Towards an MD simulation of ion currents in the alpha hemolysin channel

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***Abstract*- Experiments show that single stranded nucleic acids polymers can be transported across an alpha hemolysin channel under the action of an applied electric field. The translocation of the nucleic acid polymers causes transient blockades in the ion current. The physical and chemical details of the interactions between polymer, channel and ionic solution that lead to the blockade events are not yet fully understood. Atomistic simulations enable a realistic description of the dynamic motions of the solvated polymer-pore system as a function of time. Analysis of such calculated system trajectories leads to valuable insight possible energy barriers, ion mobilities and short time-scale interactions. This study investigates the relationship between the structural properties of the alpha hemolysin channel and the ionic current blockades that result from the translocation of single stranded nucleic acids.**

I. INTRODUCTION

The α-hemolysin protein toxin, which is produced by a spherical bacterium called *Staphylococcus aureus*, was initially used by scientists at Harvard University and the University of California at Santa Cruz as a model system to decipher ionic signature patterns in the genetic code of nucleic acids [1]. The α-hemolysin protein (33kD) spontaneously self-assembles in membranes to form a large (~100 Å) heptameric channel that allows the passage of ionic currents [2]. The protein consists of a cap and a stem section with the largest diameter in the cap cavity of 42Å and the minimum diameter in the stem of 12Å. When an electric field is applied across the open channel, negatively charged polymers, such as DNA or RNA molecules, can be drawn along the channel in extended linear conformations. The presence of a single molecule of nucleic acid polymer inside the channel results in reduced ion flow through the channel. The passage of a nucleic acid causes transient blockades in the α-hemolysin single channel current. The number, intensity and duration of the blockades is observed to be a fingerprint for various nucleic acids homopolymers. For example, a 95% reduction in the ionic current amplitude is characteristic for cytosine ribonucleotide homopolymers (poly[C]), compared to only a 85% for adenine ribonucleotide homopolymers (poly[A]), and a blockage magnitude of 85-100% for polyuridilic acid (poly[U]). The translocation rates per each nucleotide are 3μs, 20μs and 1μs, respectively. The subunit-specific variation of amplitude and duration of ionic current modulation suggests that nanopores could be used for direct reading of DNA sequence [3, 4].

However, the α-hemolysin protein pore is limited in its ability to resolve ionic signatures produced by single nucleotides. The difference in the number of ions involved in the transition between a single purine subunit adjacent to a single pyrimidine subunit is only about 100 ions per microsecond, which corresponds to an ionic signal that is lost in the noise of the measurement [3]. One solution, which requires a more robust and controllable pore, is to slow down the transport rate of the polymers through the channel [3, 4]. Thus, research has shifted to experimentation of translocation of nucleic acid polymers through a solid-state nanopore, which may enable a more robust temporal control of the transport phenomena [5].

Various computer simulations/theoretical models have been used to model ionic channels [6]. Mezo-scale models such as the Poisson-Nernst-Planck (the ionic current and the translocation of the polymer are electro-diffusion processes) and Eyring Rate Theory (processes are regarded as transitions between energy wells) allow relatively quick computations of ionic fluxes and translocation times [7, 8]. However, these models require the input of values for the diffusion/mobility coefficients of the ions and free energy profiles. Such calculations can be accurately performed using an atomistic representation of the system by employing molecular dynamics. Output from molecular dynamics simulations such as friction coefficients could be introduced in Langevin models that would allow the exploration of longer time scales (the timescale of physiological ion permeation is ~100ns-1μs).

Modeling of the nanopore system was conducted using NASA-AMES supercomputer resources, to identify the structure-function relationship between the α-hemolysin-nucleic acid polymers system and ion current signatures. Reference [13] discusses a hybrid MD-PNP approach in more detail. The present paper describes some of the details in setting up these calculations and attempts to answer some fundamental questions. Is the ionic current inhibition related to the amount of unoccupied volume in the pore? What are some of the values for the binding energies of the ions in the pore and on the polymer?

II. THE ALPHA HEMOLYSIN PROTEIN

The structure of α-hemolysin was previously determined via X-ray diffraction with a resolution of 1.9 Å at a temperature of 287K and pH=6 and was downloaded from the Protein Data Bank [2]. The protein is active as a transmembrane heptamer and contains 2051 residues (16,389 heavy atoms) organized in 7 sequence-identical chains with 27 beta-sheets and 27 helices, 180 beta- and 9- gamma turns. After correcting for the missing atoms in the residues listed in the PDB file, the structure was protonated in Amber7 [11] according to neutral pH conditions (total number of atoms 32,305) to reproduce the conditions in the polymer translocation experiments [1].

The correctness of a protein structure is usually assessed by generating a Ramachandran plot in which the phi, Φ, backbone angle is plotted versus the psi, Ψ, backbone angle in the [-180 to +180] deg range. The Ramachandran plot in Fig. 2 was generated for the α-hemolysin protein after the structure was previously minimized using the Amber7 molecular mechanics engine [11]. The blue and green regions delimit the accepted values for the phi-psi angles. The values calculated for the α-hemolysin backbone torsion angles indicate that the structure is correct.

The Protein Data Bank file also contains information on the temperature factors of each individual atom calculated from X-ray scattering. These factors characterize the amplitude of the thermal vibrations of the atoms that compose the structure. Fig.3 shows that for the α-hemolysin pore, the stem and the inside of the pore are the "dynamically active" parts which allow for more flexibility of the structure while most of the atoms that form the cap are relatively immobilized.

The α-hemolysin is a protein that self-assembles in aqueous solvent/solution. Thus, it is interesting to examine the distribution of the hydrophobic and hydrophilic residues on the protein structure, which may facilitate the assembly process. Fig.4 shows that the hydrophilic residues are positioned on the inner and outer protein walls but in alternating layers with hydrophobic residues. Hydrophobicity is also expected to influence the solvated ion dynamics because hydropylic residues form more hydrogen bonds with water increasing the local

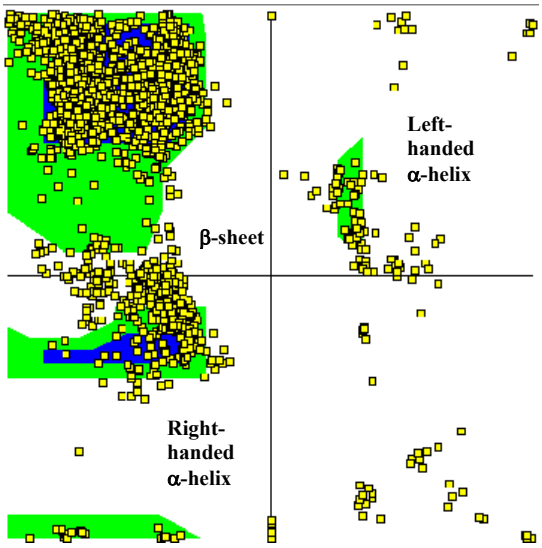

Figure 2 Ramachandran plot for the alpha hemolysin pore. The plot was rendered using VMSD (developed by the Theoretical Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign), [16].

friction coefficient.

The pdb structure of the pore is rotated such that it is parallel to the OZ axis and centered in the origin of space. The diameter at each position along the central axis (OZ) was calculated (Fig. 6). The largest diameter in the pore cavity is 42Å while at the constriction point (cap-stem joint) the diameter is reduced to 12Å, values consistent with [15]. To perform these calculations, we make use of the symmetry of the pore (the 7 chains have identical amino acids sequence). The geometric center is calculated for equivalent atom locations along the 7 chains. For equivalent atoms the radius is the average distance to the geometric center. Fig. 5 shows the plot of the diameter against the corresponding position along the pore axis. From this data the inner volume of the pore is approximated to be 56,000Å^3.

The residues located at a distance smaller than

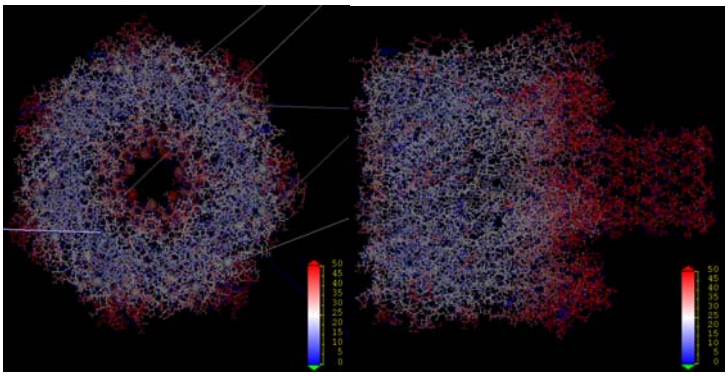

Figure 3 Temperature factors for the α-hemolysin pore. Red indicates thermally active atoms while blue colored atoms indicate reduced thermal movement. Plot generated in InsightII (Accelrys Inc.).

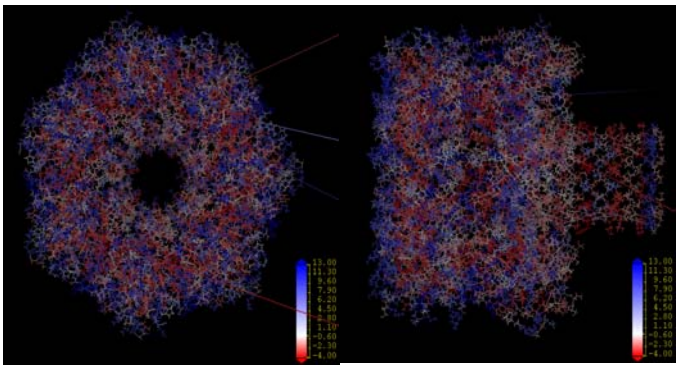

Figure 4 Aminoacids hydrophobicity on the α-hemolysin protein according to the Engleman-Steitz scale. Calculation performed in InsightII (Accelrys Inc.).

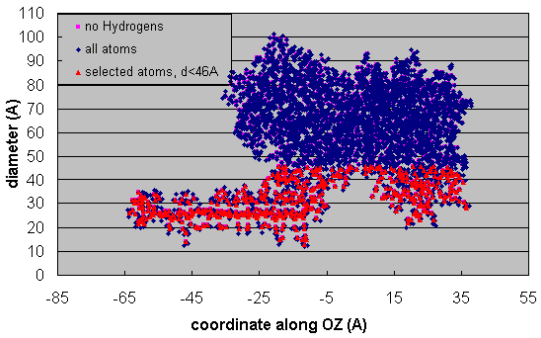

Figure 5 Internal diameter of the pore versus position on the pore central axis (OZ).

46 Å are selected to generate a reduced representation (~1/3) of the protein (Fig. 6). This figure also shows the location of the acidic and basic residues. At neutral pH, the charged residues are the N- and C- terminal ALA and ASN, ARG and LYS (positive unit charge) and ASP and GLU (negative unit charge). These charged residues are located mostly in the cap, at the constriction point and at the end of the stem (Fig. 7). Because the protein bears a total positive charge, it was neutralized (in Amber7) with Cl^- ions by calculating the Coulombic potential on a grid (1Å resolution) assuming a distance dependent dielectric in the electrostatic calculations [11]. Charges were assigned to the atoms from the parm94 set in the Cornell et al. force field [17].

The main goal in the MD calculations is to use explicit water molecules and ions. When the whole protein is included in the calculations, the total number of atoms in the unit cell (when using periodic boundary conditions) is in the order of 200,000. For such a system, 1ns of dynamics for the water molecules alone (the atoms of the protein being fixed) would require about 25,000 hours of computer time. To reach 1ns of dynamics for the reduced system (with ~100,000 atoms in the unit cell) the computer time is reduced to 10,000 hours. These results are obtained using NAMD [12], an efficient scalable open source molecular simulation package. NAMD was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign. The number of CPU's used in these calculations was 100-128. As the NAMD code scales well with large number of processors [12], the computer time required to reach 1ns on the dynamics time scale could be further reduced by increasing the number of CPU in the calculations.

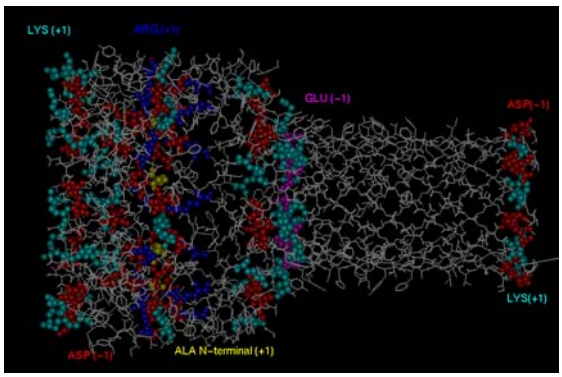

Figure 6 Reduced representation of the α-hemolysin pore. The location of the positive and negative charged residues is indicated.

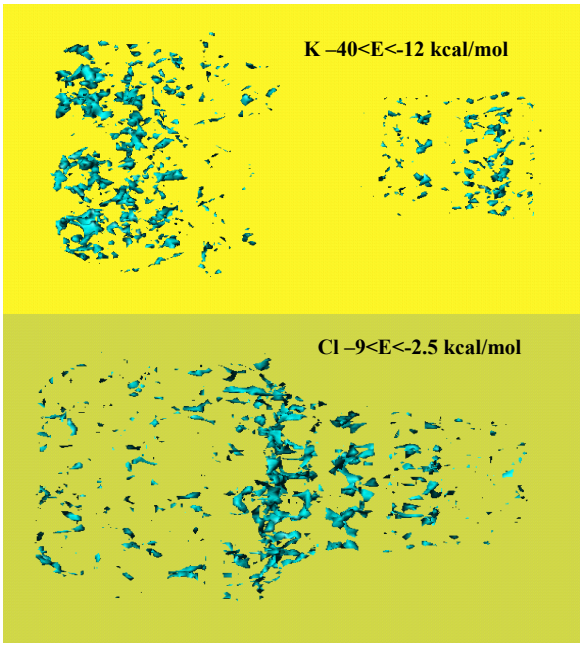

Figure 7 Binding sites for K and Cl ions inside the alpha hemolysin channel.

III. ION TRANSPORT

One of the main outcomes of the translocation experiments is the fact that the passage of various nucleic acids homopolymers generates different ionic current inhibitions. Connoly volume calculations [10] were performed in Cerius2 (Accelerys.Inc) to determine whether the magnitude of the ion current is reduced as a result of the reduction of the volume of the pore available for ion transport due to the presence of the polynucleotide. The internal volume of both the pore and the pore-stem were calculated to be 65,000$Å^3$ and 29,000$Å^3$, respectively. Cartesian coordinate models for double helical nucleic acid homopolymers with lengths comparable to the length of the pore stem (~50 Å) were generated (ABDNA or ARNA conformations) using NAB [18]. One of the two strands was then removed. Reference [14] suggests that the conformation of the polymers in the pore is linear rather than helical. Consequently, a linear configuration was also enforced for polyA and polyU using NAMD. The individual values of the volumes of the homopolymers show the following hierarchy: G>A>C>U>dG>dA>dT>dC. The fact that polyU has the smallest volume among all the ribonucleotides, could offer an explanation for the fastest translocation velocity observed in the experiments.

Given the assumption that the stem has the dominant role in the current blockade events, the pore-stem unoccupied volumes, (V_{stem}-$V_{polymer}$), were calculated by subtracting the polymer volumes from the stem volume. Table 1 lists the number of water molecules and ions that could be accommodated in the

unoccupied volumes as a function of polymer type. To calculate these numbers, it was assumed that the water molecule volume is ~30Å^3 and that a 1MKCl solution contains one K and one Cl ion for approximately every 55 water molecules. There are 18 ions of each type accommodated in the pore stem (double than estimated in [14]). The number of ions in the pore stem is reduced to 15 and 17 when homopolymers in helical respectively extended configuration are present. This indicates that the ionic current inhibition is not dominated by the amount of unoccupied volume in the pore.

Table 1 Unoccupied volume, ΔV, in the pore stem in the presence of various nucleic acid homopolymers and number of accommodated water molecules and ions. Binding energies, E_b, for K ions on the polymers are also listed.

System	$\Delta V=V_{stem}-V_{polymer}$ (Å3)	E_b (kcal/mol)	$N(H_2O)/N(K/Cl)$
Poly[dC]	25421	-51.4	847/15
Poly[dT]	25227	-52.3	840/15
Poly[dA]	25000	-47.8	833/15
Poly[dG]	24911	-59.4	830/15
Poly[U]	24388	-79.6	813/15
-extended-	27481		916/17
Poly[C]	24299	-64.0	810/15
Poly[A]	23912	-61.2	797/15
-extended-	27445		915/17
Poly[G]	23700	-74.4	790/14
Stem	29000	-39.4	967/18

However, the presence of a polynucleotide modifies the energy landscape that the ions experience when crossing the pore. Energetically favorable binding sites for the ions inside the α-hemolysin channel and on various nucleic acid homopolymers were calculated with the GRID (Molecular Discovery Inc.) software. Inside the channel, the *cis* and *trans* ends offer favorable binding sites for the positive K ions (Fig. 8) with the best binding energy of –40 kcal/mol. The locations for the best binding sites for the negative Cl ions inside the channel are at the constriction with a less negative energy (–9 kcal/mol). These sites may be correlated with the location of the positive and negative charged residues in Fig. 6. For comparison, the best binding energy for water in the channel is –16 kcal/mol. The binding energy of a Cl to a negatively charged nucleic acid polymer would be unfavorable (positive). However, for K ions some binding energies (Table 1) are comparable to the energy required to hydrated the ion (-77 kcal/mol for K+ and –87 kcal/mol for Cl^- [9]). Based on these values, the presence of the polyanions in the channel will lower the binding energies for the potassium ions and increase those for the chloride ions. For example, for the α-hemolysin-poly[dA] system, the binding energy of K is lowered to –72 kcal/mol while the binding energy for Cl is increased to –1.3 kcal/mol. For hydrated ions the above-calculated binding energies may be less negative. The calculated binding sites certainly act as a delay mechanism for the ion transport through the pore. The mobility of ions is also reduced by local friction with the water molecules, polymer nucleotides and the pore walls.

MD simulations are currently undergoing to calculate diffusion and mobility coefficients for K and Cl ions in the presence of various nucleic acid polymers and a solvated environment. These coefficients will be implemented in PNP and ERT models to explain IV profiles for the α-hemolysin pore.

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