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SIMULATION OF ION CONDUCTION IN α -HEMOLYSIN NANOPORES WITH COVALENTLY ATTACHED β -CYCLODEXTRIN BASED ON BOLTZMANN TRANSPORT MONTE CARLO MODEL

Reza Toghraee, Kyu-II Lee, David Papke, See-Wing Chiu, Eric Jakobsson, and Umberto Ravaioli

Beckman Institute for Advanced Science and Technology, University of Illinois, 405 N Mathews Avenue, Urbana, IL 61801, FAX: (217) 244-4333

Abstract

Ion channels, as nature's solution to regulating biological environments, are particularly interesting to device engineers seeking to understand how natural molecular systems realize device-like functions, such as stochastic sensing of organic analytes. What's more, attaching molecular adaptors in desired orientations inside genetically engineered ion channels, enhances the system functionality as a biosensor. In general, a hierarchy of simulation methodologies is needed to study different aspects of a biological system like ion channels. Biology Monte Carlo (BioMOCA), a three-dimensional coarse-grained particle ion channel simulator, offers a powerful and general approach to study ion channel permeation. BioMOCA is based on the Boltzmann Transport Monte Carlo (BTMC) and Particle-Particle-Particle-Mesh (P³M) methodologies developed at the University of Illinois at Urbana-Champaign. In this paper, we have employed BioMOCA to study two engineered mutations of α -HL, namely (M113F)₆(M113C-D8RL2)₁- β -CD and (M113N)₆(T117C-D8RL3)₁- β -CD. The channel conductance calculated by BioMOCA is slightly higher than experimental values. Permanent charge distributions and the geometrical shape of the channels gives rise to selectivity towards anions and also an asymmetry in *I-V* curves, promoting a rectification largely for cations.

Keywords

Ion Channel; Monte Carlo Simulation; α -Hemolysin

INTRODUCTION

Membranes are very effective barriers to the transfer of large molecules as well as smaller negatively and positively charged molecules, which are needed by the cell for various activities including growth and reproduction. Part of nature's solution to the ion transportation problem is ion channels, which are a class of membrane proteins that build a hydrophilic pathway facilitating the diffusion of ions across the membrane [1]. They are typically an assembly of multiple protein subunits closely packed to form a circular water-filled pore. There are many types of ion channels and they are usually classified based upon their ion selectivity, gating mechanism, or sequence similarity. Gating is the conformational change that opens or closes the channel, typically in response to an external stimulus [1].

From a physiological point of view, ion channels regulate the transport of ions to maintain the internal ion composition vital for cell survival and functionality. They are in charge of electrical

signaling in the nervous system, and as a result malfunctioning ion channels are associated with many diseases; thereby studying and understanding different aspects of these proteins is of great pharmaceutical importance [2].

Device-like functions of channels like gating/switching or selectivity/discriminating between different species have stimulated the engineering community to design novel bio-devices [3]. Two features distinguishing ion channels from traditional solid-state nanoscale devices, are the advantage of self-assembly and almost perfect structure duplication. Furthermore, modern biology offers the tools for channel re-engineering to alter channel functions [4-5].

Ion channels that respond directly to molecules are also found in sensory systems [6]. Stochastic sensing and DNA detection are among the very interesting potentials of ion channels. In particular, single molecule detection is an ultimate desired capability, which could revolutionize many nanoscale electronic and electromechanical applications [7]. Hemolysins are toxin proteins produced by bacteria such as *Streptococcus*. They lyse red blood cells [8]. These pore forming toxins create unregulated pores in the membranes of targeted cells as a way to obtain nutrients. α -Hemolysin (α -HL) is a β -pore forming protein and is involved in a number of human diseases [8-9]. The soluble α -HL monomers insert into the membrane and then form a heptameric transmembrane pore [8]. However, this transition from monomer to heptamer is not a trivial one. Upon this pore formation, ions and small molecules such as amino acids flow out of the cell, while water from surrounding tissue enters [9-10].

The mushroom-shaped heptamer of α -HL is about 100 Å in height and outer channel diameter, with pore diameter ranging from 15 to 46 Å [8]. The wild type (WT) channel shows weak anion selectivity [11]. Figure 1 shows the wild type α -HL composed of seven identical monomers.

Mutations of this protein channel could alter its selectivity. Along with its wide aqueous pore and capability of inserting small molecules (adaptors) in its lumen, α -HL has become an interesting protein channel for biosensor manufacturing [12-13]. Bayley, et al have used engineered version of this protein channel with molecular adaptors, such as β -cyclodextrins, inserted in its lumen for stochastic sensing of many classes of molecules [13].

Cyclodextrins comprise a family of cyclic oligosaccharides obtained from starch by enzymatic degradation [14]. Typical cyclodextrins are comprised of six to eight glucose monomers. β -cyclodextrin (β -CD) is a seven-sugar ring molecule as shown in figure 2. This water-soluble molecule can be inserted into the lumen of an ion channel like α -HL as an adaptor for sensing purposes. However, sensing with molecular adaptors could be enhanced if the adaptor could be covalently bound to the pore to prevent dissociation. Bayley, et al have successfully engineered pores to bind cyclodextrins for long periods of time [13]. They could covalently bind the α -HL mutants, (M113N) and (M113F), with β -CD in stable orientations by using a bifunctional linker. The two engineered covalent complexes are (M113F)₆(M113C-D8RL2)₁- β -CD and (M113N)₆(T117C-D8RL3)₁- β -CD.

Figure 3 shows the (M113N)₆(T117C-D8RL3)₁- β -CD complex. Note that the secondary hydroxyl face of β -CD is facing towards the *intracellular (trans)* mouth of the channel. In the other structure, (M113F)₆(M113C-D8RL2)₁- β -CD, the primary hydroxyl face of β -CD faces the *extracellular (cis)* mouth. Further details on the molecular structure of the two complexes and their manufacturing are given in reference [13].

SIMULATION ISSUES

A hierarchy of methodologies has been implemented to approach and study different systems in biology at the molecular level. The most popular simulation approach relies on Molecular Dynamics (MD), where the trajectory and evolution of the whole system is followed in full

atomic scale detail. Yet the major challenge with Molecular Dynamics is its computational cost, which still prohibits practical applications for simulation times necessary to resolve biological problems, such as current flow in ion channels [7]. In using Molecular Dynamics, current flow estimates cannot accurately be obtained since ion traversal through the channel is a rare event, and studying such macroscopic behaviors often demands simulation times lasting several micro seconds [15]. On the other hand, short-range interactions between atoms can be very strong and demand femto-second scale evolution of trajectories. Though Molecular Dynamics simulations provide essential knowledge on protein dynamics and mechanisms of ion conduction, the computational cost to study steady-state ion current through the channel is still very high, even on massively parallel supercomputers.

At the other end of the simulation hierarchy, continuum models based on Poisson-Nernst-Planck (PNP), or drift-diffusion theory, have also been implemented to study macroscopic ion currents in ion channels and nanopores [3,16-17]. Despite the relatively low computational cost of the continuum models, these paradigms deal with average densities and ignore the finite size of ions, which are of critical importance in very narrow channels, and as a result, these methods tend to overestimate the ion concentrations [18]. Coarse-grained particle approaches such as Monte Carlo (MC) models, or Brownian Dynamics (BD), help to extend the simulation times in comparison to Molecular Dynamics while retaining some atomistic level of details [17, 19-20]. In such paradigms, the ions are modeled as discrete particles with finite size, while the protein, water, and membrane are treated as continuum media with a given permittivity. Brownian Dynamics simulations in particular, have become increasingly popular to obtain macroscopic information with respect to channels conductance and selectivity [18,21-22]; nonetheless, representing ion-water interactions with a single friction coefficient and a random stochastic force may be an over-simplification in narrow regions of the channel where more complicated ion scattering mechanisms could exist [20]. The *Biology Monte Carlo* (BioMOCA), an alternative coarse-grained model for ion channels, has been developed based on Boltzmann Transport Monte Carlo (BTMC) methodology. Though a comprehensive description of BioMOCA has been furnished elsewhere [7,20], we will briefly describe the model, and address some of the advantages and drawbacks of this approach.

BioMOCA – Boltzmann Transport Monte Carlo Code

BioMOCA has been developed at the University of Illinois at Urbana-Champaign to simulate ion transport in an electrolyte environment through ion channels or nano-pores embedded in membranes [20]. It is based on two methodologies, namely the Boltzmann Transport Monte Carlo (BTMC) [23] and particle-particle-particle-mesh (P³M) [24]. The first one uses Monte Carlo method to solve the Boltzmann equation, which has been used for the past four decades to study charge transport in solid state and plasma devices. The latter, P³M methodology, splits the electrostatic forces into short-range and long-range components. To reduce computational cost, the protein, membrane, and water are treated as continuum media with given permittivities. Ions are the only particles in motion; they move according to Newtonian physics, and are damped by frequent scattering events with water molecules.

The electrostatic potential is computed at regular time intervals by solving the Poisson's equation

$$\nabla \cdot (\epsilon(r) \nabla \phi(r, t)) = -(\rho_{\text{ions}}(r, t) + \rho_{\text{perm}}(r)) \quad (1)$$

where $\rho_{\text{ions}}(r, t)$ and $\rho_{\text{perm}}(r)$ are the charge density of ions and permanent charges on the protein, respectively. $\epsilon(r)$ is the local dielectric constant or permittivity, and $\phi(r, t)$ is the local electrostatic potential. Solving this equation provides a self-consistent way to include applied

bias and the effects of image charges induced at dielectric boundaries. The ion and partial charges on protein residues are assigned to a finite rectangular grid using the cloud-in-cell (CIC) scheme [24]. Solving the Poisson equation on the grid counts for the particle-mesh component of the P³M scheme. However, this discretization leads to an unavoidable truncation of the short-range component of electrostatic force, which can be corrected by computing the short-range charge-charge Coulombic interactions.

To prevent ions from overlapping each other, the Lennard-Jones 6-12 potential has been employed to mimic the ionic core repulsion.

Dielectric coefficient

Assigning the appropriate values for dielectric permittivity of the protein, membrane, and aqueous regions is of great importance. The dielectric coefficient determines the strength of the interactions between charged particles and also the dielectric boundary forces (DBF) on ions approaching a boundary between two regions of different permittivity. However, in nano scales the task of assigning specific permittivity is problematic and not straightforward. The protein or membrane environment could respond to an external field in a number of different ways [20]. The issue of protein dielectric coefficients is addressed in detail elsewhere [25-26]. On top of that, the water molecules inside ion channels could be very ordered due to tapered size of the pore, which is often lined with highly charged residues, or hydrogen bond formation between water molecules and protein [27]. As a result, the dielectric constant of water inside an ion channel could be quite different from the value under bulk conditions. For the simulations presented here, we have adopted the same dielectric constant of 80, for all the aqueous regions to reduce the computational complications for having a gradually changing water permittivity from bulk-like baths to the channel pore.

Ion-water interactions

As a reduced particle approach, BioMOCA replaces the explicit water molecules with continuum background and handles the ion-water interactions using BTMC method. Ion trajectories are randomly interrupted by scattering events that account for the ions' diffusive motion in water [20]. In between these scattering events, ion movement is dictated by Newtonian forces. The free flight times, T_f , are generated statistically from the total scattering rate according to

$$-\ln(r) = \int_0^{T_f} \lambda(\vec{p}(t)) dt \quad (2)$$

where r is a random number uniformly distributed on the unit interval. λ , a function of momentum, is the total scattering rate for all collision mechanisms. At the end of each free flight, the ion's velocity is reselected randomly from a Maxwellian distribution. Even though BioMOCA is equipped to handle multiple scattering processes, for this work we have assumed a mechanism based on the ion's *position-dependent* diffusion coefficient in water.

Ion-protein interaction

High-resolution X-ray crystallographic measurements of complete molecular structures provide information about the type and location of all atoms that form the protein. BioMOCA uses this information in the PQR (Position-Charge-Radius) format to map the protein system onto a rectangular grid, and partition the simulation domain into continuous regions based on the Adaptive Poisson Boltzmann Solver (APBS) scheme [28].

Ions are deemed to have access to protein and lipid regions. If any point within the finite-size of ionic sphere crosses the protein or membrane boundary, a collision is assumed and the ion is reflected diffusively [20].

METHODOLOGY

The size of the α -HL channel (more than 32,000 atoms) makes running full atomic Molecular Dynamics (MD) simulations extremely expensive, especially since simulation of ion permeations requires relatively long trajectories on the timescale of hundreds of nanoseconds. Consequently, coarse-grained approaches to study ion permeation and selectivity through this channel are very appealing.

BioMOCA simulations

A total of FOUR structures were studied: the two mutants, (M113F)₆(M113C-D8RL2)₁- β -CD and (M113N)₆(T117C-D8RL3)₁- β -CD, with and without the β -CD attachment. To compare computations properly with the experimental results in [13], the pH was set to 8.0 for computation of the protonation states. Default protein partial charges were taken from CHARMM PARAM22 force fields [29], but modified as indicated by pKa calculations. The Gaussian package [30] was used to compute the partial charges of β -CD and the linking part, including the cysteine residue in α -HL. The net charge on each of the mutants is +7 *e*. The three distinct regions – lipid, protein, and electrolyte (baths and channel pore) – were assigned dielectric coefficients of $\epsilon = 5, 2$, and 80, respectively. A mesh box of $80 \times 80 \times 100$ and spacing of $\Delta = 1.5 \text{ \AA}$ was generated. The channels were embedded in a virtual membrane of $30\text{-\AA}$ thickness. The channel was positioned along the *z*-axis with the center of the membrane at *z* = 0. To generate and prepare the structures, BioMOCA-Suite - the GUI web version of BioMOCA on nanoHUB - was used [31]. Appendix A gives a short description of this suite.

Figure 4 shows the cross sections of the channel generated by BioMOCA and visualized by VMD [32].

The two buffer regions at either end of the simulation box, where a constant ion density is maintained, extend inwards from the Dirichlet planes for a distance of $25 \text{ \AA}$. The high ion-water scattering rates (total scattering rate in bulk with bulk diffusivity for K^+ and Cl^- ions are $\lambda_{\text{K}^+} \cong 3.23 \times 10^{13} \text{ s}^{-1}$, $\lambda_{\text{Cl}^-} \cong 3.46 \times 10^{13} \text{ s}^{-1}$) necessitate a short-range ion trajectory integration time-step (i.e. $\Delta t = 10 \text{ fs}$). Having said that, because the ion motion is highly damped, it generally takes on the order of 0.1-1 ps for the ion mean square displacement to approach the mesh spacing, $\Delta = 1.5 \text{ \AA}$ [7]. This would mean that the charge density assigned to the mesh nodes changes relatively slowly with regard to the ion integration time-step, which gives the green light to relax the frequency of successive solutions of the Poisson equation. Benchmark calculations on ion-ion pair correlation functions for the salt concentrations and scattering rates of interest imply that the Poisson equation can be resolved as infrequently as every *few* picosecond (i.e. 1~10 picosecond, depending on system configurations) before any significant distortion in ion radial distribution function is evident [20]. Knowing that the recurrent solution of the Poisson equation on the mesh to update the electrostatic potential is accountable for most of the computational cost, for the simulations presented in this work, the Poisson solver runs every 100 time-steps (every 1 ps). Yet it should be mentioned that although the Poisson solver is being called so infrequently, and has been implemented based on pre-conditioned Conjugate Gradient (pCG) methodology [33], it still consumes up to almost 90% of the CPU load.

At the present time, the way electrostatic potential is being updated is under further study and is being re-engineered based on Multi-Grid [34] concepts to reduce computational cost.

Position-dependent scattering rate

It is widely accepted that the ions and water molecules do not have the same mobility or diffusivity in confined regions as in bulk [35-36]. In fact, it is more likely to have a lessening in the effective mobility of ions in ion channels [37]. In reduced particle methods where the channel water is assumed as implicit continuum background, a mean ion mobility is needed to reveal how ions could diffuse due to local electrostatic forces and random events. Though approximate methods have been suggested to estimate the diffusivity of ions based on the diameter and hydrophobicity of cylindrical pores [38-39], full atomic MD simulations are required to investigate the ion mobility in different parts of the channel due to the complexity and unique geometry of each protein channel. In Transport Monte Carlo simulations, the total scattering rate (λ), is assumed to only result from ion-water interactions; it is related to ion diffusivity with the expression

$$\lambda = \frac{kT}{mD} \quad (3)$$

where m is the mass of the ion and D is its diffusion constant. As the equation indicates, reduced diffusivity of ions inside the lumen of the channel renders to increased incidence of scattering events.

Bearing in mind the relatively wide pore size in α -HL, we initially considered bulk diffusivity everywhere in the baths and channel, which as expected, led to higher flow of ions than experiments predicted [13]. The I - V curves were improved by defining a simple, yet space dependent, scattering model as shown in figure 5. This approximate diffusivity was estimated from [17], which had been used to perform Brownian Dynamics simulations on wild type (WT) α -HL.

Figure 5 (lower) shows the relative scattering rate along the z -axis for either of the ion species, K^+ , or Cl^- . The relative scattering rate (i.e. bulk value) is *one*, on either the *intracellular* or *extracellular* side, outside the channel. However, it becomes larger than the bulk value in the confined region inside the channel, due to lower effective mobility for ions. Though the space-dependent scattering rate values could be fed to BioMOCA (for different ion species) with full 3D differentiation, for the case study here, we have considered only a 1D differentiation along the z -axis. This assumption should not affect the channel conductance behavior substantially, as we expect the diffusion coefficient within the channel to be the major rate-limiting factor defining the conductance.

RESULTS

The following sections describe electrostatic and transport properties of different α -HL- β -CD mutant conformations. The current-voltage curves for symmetric and asymmetric concentrations have been produced to study selectivity, rectification, ion density, and also to make a comparison with the available experimental results. An analysis of the effect of different approximations and sources of errors is also provided.

Selectivity and Rectification

Figure 6 shows the I - V curves for symmetric 200 mM KCl . These I - V curves have separated the current for each of the ion species - the red curves are chloride ions while the black ones are the potassium ions. Additionally, the solid lines represent the mutants without the β -CD ring attached, while the dashed lines represent α -HL with the β -CD adaptor attached. Though the wild type α -HL is known to have only slight anion selectivity, the experiments performed

on the mutants exhibited strong selectivity towards anions. The selectivity becomes even greater once the adaptor is introduced into the channel [13].

In addition to reducing the current magnitude and enhancing the selectivity, the β -CD also increases the degree of rectification of the channel. Both K^+ and Cl^- currents are with pronounced current rectification larger at a given positive potential than the corresponding negative potential. Previous simulations on the wild type α -HL have also shown a similar asymmetry [17]. However, the rectification for the mutants studied here are stronger than the wild type - barely any cations enter the cell at negative potentials. Figures 7 and 8 show the one-dimensional ion concentrations. These density profiles were constructed from the time-averaged three-dimensional maps by integrating the density in two-dimensional planes parallel to the membrane, and normalized to the total ion-accessible volume in that plane. It is also noticeable that there is a peak of anion concentration in the pore near the extracellular, coupled with a decreased cation concentration. This effect is in the stem domain of the channel, at about 20 Å from the center of membrane toward the *extracellular* bath. A 2-D snapshot of the average potential and concentration densities is also shown in figure 9.

Figures 7 and 8 show two sharp and deep valleys, which correspond to the region immediately lateral to the membrane surfaces on each side. The reason this valley occurs owes to the fact that the 1-D ion concentration profiles are generated by integrating the ion density in 2-D planes parallel to the membrane normalized to the total ion-accessible volume in that plane; the center of mass of ions may not come very close to the membrane surface due to its finite size. This effect is more pronounced for chloride ions as they have a larger radius than potassium ions. Thus the valleys are not really a representation of the physics, but rather an artifact of plotting the continuum concentration on a finer grid than the size of the ions.

Residues E111 and K147 form the tightest channel passage right after the β -CD ring on *extracellular* side. E111 (Glutamic acid) has a negative charge of $-1e$ and K147 (Lysine) has a positive charge of $+1e$. Figure 10 shows how they are coordinated with respect to the β -CD ring.

The rise in anion concentration at about 20 Å from the center of the membrane to the *extracellular* side is shown in figure 11 for the M113F mutant case. A similar phenomenon is seen with the M113N mutant (with and without β -CD adapter).

It has been suggested that E111 and K147 are essential to the ion selectivity but not for the rectification on ion current [17]. The rise in anion concentration in the stem domain could surely facilitate the availability of these ions at the entrance of channel on the *extracellular* side, while the lack of cations at this entrance could be related to selectivity and also the strong rectification of cations. The same reference also indicates that the presence of D128 near the intracellular end is the molecular determinant of the asymmetric channel conductance in α -HL.

Though it is understandable that the β -CD sugar ring would block a portion of ion flux, the stronger selectivity in favor of anions might be explained by the partial charge distribution on the β -CD ring. Figure 12 shows that the partial charges on the inner side of the β -CD ring are slightly positive, which along with the narrow passage for ions through the ring, increases channel affinity for ions with negative charges.

To replicate and compare current-voltage curves with experiments [13], four sets of simulations on the two mutants, with and without the β -CD ring adaptor, were performed. Figure 13 compares the IV-curves for two sets of *KCL* concentrations (1.0/0.2M and 0.2/1.0M). Overall, the simulation results have higher currents than experiments, which are due to multiple sources of error.

Sources of Error

Among different sources of error and approximations, three issues need to be addressed here.

First are the intrinsic limitations of the methodology used in the development of this tool. Even though this method allows us to reach timescales of 100ns or more, approximations such as static modeling of lipid, protein, and water, ignores the dynamics of their molecular interactions. Moreover, the charge distribution of the protein and β -cyclodextrin are non-changing in these simulations, while in reality they could change and deform when ions are in close vicinity. These limitations are furnished in more detail elsewhere [20,40].

Another source of error is the approximate diffusion constants used for ions in baths. In the narrow parts of the pore, the diffusivity or mobility of ions is further affected by hydration and dehydration of ions, which is not included in the present model.

The same permittivity of $\epsilon=80$ has been considered for water in the baths and inside the channel. In fact, water molecules have less orientational mobility inside the protein channel and should have lower permittivity as a result. A gradual change in the permittivity of water from bulk to lower values inside the channel increases the dielectric boundary force (DBF) barrier for ions, while reducing the shield on charged particles and making the charge-charge interactions even stronger.

Figure 14 shows a sample BioMOCA ion trajectory produced by VMD. BioMOCA provides the ability to record all individual ion trajectories in a standard format. In this figure, a chloride ion is about to cross the β -CD ring in the channel.

Conclusion

While Monte Carlo based approaches have their own limitations, they allow simulations of channels on biologically related time scales thus enabling one to study current flow and ion selectivity of ion channels. Availability of such faster simulation approaches is valuable to making comparisons and predictions between different mutants before performing expensive experiments.

The results obtained here from BioMOCA are consistent with available experimental results. In general a hierarchy of different approaches, like Monte Carlo and Molecular Dynamics, is needed to study different aspects of biological systems like ion channels.

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Appendix A – NCN Nano-Devices for Medicine and Biology: BioMOCA Suite

nanoHUB was created by the NSF funded Network for Computational Nanotechnology (NCN). It is a web-based resource for researchers and students to conduct research and collaborate in nanotechnology. The resources are being used by thousands of users from all around the world.¹

We have wrapped BioMOCA in a GUI, and it is available at nanoHUB.org as the BioMOCA Suite. The BioMOCA Suite can perform ion channel flow simulations on any user-supplied channel. The suite includes: a map generator subtool, which produces protein maps for

¹www.nanohub.org

BioMOCA from the supplied PQR file; a lipid wrapper subtool, which allows the user to embed their channel in a membrane; and the boundary force potential calculator, which determines the potential energy barrier presented by the channel. The user can also download the acc and charge files produced by the map generator and lipid wrapper.

Finally, the suite contains the biology Monte Carlo simulator, which simulates ion channel flow through the user provided channel. The user has the ability to change a number of parameters, including the transmembrane voltage, intra- and extra-cellular concentrations of Na^+ , Cl^- , K^+ , Ca^{2+} , and Mg^{2+} , and the run time.

The tool is divided into four subtools; the first two subtools are designed to setup the simulations performed by the second two.

The first subtool is the map generator. Here, the user uploads a PQR file (which they can previously generate from a PDB file at pdb2pqr.sourceforge.net) and determines its mesh dimensions for simulations. The spacing between grid points in x, y, and z directions is determined by dx, dy, and dz respectively, and the number of grid points in each dimension is determined by Nx, Ny, and Nz. The user has the option of only looking at a few x and z cross-sections by choosing visualization mode 1 (to save on time), or of looking at all x and z cross-sections by choosing mode 2. An example of one of an x-axis cross-section is presented in figure 15.

The second subtool is the lipid wrapper, pictured in figure 16. Here, the user determines the appropriate placement of the intra- and extra-cellular edges of the membrane around the pore, as well as the lipid radius. The visual output is the same as the output from the map generator. Additionally included in the output of this section are the material map and charge map files; in the future, we hope to give the user the ability to skip these first two sections by uploading saved changes and material maps.

The third subtool is the boundary force calculator. This program determines the potential energy barrier encountered by a particle traversing the center of the pore. The output of this program is a graph of the potential energy vs. axial particle location as shown in see figure 17.

The final subtool is the biology Monte Carlo (BioMOCA) simulator. In this subtool, the user specifies intra- and extra-cellular concentrations of Na^+ , Cl^- , K^+ , Ca^{2+} , and Mg^{2+} , as well as the transmembrane potential and the simulation time (Figure 18). The program then simulates ion movement in these conditions, treating water as a background continuum. The output from the BioMOCA simulator is a file containing all pertinent information about individual and net ion currents, as well as the number of crossings of each ion species in each direction. In the future, this program will also output a VMD movie file, concentration maps, and electric potential distributions.

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Figure 1.

Wild type α -Hemolysin, a heptamer toxin protein, is shown here. The protein inserts into the membranes of cells to form a transmembrane pore, resulting in cell lysis.

Figure 2.

The β CD is a seven-sugar ring molecule. This donut shaped ring has two faces (a); the upper one, which is tighter, is the primary hydroxyl face; the lower wider one is the secondary hydroxyl face. The partial charges on this molecule increase the selectivity of nanopore towards anions like chloride ions.

Figure 3.

The β -cyclodextrin sugar ring is attached covalently to the mutant (M113N)₆(T117C-D8RL3)₁. Note that the secondary hydroxyl face of β CD is facing towards the *intracellular* mouth of the channel.

Figure 4. VMD visualization of channel cross-sections: (a) in the y - z plane, (b) in the x - y plane. VMD has added the feature of reading BioMOCA structure files.

Figure 5.

The relative scattering rate inside the channel is more than that in bulk. These values were roughly estimated from [17]. The upper figure is a snapshot of the channel structure, while the lower one shows the relative scattering rate along the z -axis. These values correspond to the wild type α HL and a better estimation of scattering rate, or correspondingly the diffusivity, is needed for the mutant proteins we studied here. Note that the dimension along the z -axis is in Angstroms, with the *origin* located at the center of lipid

Figure 6.

Current-voltage curves of both mutants, with and without β CD bound for the two ion species (i.e. K^+ and Cl^-). A 200mM salt concentration was assumed on both *extracellular* and *intracellular* sides. Almost no K^+ ions have passed the channel from left (*intracellular*) to the right (*extracellular*). β CD ring insertion lowers the current, as expected.

Figure 7.

Average 1-D ion concentrations of ions in both mutants with and without the β CD adaptor, in symmetric 200mM KCl baths under zero biasing potentials. The ion concentration profiles were constructed from the time-averaged 3-D density maps by integrating the ion density in 2-D planes parallel to the membrane, normalized to the total ion-accessible volume in that plane.

Figure 8.

Average 1-D concentrations of ions in both mutants, with the β CD sugar ring adaptor, in symmetrical 200mM KCl baths, under different biasing potentials. The ion concentration profiles were constructed from the time-averaged 3-D density maps by integrating the ion density in 2-D planes parallel to the membrane, normalized to the total ion-accessible volume in that plane.

Figure 9.

2-D maps of average electrostatic potential density, and $\log_{10}$ of average cation and anion densities, along the z-axis of both mutants, with the β CD adaptor, in symmetric 200mM KCl baths under different biasing potentials.

Figure 10.

Coordination of residues E111 and K47 with respect to β -CD ring (in the mutant M113N) is shown. The residues are colored based on their residue type. Red represents negative charge, and blue represents positive charge. Moreover, in VMD, green represents hydrophilic or polar, while white is associated with hydrophobic.

Figure 11.

Average ionic concentration ($\log_{10}$) for the M113F mutant with the β CD bonding, in symmetric 200mM KCl and zero biasing, for both cations (a) and anions (b) are visualized along with the protein and β -CD ring. Residues E111, K147 are colored according to their residue type. Note the rise in anion concentration along with cation diminution is shown in the stem domain

Figure 12.

The potential map in the lumen of β CD is shown. The inner side of the sugar ring has an overall positive charge density, which accounts for further selectivity towards anions like Cl^- .

Figure 13.

Current-voltage curves of both mutants, with and without the βCD adaptor, and comparison of currents with experimental results [1].

Figure 14.

A snapshot of the channel is presented here. A chloride ion is about to cross the β CD ring. Visualization was performed using VMD software [8].

Figure 15.
A cross-section of the gramicidin channel is shown.

Figure 16.
The gramicidin channel has now been wrapped in lipid.

Figure 17.

The potential energy barrier encountered by a charged particle dragged through the gramicidin channel is shown.

Figure 18.
Parameter choices and simulation options available to the user are shown here.