HYBRID MD-PNP SIMULATIONS OF THE α-HEMOLYSIN OPEN CHANNEL IONIC CURRENT

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ABSTRACT

Experiments have shown that single stranded nucleic acids polymers can be transported through the α-hemolysin ion channel under the action of an applied electric field. The translocation of the nucleic acid polymers causes transient blockade of the ionic current. The physical and chemical details of the interactions between polymer, channel and ionic solution that control the blockade events are not yet fully understood. To study such interactions, a hybrid MD-PNP model is proposed. The diffusion coefficient of the ions ($0.78 \cdot 10^{-9} m^2/s$) and pore wall charges are determined from MD simulations. These values are used in the PNP model to calculate open channel ionic currents. The calculated value for ionic current (*101 pA*) is close to the experimental value (*120 pA*) only when pore-wall charges are included in the model in addition to the pore geometry. This validates the present approach of bridging time scales by combining a microscopic and macroscopic model.

Keywords: ionic current, α-hemolysin, molecular dynamics, Poisson Nernst Planck theory, ion channel

1 INTRODUCTION

The study of biological systems at the molecular scale is useful for nanoscale engineering. One such example is the α-hemolysin protein (*33.2 kD*), which spontaneously self-assembles into a heptameric channel that inserts itself into cellular membranes causing leakage of ions and small organic molecules [1]. Recently, scientists used this protein as a model system for structural analysis of nucleic acids by deciphering ionic signature patterns [2-5].

Experimental studies show that single stranded nucleic acid polymers in extended linear configurations can be transported across the α-hemolysin pore under the action of an applied electric field. Translocation of such polymers causes partial blockades of ionic current through the channel. The level of fractional blockade differs for various nucleic acid homopolymers.

This result suggests the possibility for nanopore-based sequencing. However, the fast translocation rate (*~1-5 subunits per microsecond*) and small differences in levels of ionic current blockade presents a challenge in signal processing of the desired single nucleotide resolution. To design a nanopore that would achieve single nucleotide resolution, physical and chemical details of the interactions between polymer, channel and ionic solution need to be better understood.

Various computer simulations and theoretical models have been used to better understand structure-function relationships of ion channels. Continuum models such as the Poisson-Nernst-Planck and Eyring Rate Theory allow relatively quick computations of ionic fluxes and translocation times [6-9]. However, these continuum models do not account for fine structural details and depend on the choice of input values for the diffusion coefficients of the ions, system charges, energetic barriers and other parameters. On the other hand, it is presently not feasible with the present computation resources to reach time scales of physiological interest with full atomistic simulations[2].

For polymer translocation studies, the most important quantity to calculate and predict is the ionic current. Macroscopic scale events are influenced by the fine structural details and the time evolution of the model system (e.g. transport coefficients). The goal of the present work is to link atomistic (MD) and PNP simulations into a hybrid model to calculate the α-hemolysin open channel ionic current. An expression for the ionic flux at macro scale (electro-diffusion) is derived using the Poisson-Nernst-Planck theory that combines the solutions of both diffusion and Poisson-Boltzmann equations. Mezoscale models normally use a continuum representation for the system under study. However, in the present case information about the structural details and dynamics are implemented from MD simulations in which an atomistic representation of the system is employed. This hybrid model is thus more realistic than other mezoscale models in the ion channel literature and will be used in the future to calculate ionic currents for *organic and inorganic pores* in the presence of *pore-specific surface chemistries.*

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[2] The time scale of physiological ion permeation is in the order of ~100ns-1μs [15]. For a 100,000-atom system a reasonable upper limit with the present computer resources is about 10ns [8].

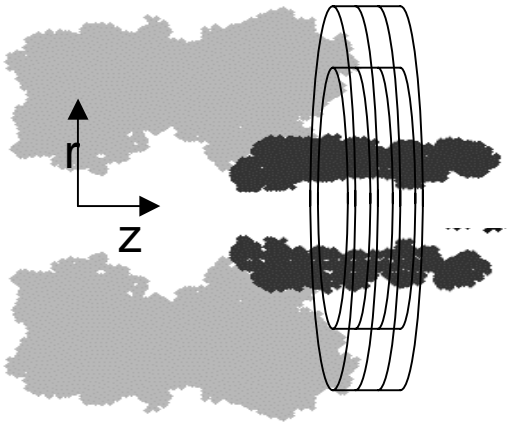

Figure 1: Schematic representation of α-hemolysin channel. In the PNP model a 2D grid (represented as concentric rings) corresponding to a cylindrical polar coordinate system is applied over the pore stem (dark area).

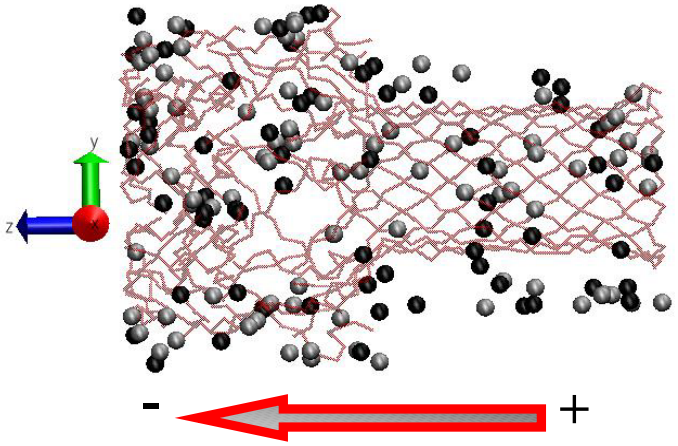

Figure 2: Snapshot from an MD simulation of the α-hemolysin system in a 1M KCl solution and an external applied electric field. The K^+ (light colored spheres) and Cl^- ions (dark colored spheres) inside and within *5 Å* of the pore are shown using the van der Waals representation. The solvent (SPC/E water) is not displayed. This figure was generated using the VMD[3] software [14].

2 MODEL AND RESULTS

The X-ray diffraction structure of the α-hemolysin channel is used as model in the present calculations (a schematic representation is given in Fig. 1). The structure is available from the Protein Data Bank with a resolution of *1.9 Å*, determined at a temperature of *287 K* and *pH=6* [1]. The protein is a transmembrane homo-heptamer and contains 2051 residues (16,389 heavy atoms).

After replacing missing atoms in the PDB file, the structure was protonated in Amber7 [13] at neutral pH (total number of atoms 32,305) to reproduce the conditions in the polymer translocation experiments [2-4]. An NH_3^+ group at the amino terminus and a COO^- group at the carboxy terminus patched the terminal amino acids. The model was then aligned with the pore axis along the *z* direction of the coordinate system, with the origin at the center of the channel. Parameters for the potential functions and partial charges on the atoms were used from the Cornell 95 et al force field [12]. At neutral pH the total charge of α-hemolysin is $+7e^-$ and it was neutralized by adding Cl^- counter ions in Amber7. The energy of the structure was then minimized in Amber7 for 250 steps using the steepest descent method followed by 1250 steps of conjugated gradient method. First the hydrogen atoms were minimized, then the side chains, then all the atoms except the α-carbons and finally all the atoms. The RMSD value calculated for all the atoms was *0.8 Å*. Thus, no significant deviation during minimization was observed.

2.1 Molecular dynamics simulations

For MD simulations, the channel was inserted into a periodic unit cell (*95Åx95Åx125Å*) with electrolyte solution (*1M KCl*) and an electric field of *0.029 kcal/mol/Å/e*$^-$ (equivalent to the experimental value of *125 mV* over the *100 Å* long pore) is uniformly applied along the *z* axis (see Fig. 2). MD simulations were conducted using the NAMD[1] software [11] on NASA-AMES Research Center SGI supercomputers.

The coordinate and topology files are generated in Amber7. The model channel consists of a "reduced" atomistic representation of the α-hemolysin pore with polar walls, obtained by calculating the diameter of the pore corresponding to each position z along the channel axis and selecting the residues that lay within *25 Å* of the channel axis (see Fig. 3).

In all present MD simulations, a switching function was used to calculate the non-bonded interactions with a switch distance of *17.5 Å* and a cutoff of *18.5 Å*. To reduce the cost of calculating the non-bonded interactions, NAMD uses a non-bonded pair list that includes all pairs of atoms within a certain distance and may be updated periodically. The pair list distance was set in the present calculations to *20 Å* and updated every 20 MD integration steps. Also, to speed up the calculations, the RATTLE algorithm [16] was used to constrain all the bonds involving hydrogen atoms, thus allowing an increase in MD integration time to *2 fs*.

[1] NAMD and VMD are developed by the Theoretical Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign.

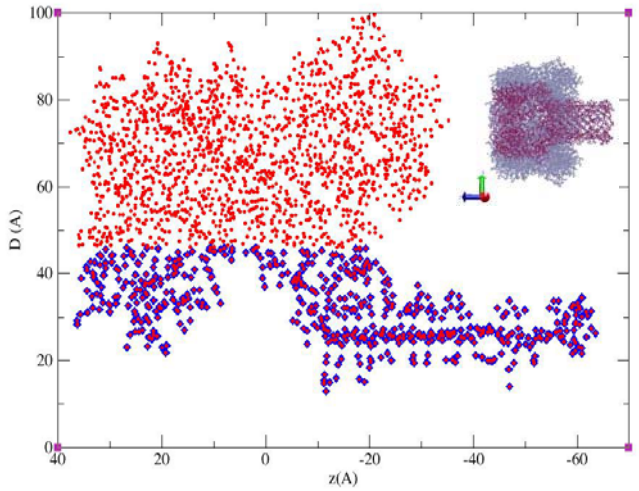

Figure 3: Projection in the axial plane of the α-hemolysin channel. The internal diameter of the pore corresponding to each position *z* along the channel axis is shown. The blue points represent the selection for the "reduced" representation of the pore used in the MD calculations of ion diffusion coefficients.

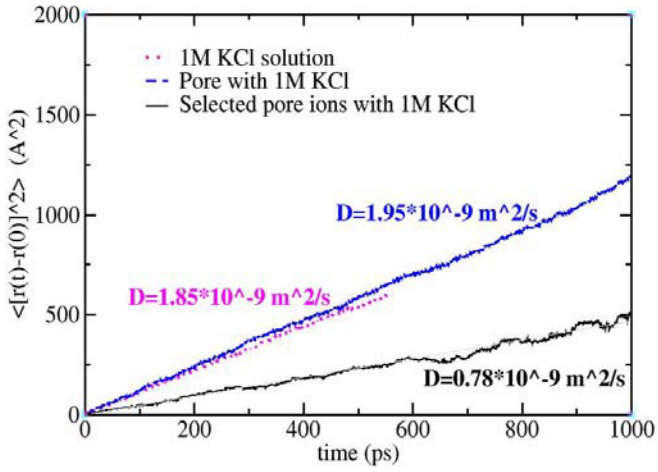

Figure 4: Mean square displacement versus time (MD calculations) for K^+ ions in: pure *1M KCl* solution (dotted line), *1M KCl* solution with α-hemolysin pore (dashed line), and *KCl* solution inside the α-hemolysin pore (solid line).

First, a periodic unit cell with ionic solution (*KCl*) was generated. The initial configuration of the K^+ and Cl^- ions originated from the KCl crystal structure, and was solvated by adding water molecules (SPC/E model) in a proportion corresponding to a 1M concentration. The system was energy minimized over 1000 steps, heated from *50 K* up to *300 K* in steps of *50 K* every *10 ps*, and equilibrated for *200 ps* at *300 K* using the NPT ensemble with the Berendsen temperature and pressure coupling. This pre-equilibrated unit cell containing KCl solution was superposed over the "reduced" representation of the α-hemolysin pore. Overlapping ions and water molecules were removed and added back randomly to the unit cell to preserve the 1M concentration. The pore was fixed and the same procedure as above is repeated. To speed up the calculations[2] and obtain longer MD timescales, a multiple time step algorithm (MTS) was used where the non-bonded interactions are evaluated periodically. Within the 20 steps cycle, the van der Waals and electrostatic interactions were updated at intervals of 2 and 4 steps, respectively. After *700 ps* of MTS-NPT dynamics a production run of *1ns* of MTS-NVE dynamics was performed with the external electric field turned on. The diffusion coefficients, *D*, of the ions inside the protein channel are calculated for the 1ns production run using Einstein's equation of diffusion:

$$2Dt = \frac{1}{3} < \left| r_i(t) - r_i(0) \right|^2 > \qquad (1)$$

with $r_i(t)$ the position vector of the particle at time t. If all the ions in the unit cell are selected, a value of $1.95 \cdot 10^{-9}$ m^2/s is determined (see Fig. 4, dashed line). This value is comparable to that determined from *550 ps* of MTS-NVE dynamics for pure *1M KCl* ionic solution ($1.85 \cdot 10^{-9}$ m^2/s, dotted line in Fig. 4). A smaller value ($0.78 \cdot 10^{-9}$ m^2/s, solid line in Fig. 4) is calculated if only the ions located inside the pore (and within *5 Å* of its boundaries) are selected and is used in the PNP calculations.

2.2 Poisson Nernst Planck calculation

In the PNP model a 2D grid (represented as concentric rings in Fig. 1) corresponding to a cylindrical polar coordinate system is applied over the pore stem (dark area). In contrast to the MD calculations where discrete ion positions are considered, the PNP approach assumes a continuum distribution of ions, where the ion concentration at a location is calculated as the time-averaged non-normalized probability of finding an ion at that location.

Following the formalism of Hollerbach et al. [10] a 2D-Poisson equation that relates the electrostatic potential ϕ to the fixed charge density ρ_F is used:

$$\nabla \varepsilon_r \nabla \phi = -\frac{q}{\varepsilon_0}(C_p - C_n + \rho_F) \qquad (2)$$

with ε_r and ε_o are the relative and vacuum permitivities. C_p and C_n are the concentrations of the positive (K^+) and negative (Cl^-) carriers, respectively, and are set initially at the bulk ion concentration (1M). In the present case, the only forces acting on the ions are the combined electrostatic forces $\nabla\phi$ resulting from the applied voltage *V* and the surrounding fixed (protein) and mobile (continuum KCl

[2] Using 200CPU's it takes 1.2 days to reach the time scale of 1ns. The modeled system contains ~120,000 atoms.

solution) charges. Under these conditions the ion flux, J, for each ion species (p for positive carriers and n for negative carriers) is approximated by the Nernst-Planck drift-diffusion equation:

$$J_i = e\mu_i C_i\left[-\nabla\phi\right] - eD_i\nabla C_i \,, i \in \{p,n\} \quad (3)$$

$$\nabla \cdot J_i = 0 \quad (4)$$

$$\mu = D\frac{e}{kT} \quad (5)$$

with D *(m^2/s)* the ion species diffusion coefficient, μ *(m^2/Vs)* the corresponding mobility calculated from Eq. 5, k (*J/K*) the Boltzmann constant, T *(K)* the absolute temperature and e^- (*C*) the unit charge. Eq. 3 relates the flux for each ion species to the force on the ion (first term) and the concentration gradients of the ion species (second term). The conservation condition given by Eq. 4 simply states that the ionic current must be constant at steady state. Eq. 2 and 3 are solved iteratively for the potential profile ϕ and the equilibrium ion concentrations C_i. For each grid section of total area ∂A (Fig. 1), the ionic current I and the number of ions per unit time dN/dt are calculated by integrating the flux of positive and negative ions:

$$I = \int\left(J_n + J_p\right)\partial A = e\frac{dN}{dt} \quad (6)$$

Values for the open channel ionic current and the ion flux are summarized in Table 1.

	I (pA)	dN/dt (ions/s)
Pore geometry	54	$3.37\cdot10^8$
Pore geometry and fixed charges	101	$6.28\cdot10^8$

Table 1 Calculated values for the open channel ionic current and the corresponding ion flux using the hybrid MD-PNP model. Diffusion coefficients and charges originate from MD simulations. The experimental determination for the open ion current is *120 pA*. [2].

In the 2D-PNP model, the ions have a diffusion coefficient ($0.78x10^{-9}$ m^2/s) determined from MD simulations (section 2.1). The open channel ionic current is *54 pA* when only the channel geometry is considered. This means that the fine structural details of the stem (see Fig. 3) but not the partial charges on the atoms are included. By including the pore-atoms charges, this value becomes *101 pA*, comparable with the *120 pA* value measured in polymer translocation experiments [2].

3 DISCUSSION

The diffusion coefficient of potassium ions in the α-hemolysin channel is reduced relative to bulk solution by a factor of 2.5 as a result of the geometry and energetic interaction with the channel. The geometry of the channel seems to be responsible for about 50% of the total ionic current. However, it is yet unclear if the surface charges act as a mean field or if the local variations in the electrostatic potential play the leading role. Future work will attempt to refine this model to separate more clearly these influences. The MD-PNP model will also account for combined polymer-dynamics to predict blockade durations for various translocating polymers and will be used to calculate ionic currents for both *organic and inorganic pores* in the presence of *pore-specific surface chemistries*. Results could be used in the experimental design of a solid-state nanopore with single nucleotide resolution for sequencing purposes.

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