NANOSCALE TRANSPORT SIMULATION OF ION CHANNELS

BY

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DISSERTATION

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ABSTRACT

Ion channels are part of nature’s solution for regulating biological environments. Every ion channel consists of a chain of amino acids carrying a strong and sharply varying permanent charge, folded in such a way that it creates a nanoscopic aqueous pore spanning the otherwise mostly impermeable membranes of biological cells. These naturally occurring proteins are particularly interesting to device engineers seeking to understand how such nanoscale systems realize device-like functions. This work is a study of such biological ion channels in terms of ion permeation and selectivity. The approach taken is based on transport Monte Carlo method. We have used BioMOCA, a three-dimensional coarse-grained simulator developed at the University of Illinois at Urbana-Champaign. In our simulations the water molecules in the system are considered as continuum background, where they interact with the ions through scattering events. The rate of scattering events is inversely related to the diffusivity of ions in the water, which itself is a function of ion position. Incorporating such position dependent diffusion coefficients for different ion species has been studied and the simulation results are compared with the experiments. Furthermore, the permittivity of water in the channels or nanopores has been studied. As it turns out, not only is the permittivity of water different in such confined regions; it is also an anisotropic parameter and can drastically change based on pore diameter. In particular we have studied the $\alpha$-hemolysin channel with covalently attached molecular adapter $\beta$-cyclodextrin. Although the wild type of this ion channel is a toxic protein in nature, its engineered mutations with molecular adapters could be key components of fabricated nanoscale biosensors.
To Father and Mother
ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

Part of nature’s solution to the problem of ion transport across cell membrane is ion channels: nanoscale water-filled pathways, built of membrane proteins, that facilitate the diffusion of ions across the impermeable lipid membrane [1]. The intriguing aspect of ion channels is that evolution has generated an endless variety of different channels with different sensing and actuating capabilities, which we have only begun to catalog and understand. Ion channels 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; therefore understanding different aspects of these proteins is of great pharmaceutical importance [2].

To a device specialist, ion channels are of extreme interest because they realize on the nanoscale single-agent detection functions like gating/switching or selectivity/discriminating between different species, and they have stimulated the engineering community to design novel bio-devices [3]. Two distinctive features of ion channels, in contrast to 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].

A comprehensive hierarchy of methodologies and device simulation approaches has been implemented and tested to approach and study different systems in biology at the molecular level. On one hand, there has been a keen interest in adapting the approaches and methods realized to study electronic charge transport in semiconductor materials and devices to describe the behavior of ion channels from a device perspective. On the other hand, a variety of physical approaches have been implemented by the biophysics community in order to simulate and explain the behavior of such systems [6, 7, 8].
The most popular simulation approach relies on molecular dynamics (MD), where the trajectories and evolution of the whole system contained in the simulation domain – ions, protein and lipid atoms, and water molecules – are followed in great detail. The major challenge with molecular dynamics is its high computational cost, which prohibits the simulation times necessary to resolve practical biological problems like current flow in ion channels [7]. In using molecular dynamics, accurate current flow estimates cannot be obtained since ion traversal through the channel is a rare event, and studying such macroscopic behaviors often demands simulation times lasting several hundred nanoseconds or even several microseconds [6]. 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 about 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.

**ION CHANNELS AS NANODEVICES**

It is useful to first compare the similarities and differences between ion channels as soft matter nanodevices and semiconductor devices as solid state ones from a charge transport point of view. The main issues are listed in Table 1.1.

Table 1.1. Comparison of charge transport in solid-state devices and electrolyte systems.

<table>
<thead>
<tr>
<th>Ion channels</th>
<th>Solid state devices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water solution</td>
<td>Crystal band structure</td>
</tr>
<tr>
<td>Carriers are ions with relatively large mass and finite volume</td>
<td>Carriers are quasi-particles with a small effective mass</td>
</tr>
<tr>
<td>Ions are scattered by interacting with water molecules</td>
<td>Carriers are scattered by phonons</td>
</tr>
<tr>
<td>Channel structures (either wild type or stable mutants) are always perfect replicas</td>
<td>Ultra nanoscale structures are troubled with fluctuations of doping and size</td>
</tr>
</tbody>
</table>
While ions as the charge carriers in an electrolyte environment have specific mass and volume, and interact strongly with polar water molecules, the charge carriers in a semiconductor system are quite different in nature. The quasi-particles in solid state environments (electrons and holes) move as if in a continuum or vacuum with an effective mass derived from the band structure. Ions are thermalized or scattered mainly by water molecules, whereas in semiconductors, the vibrations of atoms away from their ideal crystal structure, modeled in terms of phonon modes, interact with mobile charges through different scattering mechanisms. Furthermore, the effective mass and phonons together determine the mobility of quasi-particles.

While a semiconductor system can withstand relatively high temperatures, it is becoming increasingly difficult for designers to scale integrated circuits further because of the increased thermal dissipation, which eventually might even risk melting the chip. Another problem in scaling devices to the nanoscale is caused by fluctuations in geometry and doping which are becoming very difficult to control [7]. In contrast, wet biological systems seem to process information very efficiently, temperature can be readily controlled and the molecular structure of the components is replicated and self-assembled almost perfectly. This is indeed astounding, given the complexity and variety of molecular structures of proteins that form the channel pores. What is more, with the help of modern biology, ion channel proteins could be mutated, altering channel selectivity, gating, or other functionalities [4-5].

**COMPUTATIONAL ISSUES**

While the inter-atomic collisions happen in the time scale of about $10^{-15}$ second, ion channel functions follow much slower biological time scales on the order a few microseconds. In the relatively small number of channels for which the molecular structure is well known, the protein strands can have anywhere from a few hundred atoms as in gramicidin (Figure 1.1), an early antibiotic, to several thousand atoms as in $\alpha$-hemolysin ($\alpha$HL), a toxic protein (Figure 1.2). The electronic charge distribution on the protein can vary considerably with different factors in the surrounding electrolyte, like pH or salt concentration. Some protein residues could undergo protonation or de-protonation, adding or losing a proton ($H^+$) respectively. At the same time, the
pore is not a rigid structure and it may undergo significant conformational changes and fluctuations over time.

**Figure 1.1.** Gramicidin channel. It has 512 atoms and is comprised of two identical segments.

**Figure 1.2.** α-Hemolysin channel, comprised of seven identical monomers.
Simulation of ion channels poses a multiscale problem in time, so it is desirable to have available a hierarchy of simulation approaches of decreasing complexity to probe the relevant time scales of interest. Figure 1.3 shows a diagram of the simulation hierarchy of different methods and how each one could provide part of the input parameters fed to other techniques.

Figure 1.3. A hierarchy of different approaches, starting from the very ab initio quantum chemistry methods to the very high-level compact models. Arrows show how the approaches provide input parameters for each other.
Quantum Chemistry
Based on quantum mechanics and quantum field theory, quantum chemistry describes the electronic behavior of atoms and molecules, light absorption, transfer of energy, as well as electrons and protons. Such quantum calculations could also provide force-field parameters such as charge distribution, bond angles, energy parameters, and atom radii used in classical physics methods like molecular dynamics simulations. Due to the very high computational cost of such calculations, quantum chemistry can be used to study and examine only a very small number of atoms (i.e. a single amino acid, 10-20 atoms) and has to ignore their dynamics [9].

Molecular Dynamics
Based on Newtonian physics, molecular dynamics follows the dynamics of every single atom, at the highest level of detail, in the system [6, 8, 10]. However, the resulting complexity makes the computational cost very high. The overwhelming burden in MD simulations arises from the need to compute trajectories for all the atoms in the system.

Although it is possible to evaluate the charge distribution residing on the protein with quantum chemistry techniques, these can only be performed as stand-alone calculations – a coupling to a dynamic simulation is well beyond what is manageable today. Force-field parameters are derived either from quantum chemistry and/or experimental data. Some molecular dynamics packages come with their own force-fields, e.g., CHARMM [11-13], AMBER [14, 15], GROMOS [16], while other force-fields are available as stand-alone parameter sets, e.g., OPLS [17, 18] and PARSE [19].

Nonetheless, since ion channel systems typically comprise hundreds of thousands of particles, MD simulations are currently limited to time scales of the order of 100 ns even on massively parallel machines. This may be sufficient to evaluate certain thermodynamic and transport parameters, but cannot resolve measurable current flow.

Coarse-Grained Simulations
Coarse-grained or reduced particle methods are designed to reduce the computational cost by making approximations to the description of the membrane and protein, as well as to the
transport model of the electrolyte. In such methods the dynamics of the protein and lipid are ignored and instead a static configuration is chosen. The protein structure could be taken from the crystallographic measurements, though MD simulations are often used to relax the crystal structure to a more plausible conformation for specific physiological conditions. To resolve even longer time scales, the protein and lipid structures could be modeled as continuum regions delimited by hard wall boundaries, and characterized electrically by an average permittivity and static charge distribution.

For the simulation of the electrolyte, the main computational expense arises from tracking the individual water molecules. The first major approximation involves replacing thousands of water molecules with a continuum background, also electrically characterized by an average permittivity, while a suitable scattering model is introduced to account for the interaction between mobile ions and water [7, 20].

Rather than computing the Coulomb field directly as is done in MD simulations, the forces are evaluated in space by solving the Poisson equation on a grid covering the simulation domain, where one maps the permittivities and the local distribution of fixed and mobile charges. The use of the Poisson equation is very appealing to describe ion channels as device systems, because it is relatively easy to formulate boundary conditions on the electrostatic potential that represent the actual application of bias voltages across the membranes that are used in electrophysiology experiments. Also, image charges induced at dielectric interfaces are implicitly accounted for by the model. Furthermore, to capture the charge-charge interactions at close proximity, particle-particle schemes may be applied locally in addition to the particle-mesh scheme of the Poisson equation [21].

Brownian dynamics simulations in particular, have become increasingly popular to obtain macroscopic information with respect to channel conductance and selectivity [22, 23, 24]; 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. Since a comprehensive description of BioMOCA has been furnished elsewhere [7, 20], we will describe the model very briefly here, and address some of the advantages and drawbacks of this approach.

**Continuum Simulations**
At the other end of the simulation hierarchy, continuum models based on Poisson-Nernst-Plank (PNP), or drift-diffusion theory, have also been implemented to study macroscopic ion currents in ion channels and nanopores [3, 25, 26]. The assumption of a charged fluid implies that the finite size and discrete nature of the charge of the ions are neglected. For nanoscale pores, this may lead to charges entering very narrow regions, where actual ions would be excluded, and at certain locations the model may yield ion densities that are higher than would be physically possible when the finite volume occupied by each ion is considered. A possible approach for the classical ion fluid is to add a correction potential that considers the finite ion size (in terms of geometry and charge distribution) to prevent unphysical bunching of charges in restricted spaces, particularly if a strong fixed attractive field is present as in pores with highly charged protein walls [27, 28].

**BioMOCA**
Monte Carlo simulations use a range of stochastic methods that use pseudo-random numbers to generate a particular statistical distribution. They have been used to study charge transport in solid state and plasma devices for about four decades now. In an ion channel system, the collection of ions in the electrolyte baths and channel lumen represents an ensemble of particles that, for typical physiological salt concentrations, interact mostly with the water molecules. Such interactions could be modeled as scattering events that interrupt ion free flight. Such features make the ion channel system an interesting application of Monte Carlo techniques. Chapter 2 will elaborate on the BioMOCA simulator.
CHAPTER 2

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 nanopores embedded in membranes [20]. It is a 3-D particle-based Monte Carlo simulator for analyzing and studying the ion transport problem in ion channel systems or similar nanopores in wet/biological environments. The system simulated consists of a protein forming an ion channel (or an artificial nanopore like a carbon nanotube), with a membrane (i.e. lipid bilayer) that separates two ion baths on either side. BioMOCA is based on two methodologies, namely the Boltzmann Transport Monte Carlo (BTMC) [29] and particle-particle-particle-mesh (P³M) [21]. The first one uses Monte Carlo method to solve the Boltzmann equation, while the later splits the electrostatic forces into short-range and long-range components.

In full-atomic molecular dynamics simulations of ion channels, most of the computational cost owes to following the trajectory of water molecules in the system. However, in BioMOCA the water is treated as a continuum dielectric background medium. In addition, the protein atoms of the ion channel are also modeled as static point charges embedded in a finite volume with a given dielectric coefficient. So is the lipid membrane, which is treated as a static dielectric region inaccessible to ions. In fact the only non-static particles in the system are ions. Their motion is assumed classical; interactions with other ions occur through electrostatic interactions and pairwise Lennard-Jones potential. They also interact with the water background medium, which is modeled using a scattering mechanism.

The ensemble of ions in the simulation region are propagated synchronously in time and 3-D space by integrating the equations of motion using the second-order accurate leap-frog scheme. Ion positions $r$ and forces $F$ are defined at time steps $t$, and $t + dt$. The ion velocities are defined at $t – dt/2$, $t + dt/2$. The governing finite difference equations of motion are
\[ \vec{v}(t + \frac{dt}{2}) = \vec{v}(t - \frac{dt}{2}) + \vec{F}(t)dt \] (2.1)

\[ \vec{r}(t + dt) = \vec{r}(t - dt) + \vec{v}(t + \frac{dt}{2})dt \] (2.2)

where \( F \) is the sum of electrostatic and pairwise ion-ion interaction forces.

**Electrostatic Field Solution**

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

\[ \nabla \cdot (\varepsilon(r) \nabla \phi(r,t)) = - \left( \rho_{\text{ions}}(r,t) + \rho_{\text{perm}}(r) \right) \] (2.3)

where \( \rho_{\text{ions}}(r,t) \) and \( \rho_{\text{perm}}(r) \) are the charge density of ions and permanent charges on the protein, respectively. \( \varepsilon(r) \) 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 [21]. Solving the Poisson equation on the grid accounts for the particle-mesh component of the \( P^3M \) 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 Columbic interactions.

**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, at nanoscales 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, 30, 31, 32]. Field induced dipoles, reorientation of permanent dipoles, protonation and deprotonation of protein residues, larger scale reorganization of ionized side-chains and water molecules, both within the interior and on the surface of the protein, are all examples of how
complicated the assignment of permittivity is. In MD simulations where all the charges, dipoles, and field induced atomic dipoles are treated explicitly, it is suggested that a dielectric value of one is appropriate. However, in reduced-particle ion simulation programs, such as ours, where the protein, membrane, and water are continuum backgrounds and treated implicitly, and furthermore the ion motion takes place on the same time scale as the protein’s response to its presence, it is very difficult to assign the dielectric coefficients. In fact, changing the dielectric coefficients could easily alter the channel characteristics, such as ion permeation and selectivity.

The assignment of dielectric coefficient for water is another key issue. The water molecules inside ion channels could be very ordered due to the tapered shape of the pore, which is often lined with highly charged residues, or due to hydrogen bond formation between water molecules and protein [8]. As a result, the dielectric constant of water inside an ion channel could be quite different from the value under bulk conditions. To make the matter even more complicated, the dielectric coefficient of water inside nanopores is not necessarily an isotropic scalar value, but could be an anisotropic tensor having different values in different directions.

**Anisotropic Permittivity**

It has become evident that the macroscopic properties of a system do not necessarily extend to the molecular length scales. In a recent research study carried out by R. Jay Mashl and Eric Jakobsson at the University of Illinois at Urbana-Champaign [33], they used molecular dynamics simulations to study the properties of water in featureless hydrophobic cylinders with diameters ranging from 1 to 12 nm. This study showed that water undergoes distinct transitions in structure, dielectric properties, and mobility as the tube diameter is varied. In particular they found that the dielectric properties in the range of 1 to 10 nm are quite different from those of bulk water and are in fact anisotropic in nature.

Though such featureless hydrophobic channels do not represent actual ion channels and more research has to be done in this area before one can use such data for ion channels, it is evident that water properties like permittivity inside an ion channel or nanopore could be much more complicated than previously thought. While a high axial dielectric constant shields ion electrostatic charges in the axial direction (along the channel), low radial dielectric constant
increases the interaction between mobile ions and the partial charges, or the dielectric charge images on the channel, conveying stronger selectivity in ion channels. Solving the Poisson equation based on an anisotropic permittivity has been incorporated into BioMOCA using the box integration discretization method [34], which is briefly described below.

**Box Integration Discretization**

In order to use box integration for discretizing a $D$-dimensional Poisson equation

\[ \nabla \cdot (\varepsilon \nabla \phi) = \rho \quad (2.4) \]

with $\varepsilon$ being a diagonal $D \times D$ tensor, we have to reformulate this differential equation as an integral equation. Integrating (2.4) over a $D$-dimensional region $\Omega$, and using Gauss’ theorem, we then get the integral formulation

\[ \oint_{\partial \Omega} n \cdot (\varepsilon \nabla \phi) = -\int_{\Omega} \rho \quad (2.5) \]

Here we assume a two-dimensional case. Upgrading to a three-dimensional system would be straightforward and legitimate as the Gauss theorem is also valid for one and three dimensions. We assume that $\varepsilon$ is given in the rectangular regions between nodes, while $\phi$ is defined on the grid nodes (as illustrated in Figure 2.1).

![Figure 2.1](image)

**Figure 2.1.** Box integration for a two-dimensional tensor product grid. The integration region is indicated by the *dashed rectangle*. Charges are assumed to be given on the same nodes as potential.
The integration regions $\Omega$ are then chosen as rectangles centered around nodes and extending to the four nearest neighbor nodes. We then approximate the gradient $\nabla \varphi$ using centered difference normal to the boundary of the integration region $\Omega$, and average $\varepsilon$ over the integration surface $\partial \Omega$. This approach allows us to approximate the left-hand side of the Poisson equation (2.5) in first order as

$$
\oint_{\partial \Omega} \hat{n} \cdot (\varepsilon \nabla \varphi) = \frac{q_{i+1,j} - q_{i,j}}{h^x_i} \left( \frac{h^y_j}{2} \varepsilon_{i,j} + \frac{h^y_{j-1}}{2} \varepsilon_{i,j-1} \right) - \frac{q_{i,j} - q_{i-1,j}}{h^x_{i-1}} \left( \frac{h^y_j}{2} \varepsilon_{i-1,j} + \frac{h^y_{j-1}}{2} \varepsilon_{i-1,j-1} \right) + \frac{q_{i,j+1} - q_{i,j}}{h^y_j} \left( \frac{h^x_i}{2} \varepsilon_{i,j} + \frac{h^x_{i-1}}{2} \varepsilon_{i-1,j} \right) - \frac{q_{i,j} - q_{i,j-1}}{h^y_{j-1}} \left( \frac{h^x_i}{2} \varepsilon_{i,j-1} + \frac{h^x_{i-1}}{2} \varepsilon_{i-1,j-1} \right)
$$

(2.6)

where $\varepsilon^x$ and $\varepsilon^y$ are the two components of the diagonal of the tensor $\varepsilon$.

Discretizing the right-hand side of Equation (2.5) is fairly simple. We discretize $\rho$ on the same grid nodes as we did for $\varphi$.

$$
\oint_{\Omega} \rho = Volume(\Omega_i) \cdot \rho_i
$$

(2.7)

**Ion Size**

The finite size of ions is accounted for in BioMOCA using pairwise repulsive forces derived from the 6-12 Lennard-Jones potential. A truncated-shifted form of the Lennard-Jones potential is used in the simulator to mimic ionic core repulsion. The modified form of the Lennard-Jones pairwise potential that retains only the repulsive component is given by

$$
U_{LJ}(r_{ij}) = \begin{cases} 
4\varepsilon_{LJ} \left( \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^6 \right) + \varepsilon_{LJ} & r_{ij} < 2^{1/6} \sigma_{ij} \\
0 & r_{ij} > 2^{1/6} \sigma_{ij}
\end{cases}
$$

(2.8)

Here, $\varepsilon_{LJ}$ is the Lennard-Jones energy parameter and $\sigma_{ij} = (\sigma_i + \sigma_j) / 2$ is the average of the individual Lennard-Jones distance parameters for particles. Using a truncated form of the
potential is computationally efficient and prevents the ions from overlapping or coalescing, something that would be clearly unphysical.

**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. In BioMOCA the protein atoms are modeled as static point charges embedded in a finite volume inaccessible to the ions and associated with a user-defined dielectric coefficient. Moreover, a number of force-field parameters are available that provide information about the charge and radii of atoms in different amino-acid groups. The conjunction of the molecular structure and force fields provides the coordinates, radii, and charge of each atom in the protein channel. BioMOCA uses such information in the standard PQR (position-charge-radius) format to map the protein system onto a rectangular grid.

Ideally, the steric interactions between protein atoms and the ions in the aqueous medium are to use a repulsive potential like Lennard-Jones to prevent ions from penetrating the protein. As this approach could add significantly to the number of calculations, a simpler approach is chosen that treats the protein surfaces as predetermined hard wall boundaries. Many recent open source molecular biology packages have built-in facilities that determine the volume accessible to ions in a protein system. The Adaptive Poisson Boltzmann Solver (APBS) scheme [35] has been incorporated to BioMOCA to obtain the accessible volume region and thereby partition the simulation domain into continuous regions.

Ions are deemed to have access to protein and lipid regions, and if any point within the ionic sphere crosses the protein or membrane boundary, a collision is assumed and the ion is reflected diffusively.

**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, in which appropriate scattering rates should be chosen. In other words, ion trajectories are randomly interrupted by
scattering events that account for the ions’ diffusive motion in water [20]. In between these scattering events, ions follow the Newtonian forces. The free flight times, $T_f$, are generated statistically from the total scattering rate according to

$$-\ln(r) = \int_0^{T_f} \lambda(\hat{p}(t)) dt$$  \hspace{1cm} (2.9)

where $r$ is a random number uniformly distributed on the unit interval. The term $\lambda$, 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. As the correct scattering mechanism for ion-water interactions in nonbulk electrolyte solutions has yet to be developed, a position dependent scattering rate linked to the local diffusivity is used in our model. This dependency on position comes from the fact that water molecules can have different order of organization in different regions, which will affect the scattering rate.

**Position Dependent Diffusivity**

It is widely accepted that the ions and water molecules do not have the same mobility or diffusivity in confined regions as in bulk [29, 31]. In fact, it is more likely to have a lessening in the effective mobility of ions in ion channels [30]. In reduced particle methods where the channel water is assumed to be continuum background, a mean ion mobility is needed to reveal how ions could diffuse due to local electrostatic forces and random events. In transport Monte Carlo simulations, the total scattering rate ($\lambda$) is assumed to result only from ion-water interactions; it is related to ion diffusivity by the expression

$$\lambda = \frac{kT}{mD}$$ \hspace{1cm} (2.10)

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 leads to increased incidence of scattering events.

As it turns out, using bulk diffusivity values in all regions, namely the two baths and the channel, often leads to higher flow of ions than experiments predict. Even though determination of diffusivity at each specific region of the channel is as yet a very complicated task and needs MD simulations that would gather enough ion trajectory statistics, some simpler theoretical models based on the geometry of the channel are presented in literature that, as discussed in later
chapters, could lead us to current flows that are closer to experimental results. As one of the newest features of BioMOCA, we have created a program named HOLE, which is available as part of the BioMOCA repository, to determine and assign a position dependent diffusivity (or scattering rate) based on the one-dimensional wall-drag model [16, 17].

**HOLE Program**

BioMOCA generates two maps in rectangular grid form, from the input PQR protein structure. One map is called the access map, and the other is the charge map. The access map determines what areas are accessible to the ions: baths, channel, protein and membrane. The charge map saves the equivalent charge at each grid point, extracted from the permanent point charges on the protein residues. HOLE takes the access map information and uses it to render scattering maps that provide the scattering rates for each ion species at every section of the channel and baths. It initially scans the whole system and determines the lumen of the channel, and then assigns an equivalent radius to every section of the channel. It uses the one-dimensional wall-drag model to estimate the diffusion coefficients, which are dependent only on the geometric restriction in the channel.

The ratio of the diffusion coefficient inside the channel with respect to that of bulk is called the scaling factor \( k_z \) and is expressed as

\[
k_z(\beta) = \frac{1 - 2.1050\beta + 2.0865\beta^3 - 1.7068\beta^5 + 0.72603\beta^6}{1 - 0.75857\beta^3}
\]  

(2.11)

where the \( z \) direction is along the channel lumen, and \( \beta \) (function of \( z \)) denotes the ratio of the ion species, and the pore radius. The fractions in this equation come from the best fit to the tabulated values in [36]. Derivation of Equation (2.11) assumes (a) uncharged hard sphere mobile ions, (b) uncharged cylindrical hard pore (channel), (c) continuum solvent background (water), (d) one-dimensional transport of ions along the pore center axis (\( z \)-axis). The bulk diffusion coefficient itself is experimentally determined in extremely dilute conditions.

The bulk diffusion coefficient \( D^{bulk} \) is experimentally determined in extremely dilute conditions. In later chapters, we will see how such a simplified model could help us with better estimations of ionic flow.
**Hydration Shells**

In addition to having a diffusive effect on ion transport, water molecules also form hydration shells around individual ions due to their polar nature. The hydration shell not only shields the charge on ions from other ions but also modulates the ion radial distribution function, causing the formation of peaks and troughs. The average minimum distance between two ions is increased as there is always at least one layer of water molecules present between them, acting as a physical deterrent preventing two ions from getting too close to each other, in a manner that is similar to the short-range repulsive component of the Lennard-Jones potential.

The theory of hydration shells is well developed in the physical chemistry literature; however, we seek a simple model that captures the essential effects with as little computational overhead as possible. For this purpose we have implemented the same pairwise potential discussed by Im and Roux [37] to include the effect of hydration shells.

$$U_{ry} = c_0 \exp\left(\frac{c_1 - r}{c_2}\right) \cos\left(c_3 (c_1 - r) \pi\right) + c_4 \left(\frac{c_1}{r}\right)^6 \quad (2.12)$$

The coefficients $c_i$ were determined empirically for a 1 M KCl solution, using MD simulations to benchmark the ion radial distribution functions against equilibrium Monte Carlo simulations. The effect of hydration shells was found to be important in simulations at higher salt concentrations where the conductance of many ion channels, porin among them, is observed to saturate as the salt concentration in the electrolyte baths is further increased. Earlier simulations that did not include a model of hydration shells did not reproduce the conductance saturation behavior. This suggests an additional repulsive potential acting to prevent ion crowding, and hence limiting the concentration of ions and current density in the confined space of the pore even at high bath salt concentration. When the repulsive potential in Equation (2.12) was included, moderate channel conductance was observed.

**Boundary Conditions**

The electrical and physiological properties of ion channels are experimentally measured by inserting the channel into a lipid membrane separating two baths containing solutions of specific concentrations. A constant electrostatic bias is applied across the channel by immersing the
electrodes in the two baths. Formulating boundary conditions that accurately represent these contact regions may require enormously large bath regions and is a challenging task. In our approach, we assume that, beyond a Debye length from the membrane, the electrostatic potential and ion densities do not vary appreciably. This assumption has been supported by the continuum results presented earlier [38]. For typical salt concentrations used in ion channel simulations, the Debye length is of the order of 10 Å. Using the assumption, we impose Dirichlet boundary conditions on the potential at the two domain boundary planes that are transverse to the channel, taking care that these planes are sufficiently far from the membrane.

The other problem in duplicating the experimental conditions is the problem of maintaining fixed charge density in the two baths. We treat this problem by maintaining the specified density in two buffer regions extending from the boundary plane toward the membrane. The number of ions needed to maintain the density in the two buffer regions is calculated at the start of the simulations. The count of the ions in these buffers is sampled throughout the simulation and an ion is injected whenever a deficit is observed. The initial velocity of the injected particle is decided according to Maxwellian distribution. It should be noted that the ions can leave the system only by exiting through the two Dirichlet boundary planes and we do not remove an ion artificially from these buffer regions. The reflections from the Neumann boundary planes are treated as elastic reflections.

Multi-Grids and Grid Focusing Method
In almost all of the methods for simulating ion channels, the major computational cost comes from the calculation of electrostatic forces acting on the ions. In continuum models, for instance, where we have ionic density rather than explicit ions, the electrostatic potential is calculated in a self-consistent manner by solving the Poisson equation. In MD simulations, on the other hand, the electrostatic forces acting on the particles are calculated by explicit evaluation of the Coulombic force term, often splitting the short-range and long-range electrostatic forces so they can be computed with different methods. In our model as a reduced particle method, the long-range electrostatic forces are evaluated by solving the Poisson equation and augmenting the forces so obtained with a short-range component. By solving the Poisson equation it is possible
to self-consistently include the forces arising from the bias to the system, while this is a difficult issue to address in MD simulations.

Currently there are two Poisson solvers implemented in BioMOCA based on the finite difference method. One uses the pre-conditioned conjugate gradient scheme (pCG) [39] and is used by default. The later is borrowed from an APBS solver, which uses a V-multi-grid scheme. Other than the numerical approach to solve the Poisson equation, the main difference between the two solvers is how they address the permittivity in the system. In the first solver, a dielectric value is assigned to each cell in the grid, while in the APBS solver the dielectric coefficients are defined on the grid nodes. As discussed earlier we use box integration in the pCG solver, which allows us to treat the Poisson equation in the most accurate way. Even though a full multi-grid solver based on box integration has been in development, there is an effective way to reuse the already exiting code and treat the ion channel systems.

Ion channel simulations require the presence of large bath regions for accurate treatment of screening [20]. Such bath regions make the mesh domain of the Poisson equation large and lead to either a large number of grid points with fine mesh resolution or a small number of grid points with very coarse discretization. From bulk simulations we have learned that a coarse mesh is sufficient for describing the baths using the P³M scheme. However, a fine resolution is required in the channel domain because of the highly charged nature of these regions and the presence of spatially varying dielectric regions. Besides we are ultimately interested in studying the channel behavior in terms of ion permeability, selectivity, gating, density, etc. In other words, we are better off putting more computational resources in the channel region and a bare minimum in the baths to reduce the overall computational cost and speed up our simulations from weeks to perhaps days instead.

A scheme based on the grid focusing method has been developed that makes it possible to satisfy simultaneously the requirements of large bath region and fine grid resolution in the channel in a computationally effective way. This methodology also allows us to have multiple fine mesh domains, which may be needed to describe multiple pore channels like OmpF porin, or an array of ion channels sharing the same bath regions or even having yet finer meshes inside a fine mesh for relatively large channels with narrow ion passages, like a nicotine receptor channel.
The first grid is a coarse mesh spanning the entire problem domain including the bath regions and the channel region. The second grid is a much finer mesh that spans a sub-domain of the system containing the region that requires fine resolution like the channel pore. Any further grids are successively finer. The Poisson equation is first solved on the coarse mesh with all the Dirichlet and Neumann boundary conditions, taking into account the applied bias. Next the boundary conditions for the secondary meshes are obtained by interpolating from the first or previous solutions of the Poisson equation. The Poisson equation is solved again for the finer meshes using the new boundary conditions. In this way, we are able to generate electrostatic fields with different mesh discretizations for different regions.

**EMF and DBF**

The electro-motive force (EMF) is the energy needed for a charged particle like an ion to cross the ion channel embedded in a membrane. Part of this potential energy barrier is due the interaction between the crossing ion and the permanent/partial charges on the protein residues. The other part comes from the induced dipoles in the protein/membrane dielectric medium, and is referred to as the dielectric-boundary force (DBF). To compute the DBF alone, one may turn off all the static charges on the protein residues and drag the ion through the pore and compute the energy barrier using

$$P_{DBF} = \int -d\mathbf{z} \cdot \mathbf{E}$$

(2.13)

It is important to note that EMF or DBF measurements are just qualitative measurements, as an ion does not necessarily cross the channel through the center of its lumen in a straight line and it is often accompanied by other ions moving in the same or opposite directions, which dramatically changes the dynamics of the system. Moreover, unlike steered MD calculations where the protein residues dynamically reposition themselves as an ion or ions are bouncing across the channel, in our EMF or DBF calculations we model protein as a static continuum, which further affects the energy calculations in a more quantitative way. Another issue that additionally impacts the measurements is absence of water hydration molecules, which move with the ion and shield part of its charge. Having said all of the above, computing EMF or DBF is still valuable to address channel selectivity or gating. Computing either of these two energy barriers is available as an option in BioMOCA.
Visualization using VMD

In a collaborative work with VMD developers [40] in the Theoretical and Computational Biophysics Group at the Beckman Institute for Advanced Science and Technology at UIUC, VMD was equipped with the option of loading BioMOCA structures. This is a very useful feature as one could load both the protein structure (i.e. PDB or PQR file) along with the structures generated by BioMOCA to make comparisons. Figure 2.2 shows how BioMOCA has generated a structure for a gramicidin channel with a membrane wrapped around it. Furthermore, BioMOCA also dumps the ion trajectories in standard formats so they can be later loaded to molecular visualization tools such as VMD and watched frame by frame in a movie format.

![VMD visualization of Gramicidin 1MAG molecule along with the structure generated by BioMOCA, where green represents protein, red is the membrane (i.e. lipid), and purple is the channel and left and right baths.](image)

**Figure 2.2.** VMD visualization of Gramicidin 1MAG molecule along with the structure generated by BioMOCA, where green represents protein, red is the membrane (i.e. lipid), and purple is the channel and left and right baths.

Recording Trajectories in Binary

In addition to counting the number of ions crossing the channel, sometimes it is desirable to study their behavior at different regions of the channel. Such examples would be the average occupancy of ions or their average moving velocity inside the channel or a nanopore. BioMOCA has been equipped with the option of dumping every ion’s position, average and instantaneous velocities, potential and kinetic energies, average and instantaneous displacements, and other information at every step (or few steps) of the simulations in ASCII format, so such trajectory
information can be studied later on to gather further statistics. From a technical point of view, however, dumping such information for tens of ions, even at every few hundreds of time steps, could slow down the simulations and result in huge files accumulating to tens of gigabytes. Loading such files later on from disk storage is also a very time-consuming and computationally inefficient procedure. Over and above that, recoding the numerical information in ASCII format does not hold its machine precision and incurs loss of accuracy.

Solving such problems is actually an easy task and it is simple to avoid using ASCII format and use binary format instead. This not only preserves the machine accuracy but also speeds writing and reading to the file system. The computational overhead to dump the trajectories becomes negligible and the trajectory files become about two orders of magnitude smaller. The downside might be that programming and decoding the data could become very tricky, but once it is done correctly and with care, the advantages of using binary format are well worth the extra effort. BioMOCA is now equipped with the tools to record the trajectory information in binary format.

**BioMOCA and NanoHUB (BioMOCA Suite)**

NanoHUB [41] 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 on nanotechnology. The resources are being used by thousands of users from all around the world.

We have wrapped BioMOCA in a GUI, and it is available at nanoHUB.org as the BioMOCA Suite [42]. 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 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 set up 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 the 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 an x-axis cross section is presented in Figure 2.3.

Figure 2.3. Map generator showing a gramicidin channel and a cross section of the continuum structure generated by BioMOCA.
The second subtool is the lipid wrapper, pictured in Figure 2.4. 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.

Figure 2.4. Lipid wrapper showing the bilayer lipid wrapped around the channel.

The third subtool is the boundary force simulator. 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 Figure 2.5.
Figure 2.5. Boundary force simulator showing the potential energy barrier encountered by a charged particle dragged through the gramicidin channel.

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 2.6). 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.
Figure 2.6. Parameter choices and simulation options available to the user.
Hemolysins are toxic proteins produced by bacteria such as *Streptococcus*. They lyse red blood cells [43]. These pore-forming toxins create unregulated pores in the membrane of targeted cells, as a way to obtain nutrients. \(\alpha\)-Hemolysin (\(\alpha\)HL) is a \(\beta\)-pore-forming protein and is involved in a number of human diseases [43, 44]. The soluble \(\alpha\)HL monomers insert into the membrane and then form a heptameric transmembrane pore [43]. However, this transition from monomers 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 [44, 45].

The mushroom-shaped heptamer of \(\alpha\)HL displays weak anion selectivity and is about 100 Å in diameter and height, and the pore diameter ranges from 15 to 46 Å [43]. The wild type (WT) channel shows weak anion selectivity [46]. 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, \(\alpha\)HL has become an interesting protein channel for biosensor manufacturing [47, 48]. Bayley et al. have used an engineered version of this protein channel for stochastic sensing of many classes of molecules [47]. Figure 3.1 shows the wild type \(\alpha\)HL composed of seven identical monomers.
Figure 3.1. Wild type α-hemolysin, a heptamer toxin protein. The protein assembles on the membranes of cells, i.e. blood cells, to form a transmembrane pore, resulting in cell lysis.

Cyclodextrins are a family of cyclic oligosaccharides obtained from starch by enzymatic degradation [49]. Typical cyclodextrins comprise six to eight glucose monomers. β-cyclodextrin (βCD) is a seven-sugar ring molecule as shown in Figure 3.2.
The βCD is a seven-sugar ring molecule. This donut shaped ring has two faces: The upper one, which is wider, is the secondary hydroxyl face, while the lower, tighter one is the first hydroxyl face. The partial charges on this molecule increase the selectivity of the nanopore towards anions like chloride ions.

This water-soluble molecule could be put inside the lumen of an ion channel like αHL as an adaptor for sensing purposes. This sensing with molecular adaptors would be enhanced if the adaptor could be bounded inside the pore and would not dissociate from the pore. Bayley et al. have successfully engineered pores to bind cyclodextrins for long periods of time [47]. They could covalently bind the αHL mutants, (M113N) and (M113F), with βCD in stable orientations by using a bifunctional linker. The two engineered covalent complexes are (M113F)$_6$(M113C-D8RL2)$_1$-βCD and (M113N)$_6$(T117C-D8RL3)$_1$-βCD.

Figure 3.3 shows the (M113N)$_6$(T117C-D8RL3)$_1$-βCD complex. Note that the secondary hydroxyl face of βCD is facing towards the tran mouth of the channel. In the other structure, (M113N)$_6$(T117C-D8RL3)$_1$-βCD, the primary hydroxyl face of βCD faces the cis mouth. Further details on the molecular structure of the two complexes and their manufacturing are given at [47].
Figure 3.3. The β-cyclodextrin sugar ring is attached covalently to the mutant (M113N)₆(T117C-D8RL3). Note that the secondary hydroxyl face of βCD is facing towards the *tran* mouth of the channel.

The size of the αHL channel (more than 30,000 atoms) makes the computational cost of full atomic molecular dynamics (MD) simulations extremely expensive, especially because simulation of ion permeations requires relatively very long trajectories on the time scale of hundreds of nanoseconds to microseconds.

A Gaussian package was used to compute the partial charges of βCD, linking part, and the connected cysteine in αHL. Figure 3.4 shows the βCD with its linking part.
Monte Carlo and Brownian dynamics simulations, with implicit solvent, are very attractive for modeling of permeation and selectivity of ions over long time scales. The solvent is represented as a continuum with a given dielectric constant.

**METHODODOLOGY**

**Atomic Model and BioMOCA Simulations**

A total of five structures were studied: the wild type (Protein Data Bank entry: 7ahl) and two mutants, (M113F)_6(M113C-D8RL2)_1-βCD and (M113N)_6(T117C-D8RL3)_1-βCD, each with and without the βCD attachment. To reproduce the experimental results in [48], the protonation was set to 8.0, on the basis of pKa calculations. The protein partial charges were taken from CHARMM PARAM22 force fields [11, 12, 13]. The three distinct regions – lipid, protein, and electrolyte (baths and channel pore) – were assigned dielectric coefficients of $\varepsilon = 5, 2, \text{ and } 80$, respectively. A mesh box of $80 \times 80 \times 100$ and spacing of $\Delta = 1.5$ Å was generated. The channels were embedded in a lipid membrane of 30 Å thickness. Monte Carlo times steps were set to 10 femtoseconds, and the Poisson equation was solved every 100 time steps.

To generate and prepare the structures, the GUI web version of BioMOCA on nanoHUB was used [42]. Figure 3.5 shows the cross sections of the channel generated by BioMOCA and visualized by VMD [40].
Figure 3.5. VMD visualization of the channel crosses sections: (a) in the \( y-z \) plane, (b) in the \( x-y \) plane.
Position-Dependent Scattering

In BioMOCA the water molecules affect the ion trajectories by randomly interrupting the ions. This interruption or scattering event is based on Boltzmann Transport Monte Carlo (BTMC) method. Ions follow Newtonian forces in between scatterings. The free flight times are generated statistically from the total scattering rate according to

$$-\ln(r) = \int_0^{t_f} \lambda(\tilde{p}(t))dt$$

(3.1)

where $r$ is a random number uniformly distributed on the unit interval. The term $\lambda$, a function of momentum, is the total scattering rate for all collision mechanisms, where here we only have ion-water scattering events. Ions do not have the same mobility or diffusivity in all regions. The scattering rate due to water molecule collisions is related to ion diffusivity by the expression

$$\lambda = \frac{kT}{mD}$$

(3.2)

where $m$ is the mass of the ion and $D$ is its diffusion constant. Reduced diffusivity of ions inside the lumen of the channel is reflected with more scattering events happening.

In preliminary simulations, the bulk diffusivity was used uniformly everywhere in the baths and channel; as expected, this led to greater flow of ions than experiments predicted [46]. An approximation approach to estimate diffusivity has been proposed based on the diameter of cylindrical pores [50, 51] and used before to approximate the diffusivity of ions in the lumen of the $\alpha$HL channel [52].

The ionic permeability was improved by defining a simple yet space-dependent scattering model as shown in Figure 3.6.
Figure 3.6. The approximate relative scattering rate increases from the bulk value. These approximate values were taken from [46]. 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 $\alpha$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 angstrom, where zero is located at the center of the lipid. Moreover, the left side is the $trans$ side, and the right one is the $cis$ side.
RESULTS

The following sections describe electrostatic and transport properties of different αHL-βCD conformations. An analysis of the effect of different approximations and an assessment of sources of error are also provided.

Selectivity and Rectification

Overall both mutants show relatively strong selectivity toward anions, Cl\(^-\). Moreover, as shown in Figure 3.7, the channels tend to rectify, specifically the cations, in the cis to trans direction. Almost no cations have been transported from the trans side to cis. In these simulations a 200 mM concentration of KCl was assumed on either side.

Figures 3.8 and 3.9 show the one-dimensional ion concentrations. These density profiles were constructed from the time-average 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. Cations do enter the channel from the trans bath and spend more time in the lumen of channel, yet they almost never make it to the cis side. It is also noticeable that there is a peak of anion concentration at the entrance of the channel lumen on the cis side.
Figure 3.7. Current-voltage curves of both mutants, with and without βCD bonding for the two ion species (i.e. K⁺ and Cl⁻). A 200 mM salt concentration was assumed on both cis and trans sides. Almost no K⁺ ions have passed the channel from left (trans) to right (cis). The βCD ring blocks part of the current.
Figure 3.8. Average 1-D ion concentrations of both mutants with and without the βCD bonding, in symmetric 200 mM 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 3.9. Average 1-D concentrations of both mutants, with the βCD sugar ring bonding, in symmetrical 200 mM 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.
Though it is reasonable that the βCD sugar ring would block some of the current, the even stronger selectivity for cations is partly due the partial charge distribution on the βCD ring. Figure 3.10 shows that the partial charges on the inner side of the βCD ring are slightly positive, which, along with the narrow passage for ions through the ring, increases the selectivity for ions with negative charges.

Figure 3.10. The charge density distribution of βCD. The inner side of the sugar ring has an overall positive charge density, which accounts for further selectivity towards anions like Cl⁻.

Figure 3.11 shows the two-dimensional average densities of electrostatic potential and ion concentrations.
Figure 3.11. 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 βCD bonding, in symmetric 200 mM KCl baths under different biasing potentials.

To replicate and compare current-voltage curves with experiments [50], four sets of simulations on the mutants with and without the βCD ring attachment were performed. Figure 3.12 compares the IV curves for two sets of KCl concentrations (1.0/0.2 M and 0.2/1.0 M). Overall the simulations results have higher currents than experiments, which are due to multiple sources of error.
Figure 3.12. Current-voltage curves of both mutants, with and without βCD bonding, and comparison of currents with experimental results [48].
Figure 3.13 shows a sample BioMOCA trajectory of ions through the channel. Dumping the trajectories in a standard format is a feature of BioMOCA. In this figure, a chloride ion is about to cross the βCD ring in the channel.

**Figure 3.13.** Snapshot of the channel. A chloride ion is about to cross the βCD ring. Visualization was performed using VMD software [40].
**Sources of Error**
Among different sources of error and approximations, three issues need to be addressed here. The first is the intrinsic limitations of the methodology used in development of this tool. Even though this method allows us to reach timescales of 100 ns or more, approximations such as static modeling of lipid, protein, and water ignore the dynamics of molecular interactions. Moreover, the charge distributions of the protein and β-cyclodextrin are nonchanging in these simulations while in reality they could change and deform when ions are in close vicinity. These limitations are addressed in more detail elsewhere [7, 20].

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 $\varepsilon=80$ has been considered for the water in the baths and inside the channel when, in fact, water molecules become more oriented inside the protein channel and should therefore have lower permittivity. A gradual change in the permittivity of water from bulk to lower values inside the channel increases the boundary force barrier for ions.

Correcting the errors in ion diffusivity and water permittivity addressed here would lead to higher ion permeations, and the IV curves presented here represent an upper bound for ion permeation.

**Conclusion**
While Monte Carlo based approaches do have limitations, they can resolve biological timescales, thus enabling one to study current flow and ion selectivity of ion channels. Such faster simulation approaches are valuable for making comparisons among and predictions about 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.
CHAPTER 4

PERFORMANCE OPTIMIZATION USING MULTI-GRIDS

In this chapter we will revisit the αHL with βCD adapter with two new improvements: (1) use of focused grids to reduce simulation costs while achieving a more accurate solution of the Poisson equation in the channel region, and (2) use of the HOLE program as part of the BioMOCA repository to address the ion diffusivities in the channel lumen with more precision. Focused grid implementation not only provides higher resolution for force fields, but also, as it turns out, speeds up the overall computations almost one order of magnitude, which is outstanding.

Meanwhile, HOLE not only defines the ion diffusivity values with use of better formulation and estimations but also defines them very precisely in the channel region. The relatively huge mushroom-shaped heptamer of αHL with about 100 Å in height and outer channel diameter, and a pore diameter ranging from 15 to 46 Å [43], is an excellent case study for this matter.

In this work, we have focused our research on one of the mutants only, (M113F)_6(M113C-D8RL2)_1-βCD, and have compared our results with the available experimental data. Similar results would be expected for the other mutant, which we studied in the previous chapter. Figure 4.1 shows the αHL channel along with a snapshot of ion trajectories generated by BioMOCA and visualized by VMD [40].
Figure 4.1. α-Hemolysin, a heptamer (each monomer is colored differently), is a toxic protein. A snapshot of ion trajectories (1 M on each side) along with the ion channel itself has been visualized using VMD.

METHODOLOGY

To compare computations properly with the experimental results in [48], the pH was set to 8.0 for computation of the protonation states of the mutant, (M113F)(M113C-D8R12)-βCD. Default protein partial charges were taken from CHARMM PARAM22 force fields [11, 12, 13], but modified as indicated by pKa calculations. The Gaussian package [54] was used to compute the partial charges of βCD and the linking part, including the cysteine residue in αHL. The net
charge on this mutant is $+7 \, e$. A mesh box of $120 \times 120 \times 150$ and spacing of $\Delta = 1.0 \, \text{Å}$ was generated to represent the system, along with other coarse/fine focused grids to compute the electrostatic potential efficiently, as is addressed below. The three distinct regions – lipid, protein, and electrolyte (baths and channel pore) – were assigned dielectric coefficients of $\varepsilon = 5$, 2, and 80, respectively. The channels were embedded in a virtual membrane of 30 Å 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 [42].

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 Å.

The high ion-water scattering rates (total scattering rates in bulk with bulk diffusivity for $\text{K}^+$ and $\text{Cl}^-$ ions are $\lambda_{\text{K}^+} \approx 3.23 \times 10^{13} \, \text{s}^{-1}$, $\lambda_{\text{Cl}^-} \approx 3.46 \times 10^{13} \, \text{s}^{-1}$) necessitate a short-range ion trajectory integration time step (i.e. $\Delta t = 10 \, \text{fs}$). Yet the ion motion is so highly damped 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, which account for most of the computational cost. For the simulations presented in this work, the Poisson solver runs every 100 time steps (every 1 ps). To further accelerate the solution of the Poisson equation, a multi-grid method – the grid focusing method – has been implemented in BioMOCA. Although the system setup becomes more complicated, it proves to be quite effective at reducing the computational cost.

**Grid Focusing Method**

As discussed in earlier chapters, ion channel simulations require the presence of large bath regions for accurate treatment of screening. The presence of these bath regions makes the mesh domain of Poisson equation large and leads to either a large number of grid points with fine mesh resolution or a small number of grid points with very coarse discretization. A coarse mesh is sufficient for describing baths using the $P^3M$ scheme [7, 20, 55]. However, fine resolution is required in the channel domain because of the highly charged nature of these regions and the presence of spatially varying dielectric regions. A scheme based on the grid focusing method has
been developed in BioMOCA that makes it possible to satisfy simultaneously the requirements of large bath regions and fine grid resolution in the channel in a computationally efficient way [7, 20]. For the αHL channel here we have defined two discretized meshes. The first grid is a coarse mesh spanning the entire problem and has the dimensions of $40 \times 40 \times 50$ grid points with uniform mesh spacing of 3 Å. The second fine grid has dimensions of $30 \times 30 \times 60$ nodes and a mesh spacing of 1 Å, which is defined along the narrow selectivity section of the channel as depicted in Figure 4.2.

Figure 4.2. Setup of the focused grid scheme. The larger box (cyan) spans the entire region with the coarse mesh, while the smaller box (yellow) has focused on the narrow part of the channel with fine meshing.
The Poisson equation is first solved on the coarse grid using the finite difference method and then the boundary conditions are interpolated from the coarse grid to the fine grid along with some additional calculations to account for the short-range component of electrostatic potential [7, 20, 55]. Then we solve the Poisson equation on the fine mesh using the new boundary conditions. The computational cost of solving the Poisson equation depends superlinearly on the number of grid points. To benchmark the speedup, we set up the same system with a single mesh of 120 × 120 × 150 nodes and 1 Å mesh spacing. The benchmarking was done with a Xeon 5450 series processor. The simulations are conveyed significantly faster (one order of magnitude here) when using grid focusing method. The details of the benchmarking are presented in Table 4.1.

Table 4.1. The details on benchmarking the two case studies (single grid and multi-grid schemes) are addressed here. A reduction in the number of grids reduces the computational cost by about 15 times.

<table>
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<th>Meshes</th>
<th>Number of grid points</th>
<th>CPU-hour per 1 nanosecond simulations</th>
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<td><strong>Multi-grid scheme</strong></td>
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<td></td>
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<tr>
<td><strong>Single-grid scheme</strong></td>
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</table>

**HOLE and Position-Dependent Diffusivity**

Bearing in mind the relatively wide pore size in αHL, we initially considered bulk diffusivity everywhere in the baths and channel for the sake of comparison, which, as expected, led to higher flow of ions than experiments predicted [48]. Next we used the HOLE program, which is based on the one-dimensional wall-drag model [56, 57], where the diffusion coefficient is dependent only on the geometric restriction in the channel. As discussed in Chapter 2 along with the BioMOCA description, remember that the ratio of the diffusion coefficient inside the channel with respect to that of bulk is called the scaling factor (kₗ) and is expressed as

\[
k_\text{c}(\beta) = \frac{1 - 2.1050\beta + 2.0865\beta^3 - 1.7068\beta^5 + 0.72603\beta^6}{1 - 0.75857\beta^5}
\] (4.1)
where the $z$ direction is along the channel lumen, and $\beta$ (function of $z$) denotes the ratio of the ion species, and the pore radius. The bulk diffusion coefficient ($D_{\text{bulk}}$) is experimentally determined in extremely dilute conditions. Also, note that this equation does indeed ignore charges on the ion or channel walls. Moreover, it assumes a one-dimensional transport of ions along the pore center axis ($z$-axis). Figure 4.3 shows the scaling factor for both potassium and chloride ions along the mutant $\alpha$HL with $\beta$CD.

Figure 4.3. The relative diffusivity for each of the ion species along the $\alpha$HL channel. Zero on the $z$-axis is at the protein center of mass. Note that chloride has a smaller relative diffusion coefficient along the pore due its larger radius compared to potassium.

Figure 4.4 shows how such scaling factor has been implemented inside the channel. The beauty of using HOLE is that it automates the assignment of the diffusivity (and accordingly the scattering rate) for each ion species automatically, as is seen in Figure 4.4.
Figure 4.4. A 2-D snapshot of modified diffusivity implementation inside the channel. The color red, which covers all regions outside the channel, has a value of one, representing bulk diffusivity/mobility.

RESULTS

Two sets of simulations were carried out, one using bulk diffusivity for each ion species in all regions and one with modified diffusivity inside the pore as discussed earlier. For each bias point, five 100 ns simulations were conveyed and averaged out, which is equivalent to running one longer 500 ns simulation per bias point. Figure 4.5 shows the IV curves for symmetric 1000 mM KCl salt concentrations and compares the simulation results with the available experimental data in [48].
Figure 4.5. IV curves for the total currents: Solid black is extracted from experimental data, dashed red is the simulations using the modified diffusivity, and dashed-dot blue is simulations with bulk diffusivity in all regions including inside the channel. There is a very good agreement between the experiments and simulations with modified diffusivity of ions, while the current overshoots strongly when bulk diffusivity is used.

Using bulk diffusivity inside the channel produces huge currents (dash-dot blue line) compared to experiments (solid black line). However, good agreements with experiments are achieved using the modified diffusivity (dashed red line), which seems to be diverging from the experiments in larger negative biases.
Conclusion

As discussed in the previous chapter, even though Monte Carlo or coarsed grained methods do have limitations, they allow simulations of channels on biologically relevant time scales, which allow us to measure current flow and ion selectivity of ion channels. That is why such approaches are valuable for making comparisons among and predictions about different mutants before performing expensive experiments. The results obtained here from BioMOCA are in good consistency with available experimental results.
As discussed in Chapter 2, we know that the macroscopic properties of a system do not necessarily extend to the molecular length scales. In case of water molecule properties, the research study carried out by R. Jay Mashl and Eric Jakobsson at the University of Illinois at Urbana-Champaign [33], showed how water undergoes distinct transitions in structure, dielectric properties, and mobility in featureless nanotubes of different diameters. In particular they found that the dielectric properties in the range of 1 to 10 nm are quite different from those of bulk water and are in fact anisotropic in nature. Figure 5.1 shows the relative anisotropic permittivity of water in such effectively infinitely long nanotubes.

**Figure 5.1.** Relative axial or radial permittivities for effectively infinite hydrophobic nanotubes; effective diameter is essentially the diameter that the water molecules occupy, and is slightly smaller than the pore diameter.
Though such featureless hydrophobic channels do not represent actual ion channels and more research has to be done in this area before one can use such data for ion channels, it is evident that water properties like permittivity inside an ion channel or nanopore could be much more complicated than previously thought. While a high axial dielectric constant shields ion electrostatic charges in the axial direction (along the channel), low radial dielectric constant increases the interaction between mobile ions and the partial charges, or the dielectric charge images on the channel, conveying stronger selectivity in ion channels. The anisotropic permittivity has been incorporated into BioMOCA using the box integration method [34], which was briefly discussed in Chapter 2.

A good starting point, before one could use such values for water permittivity inside ion channels, is to use them in simple hydrophobic nanotubes and study the ion permeation.

Case Study: NANOPORES

To further realize the effect of the anisotropic permittivity values given in Figure 5.1, we have constructed four nanopores with diameters of 6.75, 7.6, 9.6, and 10.4 nm and named them Pore 1 through 4, respectively. The corresponding relative axial and radial permittivity values are listed in Table 5.1. Figure 5.2 also schematically shows the four pore selections.

**Table 5.1.** Approximate axial and radial relative permittivities for each of the four pores with their given diameters. These values were approximated from Figure 5.1.

<table>
<thead>
<tr>
<th>Pore</th>
<th>Diameter</th>
<th>Relative Axial Permittivity</th>
<th>Relative Radial Permittivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.75</td>
<td>600</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>7.6</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>9.6</td>
<td>2.9</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>10.4</td>
<td>130</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Figure 5.2. Selections of Pores 1 through 4 are highlighted.

A box of size $L_x, L_y, L_z = 20, 20, 70$ Å was chosen for all pores with 0.5 Å grid spacing in each dimension resulting in a $N_x, N_y, N_z = 40, 40, 140$ mesh. A pore of length 40 Å was then inserted along the $z$ direction at the center of the box. The pore was then wrapped in a membrane with dielectric constant of 5. We chose the value of 5, as it is often the value chosen for protein permittivity in coarse-grained or continuum models. If an ion runs into the pore wall, it will be reflected diffusively, as it has run into protein. Figure 5.3 schematically shows the system design.
Simulations were carried out with 1 M K\(^+\)Cl\(^-\) concentration on each side (baths left and right), 1 V bias, and a total simulation time of 100 ns with 10 fs Monte Carlo time steps.

![Diagram](image)

**Figure 5.3.** Illustration of the system setup for BioMOCA transport simulations.

For each pore we ran two simulations, one with isotropic relative permittivity of 80 for all the aqueous regions (baths and channel), and the other one with the given anisotropic dielectric constants inside the channels while having an isotropic permittivity of 80 for the baths. For the case of anisotropic permittivities, we have smoothed out the axial values at the border of the bulk and channel entrance so the electric field along the \(z\)-axis changes gradually, not abruptly. Figure 5.4 shows the relative axial permittivity for Pore 1.

The total crossings of both ion species are counted as they traverse the pore midpoint (across the pore at \(z = 0\) plane). The total counting for 100 ns simulation run time is shown in Table 5.2. In this table the number of crossings counts only for potassium ions from the right bath to the left one, and the opposite direction counts only for the chloride ions, as the strong 1 V bias applied almost excludes the traversal of potassium ions from left to the right and chloride ions from right to left. Figure 5.5 shows the total current in pico-amperes for the pores.
Table 5.2. Ion crossing counts over the course of 100 ns for each of the four pores, with either anisotropic or isotropic dielectric constant inside the channel.

<table>
<thead>
<tr>
<th>Pore</th>
<th>Anisotropic Pores</th>
<th>Isotropic Pores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl⁻ (left → right)</td>
<td>K⁺ (right → left)</td>
</tr>
<tr>
<td>Pore 1</td>
<td>409</td>
<td>736</td>
</tr>
<tr>
<td>Pore 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pore 3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pore 4</td>
<td>20</td>
<td>15</td>
</tr>
</tbody>
</table>

Figure 5.4. Comparison of relative axial permittivity to the bulk value of 80 in Pore 1. The axial permittivity has been changed gradually from the bulk value of 80 to the channel value of 600.
As expected, in the pores with isotropic bulk permittivity, the flow of charges increases linearly with pore diameter. However, the current flow in pores with anisotropic permittivity has a completely different behavior. Pores 2 and 3, with very low relative permittivities (both radial and axial), do not experience any crossings over the course of 100 ns. In other words, no ions happened to have enough kinetic energy to overcome the energy barrier due to the dielectric boundary forces (DBF) and enter the pores in these simulations. Relatively high axial permittivity in Pore 4 accounts for the small current observed here. Pore 1 shows an outstanding high current due its very high axial permittivity. Computing the DBF potential energy could shed light on part of this nonlinear behavior in pores with anisotropic dielectric constants.

![Total Current](image)

**Figure 5.5.** Total current for each of the four pores (marked from 1 to 4). Dashed black line is the current when we use bulk dielectric constant inside the channels, while the solid red line represents the total current with given anisotropic dielectric constants inside the channels.
Dielectric Boundary Force Measurements

To compute the DBF related potential energy, we steered an ion (i.e. $K^+$) through the center of the pore from $z = -10 \, \text{Å}$ to $z = +10 \, \text{Å}$, with 0.1 Å steps and computed the potential energy with the formula

$$P_{DBF} = \int -d\vec{z} \cdot E$$

(5.1)

The electric field ($E$) is computed at the position of the ion as we drag it through the pore. Figure 5.6 schematically shows the charge steering process.

![Figure 5.6](image)

Figure 5.6. Illustration of how an ion is being steered through the pore, in absence of an applied field (i.e. bias = 0).

The potential energy barriers of Pore 1 are shown in Figure 5.7. In this figure, the solid blue curve is the energy barrier when we have an isotropic dielectric constant of 80 in all the aqueous regions. Changing the radial component of permittivity from 80 to 600, while keeping the radial part the same as the bulk, would actually produce a potential well for charged particles, as shown by the dashed green curve. Setting the radial component of the permittivity to a very low value of 3.5 would increase the potential barrier to about $50kT$ (solid black curve). Figure 5.8 shows all the DBF potential energies for all four pores with the given anisotropic permittivities. As is seen in this figure, Pore 1 has the lowest potential barrier, while Pores 2 and 3 have the highest ones, which explains why they have no ion flux.
Figure 5.7. The DBF potential energy for Pore 1. Solid blue: Potential barrier when we use bulk relative permittivity of 80 inside the pore. Dashed green: Potential well, as a result of setting the axial relative permittivity to 600 inside the channel, while keeping 80 in either the $x$ or $y$ direction. Solid black: Potential barrier with axial relative permittivity of 600, and radial relative permittivity of 3.5.
Figure 5.8. Comparison of all DBF potential energies for each of the four pores with the given anisotropic permittivities.

Pore 1 with anisotropic permittivity has to be further investigated, as it has a higher potential barrier compared to the one with isotropic bulk dielectric constant (~50kT compared to ~7kT); yet it manages to have an ion flux of almost 3 times bigger in size. To do so, we may look at the ion trajectories at every single step inside the channel and measure their density, average velocity, or displacement. Following the trajectory of ions inside the channel reveals that ions in the pore with isotropic bulk permittivity move in the z direction an average of 8.6 times faster than those in the pore with anisotropic permittivity. However, the number of ions inside the pore with anisotropic permittivity is more than 18 times larger. Table 5.3 lists the average velocity and number of ions inside the pore for the two case studies.
Table 5.3. Average estimates of pore occupancy and ion speed inside the pore, over a 10 ns trajectory analysis.

<table>
<thead>
<tr>
<th></th>
<th>Average velocity of ions inside the channel</th>
<th>Average number of ions inside the channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore 1 (isotropic $\varepsilon$)</td>
<td>18.2601 (m/s)</td>
<td>0.741</td>
</tr>
<tr>
<td>Pore 1 (anisotropic $\varepsilon$)</td>
<td>2.11361 (m/s)</td>
<td>13.622</td>
</tr>
</tbody>
</table>

Figure 5.9 also shows two snapshots of the trajectories visualized with VMD software [40]. This explains why there is larger flux in the Pore 1 with anisotropic permittivity than in the corresponding pore with bulk value dielectric constant, even though the DBF potential barrier is quite larger in the first one. High axial permittivity shields the ion charges from each other in the axial direction, allowing many of them to trail along the channel.

Discussion

At first it might seem that such anisotropic permittivity values for nanopores are incoherent. However, MD simulations of electrolyte transport through carbon nanotubes suggest otherwise. Sony Joseph et al. showed that for a number of uncapped carbon nanotubes of various diameters ranging from 8.1 Å to 21.7 Å, in a solution of K$^+$CL$^-$, with zero bias applied, over the course of 3 ns, the ion occupancy was observed to be largely 0 or 1. Moreover, they found that the ions that enter the tube from either side do not travel across the length of the tube [58]. In fact, transport of ions in ion channels is stabilized by polar interactions with surrounding residues, which is a feature missing in hydrophobic nanotubes. Zero to very low ionic permeation in Pores 2, 3, and 4 does agree with these MD simulations, and naively assigning bulk dielectric constants to the water inside hydrophobic nanopores is quite debatable.
Figure 5.9. Two snapshots of the pore occupancy: (a) Pore 1 with isotropic dielectric constant (bulk value), (b) Pore 1 with the given anisotropic dielectric constants.
Conclusions

In conjunction with molecular dynamics, the transport simulation tools adapted from computational electronics provide a hierarchy of methods to describe ionic permeation in nanoscale biological systems such as ion channels. While the use of an implicit water model inherits its own limitations, biological time scales can be resolved, thus enabling one to explore current flow, selectivity, and gating mechanisms. These faster simulation tools could be particularly valuable in predicting the behavior of different mutant structures of a given ion channel, and provide guidance for experimentalists before they initiate costly laboratory procedures.

What is more, water molecules could surprise us in nanoscale confinements by showing very distinct structural conformations that drastically affect the ionic permeation and selectivity, demanding careful attention in assigning physical properties such as dielectric constant to the implicit water in coarse-grained approaches. Moreover, simple DBF or EMF potential energies computed from single ion steering, though useful in shedding light on some physics of the channel, are not enough to describe channel behavior, as was shown for the case of nanopores with very high axial permittivity.
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