Simulation Studies of Biological Ion Channels

Ben Alexander Corry

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Declaration

The material presented in this thesis is my own original research, conducted as part of a collaborative project with my supervisors Dr Shin-Ho Chung and Dr Serdar Kuyucak. Glenn Moy assisted with some of the programming and runs presented in chapter 3, in particular with extending my Poisson-Boltzmann solver to cope with some of the channel geometries discussed. The work presented in chapter 7 originated from an honours project undertaken by Scott Edwards supervised by Dr Shin-Ho Chung and myself, which I then extended substantially into the form presented here.

Ben Corry

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Abstract

Biological ion channels are responsible for and regulate the communication systems of the body. In this thesis I develop, test and apply theoretical models of ion channels that can relate their structure to their functional properties. In particular, Brownian dynamics simulations are introduced, in which the motions of individual ions are simulated as they move through the channel and in baths attached to each end. The techniques for setting boundary conditions which maintain ion concentrations in the baths and provide a driving potential are tested. Provided the bath size is large enough, all boundary conditions studied yield the same results.

Continuum theories of electrolytes have previously been used to study ion permeation. However, I show that these continuum models do not accurately reproduce the physics taking place inside ion channels by directly comparing the results of both equilibrium Poisson-Boltzmann theory, and non-equilibrium Poisson-Nernst-Planck theory to simulations. In both cases spurious shielding effects are found to cancel out forces that play an important role in ion permeation. In particular, the 'reaction field' created by the ion entering the narrow channel is underestimated. Attempts to correct these problems by adding extra force terms to account for this reaction field also fail.

A model of the L-type calcium channel is presented and studied using Brownian dynamics simulations and electrostatic calculations. The mechanisms of permeation and selectivity are explained as the result of simple electrostatic interactions between ions and the fixed charges in the protein. The complex conductance properties of the channel, including the current-voltage and current-concentration relationships, the anomalous mole fraction behaviour between sodium and calcium ions, the attenuation of calcium currents by monovalent ions and the effects of mutating glutamate residues, are all reproduced and explained.

Finally, the simulation and electrostatic calculation methods are used to study the gramicidin A channel. It is found that the continuum electrostatic calculations break down in this narrow channel, as applying a uniform dielectric constant is not accurate in this situation. Thus, the permeation properties of the channel are examined using Brownian dynamics simulations without electrostatic calculations. Future applications and improvements of the Brownian dynamics simulation technique are also described.

List of Publications

Portions of the work presented in this thesis have been published in the following papers:

Chapter 4:

- B. Corry, M. Hoyles, T.W. Allen, M. Walker, S. Kuyucak and S.H. Chung. Reservoir Boundaries in Brownian Dynamics Simulations of Ion Channels. *Biophysical Journal*, **82**: 1975-1984, 2002.

Chapters 5 and 6:

- B. Corry, S. Kuyucak and S.H. Chung. Test of Poisson-Nernst-Planck theory of ion channels. *Journal of General Physiology*, **114**: 597-599, 1999.
- B. Corry, S. Kuyucak and S. H. Chung. Invalidity of continuum theories of electrolytes in nanopores. *Chemical Physics Letters*, **320**: 35-41, 2000.
- G. Moy, B. Corry, S. Kuyucak and S.H. Chung. Tests of continuum theories as models of ion channels: I. Poisson-Boltzmann theory versus Brownian dynamics. *Biophysical Journal*, **78**: 2349-2363, 2000.
- B. Corry, S. Kuyucak and S.H. Chung. Tests of continuum theories as models of ion channels: II. Poisson-Nernst-Planck theory versus Brownian dynamics. *Biophysical Journal*, **78**: 2364-2381, 2000.

Chapter 8:

- B. Corry, T.W. Allen, S. Kuyucak and S.H. Chung. A Model of Calcium Channels. *Biochimica et Biophysica Acta - Biomembranes*, **1509**: 1-6, 2000.
- B. Corry, T.W. Allen, S. Kuyucak and S.H. Chung. Mechanisms of Permeation and Selectivity in Calcium Channels. *Biophysical Journal*, **80**: 195-214, 2001.

Chapter 9:

- S. Edwards, B. Corry, S. Kuyucak and S.H. Chung. Continuum Electrostatics fails to describe permeation in the Gramicidin Channel. *Biophysical Journal*, **82**: in press, 2002.

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Nomenclature:

PNP	Poisson - Nernst - Planck
NP	Nernst - Planck
PB	Poisson - Boltzmann
BD	Brownian dynamics
MD	Molecular dynamics
GA	Gramicidin A
PMF	Potential of mean force
GCMC	Grand Canonical Monte Carlo
DFT	Density functional theory
NMR	Nuclear-magnetic-resonance
DSE	Dielectric self energy

Commonly used symbols:

ϵ	Dielectric constant
ϕ	Electric potential
$\mathbf{E}$	Electric field
C	Concentration of an electrolyte
n	Number density
T	Temperature
q	Charge
z	Ion valence
I	Current
V	Applied potential across a channel

Constants:

Symbol	Name	Value
k	Boltzmann's constant	$1.38 \times 10^{-23} JK^{-1}$
e	elementary charge	$1.602 \times 10^{-19} C$
N_A	Avogadro's number	6.02×10^{23}

Introduction

All electrical activities in the body are regulated by ion channels. These channels provide pathways for the movement of charged particles that are responsible for the communication between cells. They underlie such diverse processes as neural signaling and the conversion of sensory inputs - such as light striking the eye - into a form that can be interpreted by the brain. Channels are also targets for many drugs, hormones and toxins, yet surprisingly they are still poorly understood. For this reason, uncovering the mechanisms of channel function at a molecular level is a fundamental challenge in biology.

Cells in living organisms are surrounded by a membrane which forms a barrier, preventing the contents of the cell spilling into the outside world. But, for the cell to be able to function it must be able to interact with its environment. Ion channels are protein molecules that provide one of the means for this interaction. They sit in the membrane and are shaped such that there is a central pore through which the ions that exist in the solution inside and outside of the cell, can pass. This enables the movement of ions from outside to inside the cell or vice versa.

When the brain sends a signal telling the muscles in the hand to clench, the message is carried through the nervous system from cell to cell in a chain reaction of events. At each step, millions of ions cross the cell membrane in a millisecond, creating an electric signal, which activates a process that starts the flow of ions into a neighbouring cell. Finally, when the message reaches the muscle fibres, the electric signal prompts them to contract. This whole process, of course, is regulated by the opening and closing of ion channels to allow the correct message to pass. Like an elaborate telephone exchange, to send the correct signal the channels have to open and close at the appropriate times, allow only the correct types of ions to pass, and move them through as quickly as possible so that there is minimal delay.

Over the last twenty years, developments have enabled the measurement of currents passing through a single ion channel. Very recently, the molecular structure of a few ion channels has been determined. However, the link between the channel

structure and conductance is not well known, and making this link is essential if we are to claim that we know how ion channels work. In this thesis I aim to develop a method that describes the conduction of ions through an ion channel and to apply it to a real biological channel. I aim to be able to relate the current of ions that pass through the channel to its structure. I hope to elucidate the process through which channels differentiate between ions as well as how they transport them.

The difficulty in developing theories of channel conductance lies in the fact that their size (around 50 to 100 Å long) places them in the complex mesoscopic regime. They are bigger than the scale in which the detailed atomistic laws of physics can be applied, but small enough that many of the statistical properties of bulk materials break down. Thus, a very large part of this thesis involves developing models and testing their range of validity to determine if they can be appropriately applied to ion channels. Ideally, to test a theory we need to be able to compare its predictions with experimentally measurable properties, primarily the channel current. However, the complexity of some theories means that they cannot predict a channel's current. Due to the complexity of the systems involved, an appropriate analytic theory is unlikely to be found. So, all the models described here involve computer calculations or simulations. A model of channel conductance should be detailed enough that it captures all the important physics taking place, providing a good description of the physical reality. It is desirable, however, that the theory not be too detailed, such that the essence of the situation becomes lost in a sea of data and calculation. The trick is to find a balance between accuracy and simplicity. Here I primarily use and develop Brownian dynamics simulations, a technique that mostly fits this criteria and can be used to predict currents from a channel structure.

I describe ion channels further in chapter 1, touching on their biological role and their functional and structural properties. In chapter 2 I introduce a number of theories that have been used to model channel conductance. The simplest theories do not provide a way to link the physical structure of a channel to its conductance, and so do not suit our purpose. The most complex contains a great deal of physical detail, but involves too many calculations to be able to predict currents. This means that some sort of middle ground must be found.

In chapter 3 I introduce the details of Brownian dynamics simulations, a simplified simulation technique that falls in this middle ground, and can be used to model channel currents. The following chapter then presents a test of the boundary conditions used in these simulations.

I consider the possibility of using theories simpler than Brownian dynamics in chapters 5 and 6. These candidates are continuum theories whose application to

channels has become common in the last 10 years. But, a comparison with simulations shows that in their present form continuum theories designed for both equilibrium (Poisson-Boltzmann) and non-equilibrium (Poisson-Nernst-Planck) situations break down when applied to ion channels. Because of the simplicity of continuum models compared to simulations, I examine an attempt to salvage them by adding some correction terms in chapter 7, but find that there is no simple way to do so.

Chapter 8 describes an application of the Brownian dynamics technique to examine the mechanisms of permeation and selectivity in a real biological channel: the L-type calcium channel. The model of the calcium channel developed proves to be very informative in describing the selectivity and permeation mechanisms of the channel, as well as predicting experimentally measurable properties with remarkable accuracy.

Finally, in chapter 9, I study the gramicidin A channel. In this case the Brownian dynamics modelling is less successful and demonstrates the failure in this narrow channel of the continuum electrostatic calculations that have been used to calculate electric forces. The Brownian dynamics model, however, can predict the correct conductance properties of the channel when the forces acting on ions are calculated in a different way.

The modelling techniques developed in this thesis have proven to be extremely successful in describing the properties of the calcium, Gramicidin and KcsA potassium channel. As more channel structures are found this approach will find many new applications, particularly in conjunction with detailed molecular simulations.