Combining Computational Chemistry and Computational Electronics to Understand Protein Ion Channels.

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ABSTRACT

Nanoscale computational engineering can be achieved by combining atomic and molecular scale methods of computational chemistry with coarser-grained continuum theories used in computational electronics. Each computational discipline is quite different; yet both must give the same results if they are done correctly. Here we apply both classes of simulation technique to the specific problem of computing ion current in protein channels. In the biological tradition, ion channels are transport enzymes, catalyzing the movement of ions from one side of a membrane to the other. In the tradition of nanodevices, ion channels can be viewed as transistors with unusual properties_exquisite sensitivity to specific environment factors and ability to self-assemble.

Keywords: ion channels, computational electronics, molecular dynamics, electrostatics, electrodiffusion

1 INTRODUCTION

Ion channels are a class of proteins that form nanoscopic aqueous tunnels controlling ion transport through the otherwise almost impermeable membranes of all biological cells. Each channel consists of a chain of amino acids carrying a strong and rapidly varying localized permanent charge. Molecular dynamics (MD) simulations can resolve channel physics in atomic detail, however at present the computational costs of such large-scale simulations prohibits the direct calculation of steady-state channel currents. Alternatively, drift-diffusion theory, used widely in the device engineering community to describe charge transport in semi-conductor devices, can be used to compute macroscopic current with a reasonable amount of computational effort. Although drift-diffusion models sacrifice resolution of molecular detail, when combined appropriately with MD and other atomistic simulations, they have been found to describe ion permeation through ion channels surprisingly well.

In this paper we combine traditional computational chemistry methodologies with a coarser-grained drift-diffusion model to compute ion current-voltage (*IV*) relations for potassium-chloride (KCl) permeation in *ompF*, a trimeric porin channel that resides in the outer membrane of the *e-coli* bacterium. Each hourglass-shaped monomer of *ompF* carries a net permanent charge of approximately , where *e* is the electron charge, and is moderately selective for positive ions. *omp*F can be mutated by replacing or deleting one or more of the amino acids, altering the charge distribution along the channel. Engineering channels with specific conductances and selectivities is thus conceivable.

In section 2 we outline the various computational methods used in the simulation hierarchy. In section 3 we demonstrate how the results from these different methods, can be combined to provide an overall picture of ion transport in the porin channel. Section 4 concludes with a discussion of this work and future plans.

2 METHODS

The overall strategy of this paper is to use MD simulations, a tool of computational chemistry, to determine the mobility of ions in the *ompF* channel (bacterial porin [1]), use continuum electrostatics calculations to determine the ionization state of titratable residues in the protein (hence the charge distribution in the protein), and use the protein charge distribution and ion mobilities as parametric inputs to a drift-diffusion calculation to compute fluxes through the channel. In the MD simulations molecules are modeled as charged balls (atoms) connected by springs (chemical bonds), brought to simulated experimental temperature and equilibrated by initially applying random velocities with a Maxwellian distribution and then permitting the system to evolve according to Newtonian mechanics. For this work we have used Gromacs [2], an efficient scalable open source molecular simulation package, to simulate the *ompF* channel in a decane membrane solvated by KCl electrolyte (Figure 1). For the continuum electrostatics computations to determine the protein ionization states, the computational chemistry software package UHBD [3] was used.

The three-dimensional (3D) self-consistent drift-diffusion theory is based on the simultaneous solution of Poisson's equation, which describes Coulomb interactions between all charges in the system including those

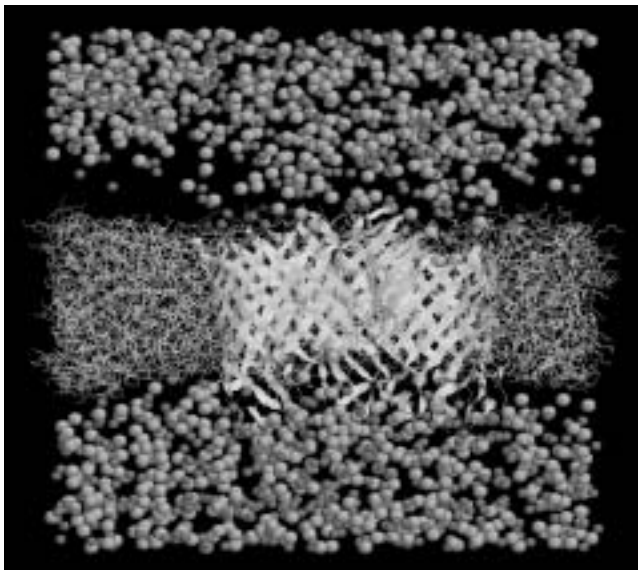

Figure 1. Snapshot of the MD simulation of *ompF*. Ribbon structure is the porin channel protein, small balls of different shading are potassium and chloride ions, strands at the sides of the porin are decane molecules that emulate the lipid membrane.

permanently residing on the protein, and a continuity equation for each ion species, describing permeation down an electrochemical gradient [4] Water is treated as a uniform background medium with a specific dielectric constant ˘ . For the results presented here we use ˘ = 80, 20 and 2, for the aqueous, protein and membrane regions, respectively. Macroscopic current flow is resolved by assigning an appropriate mobility and diffusion coefficient to each ionic species. The drift-diffusion model is implemented using the PROPHET simulator [5], a computational platform developed at Lucent Technologies and Stanford University. The PROPHET simulator uses the "dial-an-operator" methodology to construct systems of partial differential equations by combining existing differential operators described using a scripting syntax. After the channel geometry is defined on a customized mesh provided by the user (Figure 2), the drift-diffusion equations are discretized and the linear system is solved using iterative methods.

One of the critical features of proteins is that the side chains of the amino acids (hereafter referred to as residues) are ionizable via the addition or subtraction of protons (protonization). The pKa value of a residue is the pH at which the probability of ionization is one half. Standard pKa values calculated for most ionizable residues floating freely in bulk electrolyte are far from neutral pH. This implies that in neutral electrolyte an isolated residue is either always or never protonated, depending whether the pKa value is above or below the electrolyte pH. However, the dielectric environment, proximity of neighboring residues in the folded of the protein, and concentration of the electrolyte solution can shift the pKa from its standard

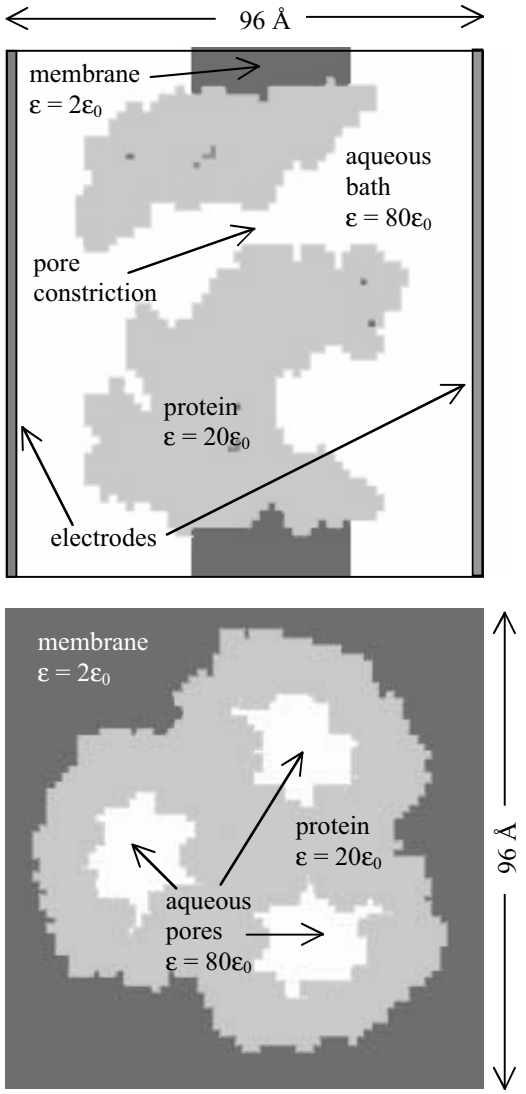

Figure 2. PROPHET mesh representation of the *ompF* trimer *in situ* in a membrane, immersed in an aqueous bath of *KCl* - (a) longitudinal and (b) cross-sectional slices through the 3D computational domain generated on a uniform rectilinear grid (1.5Å spacing). Electrodes immersed in the baths maintain a fixed bias across the channel/membrane system.

value. Hence the ionization state for every ionizable side chain in a protein must be calculated for that protein in the ion concentration and pH of experimental interest. This is done by solving the nonlinear Poisson equation for the electrostatic potential at all points on the surface of the protein, and postulating that the local pH varies from the bulk pH by a Boltzmann factor. A detailed description of the method is given in [6]. An example of the significance of this calculation is shown in Figure 3, which demonstrates that at neutral pH several residues in *ompF* effectively carry a non-integral charge. Note that the curves for the residues glutamate 117 and glutamate 296, chemically identical, are very different. At the neutral pH of 7 glutamate 296 has

practically no charge while glutamate 117 carries a full electronic negative charge. Note also that the charge on histamine 21 varies a lot with just small pH changes near neutrality.

The ionization states for each of the residues in *ompF* were computed as outlined above and used to construct a charge density profile in the protein. In the next section we describe how MD simulations were used to compute the ion diffusion coefficients inside the channel pore. The charge distribution and diffusion coefficient profiles *D(z)* provide inputs for the drift-diffusion simulation.

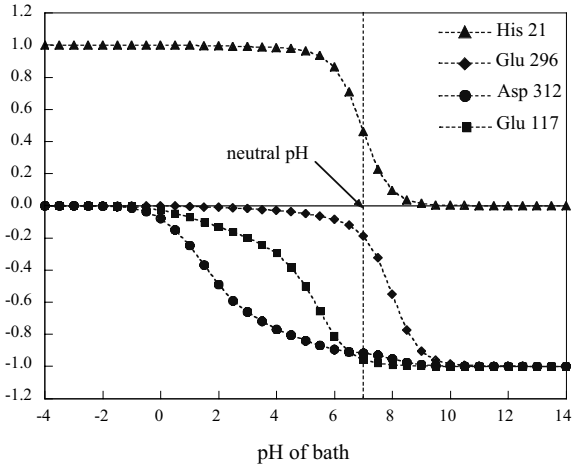

Figure 3. Titration curves for selected amino acids in the *ompF* porin channel at 150mM KCl. Vertical axis is net charge on the amino acid (electronic units).

3 RESULTS

3.1 Molecular dynamics of *ompF* Porin

In these calculations both potassium and chloride ions were permitted to move freely in the electrolyte and in the porin channel. The mobility of ions in different parts of the channel is inferred from the mean-square deviation correlation function (MSD) = $\left\langle \quad \right\rangle$. Samples of the MSD curves for K^+ are shown in Figure 4. Each curve was calculated from position fluctuations of ions residing in a 2 angstrom-long "slice" within the channel pore. The effective diffusion coefficient is one half the slope of the curve at large _ [7].

Figure 4 shows that the local friction, represented by the initial slope from the origin of the curve, is less than what would correspond to the effective diffusion coefficient, represented by the sustained slope of the curve. The irregular deviation of the MSD curves from linearity suggests that the 5 nanosecond simulation is not sufficient to compute the diffusion coefficient to high accuracy. However the results definitely show that the mobility is much lower in the channel than in the bulk. Similar results are found for chloride ions.

Figure 5. shows the K^+ and Cl^- diffusion coefficients, calculated from the MSD curves, as a function of axial position inside the channel pore. The protein channel is located between z=5Å and z=65Å, with the pore constriction located at approximately z=30-37Å. Note that for both species the diffusion coefficient inside the pore is suppressed from its value in bulk electrolyte solution (D_{bulk}~2.0×10^{-5} cm^2/sec), decreasing by roughly an order of magnitude in the narrowest section of the channel. Diffusion coefficient profiles used in a one-dimensional (1D) drift-diffusion theory are also shown [8]. In contrast to the MSD results, these profiles, which were "reverse-engineered" to fit experimentally measured *IV* curves, suggest a large increase in the diffusion coefficient in the pore constriction. However, due to low ion occupancies in pore constriction region (i.e. the ions are rarely found there), the MSD diffusion coefficients are not well resolved in that region. Reliable estimates of the ion occupancies and diffusion coefficients require longer simulation times.

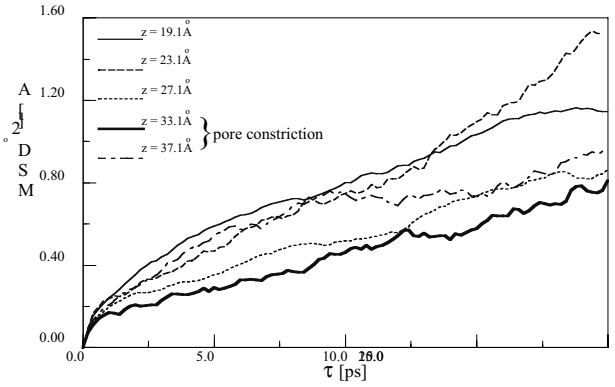

Figure 4. Mean-square-deviation (MSD) curves for K^+ position fluctuations inside the *ompF* porin channel.

3.2 Current-voltage relations

Figure 6 shows the measured *IV* curve for ion permeation through the open porin channel immersed in 100mM KCl, together with those computed from the drift-diffusion model for various *D(z)*: (a) Axially varying diffusion coefficients (shown in Figure 5), found by "reverse-engineering" a 1D drift-diffusion model to fit experiments, (b) A modified form of these profiles, in which the large peak in the pore constriction is removed, resulting in diffusion coefficients that are both qualitatively and quantitatively similar to that computed from MD simulations, and (c) a spatially uniform diffusion coefficient, the same for both ionic species, the value of which is chosen to give the best fit to the measurement and turns out to be approximately one third of the experimentally determined values for K^+ and Cl^- in bulk solution. Mobilities were assigned using the Einstein

relations. Of the three *D(z)* profiles both (a) and (c) give reasonable agreement with the measured *IV* curve, while (b) underestimates the current. This discrepancy is discussed in the next section.

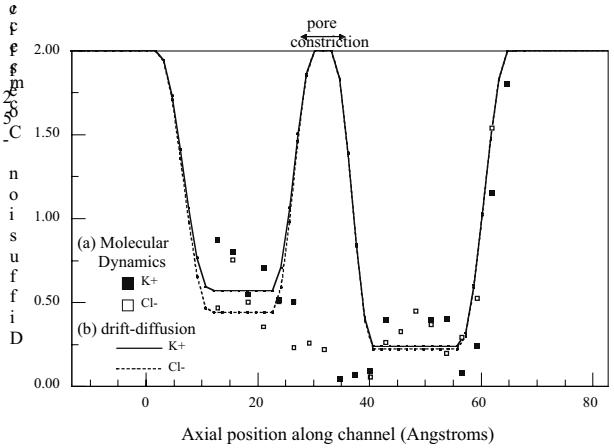

Figure 5. Diffusion coefficient as a function of axial position in the *ompF* porin channel computed by (a) MD and (b) "reverse-engineering" 1D drift-diffusion theory to fit experimental data.

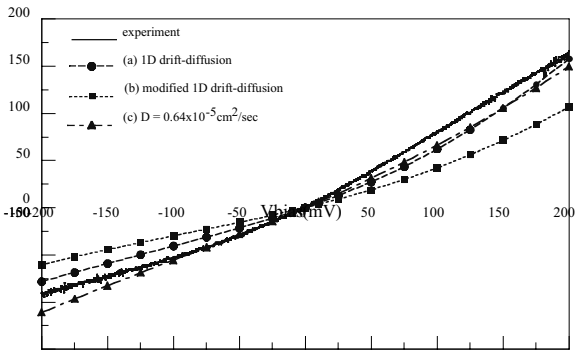

Figure 6. Comparison of measured and computed *IV* curves for ompF in 100mM KCl, for various *D(z)*: (a) reverse engineered from a 1D drift-diffusion model, (see Figure 5) (b) a modified form of (a) in which the peaks in the diffusion coefficients in the pore constriction are removed (c) uniform diffusion coefficient.

4 DISCUSSION AND SUMMARY

We have simulated ion permeation through porin channels using software and algorithms from the fields of computational biochemistry and computational electronics. The different calculations are complementary in various ways. Ionization state calculations are essential to provide the correct input for computing any process involving interactions of ionic charges and proteins. MD computations provide detailed views of atomic scale motions but require heroic amounts of computer time as well as statistical mechanical analysis to provide information relevant to computing fluxes in channels. However, they can be used to infer ion transport parameters *ab initio,* which can be fed into the more coarse-grained drift-diffusion calculations to compute ion fluxes in modest amounts of compute time.

Figure 5 is particular noteworthy in showing the complementarity between the computational chemistry molecular dynamics approach and the semiconductor drift-diffusion approach. To interpret the figure, two facts are needed; 1) The channel gets narrower towards its midpoint, and 2) There are theoretical reasons to believe that the drift-diffusion theory will lose validity when the channel radius becomes less than a Debye length [9]. We see that in the wide part of the channel, the two approaches agree to within the "noise" of the molecular dynamics results. In the narrow area of the channel, where the drift-diffusion approach is no longer valid, the results obtained by the two methods diverge. Each has a contribution to the total picture. In the wide part of the channel, the drift-diffusion theory gives a precise number with relatively modest computational resources compared to molecular dynamics. In the narrow part of the channel, the molecular dynamics can provide an answer that is not obtainable from the drift-diffusion approach. This result suggests the desirability of developing hybrid methods in which a particle model (e.g. Monte-Carlo or Brownian Dynamics) of ion transport in the narrow region is coupled to a continuum description elsewhere. Alternatively, it might be possible to modify the drift-diffusion model to include a phenomenological term describing the effects of ion volume. Both approaches are currently being pursued.

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