experimentally determined structure of the KcsA potassium channel from which a conductance in reasonable agreement with the experimental result is predicted.

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As the primary conduit for ions passing across cell membranes, ion channels underlie many basic biological functions (nerve signal propagation, pacemaker regulation) and are implicated as the cause of a number of diseases when occurring in a malfunctioning state [1,2]. In addition to the obvious medical applications, details such as the mechanism of ion selectivity make the structure and function of these membrane proteins of great interest. This paper presents molecular dynamics (MD) simulations of ion currents through a realistic ion channel model done at atomic scale. Previous nonequilibrium (with a field applied to the system) MD simulations of ion currents [3,4] were based on simplified models of membrane pores, or Brownian dynamics simulations with adjustable parameters [5,6]. Since ion permeation even through the most conducting channels (e.g., the BK potassium channel) occurs on a 1 ns time scale, and a realistic model of the channel involves thousands of atoms, we must limit the system size (described later) and apply a larger than physiological electric field in order to collect ion current data in a reasonable time. Larger fields than normal are not a problem provided one stays within the linear response (Ohm's law) regime where $I=gV$. To avoid nonlinear effects connected with protein distortion by the large electric field, the field was applied to the ions only. We have developed the theory to justify this approach and in this paper we present the results for a simple test system (two ions in a water box) as well as a system based on the known structure of KcsA [7,8].

To prove that the correct result can be obtained with the field applied to the conducting ions only, we use the basic Kubo theory of the electrical conductivity tensor $\vec{\vec{\sigma}}$. In our system σ_{zz} is the relevant component, where z is the longitudinal direction along the channel. Also σ_{zz} and g differ by geometric factors that are not important for the following argument. We write the Kubo expression for two cases: (i) the external field applied to all charged species in the system and (ii) the external field applied to the conducting ions alone. In the first case, the conductivity is given by:

$$\vec{\vec{\sigma}}_{all} = \beta \int_0^\infty d\tau \langle \vec{j}\vec{j}(\tau) \rangle \tag{1}$$

where $\beta = 1/kT$ with T the temperature, and $\langle \vec{j}\vec{j}(\tau) \rangle$ is the equilibrium autocorrelation function of the microscopic current $\vec{j}$, which is defined by the sum of the charge times the velocity of all charged species in the system. This is an example of the fluctuation-dissipation theorem where a transport coefficient (the conductance) can be calculated from fluctuations of an equilibrium property (the microscopic current). Carrying out the derivation for the field applied to the ions alone, we obtain the result

$$\vec{\vec{\sigma}}_{ion} = \beta \int_0^\infty d\tau \langle \vec{j}_k \vec{j}_k(\tau) \rangle. \tag{2}$$

Here, $\vec{j}_k(\tau)$ is the microscopic current corresponding to the ions only.

To demonstrate the equivalence of (1) and (2), we break up the microscopic current into contributions from the ions ($\vec{j}_k$) and from all other charged species ($\vec{j}_o$). The latter include the partially charged atoms of the water and the protein. The microscopic current then becomes

$$\vec{j} = \sum_{i=1}^{N_k} q_i \vec{v}_i + \sum_{j=1}^{N_o} q_j \vec{v}_j \equiv \vec{j}_k + \vec{j}_o \tag{3}$$

where q_i is the charge and v_i is the velocity of the charged species i, N_k is the number of ions, and N_o is the number of all other charged species in the system. Using this in (1) and (2) we obtain

$$\vec{\vec{\sigma}}_{all} = \beta \int_0^\infty d\tau \langle \vec{j}_k \vec{j}_k(\tau) \rangle + \beta \int_0^\infty d\tau \langle \vec{j}_o \vec{j}_o(\tau) \rangle + 2\beta \int_0^\infty d\tau \langle \vec{j}_k \vec{j}_o(\tau) \rangle \tag{4}$$

and

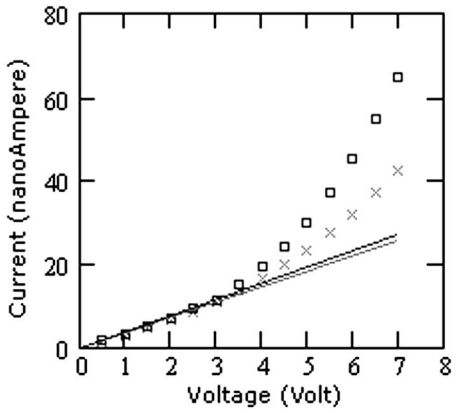

FIG. 1. Potassium ion current vs voltage for the water box system with an external field applied to all charged species (boxes) and the ions alone (crosses).

$$\vec{\vec{\sigma}}_{ion} = \beta \int_0^\infty d\tau \langle \vec{j}_k \vec{j}_k(\tau) \rangle + \beta \int_0^\infty d\tau \langle \vec{j}_o \vec{j}_k(\tau) \rangle. \tag{5}$$

In the above, we have contributions from three terms: the ion current autocorrelation term (ion-ion term), the autocorrelation of the current of all other atoms (other-other) term, and a term for the correlation of the ion current with the current of all other atoms (cross term).

Recall that the right-hand sides of the above equations are calculated at equilibrium. Considering that $\vec{j}_o$ receives contributions from bound charges and neutral molecules only, there will be no net contribution to the conductivity. To see this formally, we rewrite $\vec{j}_o$ as

$$\vec{j}_o = \frac{d}{dt} \sum_{j=1}^{No} q_j \vec{x}_j \equiv \dot{\vec{\mu}} \tag{6}$$

where $\vec{x}_j$ is the position of the jth charged species and $\dot{\vec{\mu}}$ is the time derivative of the net dipole moment of all species other than the conducting ions. We can recast the term containing the autocorrelation of all other species by using the following identity:

$$\int_0^\infty d\tau \langle \dot{\vec{\mu}} \dot{\vec{\mu}}(\tau) \rangle = \lim_{t \to \infty} \frac{1}{2t} \langle |\vec{\mu}(t) - \vec{\mu}(0)|^2 \rangle. \tag{7}$$

The fluctuations of the net dipole moment of the system (excluding the ions) given by $\langle |\vec{\mu}(t) - \vec{\mu}(0)|^2 \rangle$ will remain finite. Consequently, as $t \to \infty$ the other-other term will go to zero.

For the cross term, one can use the Schwarz inequality to show that the square of the cross term will never be greater than the product of the ion-ion term with the other-other term. As the other-other term goes to zero and the ion-ion term remains finite, the cross term will also go to zero. Consequently, both (4) and (5) reduce to

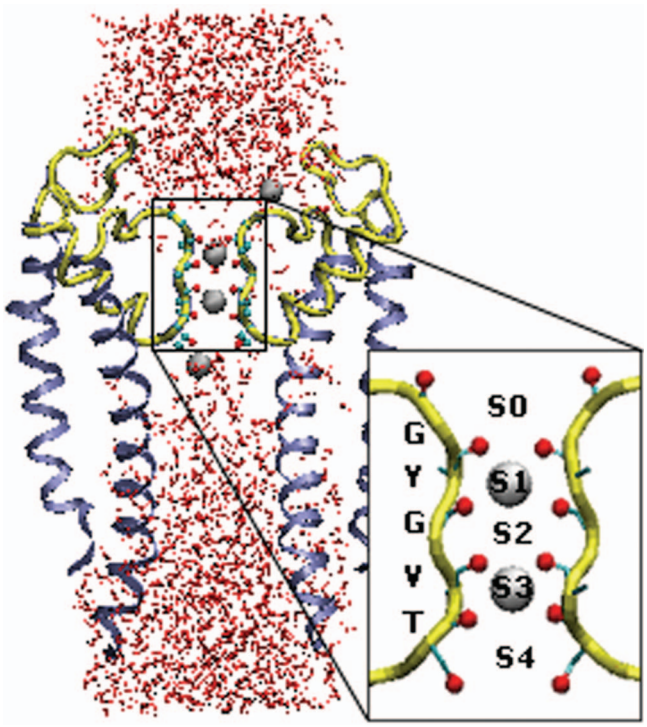

FIG. 2. (Color) System setup containing the KcsA channel (fixed TM helices as blue ribbons, free regions as yellow tubes), potassium ions (large spheres), and the cylinder of water. Two subunits are displayed; the subunits above and below the page are removed for clarity. The inset is a closeup of the selectivity filter showing the carbonyl and carboxyl oxygens of the TVGYG residues that form the binding sites labeled S0–S4.

$$\vec{\vec{\sigma}} = \beta \int_0^\infty d\tau \langle \vec{j}_k \vec{j}_k(\tau) \rangle. \tag{8}$$

Thus, in the linear regime, we expect to get the same conductivity whether we apply the field to all charged species in the system or to the ions only.

To directly test this result, we constructed a simple test system consisting of 256 water molecules (SPC model [9]), a potassium ion and a chloride ion in a 20 Å cubic box. The molecular dynamics simulations were performed using GROMACS [10,11]. The long-ranged interactions were handled by a particle mesh Ewald (PME) algorithm and the temperature was controlled by coupling the system to an external bath at 298 K [12]. An external field, ranging from 0.5 to 7 V across the 20 Å box, was applied in one case to all partial charges and ions and in the second to the ions only. The resulting potassium ion current vs voltage data are displayed in Fig. 1.

From Fig. 1, the linear regime is seen to extend from zero to 3 V. In this region, the conductance for both cases is very similar, validating our theory. The conductance as determined from the linear regime for both cases can be converted into a diffusion coefficient D using the Nernst-Einstein relation [13] $D = \sigma / q^2 \beta \rho$, where ρ is the density of the charged particles and the other quantities are as previously defined. The result for the field applied to all charged species is a

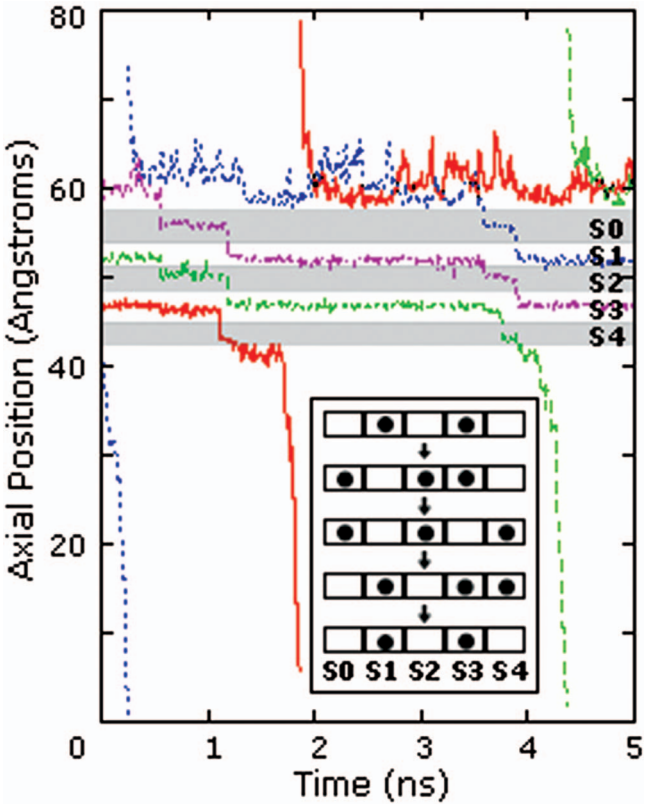

FIG. 3. (Color) 5 ns sample trajectory for 450 mV applied across the selectivity filter. The different ion positions are labeled S0–S4 and are separated by shading. The inset shows a schematic representation of the simplified kinetic sequence followed by the events shown in the main figure.

diffusion coefficient of 2.54×10^{-9} m^2/s while for the field applied to the ions only it is 2.41×10^{-9} m^2/s. For comparison, an equilibrium simulation was performed on the same system. The diffusion coefficient of the potassium ion, as determined via the Einstein relation [14] from the mean square displacement, is 2.50×10^{-9} m^2/s, in good agreement with both results and in reasonable agreement with the experimental value of 1.96×10^{-9} m^2/s [1]. A notable feature of Fig. 1 is that when the field is applied to the ions only, the deviation from linearity after 3 V is less severe than when the field is applied to all charged species. We expect that this may be amplified in the channel, where any distortion of the selectivity filter due to the applied field could drastically affect conductance.

We have used this method to perform MD simulations on the KcsA potassium ion channel. KcsA is a membrane-spanning protein which consists of four identical subunits. The narrowest portion of the channel, the selectivity filter, is formed by the highly conserved sequence TVGYG. The ion must be at least partially dehydrated to pass through the filter. The energy cost of dehydration is compensated by the ion interacting with the partially charged protein backbone carbonyl oxygens forming binding sites S0–S4 [7,15,16] along the filter. These sites are labeled in the inset in Fig. 2.

In the structure resolved by Mackinnon *et al.* [15,16], the intracellular mouth of the channel is not in a conducting

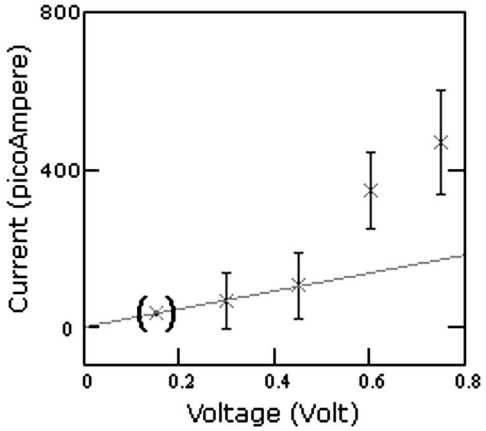

FIG. 4. Current vs voltage across the selectivity filter (12 Å) for the KcsA simulation system.

(open) configuration. To alleviate this, the inner mouth was widened to achieve a 10 Å diameter by applying a force to the transmembrane α-helices. Potassium ions were then added and water was overlaid to complete the system. To limit the system size, a membrane was not included in the simulations. To retain the open protein structure, all eight membrane-spanning helices were frozen in place. All other regions, including the selectivity filter, pore helices, and connecting regions were free to move. As a further step to minimize the system size, all water not within 10 Å of the pore axis was deleted. The resulting cylinder of water was held together by a harmonic constraint scheme (the position restraint algorithm in GROMACS was modified for this purpose). The final system, consisting of 7148 atoms (the protein, four potassium ions, and 1060 water molecules in an 80 Å cubic box), is shown is Fig. 2.

The free portions of the protein as well as the water were coupled to an external thermal bath to maintain temperature at 298 K. Long-range forces were again handled through the PME algorithm. For convenience, periodic boundary conditions were used in all three dimensions, but were only relevant in the axial direction (along the pore axis) as ions exiting the bottom of the system reenter at the top. After equilibration, an external electric field ranging from 1 to 5 V across the 80 Å box was applied. It is necessary to convert our field, applied to the ions only, to an equivalent transmembrane voltage. As it is seen in Fig. 3, the ions spend most of their time in the selectivity filter region. Thus, this region is the most resistant part of the open channel. This is in agreement with the estimation of the applied voltage profile across KcsA made by Berneche and Roux [5], which showed that for an open potassium channel, virtually all the voltage drop occurs across the filter. Therefore, to calculate the KcsA conductance we use the electrical potential difference for the ion in the applied field at the beginning and at the end of the selectivity filter (12 Å length) as an estimation for the equivalent voltage. Hence, the 1–5 V applied across the entire system translates to an equivalent 150–750 mV applied across the selectivity filter. These are the relevant voltages and are used in the following discussion. MD trajectories of the length of 15 ns to over 50 ns were generated on a large parallel computing system. A sample trajectory is shown in Fig. 3.

Figure 3 shows the axial positions of all four potassium ions for a 5 ns run calculated with 450 mV. For any open ion channel, the selectivity filter represents the region of highest resistance. In Fig. 3 the binding sites of the filter, labeled S0–S4, are indicated by shading. Note that the movement of an ion into the selectivity filter or jumping to a consecutive binding site is usually correlated with similar motion of a neighboring ion. Furthermore, in analyzing the trajectories generated at 300 and 450 mV, one can see a general pattern emerging for the passage of an ion across the channel. A simplified version of the observed conduction events is shown as a schematic in the inset in Fig. 3 and this pattern can be traced out in the two events occurring in the main figure.

The resulting current vs voltage graph for the simulations is shown in Fig. 4. The data points in Fig. 4 represent the current calculated from the average number of permeation events per 5 ns run. The 300 mV data point is calculated from ten events over 50 ns and the 450 mV data point from 15 events over 50 ns. The 600 mV data point was determined from 27 events over 20 ns and the 750 mV point from 22 events over 15 ns of data. The 150 mV data point is calculated from 20 ns of data in which only two events were observed. Clearly, this point is tentative and we are continuing to collect data at this voltage.

Examining Fig. 4, we see that the linear regime extends up to 450 mV (applied) and the resulting conductance from fitting over these points is 113 pS. To compare this to experimental results [17], it is necessary to estimate the effective concentration of the bathing solutions. At 450 mV, we estimate there are on average 2.1 ions in the channel. Morais-Cabral *et al.* [15] found an average of 2.1 ions in the selectivity at a concentration of 200 mM. This corresponds to a conductance of 125 ps [17]. The dependence of the conductance on the concentration at these values is not very steep and the range of concentration from 100 to 300 mM corresponds to a conductance range of 100–150 pS. Hence, even with the uncertainties due to the system setup and the estimation of concentration, it is clear that the results are in good agreement with laboratory measurements. To summarize, we have given a fully atomistic simulation for ion channel conductance.

Images of the channel included in this paper were created using VMD [18]. Movies of the simulations are available on our website at www.physics.uoguelph.ca/~hdh

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