Mathematical Models of Ionic Flow
Through Open Protein Channels

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by

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Abstract

The subject of this dissertation is a mathematical and physical study of ion flow through open protein channels of biological membranes. Protein channels are macromolecules embedded in biological membranes, through which almost all transfer of ions into and out of living cells is done. There are hundreds of different protein channels with diverse functions, ranging from the transfer of electrical signals in the nervous system and coordination of muscle contraction, to secretion of urine and transfer of hormones. Over the past twenty years a vast amount of experimental data concerning the permeation characteristics of various channels has been accumulated. However, much theoretical work remains to be done to understand and interpret these data. Therefore, the study of ionic permeation through protein channels is one of the central problems of theoretical biology today.

To quantitatively study channels, mathematical and physical models of ionic permeation need to be derived. In this dissertation we consider mathematical models for the determination of the ionic current flowing through a single protein channel, given its structure, the applied electric potential and the ionic bath concentrations. We present three mathematical models of permeation on different scales of resolution. First, we derive coarse grained continuum equations of ionic permeation through a channel from a microscopic model of motion, with long and short range interactions. Then, on a coarser level, we propose a semi-Markovian model, with general probability distributions of residence times, which generalizes Markovian rate theories of channel permeation. Finally, we analyze a Langevin simulation of ionic motion in a region possibly containing a protein channel.

The main results of this dissertation are as follows: The first result is a new coupled system of partial differential equations for the ionic densities and electrostatic fields that describe ionic permeation in an electrolyte bath containing a protein channel. These equations are derived from a non equilibrium molecular model by a novel averaging procedure. Starting from a stochastic Langevin description of the coupled motions of all ions in a finite system containing an electrolytic solution, two electrodes, a membrane and a channel, a mathematical averaging procedure is devised to describe the system with averaged electrostatic
potentials and averaged charge concentrations. The resulting equations form a coupled system of Poisson and Nernst-Planck equations involving *conditional and unconditional charge densities* (C-PNP). While derivations of continuum equations from equilibrium molecular models are common in the statistical physics literature, this is the first derivation of Poisson Nernst Planck type equations from a *non equilibrium* molecular model.

The resulting equations differ in many aspects from the standard PNP equations used so far. To start with, the force in the resulting NP equations has two components instead of one. The first component of the force is the gradient of a conditional electric potential, which depends on ionic densities at one location, conditioned on the presence of an ion at another location. In contrast, in previous PNP theories only unconditional densities and an unconditional potential enter the equations. Our conditional densities take into account short range forces and excluded volume effects, which are important for ionic flow through confined regions such as a protein channel. The second component of the force, not present at all in standard treatments, is the self induced force on a single discrete ion due to surface charges induced by that ion at dielectric interfaces. This component is important near dielectric interfaces, for example near all interfaces between the channel, the ionic baths and the bilipid membrane. Another difference from the standard PNP system is that the proposed conditional PNP system is not complete, because Poisson’s equation for the electric potential depends on conditional charge densities, while the NP equations contain unconditional densities. The conditional densities present in our equations are the non equilibrium generalization of the well studied pair-correlation functions of equilibrium statistical mechanics.

According to the mathematical derivation, the charge densities in the NP equations are interpreted as time averages over long times, of the motion of a quasi-particle that diffuses with the same diffusion coefficient as that of a real ion, but driven by an averaged force. In this way, our derivation explains how continuum equations with averaged charge densities and mean-fields can be used to describe permeation through a microscopic protein channel. The importance of our derivation lies in the fact that the description of a complex molecular bath and channel system with averaged continuum equations reduces dramatically the number of degrees of freedom, rendering the problem computationally feasible.

The second main result in this dissertation is the presentation of semi-Markov chain models, with arbitrary probability distributions of residence times, which generalize Markovian rate models of ionic permeation through a protein channel. A mathematical procedure for
the determination of the average unidirectional and net currents as a function of the Markov chain parameters is presented, and its outcome is compared to the analogous rate type models. Markovian rate models, which are the most common theories for ionic permeation, rely on many unphysical and unrealistic assumptions, such as the existence of well defined binding sites inside the channel, with exponential jumping rates between these sites. In Markovian theories, a fit of the rate model parameters is performed to match experimental data. However, as there is only a vague connection between the model states and the actual three dimensional structure of the channel, the physical meaning of these parameters is unclear. The proposed semi-Markov chain models are free from these non-physical assumptions of rate models. The inputs needed for the semi-Markov model are the average lifetimes of the different occupancy states of the channel and the transition probabilities between the different occupancy states. These parameters should and can be computed from more refined physical microscopic theories, including the possibility of coupled motion of many mobile ions inside the channel, and without assuming the existence of well defined affinity sites. In this way, semi-Markov chain models provide a method to bridge between computationally intensive molecular dynamics simulations or other physical microscopic theories of ionic motion inside the channel, and the actual computation of macroscopic quantities such as the net and unidirectional currents through the channel.

The third main result in this dissertation is a mathematical theory for Langevin trajectories between fixed concentrations, with an application to a Langevin simulation of a bath and channel system. Due to limited computer resources, all simulations of ionic motion through a protein channel are done in a region containing the channel and two small parts of the practically infinite surrounding electrolyte baths. The simulation region must therefore be connected to the surrounding non simulated baths. Specifically, the boundary conditions of simulated trajectories, as well as the injection process of new trajectories, must be specified. In the case of ionic permeation through a protein channel, the net current through the channel is so small that the concentrations in the two baths are essentially fixed. Therefore the non simulated surrounding baths serve both as sources for ingoing trajectories and as absorbers of outgoing trajectories from the simulated region, and the fixed average concentrations at the boundaries can be viewed as the result of sources and absorbers of trajectories there. We calculate the strengths of the sources needed to maintain the given average concentrations. This description is applied to the design of a Langevin simulation
of the system, both for interacting and non-interacting particles. In particular, we propose to apply our method for the simulation of ionic diffusion through protein channels across biological membranes separating salt solutions of different concentrations. While other simulation algorithms inject trajectories according to ad-hoc methods, some of which are based on equilibrium assumptions, the strengths of the sources in our derivation depend on the current flowing through the system. The proposed simulation procedure enables the simulation of Langevin trajectories in a region between fixed but different concentrations, which is a typical experimental setup in single channel current measurements. Such a simulation is not possible with standard simulation procedures.
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Chapter 1

Introduction

The subject of this dissertation is a mathematical and physical study of ion flow through open protein channels of biological membranes. Ion channels are cylindrical hollow proteins embedded in biological membranes of all living cells. These channels control the passage of ions across otherwise impermeable bi-lipid cell membranes [72]. As such, they play key roles in many important physiological life processes, ranging from the transfer of electrical signals (action potentials) in the nervous system and coordination of muscle contraction, to secretion of urine and the regulation of hormone release from endocrine cells [85]. A substantial fraction of drugs, including valium and phencyclidine, act directly or indirectly on channels, altering their structure and thus their function [144]. Therefore, the study of channel proteins is of vital importance to both the biological and pharmaceutical community [6, 135].

In the past thirty years there have been major advances in the experimental study of protein channels. The invention of the patch clamp technique enabled the measurement of the current flowing through single channels under controlled conditions, such as the applied voltage and the surrounding bath concentrations [128]. Since then, hundreds of functionally different channels have been identified and their amino acid sequences have been determined with the tools of molecular biology. In addition, in the past few years the three dimensional structure of a few channels has been resolved as well [45, 89, 127, 155]. Nowadays, there exists a vast collection of experimental data concerning the permeation characteristics of dozens of channels. Despite these experimental advances, on the theoretical level, the underlying microscopic mechanisms of ionic permeation through protein channels and the relation between channel structure and function are not yet fully understood. Thus, ionic
permeation through protein channels is a treasure trove of new problems in mathematical physics, computational mathematics and mathematical modeling.

This dissertation is concerned with the theoretical problem of determining the function of a channel given its structure. Specifically, we consider the problem of determining the ionic current through a single channel, given its structure and the experimentally controlled applied electric potential and ionic bath concentrations. To this end, mathematical and physical models of ionic motion in the channel and in the surrounding electrolyte bath need to be derived. The importance of these models is that they can serve as a computational or analytical platform for the study of channel function. Nowadays, artificially engineered channel mutations or the effects of different substances on protein channels are studied only experimentally, due to lack of a reliable mathematical physical model of ionic permeation through the channel. The development of a trustworthy model of ionic permeation through a channel could facilitate these lab experiments by first running faster and cheaper computer experiments. Thus, a reliable computational model will have an enormous impact on the pharmaceutical community and on the design of new drugs.

The most common approaches to the study of channel function given its structure are i) application of continuum drift-diffusion type equations, ii) stochastic Langevin or Brownian dynamics simulations of the motion of ions through a single channel, iii) application of Markovian rate models, and iv) molecular dynamics and Monte Carlo simulations. In this dissertation we present some new results about the first three methods.

We start with type (i) models. The description of ionic flow through a single open channel by continuum drift-diffusion Nernst-Planck (NP) equations for the concentrations and Poisson’s equation for the electric potential, which is a common practice in semiconductor device modeling [140, 149], has been successful in the prediction of current-voltage relations for a few channels, under a wide range of experimental conditions [27, 30, 31, 33, 77, 116]. However, for other channels the PNP equations have been successful in fitting only some of the experimental data, and failed in reproducing other parts of the data. Moreover, some non linear phenomena of channels, such as the anomalous mole fracation effect, cannot be recovered at all with the standard PNP system [30]. This raises the question of the validity of the PNP model in describing ionic permeation through a single channel. The application of the NP equations with continuum concentration profiles as state variables to the flow of discrete charges through a microscopic channel (which can contain only one or two ions at
a time) requires justification. Otherwise, the mere concept of concentration in a single ion channel is at best problematic. The successes of the PNP theory raise the theoretical question of why this ad hoc model fits so many experimental data, and how can these equations be derived from a microscopic description. This is merely an example of a general fundamental problem in mathematical physics: the derivation of effective (continuum) equations to describe phenomena in mesoscopic systems. The importance of such a derivation lies in the fact that mesoscopic systems with many particles have a prohibitively large number of degrees of freedom, rendering computer simulations intractable. The description of such systems by continuum equations greatly reduces the number of degrees of freedom and makes the problem computationally feasible. It also becomes clear from such a derivation which phenomena are retained and which are lost.

In Chapter 3 we consider the description of the structure of an ionic solution carrying a steady current by partial differential equations. Standard statistical mechanics describe systems of interacting particles in equilibrium by a partition function [21, 71]. Then, by averaging out the partition function, it is possible to obtain equilibrium continuum equations to describe the microscopic system. The Poisson-Boltzmann equations, for example, are one the most common continuum approximations to describe electrolyte baths in equilibrium. However, a system carrying a steady current, such as bath containing an open protein channel, is not in equilibrium and its partition function is generally unknown. Therefore, while macroscopic continuity equations are often used to describe such systems, it is not even clear how to obtain approximate continuum representations of such non equilibrium systems from a molecular model [10]. In Chapter 3 we present a derivation of new effective Poisson-Nernst-Planck type equations for ion flow through a protein channel, from a non equilibrium molecular model.

Starting from a stochastic Langevin description of the coupled motions of all ions in a finite system, containing an electrolytic solution, two electrodes, a membrane and a channel, a mathematical averaging procedure is devised to describe the system with only a few state variables: averaged electrostatic potentials and averaged charge concentrations for the different ionic species. The resulting new Poisson and Nernst-Planck equations involve conditional and unconditional charge densities. While derivations of continuum equations from equilibrium molecular models are common in the statistical physics literature, this is the first derivation of Poisson Nernst Planck type equations from a non equilibrium molecular
model. In contrast to equilibrium derivations of statistical physics, that depend on the explicit knowledge of the partition function, our derivation is based on the full Fokker-Planck partial differential equation for the joint probability density of all particles. Thus, while partition functions are generally not known in general geometries with arbitrary boundary conditions, these can be easily accounted for in our formulation, which is based on partial differential equations. Therefore our method can be applied also to general equilibrium systems for which the partition function is not known.

The resulting continuum equations differ in many aspects from the standard PNP equations used so far. First, the force in the new NP equations has two components instead of one. The first component of the force is the gradient of a conditional electric potential at one location, conditioned on the presence of an ion at another location. This electric potential satisfies a conditional Poisson equation, that depends on conditional charge densities. Thus, the new system is a conditional PNP, with conditional charge densities and conditional electric potentials, in contrast to the standard PNP system, in which the state variables are unconditional charge densities and one unconditional potential. While in the PNP system the charge carriers have zero size with no short range forces, our conditional densities take into account short range forces, for example Lennard-Jones forces, and excluded volume effects. Short range forces have been long known to be a first order effect in the determination of the thermodynamical properties of electrolytes in equilibrium [21]. Short range forces are also important in the determination of transport processes in electrolytes [10]. Our work shows that they also play an important role in the study of ionic flow through confined regions, such as protein channels. The second component of the force, not present at all in standard treatments, is the self induced force on a single ion due to surface charges induced by that ion at dielectric interfaces. This component is important near dielectric interfaces, for example near all interfaces between the channel, the ionic baths and the bilipid membrane. Another difference from the standard PNP system is that the new conditional PNP system is not complete, because Poisson’s equation for the electric potential depends on conditional charge densities, conditioned on the location of an ion, while the NP equations contain unconditional densities. The conditional densities present in our equations are the non equilibrium generalization of the well studied pair-correlation functions of equilibrium statistical mechanics. In order to close the system, we examine a specific closure relation, which replaces the conditional charge densities by the unconditional
ones in the Poisson equation on the one hand, and replaces the self induced force in the NP equation by an effective force on the other hand. The effective force is nearly zero in the baths, but is approximately equal to the self induced force in and near a channel. This closure relation recovers the standard PNP system, with the addition of this effective force in the NP equations. According to the mathematical derivation, the charge densities in the NP equations are interpreted as long time averages of the motion of a quasi-particle that diffuses with the diffusion coefficient of a real ion but is driven by an averaged force. In this way, our derivation explains how continuum equations with averaged charge densities and mean-fields can be used to describe permeation through a microscopic protein channel. Under this approximation the average net current is recovered by the resulting PNP equations, even though other phenomena, such as single filing, are lost.

Modern PNP models of ionic permeation through protein channels are mostly three dimensional [27, 77]. However, even simpler one dimensional PNP models have been successfully applied to various channels [30, 31, 32]. Thus, an interesting question is to explain the success of these models in fitting experiments of some channels, that is, to study the mathematical features of the solutions to these equations. In Chapter 4 we present an asymptotic analysis of the one dimensional PNP system of equations used to fit current voltage relations of various channels, under the assumption of large permanent charge inside the channel. In this setting both the Poisson equation and the boundary conditions are singularly perturbed, and we apply the tools of boundary layer theory to compute the asymptotic solution of the equations. The main result of this chapter is an explicit formula for the net current flowing through the channel as a function of applied voltage, permanent charge profile and bath concentrations. The resulting current is linear in the applied voltage, thus recovering Ohm’s law for charged channels. This result explains the success of the 1D-PNP theory in fitting linear current-voltage relations (I-V curves) of some channels.

Markovian rate theories of ionic permeation in a protein channel have been in wide use for the past twenty years [12, 69, 72, 73, 74, 158]. These theories assume the existence of binding sites inside the channel with exponential residence times at each site. Exponential distributions of residence times are the result of diffusive motion in the presence of high barriers, as predicted by Eyring theory [94]. However, while the standard form of Eyring theory contains a pre-exponential factor of \( k_B T/h \), most rate models let this factor be an additional fit parameter. Since the resulting values for this fit pre-exponential factor were
usually orders of magnitude larger than $k_B T / h$, this point has raised criticism against rate models [38]. Other criticisms against barrier type models are concerned with the assumption of high barriers inside the channel. However, these potential barriers cannot be high, given the large currents flowing through them. Moreover, yet other assumptions of rate models, such as the existence of well defined binding sites, are also questionable [42, 148]. In Chapter 5, a generalization of rate type models to general semi-Markov chain models with general (non-exponential) distributions of residence times is formulated and analyzed. A procedure for the determination of the average unidirectional and net currents as a function of the Markov chain parameters is devised, and its outcome is compared to the analogous rate type models. The main result of this chapter is that the assumption of exponentially distributed residence times of rate type models can be relaxed, with the major features of these models unchanged. Another new result of this chapter is the application of Markov chains to coupled motion type models of multiply occupied channels, in which it is assumed that the motion of two or more ions inside a channel is coupled not necessarily confined to the binding sites assumed in rate models. Comparison of these models to the analogous multiple occupancy rate models reveals that some permeation characteristics can be recovered with coupled motion type models as well. Therefore, drawing conclusions about the number of ions and affinity sites inside a channel based on the ability to fit data with specific rate type models should be considered with great care. In addition, there is another important difference between the proposed semi-Markov chain model and rate type models. In the latter, there is only a vague connection between the actual three dimensional structure of the channel and the affinity sites of the model. Thus, while a fit of the Markovian rate model parameters is usually performed to match the experimental data, the physical meaning, if any, of these parameters is not clear. However, the proposed semi-Markov chain models depend on \textit{computable} quantities directly connected to the physical features of the channel. The input parameters for the semi-Markov chain are the average lifetimes of the different occupancy states of the channel and the transition probabilities between the states. These parameters can be computed from more refined physical models of ionic motion inside the channel, such as molecular dynamics or Brownian dynamics simulations. Thus, semi-Markov chain models present a bridge between molecular dynamics or other computationally intensive microscopic models, that are unable to compute macroscopic quantities, to coarse grained models that are able to compute macroscopic experimentally measured quantities, such as the net and
unidirectional currents through the channel.

As described above, nowadays that the three dimensional structure of some channels has been resolved, detailed Brownian dynamics (Langevin) simulations of ionic motion through these channels is one of the most promising new areas of research in this field [39, 79, 80, 100]. In Brownian dynamics (BD) simulations, the ionic motions and their interactions both inside the channel and in its vicinity are followed in time according to a Langevin system of equations. Due to limited computer resources, however, ionic trajectories are simulated only inside the channel and in a small region of the two surrounding electrolytic baths. Thus, a realistic simulation must deal with two major questions: One concerns the injection and removal of ions from the simulation, and the other concerns the correct computation of the electric field acting on the simulated ions. In Chapter 6 we are concerned with the first issue, and present a mathematical theory of Langevin trajectories between fixed concentrations. The theoretical question is how to describe Langevin trajectories, and specifically their boundary behaviour, in a region that separates two baths of constant, although possibly different, solvent concentrations. This is exactly the case in BD simulations, in which the current flowing through the channel is so small that the concentrations in the two baths far away from the channel are essentially fixed. First, we note that the non simulated baths surrounding the simulation region serve both as sources for ingoing trajectories and as absorbers of outgoing trajectories from the simulated region. Thus, the fixed average concentrations at the boundaries can be viewed as the result of sources and absorbers of trajectories there. We calculate the strengths of the sources needed to maintain the given average concentrations, when taking into account the crossing and possible recrossings of ions from the simulation region to the surrounding baths and vice versa. This description is applied to the design of a Langevin simulation of the system, both for interacting and non-interacting particles. In particular, we propose to apply our method for the simulation of ionic diffusion through protein channels across biological membranes separating salt solutions of different concentrations. Most simulation algorithms use either reflecting or periodic boundary conditions, whose limitations have been discussed at length in the literature [2]. Specifically these algorithms are unable to simulate Langevin trajectories between different ionic concentrations. Other methods inject new trajectories into the simulation according to ad-hoc methods, some of which are based on equilibrium assumptions (for a non-equilibrium simulation). In contrast, the strengths of the sources in our theory are derived from the
underlying mathematical theory of diffusion in a non equilibrium system, and depend on the net current flowing through the system. The proposed procedure enables the simulation of Langevin trajectories in a region between fixed but different concentrations, which is a typical experimental setup in single channel current measurements. Such a simulation is not possible with standard simulation procedures.

Chapter 7 is a theoretical chapter, devoted to the problem of steady state diffusion into an absorbing boundary. In this chapter we consider a system with an infinite number of independent diffusers, and show that the resulting arrival process at an absorbing boundary is Poissonian. This theoretical result is used in the mathematical analysis of the time course of a Langevin simulation with a buffer region [107, 110].

Finally, we conclude in chapter 8, in which a summary of the main results of this dissertation is presented, as well as directions for future research. It is often said that molecular biology is the science of the future. In this context, protein channels are one of the most basic and well defined entities in molecular biology. Their structure is known, and their function is easily measured experimentally. The missing piece nowadays is a quantitative physical mathematical theory to link structure to function. Its inputs should be the channel structure, applied voltage, bath concentrations and ionic characteristics such as the diffusion coefficients, ionic radii, charge, etc. Its output should be the net current flowing through the channel. This dissertation makes a few steps towards this goal.
Chapter 2

Channel Phenomenology: Experiments and Theories

Ionic channels are macromolecules embedded in biological cell membranes. The membrane surrounding a cell consists of a lipid bi-layer which is impermeable to ions, so almost all transfer of ions and other larger molecules occurs through these protein channels. Protein channels are thus responsible for most transfer processes from and into the living cell, and their study is of considerable importance for the biological community.

This introductory chapter provides a short biophysical review of protein ionic channels, from both the experimental and theoretical side. For a comprehensive review on channels, channel biology, their crucial function in the various life processes, their diversity and evolution, the interested reader is referred to Hille’s book [72] and references therein. The rest of the chapter is organized as follows: In section 2.1 we give a brief description of channel experiments, starting from the early voltage clamp experiments and theories, and ending with the modern patch clamp experiment. Following, in section 2.2 some of the vast diversity of single channel characteristics and phenomena is described. Section 2.3 outlines various applications of modern molecular biology to the study of channels, ranging from channel sequencing and mutations to resolution of channel structure with NMR spectroscopy. Finally, in section 2.4, we describe some of the different theoretical models of ionic permeation through a single open channel, which is the subject of this dissertation.
2.1 Channel Experiments

2.1.1 Early Channel Experiments - The Voltage Clamp

Channels embedded in biological membranes have been studied since the beginning of the 20th century, mainly by physiological experiments in living cells. The invention of the voltage clamp technique in the late 1940’s enabled experimentalists to study the collective permeation characteristics of a very large ensemble or cluster of (different) channels found on the membrane of specific types of cells. In voltage clamps, the experimentalists controlled the ionic concentrations on both sides of a membrane and the applied potential across it, and measured the net current flowing through the membrane as a function of these parameters. In those days, it was conjectured that ions permeated the membrane through channels, since there was no direct experimental evidence for their existence. With these experiments, a vast amount of data of current-voltage-concentration relations was collected for many membranes of different cell types. The concept of the reversal potential, defined as the potential at which the net current of one ionic species is zero, was introduced and with it a quantitative definition of the ionic selectivity of cell membranes to different ionic species. On the theoretical side, two of the most successful theories to explain the results of voltage clamp experiments were the GHK theory (Goldman, Hodgkin and Katz) and the HH theory (Hodgkin and Huxley) [72, 75]. The GHK theory predicted the current voltage relations and the reversal potential as a function of the channel selectivity to different ions and their respective concentrations on both sides of the membrane. The HH theory successfully modeled with differential equations the time dependent permeability changes of the entire membrane due to the random openings and closings of single channels, in response to the time dependent voltage pulses applied across the membrane. One of the great successes of these theories was the identification of two separate systems for $Na^+$ and $K^+$ permeation through the membranes of squid axon cell, and the study of their properties. Today we know that indeed these two permeation processes are nearly independent as the relevant ions flow through different channels.

Despite the success of these theories in predicting and explaining the results of voltage clamp experiments, these theories were macroscopic in nature, considering the collective averaged behavior of a large ensemble of channels. They did not consider microscopic physical models for the function or permeation mechanisms of single channels, since single channels
were not known to actually exist. Both the GHK and the HH theories were thermodynamical black box theories that successfully fitted the experimental data available in those days.

2.1.2 Modern Experiments - The Patch Clamp

A major breakthrough in the experimental study of biological membranes came with the invention of the patch clamp experiment in 1976, by Neher and Sakmann [128, 129, 130]. The goal of the patch clamp experiment was to measure the current flowing through a tiny area of a membrane. This was accomplished by allowing a very narrow pipette to fuse into the membrane of a living cell, and then tearing the pipette from the cell (see figure 2.1). This resulted in a tiny patch of cell membrane strongly glued to the pipette, forming a seal of high mechanical stability and electrical resistance. Similar to the voltage clamp experiment, a typical patch clamp experiment consisted of placing this seal between two electrolytic solutions, one outside the pipette and one inside it, inserting electrodes into the two solutions, applying a voltage between them and recording the resulting current flowing through the seal. In some cases, the resistance of the seal was unexpectedly high, of the order of tens of gigaohms, thus giving it its name - gigaseal. In other cases, the resistance was orders of magnitude lower and the recorded current fluctuated between two mean values (see figure 2.2). Yet in other cases, the recorded current fluctuated between three or more values, where all differences between consecutive ordered values were equal to the same constant. These results were interpreted as follows: A measurement of a very high seal resistance was interpreted as the case in which no channels were captured in the tiny patch of the membrane. A measurement of exactly two values for the current was interpreted as exactly on channel captured inside the patch, with a single open state and a single closed state. A measurement of more than two values was interpreted as a capture of more than one channel in the patch. The fluctuations in the current levels were explained in terms of the number of open channels at any given time.

The results of patch clamp experiments were recognized in the scientific community as the first clear experimental evidence for the existence of channels in biological cell membranes. In addition, the patch clamp technique allowed experimentalists, for the first time, to record the current flowing through a single protein channel under experimentally controlled conditions. Moreover, the results of patch clamp experiments were reproducible. For a specific type of channel, patch clamp experiments conducted in different labs around the world would always
Figure 2.1: The patch clamp technique. (a) A pipette is allowed to fuse into the membrane of a living cell. (b) the pipette is separated from the cell, tearing with it a small patch of membrane forming a gigaseal.

give the same measurement, up to instrumentation noise, of the mean net current flowing through the channel, given all other variables kept fixed. Therefore, in contrast to voltage clamp experiments, that measured permeation through an (unknown) ensemble of different channels, patch clamp experiments enabled the definition and measurement of precise and definite characteristics of single specific channels. Since their discovery, hundreds of different types of channels with inherently different characteristics have been identified and studied.

### 2.2 Single Channel Characteristics

In a typical patch clamp experiment, a small patch of membrane containing a single channel is placed between two electrolytic baths. Two electrodes are placed in the bath, a constant voltage is maintained between them and the resulting current is recorded. Typical bath concentrations for this experiment are in the range of 10 mM to 2 Molar, with both symmetric and asymmetric solutions, and typical values for the applied voltage range from -200 mV to +200 mV, with resulting currents of the order of 1 pA up to 500 pA, depending on the channel and the experimental conditions. These numbers correspond to a passage of $10^7 - 10^9$ ions/sec through the channel.
The radius of a protein channel can be estimated by patch clamp experiments by recording the sizes of ions that can pass through it. Most channels have a narrow region of radius $2 - 4\text{Å}$, a finding that has been confirmed by other methods as well.

A typical recording of the current vs. time from a single channel in a patch clamp experiment is shown in figure 2.2. There are two evident features in this figure. One is the fluctuation of the net current between only two average values, interpreted as an open or closed channel state. The other is that the channel opens and closes abruptly at random times. Performing the same experiment on the same channel under the same experimental conditions reproduces the same mean values for the net current, although the openings and closings of the channel occur at different (random) times.

### 2.2.1 Channel Gating

The random opening and closing of a channel is called *gating*. Channel gating is believed to be a series of one or more conformational changes of the channel’s three dimensional structure. While some channels have more than one open state, most channels have only one open state, identified by a unique mean current, that depends of course on applied voltage and ionic contents of the surrounding solutions. Since the invention of the patch clamp experiment, the probability distributions of the open and closed times of channels have been extensively studied. Experimentally, it has been found that the gating characteristics of a channel depend on the applied voltage, contents of the ionic solution, and in particular on the introduction of various larger molecules into the surrounding solutions [72]. For example,
insertion of 300 µM of N-bromoacetamide into the electrolytic solution increases the mean open time of Na channels almost tenfold [121].

On the theoretical side, many different gating mechanisms and models have been proposed [72]. While some of them have been rejected, none of the remaining models has been conclusively confirmed. The gating of channels is of vital importance to the living organism. For example, it controls the transfer of electric signals in the nervous system. This thesis is concerned with the permeation characteristics of an open channel, and not with the mechanisms that open or close it. Therefore, the study of channel gating is not treated in this thesis, and the interested reader is referred to [72] and references therein.

2.2.2 Current / Voltage / Concentration Relations

One of the basic quantities measured in patch clamp experiments is the net ionic current through a single open channel, as a function of applied voltage and concentrations on both sides of the channel. For many channels the resulting I-V curves are almost linear, although there are channels that exhibit sub-linear and even super-linear increase of the current as a function of applied voltage. In figure 2.3 the current voltage relations of both wild type Porin and its mutation G119D are shown, for symmetric bath concentrations of 1M. Note that while the wild type Porin has an almost linear I-V curve, the mutation G119D has a slightly super-linear curve.

Some channels exhibit saturation of the current, either at large applied voltages or at high concentrations. As mentioned above, the potential at which the net current vanishes is denoted the reversal potential or the resting potential.

An important characteristic of a single channel, derived from its I-V relation is its unitary conductance. For the majority of studied channels, for small applied voltages away from the resting potential, the total current $I$ is linear with respect to the applied voltage $V$, that is

$$I = gV.$$  

The proportionality factor, denoted $g$, is defined as the unitary conductance of the channel. This conductance depends of course on ionic bath concentrations, with different channels exhibiting very different concentration dependencies.
2.2.3 Unidirectional Currents, Ussing’s Ratio

A different set of patch clamp experiments involves radioactive tracers. A radioactive tracer is placed only on one side of the membrane so its unidirectional flux to the other side can be measured. In these experiments it is common to measure the ratio of the unidirectional fluxes, also known as Ussing’s ratio.

Early descriptions of ionic permeation through membranes used the formalism of bulk electrodiffusion or the conductance approximation (the HH Theory). These theories assumed the principle of independence, i.e., independence of the unidirectional flows across the membrane. However, as early as 1955, the work with tracer fluxes of Hodgkin and Keynes [76], revealed deviations from the principle of independence for ionic flow across membranes. The experimental results showed that for certain membranes ions diffuse in a single-file coupled motion. In order to quantitatively measure the amount of single-file coupling, Hodgkin and Keynes introduced the flux ratio exponent \( n' \), also known as the Ussing exponent. This exponent is defined according to the following equation

\[
\frac{I_{LR}}{I_{RL}} = \exp \left( n' \frac{ze(V - V_N)}{k_B T} \right),
\]

where \( I_{LR} \) and \( I_{RL} \) denote the unidirectional currents from left to right and right to left, respectively, \( V_N = (k_B T / ze) \ln(C_R / C_L) \) is the Nernst reversal potential associated with the permeating ion of valence \( z \), and \( V \) is the applied potential, so \( ze(V - V_N) \) is the electrochemical energy difference. For ions moving independently across the membrane, the Nernst-Planck theory predicts a value of exactly \( n' = 1 \) for the flux exponent. However,
experiments reveal that while for certain channels the flux exponent is roughly one, other channels have flux exponents larger than one, ranging from 1 to even 3.5 [26, 56, 145]. Moreover, in some channels the flux ratio exponent depends on the bulk concentrations [13].

Various barrier type models have been proposed to explain the measured Ussing flux ratio exponents [72, 73]. In these models, the exponent \( n' \) is intimately connected to the number of mobile ions located inside a single channel [136]. Therefore, it is common practice to assume that an Ussing exponent \( n' \) larger than one is a direct evidence of a multiply occupied channel.

### 2.2.4 Anomalous mole-fraction effect

Another phenomena identified with multiple ionic occupancy inside a protein channel is the **anomalous mole-fraction effect**. The basic experiment is as follows: In a patch clamp experiment a membrane containing a single channel separates two ionic solutions. In each of the two solutions a mixture of two permeant ions is placed, for example K\(^+\) and Tl\(^+\). A small voltage is applied and the channel unitary conductance is measured as a function of the permeant ions concentration ratios, e.g. \( C_K/(C_K + C_{Tl}) \) in the example. Surprisingly, for mixed solutions the resulting conductance of the channel is smaller than for a pure solution of only one permeant ion. Such behavior, where the conductance \( g \) goes through a minimum as a function of the ratio of ionic concentrations, is called anomalous mole-fraction dependence. Among others, one of the reasons why this phenomena has been identified with multiple ionic occupancy is that it can be easily recovered with multi-ion barrier models of channel occupancy [72]. However, it should be noted that this behavior can also be recovered from continuum models such as PNP, with the inclusion of an ion specific chemical potential (binding potential) into the model [114].

### 2.2.5 Selectivity

One of the interesting and most important properties of ionic channels is their **selectivity** to different ions. Some channels have a conductance for a specific ion which is tenfold or even a hundred times higher than for other ions of roughly the same size and valence.

Many mechanisms have been proposed to explain channel selectivity. Some assume it is due to the structure of the hydration shell surrounding the ion as it enters the channel.
[72], others assume it is caused by the coordinated motion of the mobile ion with the flexible protein channel. Other theories attempt to explain this phenomena by a selectivity filter located inside the channel, and study the ionic motion of different species near this filter using molecular dynamics simulations [2]. Yet other theories explain selectivity by simple geometric constraints or excluded volume effects [23]. All in all, this feature, crucial for the normal action of the cell, still awaits a satisfactory explanation.

2.3 Molecular Biology Tools Applied to Channels

While patch clamp experiments allow the measurement of the function and permeation characteristics of single channels, they give no direct information about the underlying permeation mechanisms. An important step in this direction was the resolution of the three dimensional structure and atomic sequence of some protein channels. This information was found by application of the advanced tools of molecular biology, such as gene coding, gene manipulation, NMR spectroscopy and X-ray crystallography. Nowadays, that both the sequence and three dimensional structure of a few channels has been resolved, scientists can attempt to answer one of the fundamental questions of cell biology: Determination of the function of a protein channel, given its structure.

2.3.1 Resolution of Channel Structure

An important breakthrough in the resolution of channel structure was the ability to crystallize a protein channel and then study its three dimensional structure with Nuclear Magnetic Resonance (NMR) spectroscopy, X-ray crystallography and other methods [127, 131, 159]. So far, the structure of only very few channels has been resolved, the main obstacle being the difficulty to crystallize a channel without breaking it. The Gramicidin A channel is one of the few channels whose three dimensional structure is known [155, 156], and whose permeation characteristics have been extensively studied in great detail [60]. Another family of well studied channels, with known three dimensional structure are the Porin channels [89, 133]. Yet another channel, whose structure has been resolved only recently is the KcsA K⁺ channel [45].
2.3.2 Channel Sequencing and Mutations

Yet another advance in the study of channels was the identification of genes in the DNA sequence that code specific protein channels. Once the gene that codes a specific channel is identified, the atomic sequence of the channel is known. The knowledge of the atomic sequence of a channel, combined with its three dimensional structure, is the starting point for the determination of channel function given its structure.

Regretfully, while the atomic sequence of many channels is nowadays known, the protein folding problem, that is the determination of the three dimensional structure of a channel and the residual charges of all its atoms given its coding sequence, is a yet unsolved problem [58, 64].

Last but not least, once the genes that code channels have been identified, the ability to manipulate them and thus change the molecular sequence of the resulting channel has also been developed in the past few years. Nowadays, with the advanced tools of molecular biology, it is possible to mutate a channel by changing its atomic sequence at one or more specific locations, chosen by the experimentalist, and then to study the consequences of this mutation on the permeation characteristics of the channel. This process is of vital importance to channel study as it enables to study the effects of specific atoms in the protein on its permeation characteristics. Therefore, mutations can also be used as tests of theories that attempt to predict the function of a channel given its structure.

One example of a man-made channel mutation is the pair of the wild type Porin channel, denoted OmpF, and its mutation G119D, both of known crystallographic structure. In figure 2.3 the current voltage relation of both OmpF and G119D are shown for symmetric 1 Molar bath concentrations [150]. While this mutation consists of a single substitution of an aspartate in the channel sequence, this substitution has large effects on the permeation characteristics of the channel, decreasing the net current by nearly half its original value.

2.4 Theories of Channel Permeation

Theories of channel permeation fall into one of the following four groups: i) Barrier models, ii) Molecular dynamics or Monte Carlo simulations iii) Langevin models and simulations iv) Continuum models. In this section we describe briefly the main features of the theories in
each of these four groups.

2.4.1 Barrier Models

Theories of ionic permeation through single channels were proposed long before the invention of the patch clamp experiment, that actually measured permeation through a single channel, and showed that channels really existed. The earliest models described the (unknown at the time) structure of a channel as a sequence of binding sites [69, 72, 73, 74]. These models, also known as barrier models, rely on the assumption that at any given time the channel can be in only one of a finite number of well defined states, corresponding to different occupancy of ions at the binding sites inside the channel. Transition rates corresponding to high barriers are assigned between the different sites and the resulting net and unidirectional currents are computed as a function of applied voltages and bath concentrations.

The simplest of these barrier models is the 2B1S model [72], in which it is assumed that the channel can be represented as a free energy profile with two barriers and one deep well (binding site) between them, as shown schematically in fig. 2.4 (a). This model assumes that, regardless of bath concentrations and applied voltages, the channel can be occupied by at most one ion at a time, attached to the binding site in the middle of the channel pore. For each permeant ion, entrance rates into the channel from the left and right baths are assumed, with a linear dependence on bath concentrations. In addition, two exit rate constants are assumed for each permeant ion that resides at the binding site inside the channel, to exit on either the left or right side of the channel respectively. These rate constants are assumed to scale exponentially with the applied voltage $V$ multiplied by another proportionality factor. Thus, the 2B1S model has five (!) adjustable parameters for each permeant ion. The unidirectional currents and the net flux flowing through the channel can be derived from these parameters by application of chemical reaction rate theory [69, 72]. In most applications of barrier models, these parameters are set to fit the experimental data.

For the case of a single species of permeating ions, regardless of the exact values of the five parameters, the simple 2B1S model predicts a value of exactly one for the exponent $n'$ in the Ussing flux ratio equation (2.1). Since many channels exhibit a flux ratio exponent larger than one, this finding has lead to the formulation of more complicated rate models for multi ion channels, that can hold more than one ion at a time [72]. These models predict an
Figure 2.4: Schematic potentials along the channel axis for: (a) the 2B1S model, (b) the 3B2S model.

exponent \( n' \geq 1 \), that also depends on applied voltage and bath concentrations. The most common of these models is the 3B2S model for a two ion channel, with three barriers and two ion sites inside the channel [60, 73]. The underlying schematic picture of the potential along the channel axis for this model is shown in figure 2.4 (b). Based on the predictions of multi ion rate models concerning the flux ratio exponent, an experimentally measured value of \( n' > 1 \) is considered a definite criterion for the presence of multiple ions in a channel [72, 136].

Rate models were a generally accepted technique due both to their relative simplicity, and to their ability to fit at least some of the experimental data. However, rate models have been criticized for relying on many ad-hoc, unrealistic and unphysical assumptions [38, 42, 50, 51, 97, 115, 148]. As described above, the main assumption of barrier models is the existence of binding sites inside the channel. Another implicit assumption is that the corresponding barriers between sites are high, thus enabling the application of Eyring rate theory to describe the transition between different sites with rate constants. However, most rate models did not use the Eyring rate theory in a self consistent manner, since instead of the fixed pre-exponential factor present in Eyring theory, of \( kT/h \), these models let this factor be another fit parameter, and the resulting value for this fit was orders of magnitude larger than \( kT/h \). Moreover, not only that such sites need not necessarily exist in channels, the actual potentials barriers for ions are probably not high, given the large currents passing through the channel. Finally, barrier models either assume that these transition rates are independent of concentration and applied voltage, or that the exact dependence on these
quantities is postulated rather than derived. Consider for example the simple exponential
dependence of rate constants on the applied voltage. In reality, the force exerted on a mobile
ion at a given location inside the channel depends not only on the applied voltage but also
on all its interactions with all other charges as well. In particular, the force depends on
the arrangement of surrounding bath ions on the two sides of the channel, which by itself
depends on the applied voltage, on the average flux flowing through the channel and on the
presence of the mobile ion itself inside the channel. Thus, all of these quantities are coupled
and the simplistic assumptions of barrier models are regretfully unphysical in their nature.
Another point rarely treated in multi ion barrier models is coupled motion of two or more
ions inside the channel. While multi ion models contain transition rates that depend on the
occupancy of the channel, they rarely consider the case of two ions hopping in a concerted
or coupled motion from their original configuration to a new configuration. Finally, we note
that traditional barrier models are not very useful in the study of channel function given its
structure as their states and transitions are only vaguely related to the actual structure of
the channel.

In chapter 5 we generalize the traditional Markovian rate models to general semi Markov
chain models with general probability distributions of the residence times at different states.
We also consider multiple occupancy models with coupled motions of the ions inside the
channel, not necessarily confined to well defined affinity sites and compare them to the
traditional rate models.

To conclude, it is generally accepted nowadays that rate models by themselves are inade-
quate as a description of ionic permeation through protein channels. Still, in spite of the vast
criticism against barrier models, their basic underlying picture of ionic permeation remains
intact. Protein channels are narrow pores embedded in membranes, that can contain more
than one ion at a time. Due to electrostatic repulsions and the narrow radius of the channel,
in most channels ions cannot cross each other and thus move inside the channel in single file.

2.4.2 Molecular Dynamics

The determination of channel structure on the one hand, as well as the appearance of powerful
computers and simulation techniques on the other, enabled the application of much more
sophisticated approaches than barrier models to the study of ionic permeation. One of
the most ambitious attempts to study ionic permeation is with molecular dynamics (MD) simulations. In MD simulations, the locations and velocities of all mobile ions, protein channel atoms and water molecules in a small finite domain surrounding the channel are included in the model as state variables. At each time step of the simulation, the electric potential and the forces on each of these atoms is computed, and their positions and velocities are updated by direct integration of Newton’s equations of motion. In MD simulations, the channel atoms are allowed to move as an ion passes through the channel. Therefore, in contrast to barrier models, there is no notion of a fixed free energy profile of the channel. Another difference from barrier models concerns the underlying assumption about the motion of mobile ions. While barrier models are based on a picture of ionic diffusion with effective friction and diffusion coefficients in a channel described by a potential with an effective dielectric constant, these are non-existent in MD simulations. The dielectric constant is one, and all electrostatic shielding is done by the explicit inclusion of mobile water molecules into the model.

To run an MD simulation, the structure of the channel must be known. Until recently, this of course limited most MD simulations to the Gramicidin family of channels [123, 124]. As discussed above, in the past few years the 3-D structure of other channels has been resolved, and naturally MD simulations on these channels have been performed as well [20, 66, 132].

In a sense, MD simulations present a "brute force" study of ionic permeation, and this is their main limitation. The time step in a molecular dynamics simulation is of the order of $10^{-16}$ sec. Simulations with coarser time step are not pure MD, but rather rest on additional assumptions concerning the effective motion of water molecules or other ions. Currently, with such small time steps it takes a good few days of a supercomputer to run the atomic trajectories for only one nanosecond. Since the time order of permeation of an ion through a channel is of the order of $10^{-7}$sec, to the author’s knowledge, so far no molecular dynamics simulation has been able to follow the trajectory of a mobile ion since its entrance on one side of a channel till its exit on the other side. Thus, while molecular dynamics simulations can be useful in the study of the details of ionic motion inside the channel, they are yet unable to predict the macroscopic measured ionic currents flowing through it. This situation is not bound to change in the foreseeable future.

There are many other limitations to MD simulations [115]. One of them is that while channels function in conditions away from equilibrium, with non-vanishing applied potentials
and concentration gradients, MD simulations are almost always done at equilibrium with a computation of the electric field that allows no transmembrane potentials to be present. Another problem is that while channel function depends critically on the surrounding bath concentrations, almost all MD simulations do not include actual bath concentrations, but rather study the motion of only one or two ions and a few water molecules inside a channel. MD simulations with larger domains and inclusion of bath ions and their surrounding water molecules are orders of magnitude more complex. Since statistical errors scale like the square root of the number of samples, about 1000 simulated ions are needed to define a bath concentration within an error of 3%. At a concentration of 100 mM, there are about 550 water molecules per ion, which gives a total of more than half a million simulated atoms. These requirements make an MD simulation infeasible to run for a reasonable physical time even on a supercomputer. Thus, MD simulations are unable to compute channel characteristics that depend on bath concentrations. In this context, we note that for some channels, an introduction of only 1 μM Ca\textsuperscript{2+} into the solution changes drastically the channel permeation characteristics. Needless to say, it seems hopeless for molecular dynamics simulations to capture or study this effect.

2.4.3 Langevin Models and Simulations

Due to the severe limitations and enormous computer resources of molecular dynamics on the one hand, and the unrealistic ad-hoc assumptions of barrier models on the other, various other coarse grained approaches for ionic permeation through a channel have been devised. One of these approaches is based on the assumption that on a coarse enough time scale, say 1000 times larger than the characteristic 10^{-16} sec time step of MD, the motion of a mobile ion inside the channel can be approximated as overdamped diffusion in an electrostatic potential field.

In contrast to MD simulations where the motion of all ions and water molecules is followed, in these stochastic Langevin models, only the motion of the mobile ions is simulated, with the structure of the protein channel usually assumed fixed. Also, while water molecules are not explicitly present in Langevin models, they are implicitly present as the source of noise and friction for the motion of the mobile ions. In addition, the non-simulated water molecules are assumed to contribute an effective dielectric coefficient in the computation of electrostatic forces between ions.
In stochastic Langevin models, the motion of each mobile ion is described by a Langevin equation
\[ \dot{x}(t) = \frac{F(x)}{m} + \sqrt{2D(x)} \dot{w}(t), \] (2.2)
where \( x \) is the coordinate of the ion, \( m \) is its mass, \( F(x) \) the force exerted on it, \( D(x) \) is the location dependent diffusion coefficient, \( w(t) \) is a standard white noise process and a dot on a variable denotes differentiation with respect to time.

Early stochastic models, most notably the work of Levitt [97, 98, 99], considered only the mobile ions inside the channel, and assumed that their motion is purely one dimensional, on the channel axis. Some of these models assumed that the force \( F(x) \) exerted on the mobile ion can be computed from a fixed potential profile of the channel superimposed on a linear profile resulting from the applied potential. Absorbing boundary conditions are imposed at the two channel ends for the ion trajectories, and new particles are introduced into the channel by means of entrance rate constants, by the same mechanism as in barrier models. In the case of a channel that can hold at most one ion at a time, the corresponding Nernst-Planck equation for the probability density of the ion inside the channel can be solved analytically given the potential profile of the channel, and a generalization of rate models, referred to as continuum solution or Nernst-Planck model is formulated and solved in order to compute the flux through the channel. The same method has also been extended to the case of two ions inside the channel [99]. However, these models suffered from many of the same ad-hoc assumptions of rate theory as described above.

The first Brownian dynamics simulations of ionic permeation, mainly by Jakobsson and coworkers, also considered only the motion of the mobile ions present inside the channel, usually only one or two ions [15, 35, 82], and assumed that their motion is one dimensional along the channel axis. The "entrance tube algorithm" developed by Cooper & al. [37] was used to connect the channel to the surrounding baths by deploying boundary conditions for particle trajectories at ends of two tubes located at the edges of the channel. The length of this tube that extends into the bath is derived under the assumption that the channel end is in equilibrium with the surrounding bath. In most of these early models, the electrostatic potential is not computed from the solution of the full three dimensional Poisson equation, but rather various one dimensional or spherical approximations are made in order to simplify the computation [15, 35]. In particular, the effect of the surrounding bath ions outside the channel is rarely taken into account.
A three dimensional Brownian dynamics simulation of ion flow through Porin Channels with known structure is reported in [134]. In this model, there is no applied potential, and only one ionic trajectory is simulated at any given time, since its start at a random location on a planar plate far away from the channel entrance till the passage of the ion to the other side, or till its exit at the boundary of a hemisphere far away from the channel entrance.

In the past few years advanced Brownian dynamics simulations with many diffusing particles have been performed [39, 40, 79, 80, 100, 104]. In these models, the simulation region consists of a membrane with a protein channel and two parts of the surrounding baths. The motions of all ions present in this region, of the order of 100 particles, is followed in detail. In addition, at each time step of the simulation, the force exerted on each ion is computed from the solution of Poisson’s equation including all interactions with the other mobile ions, the fixed and induced charges of the protein channel and the applied voltage. These simulations are the first attempt to study the permeation process through ionic channels in a self consistent manner, in which the electric potential is not assumed fixed, but rather is computed at every time step from the distribution of all charges in the system.

There are three main issues to be resolved in Langevin simulations. The first issue is the determination of the diffusion coefficient $D(x)$ in equation (2.2). In principle, this quantity could be estimated from a finer MD simulation of the motion of the ion inside the channel [142]. While some works use this approach, other take the diffusion coefficient inside the channel to be the same as the diffusion coefficient in bulk solution, and yet other theories let the diffusion coefficient be a fit parameter of the model.

The second issue is the computation of the force $F(x)$ acting on the mobile ion at location $x$. This force is usually given by $-ze\nabla U(x)$ where $U(x)$ is the electrostatic potential. In principle, this potential must be calculated from the solution of Poisson’s equation including all charges in the system and the applied voltage as a boundary condition. Two prominent problems concerning this computation are the description of water molecules by some effective dielectric constant, and the incorporation of the surrounding baths and appropriate boundary conditions into the computation. Much work has been done on the effective dielectric constant of a chain of water molecules inside a narrow pore [86, 120], although in a very narrow region like a channel pore the description of water as an effective dielectric constant may simply be inadequate.

The last issue concerns the connection of the simulation to the surrounding baths. In
particular, the injection and absorption mechanisms of ions must be derived, or equivalently
the boundary conditions of exiting and entering ionic trajectories. This problem occurs
not only in the simulation of ionic motion inside a channel, but also in the more general
setting of ionic motion in an electrolyte bath. Some simulations solve this problem by
employing reflecting or periodic boundary conditions for the ionic trajectories at the edges
of the simulation box [151]. However, simulations with such boundary conditions suffer from
serious limitations, as discussed in the literature. For example, it is not possible to simulate
diffusion between different fixed concentrations. Other simulations with a variable number
of simulated ions consider a larger buffer region in which new ions are injected, and all
trajectories are absorbed at its boundary. However, the injection mechanism of new ions is
usually ad-hoc. In [107, 110] and in chapters 6 and 7 a first attempt to mathematically model
this connection problem, and develop the mathematical law for injection of new particles
into a simulation is presented.

2.4.4 Continuum Models

Last but not least, continuum models have also been applied to study the permeation char-
acteristics of ionic channels. Continuum models describe the permeation process through a
protein channel not with discrete ions, but rather with continuum concentrations and elec-
trostatic potentials. The channel contents are described by concentrations of the different
ionic species, that satisfy Nernst-Planck (drift-diffusion) equations with a force term derived
from an electrostatic potential. This potential is assumed to satisfy the Poisson equation,
which is coupled to the concentrations. The resulting system of equations is denoted in
shorthand the PNP system.

The first applications of the PNP system to channel permeation were done by Eisenberg,
Chen and coworkers [29]-[33], [50]-[52]. In these early treatments, the PNP equations were
solved only inside the channel in a one dimensional setting, with the channel represented as
an effective one dimensional fixed charge profile $P(x)$ along the channel axis. At the two
ends of the channel built in (Donnan) potential boundary conditions were imposed on the
potential and concentrations. Furthermore, as in barrier models, it was assumed that the
potential drop in the surrounding baths is negligible so the potential drop across the channel
is assumed equal to the applied voltage between the electrodes. The diffusion coefficients
of the different permeant ions as well as the permanent charge profile of the channel were
fit parameters of the model. With this simple model it is possible to fit many I-V curves of
different channels at many symmetric and asymmetric concentrations.

Recently, the same set of equations but in three dimensions, denoted as 3D-PNP, was also
applied to channel proteins with known 3-D structure [27, 77, 93]. In these more detailed
models, the PNP equations are solved in a slightly larger region surrounding the channel
which contain a small part of the two surrounding baths. Therefore, natural boundary
conditions for the concentrations and potential are used rather than the previously used
built-in potential boundary conditions. Obviously, computations with these models include
the actual three dimensional (known) channel structure with its fixed charges, instead of
the one dimensional approximation of the channel fixed charge in 1D-PNP. In these models
the only fit parameters are the diffusion coefficients of the permeating ions and the effective
dielectric constant inside the channel.

One of the criticism against the application of the PNP system of equations to channel
permeation concerned the validity of these continuum equations in the narrow microscopic
region of the channel. In chapter 3, we present a derivation of an averaged continuum PNP
type model from an underlying coupled Langevin system of equations of all the ions present
in the system. Thus, while we obtain a different set of PNP equations, this work shows how
continuum equations can describe permeation through a microscopic region.

2.5 Summary

The invention of the patch clamp technique enabled the experimental study of many diverse
characteristics of single channels, ranging from their gating and selectivity properties and
their I-V relations to their response to various macromolecules. Since its invention, a vast
experimental data has been collected for many different channels. However, no satisfactory
theory to explain and predict these measurements has been found yet.

Nowadays, that the structure of quite a few channels has been resolved, there is a need for
a quantitative physical mathematical theory to link structure to function. Its inputs should
be the channel structure, applied voltage, bath concentrations and ionic characteristics such
as the diffusion coefficient, ionic radii, charge, etc. Its output should be the net current
flowing through the channel. In the following chapters we present another few steps towards
this goal.
Chapter 3

Derivation of PNP equations of ion flow through open channels

In this chapter, a derivation of effective PNP equations is presented, starting from a molecular description of the bath and channel. The results of this chapter were published in [138].

3.1 Introduction

As discussed in previous chapters, permeation of ions from one electrolytic solution to another through a protein channel is a biological process of considerable importance. Permeation occurs on a time scale of micro- to milliseconds, far longer than the femtosecond time scales of atomic motion. Direct simulations of atomic dynamics are not yet possible for such long time scales; thus, averaging is unavoidable. The question is what and how to average. In this chapter, we average a Langevin model of ionic motion in bulk solution and protein channel. The main result is a coupled system of averaged Poisson and Nernst Planck equations (CPNP) involving conditional and unconditional charge densities and conditional potentials. The resulting NP equations contain the averaged force on a single ion, which is the sum of two components. The first component is the gradient of a conditional electric potential, which is the solution of Poisson’s equation with conditional and permanent charge densities and boundary conditions according to the applied voltage. The second component is the self induced force on an ion due to surface charges induced only by that ion at dielectric interfaces. The ion induces surface polarization charge that exerts a significant force on the ion itself, not present in earlier PNP equations. The proposed CPNP system, is not complete, however, because the electric potential satisfies Poisson’s equation with conditional
charge densities, conditioned on the location of an ion, while the NP equations contain unconditional densities. The conditional densities are closely related to the well studied pair correlation functions of equilibrium statistical mechanics [21, 71]. We examine a specific closure relation, which on the one hand replaces the conditional charge densities by the unconditional ones in the Poisson equation, and on the other hand replaces the self induced force in the NP equation by an effective self induced force. This effective self induced force is nearly zero in the baths but is approximately equal to the self induced force in and near the channel. The charge densities in the NP equations are interpreted as time averages over long times, of the motion of a quasi-particle that diffuses with the same diffusion coefficient as that of a real ion, but driven by the averaged force. In this way, continuum equations with averaged charge densities and mean-fields can be used to describe permeation through a protein channel.

The Poisson-Nernst-Planck (PNP) equations have been used for the description of macroscopic properties of electro-chemical systems, usually without current flow, and also for the description of currents in semi-conductor devices [112, 140]. These equations have been also quite successfully applied to the description of ionic currents in protein channels of biological membranes [27, 51, 77, 93]. The state variables in the PNP equations are the electrostatic potential and the charge densities of the different ionic species. The PNP equations are usually derived from conservation laws of a continuum formulation [140].

The application of the PNP equations inside narrow channels that can contain only a small number of ions at a time, or in channels where the ions are arranged in a single file, raises interesting conceptual and mathematical problems. For example, what is the meaning of concentration in such context? The PNP equations predict quite accurately the current-voltage response of narrow channels over a wide range of salt concentrations in the bath. However, many phenomena, usually associated with single filing of ions, are missed by the PNP system [51]. The record of current vs time of a single channel in patch clamp and bilayer experiments [72], ranges from noisy to very noisy, depending on the type of channel. Although this is not captured by the PNP system at all, still the average net currents predicted by PNP in an open channel are quite accurately reproduced in several channels whose structure is known [77].

The partial success and partial failure of the PNP system poses the question why? Which results of the PNP model can be accepted and which cannot? The purpose of this chapter is
to partially answer these questions by deriving the PNP equations from a molecular model of ionic motion in bath and channel. As discussed below, the resulting (exact) PNP equations differ from the standard ones used so far.

The point of departure for our derivation is the classical view that ionic motion in solution is governed by electrostatic forces and thermal fluctuations of the solvent [21]. The fluctuations give rise to the diffusive motion of ions in the bath as well as inside the channel. The prediction of macroscopic properties of ionic solutions from the microscopic Brownian motion of their components raises interesting mathematical issues illustrated here with a primitive Langevin model of ionic motion. In this model the electrostatic forces acting on the diffusing ions consist of the Coulombic interactions between the ions, the permanent charges, such as charges on molecules immersed in the solution, induced surface charges on dielectric boundaries, and the effects of an external field. Our simple model of ionic motion assumes that the fluctuating force exerted on an ion by the solvent can be represented as friction and a zero-mean Gaussian stationary force (noise) satisfying Einstein’s (generalized) fluctuation-dissipation principle, and that noises acting on different ions are independent of each other.

Our derivation follows traditional methods of statistical physics, where continuum equations for densities are derived from microscopic laws governing the motion of individual particles (see [44] and the more recent [95]). The case at hand differs from the standard models in that the force term in the equations of motion is governed by a separate Poisson equation. The assumptions on the independence of the noises in the Langevin equations and on the representation of the solvent as a dielectric constant in Poisson’s equation need further examination in concentrated solutions and in multiply occupied protein channels. For other theories of multiply occupied narrow channels see [72] and references therein and the more recent paper [34].

The results of this chapter are the derivation of a coupled system of NP equations for the charge densities and Poisson equations for averaged electric potentials, from a Langevin model of ionic motion. The force in the NP equations has two components, the gradient of an electric potential, and a self induced force of an ion produced by surface charges induced by that ion at dielectric interfaces. Similar NP equations have been derived from hydrodynamical continuity considerations, in the context of macroscopic transport processes in electrolytes [10, 49]. The latter component in our NP equations, e.g. the dielectric self
induced force, seems to be new, although the existence of such a force has been considered in simulations [39]. The proposed PNP system, however, is not complete (i.e. ‘closed’), since the electric potential depends on conditional charge densities, given the location of an ion, while the NP equations contain unconditional densities. Closure relations, mainly applied to equilibrium Poisson-Boltzmann systems, have been the subject of extensive study in the literature [70, 71, 21, 118], and references therein. The proposed theories are based on various physical and excluded volume assumptions, and sometimes lead to different results, especially at high concentrations [125]. In recent years, some work has also been done on non-equilibrium closure relations, mainly in the context of transport processes [10, 46, 47]. However, non-equilibrium closure relations for a non-equilibrium finite system, e.g. for a finite system carrying a steady current, and specifically permeation through confined geometries, are still an open problem. In this chapter we consider a closure relation for the particular case of an electrolytic solution in the presence of a dielectric interface. In this closure relation the conditional charge densities are replaced by the unconditional ones, and the self induced force in the NP equation is replaced by an effective force.

Our derivation of PNP equations is essentially an averaging procedure of a finite but large discrete system. The charge densities in the NP equations are interpreted as time averages over long times of the number of particles per unit volume in the discrete system. This interpretation gives, among others, meaning to densities even in narrow channels that can contain at most one or two ions at a time. Although in the averaging procedure all microscopic phenomena in a narrow channel may be lost—including finite size of the ions, blocking, single filing, noise and so on [72]—still the average net flux is preserved. The average net flux is the variable most relevant to the biological function of most channels.

3.2 Formulation

We consider an electrostatically neutral binary solution confined in a finite volume, between electrodes and impenetrable hard walls. The two electrodes are connected to a feedback apparatus, external to our model, that maintains constant average voltage and concentrations at the electrodes. The feedback mechanism achieves this by removing and feeding back ions as current flows through the system. Thus, on average, the number of ions in the solution is constant. In reality, the voltage, concentrations, flux, and number of ions are not constant.
but rather fluctuate in time. These fluctuations are caused by the random motion of the ions, by the deterministic and random time-delays of the feedback mechanisms, by the finite precision of the measuring devices, and so on.

In our treatment, we neglect these fluctuations and assume that the number of both positive and negative ions in the solution is constant at all times. In particular, we assume that the feedback mechanism is instantaneous; that is, when an ion reaches one electrode, it is immediately reinjected into the solution, either at this electrode or at the opposite one. Thus, the total flux of particles on the boundary of the system vanishes at all times. This approximation reduces the resolution of our analysis but is a reasonable representation of the typical experimental situation. These fluctuations are indeed negligible for an experimental system in which the electrodes are placed far away from the region of biological or chemical interest. Fluctuations are significant in computer simulations, in which the total number of ions is relatively small, and thus must be taken into account [110].

We consider a solution containing $N$ positive and $N$ negative ions. We denote the coordinates of a point by $\mathbf{x} = (x, y, z)$. We number the ions in the solution at time $t = 0$ and denote the vectors of coordinates and velocity of the $j$-th positive ion at time $t$ by $\mathbf{x}_j^p(t)$ and $\dot{\mathbf{x}}_j^p(t)$, respectively, and those of the $k$-th negative ion by $\mathbf{x}_k^n(t)$ and $\dot{\mathbf{x}}_k^n(t)$. The coordinate vector of all ions in the $6N$-dimensional configuration space is denoted $\mathbf{\tilde{x}} = (\mathbf{x}_1^p, \ldots, \mathbf{x}_N^p, \mathbf{x}_1^n, \ldots, \mathbf{x}_N^n)$, while in analogy, the vector of all velocities is denoted as $\mathbf{\tilde{v}}$ or $\dot{\mathbf{\tilde{x}}}$. For future reference, the vector of coordinates of all $2N - 1$ ions, excluding the $j$-th positive ion is denoted $\mathbf{\tilde{x}}_j^p$.

### 3.2.1 Equations of motion

Ionic solutions nearly always contain many more water molecules than ions, even when they are nearly saturated, at their solubility limit. For example, there are about 55 water molecules per ion in a 1 M salt solution, and at biological concentrations of 100 mM, there are about 550 water molecules per ion. Thus, the collective motion of only the ions (without the water) is a lower dimensional projection of the joint motion of all water and salt molecules in the solution, that can be approximated by a system of generalized Langevin equations [18]. These equations assume that the thermal motion of ions is due mainly to the thermal motion of the surrounding water molecules. There is of course another contribution to the diffusive motion of each ion, from its interactions with the other ions, as in an ionic plasma. This effect decreases as the solution becomes more dilute.
The generalized Langevin equations involve a friction memory kernel and correlated noise, satisfying a generalized fluctuation-dissipation principle [18]. As the motion of ions in solution is strongly overdamped, the correlation time of the noise is much shorter than the characteristic diffusion time scale considered here. On a sufficiently coarse time scale, much longer than the relaxation time of the solution, memory effects can be neglected [48]. Thus, our point of departure is the memoryless system of Langevin equations,

\[
\ddot{x}_j^p + \gamma^p(x_j^p)\dot{x}_j^p = \frac{f_j^p(\bar{x})}{m^p} + \sqrt{2\gamma^p(x_j^p)k_BT/m^p} \dot{w}_j^p, \quad (j = 1, 2, \ldots, N),
\]

\[
\ddot{x}_k^n + \gamma^n(x_k^n)\dot{x}_k^n = \frac{f_k^n(\bar{x})}{m^n} + \sqrt{2\gamma^n(x_k^n)k_BT/m^n} \dot{w}_k^n, \quad (k = 1, 2, \ldots, N),
\]

where a dot on a variable denotes differentiation with respect to time, \(f_j^p(\bar{x})\) and \(f_k^n(\bar{x})\) denote the electrostatic forces acting on the \(j\)-th positive ion and on the \(k\)-th negative ion, respectively, \(\gamma^c(x)\) is the location dependent friction coefficient per unit mass of the ionic species of type \(c\), \((c = p, n)\), and \(\dot{w}_j^c\) are, by assumption, independent standard Gaussian white noises. The parameter \(k_B\) is Boltzmann’s constant, \(T\) is absolute temperature, and \(m^c\) is an effective mass of an ion of type \(c\).

In addition we assume that positive and negative ions have radii \(a_p\) and \(a_n\), respectively, and that there are hard wall potentials between ions, preventing oppositely charged ions from collapsing into each other.

The physical three-dimensional domain in which our system is confined, called \(\Omega\), is shown in figure 3.1. Its boundary, \(\partial\Omega\), consists of two parts. One is the electrodes, and the other one is impermeable walls. The \(6N\)-dimensional configuration space of the trajectories of all \(2N\) particles in the Langevin system (3.1) is confined to the domain

\[
\tilde{\Omega} = \bigotimes_{j=1}^{2N} \Omega_j,
\]

where \(\bigotimes\) denotes Cartesian product, and \(\Omega_j\) is the three dimensional physical domain of the \(j\)-th particle, identical to \(\Omega\). The boundary of the domain \(\tilde{\Omega}\) is

\[
\partial\tilde{\Omega} = \bigcup_{j=1}^{2N} \Omega_1 \times \Omega_2 \times \cdots \times \Omega_{j-1} \times \partial\Omega_j \times \Omega_{j+1} \times \cdots \Omega_{2N},
\]

which consists of all points in the \(6N\) dimensional space, where at least one of the \(2N\) components is on the boundary of the corresponding region \(\Omega_j\).
The boundary behavior of the random trajectories of the Langevin equations (3.1) reflects the boundary conditions imposed on the system at the boundary $\partial \Omega$. Every trajectory that reaches an electrode is instantaneously injected at the opposite one. Trajectories that reach other boundaries of the domain are reflected [51], [54]. The fluctuations removed by this idealized electrode (voltage clamp) boundary conditions will be studied elsewhere.

### 3.2.2 The electrostatic forces

As stated in the introduction, we assume that the electrolytic solution is a fast bath, namely that the relaxation time of the solvent water is very fast so that the dielectric coefficient of the pure solvent is time independent. We also assume that the potential in the bath can be represented as the solution of Poisson’s equation, and not of the time dependent Maxwell equations. The effects of displacement current and possible radiation will be examined elsewhere. The electrostatic force acting on an ion is then computed from the electrostatic potential $\phi(x)$, a solution of Poisson’s equation

$$\Delta \phi(x) = -\frac{1}{\varepsilon_0} \left[ e\rho_{\text{perm}}(x) + e \sum_j \delta(x - x_j^p) - e \sum_k \delta(x - x_k^n) \right] + \nabla \cdot P(x), \quad (3.2)$$

where $\rho_{\text{perm}}(x)$ is the permanent charge density, and $P(x)$ is the polarization field, that describes the charge induced by the electric field in matter that otherwise would be charge neutral [81]. In an isotropic medium with linear response, the polarization field is connected to the local field by

$$P(x) = \chi E(x) = -\chi \nabla \phi(x), \quad (3.3)$$

where $\chi(x) = \varepsilon(x) - 1$ is the dielectric susceptibility of the medium [81]. Combining (3.3) and (3.2), we obtain the familiar form of Poisson’s equation

$$\nabla \cdot \varepsilon(x) \nabla \phi(x) = -\frac{1}{\varepsilon_0} \left[ e\rho_{\text{perm}}(x) + e \sum_j \delta(x - x_j^p) - e \sum_k \delta(x - x_k^n) \right].$$

In addition, the potential $\phi(x)$ satisfies boundary conditions on the electrodes and the standard continuity conditions of the electric displacement vector across surfaces of discontinuity of the dielectric coefficient [81].

The electrostatic force on the $j$-th positive ion at position $x_j^p$, denoted $f_j^p$, is calculated from the potential $\phi(x)$ as follows

$$f_j^p = -e \nabla \left( \phi(x) - \frac{1}{4\pi\varepsilon_0\varepsilon(x_j^p)} \frac{1}{|x - x_j^p|} \right) \bigg|_{x = x_j^p}. \quad (3.4)$$
The last term on the right hand side of equation (3.4) removes the singularity of the potential \( \phi(x) \) at the location of the ion, (see Appendix for details).

This force, which in general depends on the coordinate vector \( \bar{x} \) of all the charges in the system, can be decomposed into two components,

\[
f_j^p = f_j^p(\bar{x}, P) + f_D(x_j^p).
\]  

(3.5)

The first component, \( f_j^p(\bar{x}, P) \), includes the interaction forces of the \( j \)-th positive ion with all other ions in the solution, with the permanent charge, with the charges on the electrodes that maintain a fixed applied voltage, and with the surface charges induced by these charges. The second component, \( f_D(x_j^p) \), is a self induced force produced by the surface charges induced at the dielectric interfaces by the ion at \( x_j^p \). If there are no dielectric interfaces, this induced force is zero. Note that this force component is proportional to the square of the ion’s charge, regardless of its sign. For example, it is equal for monovalent anions and cations (see Appendix for details).

While the first component depends on the locations of all charges in the system, the second component depends only on the location of the \( j \)-th positive ion, and is independent of the location of all the other charges in the system, and in particular of the applied voltage. The computation of this self induced force component is described in the Appendix. The decomposition (3.5) of the total force into these two components is particularly important in the averaging process described below.

The first component of the force, \( f_j^p(\bar{x}, P) \), is obtained from the solution of Poisson’s equation (3.2), with the charge of the \( j \)-th positive ion removed from the right hand side. We denote the resulting electric potential by \( \phi_j^p(x, \bar{x}_j^p) \), determined from

\[
\nabla_x \cdot \varepsilon(x) \nabla_x \phi_j^p(x, \bar{x}_j^p) = -\frac{1}{\varepsilon_0} \left[ e \rho_{\text{perm}}(x) + e \sum_{j' \neq j} \delta(x - x_{j'}^p) - e \sum_k \delta(x - x_k^n) \right],
\]  

(3.6)

with the same boundary and continuity conditions mentioned above for the potential \( \phi(x) \). Here \( \nabla_x \) denotes the gradient with respect to the variable \( x \),

\[
\nabla_x = \left( \frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z} \right).
\]

In terms of this potential, the first component of the force, \( f_j^p(\bar{x}, P) \), is given by

\[
f_j^p(\bar{x}, P) = -e \nabla_x \phi_j^p(x, \bar{x}_j^p) \bigg|_{x = x_j^p}.
\]  

(3.7)
The second component of the force, \( f_D(x_j^p) \), is given by
\[
f_D(x_j^p) = -e \nabla_x \left( \phi_D(x, x_j^p) - \frac{1}{4\pi \varepsilon(x_j^p)} \frac{e}{|x - x_j^p|} \right) \bigg|_{x = x_j^p},
\] (3.8)
where \( \phi_D(x, y) \) satisfies Poisson’s equation
\[
\nabla_x \cdot \varepsilon(x) \nabla_x \phi_D(x, y) = -\frac{e}{\varepsilon_0} \delta(x - y),
\] (3.9)
with zero potential boundary conditions at the electrodes, (see Appendix for derivation).

### 3.3 Derivation of the PNP equations

Equations (3.1)-(3.4) form a discrete high-dimensional system of coupled stochastic and partial differential equations that describe the time evolution of this many particle system. We derive a simplified, though approximate, description of this system in which ions are described as continuum charge densities. In this approximation the description of the system is reduced to averaged charge densities and electric potentials governed by Nernst-Planck and Poisson equations.

The outline of the computation is as follows. First, the connection between the positive ion concentration and the stationary probability density function (pdf) of a single ion is derived in Section 3.1. In Section 3.2, the Fokker-Planck equation for the joint pdf of all ions in the system is formulated. The probability density of a single positive ion is obtained by integration of the joint pdf over the phase space of all ions, excluding a single positive ion. This procedure results in a Fokker-Planck type equation (FPE) for the pdf of the phase space coordinates of a single ion. The resulting equation, however, is not a standard FPE because it contains an average force term that depends on the probability density of all other ions. Since this probability density depends on the unknown solution of the full FPE for all ions, the force term in the FPE equation is in general unknown. In Section 3.4 we consider the large friction limit of this FPE, resulting in a reduced Smoluchowski equation for only the pdf of the location of the ion, with a still unknown force function. According to the results of Section 3.1, this Smoluchowski equation turns into a Nernst-Planck equation for the averaged charge density.

The average force appearing in these equations, which depends on the probability density of all other ions, is evaluated in Section 3.5. We separate the average force into two
components. One is the self induced force that an ion exerts on itself arising from charge induced by the ion at boundaries between regions of different dielectric coefficient. It might be called a dielectric boundary force. The other component is an average force due to the interaction of the ion with all other charges in the system, e.g., the permanent charge on the protein, the ions in the bath, and the charges on the electrodes that maintain the voltage clamp boundary condition. The second term can be written as the gradient of an averaged potential, described by an averaged Poisson equation. The averaged Poisson equation, however, contains conditional charge densities, rather than the unconditional charge densities present in the Nernst-Planck equation just described. This key result is a direct consequence of the averaging procedure, and is not an assumption. Thus, the resulting system, denoted CPNP, is not closed, i.e., it is incomplete. In Section 3.6, we examine one specific closure relation and its consequences. This closure relation replaces the conditional charge densities by the unconditional ones, neglecting the finite size of ions. This approximation leads to a (closed) PNP-type system of equations, but with an additional effective induced force term in the NP equations.

### 3.3.1 Charge density and probability density

We use $c_p(x)$ to describe the time-averaged steady state charge density of the positive species at location $x$, and $p^p_j(x)$, $j = 1, \ldots, N$, to define the stationary probability density function (pdf) of the location of the $j$-th positive ion.

These two quantities are related as follows. By definition, for a small volume $\Delta x$ around the point $x$, the product $c_p(x)\Delta x$ denotes the time-averaged number of ions in this volume. We introduce $\chi(x, \Delta x)$ as the indicator function of the volume $\Delta x$,

$$
\chi(x, \Delta x) = \begin{cases} 
1 & \text{if } x \in \Delta x, \\
0 & \text{otherwise}. 
\end{cases}
$$

We abbreviate $\chi_j = \chi(x^p_j(t), \Delta x)$. By definition,

$$
c_p(x)\Delta x = \sum_j E\{\chi_j\} = \sum_j \Pr\{\chi(x^p_j(t), \Delta x) = 1\},
$$

where $E\{\cdot\}$ denotes the expected value operator.

In the steady state, for small volumes $\Delta x$

$$
\Pr\{\chi(x^p_j(t), \Delta x) = 1\} = p^p_j(x)\Delta x + O((\Delta x)^2).
$$
Moreover, for a stationary system in steady state with a single species of positive ions, from symmetry considerations the probability of finding the $j$-th positive ion at location $x$ is the same as that of finding any other positive ion there. Thus, the probability density $p_j^p(x)$ is independent of the index $j$, and we denote it simply by $p(x)$. Hence, in the limit $|\Delta x| \to 0$,

$$c_p(x) = N p(x). \quad (3.10)$$

Thus, the density $c_p(x)$ is $N$ times the stationary pdf of the $j$-th positive ion. Similarly, the charge density of negative ions $c_n(x)$ is $N$ times the stationary marginal, i.e. integrated, pdf of the $k$-th negative ion. These densities are independent of the indices $j$ and $k$, respectively. The density $c_p(x)$ is the physical density of positive ions and it integrates to the total number of positive ions in the solution.

In mixed solutions, for example Na$^+$, Ca$^{2+}$ and Cl$^-$, the analog of equation (3.10) holds for each species separately. This will lead to different Nernst-Planck equations for each species and other complexities in the analysis, as discussed later on.

### 3.3.2 The multi-dimensional Fokker-Planck equation

Equation (3.10) shows the connection between the stationary pdf of a single positive ion $p(x)$ and the macroscopic charge density $c_p(x)$. Therefore, we now derive an equation for $p(x)$, which by relation (3.10) readily turns into an equation for $c_p(x)$. We start from an equation for the stationary joint probability density of all ions, and integrate it over the phase space coordinates of all ions, excluding a single positive ion, to obtain an equation for the marginal, i.e. integrated, density of this ion.

The joint transition probability density of all ions is defined as

$$p(\tilde{x}, \tilde{v}, t \mid \tilde{\xi}, \tilde{\eta}, s) = \Pr \left\{ \tilde{x}(t) = \tilde{x}, \tilde{v}(t) = \tilde{v} \mid \tilde{x}(s) = \tilde{\xi}, \tilde{v}(s) = \tilde{\eta} \right\}, \quad (3.11)$$

where $\tilde{\xi}$ and $\tilde{\eta}$ are the phase space coordinates of all ions at some initial time $s$. The stationary pdf of all ions is defined as the long time limit of the transition pdf (3.11),

$$p(\tilde{x}, \tilde{v}) = \lim_{t \to \infty} p(\tilde{x}, \tilde{v}, t \mid \tilde{\xi}, \tilde{\eta}, s), \quad (3.12)$$

and is independent of the initial values $\tilde{\xi}, \tilde{\eta}$ at time $s$. 39
The motion of all ions is governed by the Langevin system (3.1), so the stationary pdf (3.12) satisfies the multi-dimensional stationary Fokker-Planck equation [137]

\[ 0 = \sum_j \mathcal{L}^p_j p + \sum_k \mathcal{L}^n_k p, \tag{3.13} \]

where \( \mathcal{L}^p_j \) and \( \mathcal{L}^n_k \) are the Fokker-Planck operators acting on the phase space coordinates of the \( j \)-th positive and \( k \)-th negative ion, respectively. They are given by

\[
\mathcal{L}^c_j p = -v^c_j \cdot \nabla x^c_j p + \nabla v^c_j \cdot \left( \gamma^c_j(x^c_j) v^c_j - \frac{f^c_j}{m^c} \right) p + \Delta v^c_j \frac{\gamma^c_j(x^c_j) k_B T_j}{m^c} p, \quad (c = p, n),
\]

where the operators \( \nabla x \) and \( \Delta x \) denote the gradient and the Laplacian with respect to the variable \( x \), respectively. Equation (3.13) is defined in the \( 12N \) dimensional region \( \tilde{x} \in \tilde{\Omega} \) and \( \tilde{v} \in \mathbb{R}^{6N} \).

The FPE (3.13) can be written as a conservation law

\[
0 = - (\nabla \tilde{v}, \nabla \tilde{x}) \cdot (J_{\tilde{v}}, J_{\tilde{x}}),
\]

\[
= - \sum_{j=1}^{N, c=p,n} \left( \nabla v^c_j \cdot J v^c_j + \nabla x^c_j \cdot J x^c_j \right), \tag{3.14}
\]

where \( J_{\tilde{v}} = (J v^p_j, J v^n_k) \), and \( J_{\tilde{x}} = (J x^p_j, J x^n_k) \), \( (j = 1, \ldots, N, k = 1, \ldots, N) \), are 2\( N \)-dimensional flux density vectors whose components are the three dimensional flux densities

\[
J v^c_j = - \left( \gamma^c_j(x^c_j) v^c_j - \frac{f^c_j(x^c_j, \tilde{v})}{m^c} \right) p(\tilde{x}, \tilde{v}) - \nabla v^c_j \frac{\gamma^c_j(x^c_j) k_B T_j}{m^c} p(\tilde{x}, \tilde{v}),
\]

\[
J x^c_j = v^c_j p(\tilde{x}, \tilde{v}). \tag{3.15}
\]

These three-dimensional components of the flux density vector represent the probability flux densities of the individual ions [111].

The boundary conditions for the FPE (3.13) are determined from the boundary behavior imposed on the trajectories of the Langevin equations (3.1). They can be expressed in terms of the three dimensional components of the flux vector \( J = (J_{\tilde{x}}, J_{\tilde{v}}) \). Specifically, on the insulating (reflecting) part of the boundary where particle trajectories are reflected, the following condition holds,

\[
\left. J x^c_j(\tilde{x}, \tilde{v}) \cdot n \right|_{x^c_j \in \partial \Omega} = -J x^c_j(\tilde{x}, \tilde{v}) \cdot n \left|_{x^c_j \in \partial \Omega} \right. \quad \left. v^c_j \cdot n = v \right|_{x^c_j \in \partial \Omega} \quad \left. v^c_j \cdot n = -v \right|_{x^c_j \in \partial \Omega} \tag{3.16}
\]
where \( n \) denotes the unit normal to the boundary.

On the electrode boundaries, where particles are recycled and the electric potential is controlled, the boundary condition is that the influx at one electrode equals the efflux on the other for each component of the probability flux density. Therefore, the total flux on the boundary vanishes for each one of the \( 2N \) components of the flux vector \( \mathbf{J}_{\mathbf{x}} \),

\[
\int_{\partial \Omega} \mathbf{J}_{\mathbf{x}}^c \cdot \mathbf{n} dS = 0, \tag{3.17}
\]

where \( dS \) is a surface element on the boundary.

Last but not least, the hard wall potentials at a finite distance from the center of each ion also transform into reflecting boundary conditions for the fluxes of other ions, thus preventing the collapse of positive and negative ions onto each other.

### 3.3.3 The probability density of a single ion

We now consider the probability density of the \( j \)-th positive ion. This is the marginal (integrated) density of the joint probability density of all ions,

\[
p(x_j^p, v_j^p) = \int \int \hat{\mathcal{O}}_j^p \times \mathbb{R}^{6N-3} \ p(\mathbf{x}, \mathbf{v}) \ dx_j^p \ dv_j^p, \tag{3.18}
\]

where \( \hat{\mathcal{O}}_j^p \) is the configuration space of all ions in \( \Omega \), except the \( j \)-th positive ion and \( \mathbb{R}^{6N-3} \) is the space of velocities of all ions except the \( j \)-th positive ion. That is, the right hand side contains an integration over the \( 12N - 6 \) positions and velocities of all particles, excluding the \( j \)-th positive ion.

To obtain an equation for \( p(x_j^p, v_j^p) \), we integrate equation (3.14) with respect to the phase space coordinates of all ions except those of the \( j \)-th positive ion,

\[
0 = - \int \int \hat{\mathcal{O}}_j^p \times \mathbb{R}^{6N-3} \ \nabla \cdot \mathbf{J}(\mathbf{x}, \mathbf{v}) \ dx_j^p \ dv_j^p. \tag{3.19}
\]

We separate the integrand \( \nabla \cdot \mathbf{J}(\mathbf{x}, \mathbf{v}) \) into the component of the \( j \)-th positive ion, and to the remaining \( 2N - 1 \) other components. For these \( 2N - 1 \) components we apply the divergence theorem in the \( 12N - 6 \) dimensional phase space of the integration. First, we consider the velocity flux components, \( \mathbf{J} v_j^p \). For these components, the integration is over all possible velocities. These terms vanish after the application of the divergence theorem because the
velocity flux decreases exponentially fast for large absolute velocities, simplifying (3.19) to

\[0 = -\int \int \tilde{\Omega}_j^p \times \mathbf{R}^{6N-3} \left( \nabla \mathbf{v}_j^p \cdot \mathbf{J} \mathbf{v}_j^p + \nabla \mathbf{x}_j^p \cdot \mathbf{J} \mathbf{x}_j^p \right) d\hat{x}_j^p d\hat{v}_j^p - \sum_{i \neq j} \int \int \partial \Omega_i^p \times \mathbf{R}^{6N-3} \mathbf{J} \mathbf{x}_i^p(\tilde{x}, \tilde{v}) \cdot \mathbf{n}(\mathbf{x}_i^p) dS_{\mathbf{x}_i^p} d\hat{v}_i^p - \sum_k \int \int \partial \Omega_k^p \times \mathbf{R}^{6N-3} \mathbf{J} \mathbf{x}_k^p(\tilde{x}, \tilde{v}) \cdot \mathbf{n}(\mathbf{x}_k^p) dS_{\mathbf{x}_k^p} d\hat{v}_k^p, \tag{3.20}\]

where \( \mathbf{J} \mathbf{v}_j^p \) and \( \mathbf{J} \mathbf{x}_j^p \) are given by equation (3.15) and \( dS_{\mathbf{x}_k^p} \) denotes integration over the surface of the boundary. By definition, the last two terms in eq. (3.20) are the total probability flux on the boundary \( \partial \Omega \) of all ions besides the \( j \)-th positive ion. Due to the specified boundary conditions (3.16) for the Fokker-Planck equation, the total probability flux of each ion on the boundary is zero, eq. (3.17), so the last two terms in equation (3.20) vanish.

Consider the remaining first term on the right hand side of (3.20). Inserting (3.15) into (3.20) gives

\[0 = \int \int \tilde{\Omega}_j^p \times \mathbf{R}^{6N-3} \nabla \mathbf{v}_j^p \cdot \left( \gamma^p(\mathbf{x}_j^p) \mathbf{v}_j^p - \frac{f_j^p(\tilde{x})}{m^p} + \frac{\gamma^p(\mathbf{x}_j^p) k_B T}{m^p} \nabla \mathbf{v}_j^p \right) p(\tilde{x}, \tilde{v}) d\hat{x}_j^p d\hat{v}_j^p + \int \int \tilde{\Omega}_j^p \times \mathbf{R}^{6N-3} \nabla \mathbf{x}_j^p \cdot \mathbf{v}_j^p p(\tilde{x}, \tilde{v}) d\hat{x}_j^p d\hat{v}_j^p.\]

Note that since integration is over the phase space coordinates of all ions excluding the \( j \)-th positive ion, the differentiation operators \( \nabla \mathbf{x}_j^p \) and \( \nabla \mathbf{v}_j^p \), as well as all other terms that depend only on \( \mathbf{x}_j^p \) or \( \mathbf{v}_j^p \) can be taken out of the integration. Using definition (3.18), we obtain

\[0 = \nabla \mathbf{v}_j^p \cdot \gamma^p(\mathbf{x}_j^p) \mathbf{v}_j^p p(\mathbf{x}_j^p, \mathbf{v}_j^p) - \nabla \mathbf{v}_j^p \cdot \int \int \tilde{\Omega}_j^p \times \mathbf{R}^{6N-3} \frac{f_j^p(\tilde{x})}{m^p} p(\tilde{x}, \tilde{v}) d\hat{x}_j^p d\hat{v}_j^p - \frac{\gamma^p(\mathbf{x}_j^p) k_B T}{m^p} \Delta \mathbf{v}_j^p p(\mathbf{x}_j^p, \mathbf{v}_j^p) - \mathbf{v}_j^p \cdot \nabla \mathbf{x}_j^p p(\mathbf{x}_j^p, \mathbf{v}_j^p). \tag{3.21}\]

The only term for which the marginal pdf of the \( j \)-th positive ion \( p(\mathbf{x}_j^p, \mathbf{v}_j^p) \) could not be recovered from the integration is the mixed term \( f_j^p(\tilde{x}) p(\tilde{x}, \tilde{v}) \). To recover the pdf \( p(\mathbf{x}_j^p, \mathbf{v}_j^p) \) from this term as well, we denote by \( p(\tilde{x}_j^p, \tilde{v}_j^p | \mathbf{x}_j^p, \mathbf{v}_j^p) \) the conditional pdf of all ions excluding the \( j \)-th positive ion, given the phase space coordinates of the \( j \)-th positive ion. We write

\[p(\tilde{x}, \tilde{v}) = p(\tilde{x}_j^p, \tilde{v}_j^p | \mathbf{x}_j^p, \mathbf{v}_j^p) p(\mathbf{x}_j^p, \mathbf{v}_j^p),\]
and set
\[ \bar{f}^p(x^p_j, v^p_j) = \int_{\tilde{Q}^p_j \times \mathbb{R}^{6N-3}} \int f_{jp}^p(\tilde{x}) p(\tilde{x}^p_j, \tilde{v}^p_j | x^p_j, v^p_j) \, d\tilde{x}^p_j \, d\tilde{v}^p_j. \]  
(3.22)

In equation (3.22), the integration over the velocity vector \( \tilde{v}^p_j \) can be carried out since the force \( f_{jp}^p \) depends only on the locations of the ions, and not on their velocities. This simplifies the last equation to
\[ \bar{f}^p(x^p_j, v^p_j) = \int_{Q^p_j} f_{jp}^p(\tilde{x}^p_j) p(\tilde{x}^p_j | x^p_j, v^p_j) \, d\tilde{x}^p_j, \]  
(3.23)
where \( p(\tilde{x}^p_j | x^p_j, v^p_j) \) denotes the conditional density of the locations of all other ions, excluding the \( j \)-th positive ion, given the location and velocity of the \( j \)-th positive ion.

With these definitions, and suppressing the index \( j \), equation (3.21) becomes
\[ 0 = -v \cdot \nabla x p(x, v) + \nabla v \cdot \left( \gamma^p(x) v - \frac{\bar{f}^p(x, v)}{m^p} \right) p(x, v) + \Delta v \frac{\gamma^p(x) k_B T}{m^p} p(x, v). \]  
(3.24)

Equation (3.24) is a Fokker-Planck equation for the probability density of a single positive ion. It contains an average force \( \bar{f}^p(x, v) \) whose calculation, according to eq.(3.23), depends on the conditional probability density of all ions, given the phase space coordinates of the \( j \)-th positive ion. However, this conditional density depends on the solution of the full Fokker-Planck equation (3.13). Therefore all of these quantities are coupled, and the Fokker-Planck equation for the stationary probability density of a single ion cannot be solved independently of the full Fokker-Planck equation of all other ions. Obviously, equation (3.24) is not very useful as long as its force term is not known. Note that although the forcing function \( \bar{f}^p(x, v) \) is not known, equation (3.24) is exact.

### 3.3.4 The overdamped limit

We consider the Smoluchowski limit of large friction, because the motion of a single ion is strongly overdamped. The first approximation assumes that (in this limit) the conditional probability density of the locations of all ions, excluding the \( j \)-th positive ion, given its phase space coordinates, depends only on the position of the ion and not on its velocity,
\[ p(\tilde{x}^p_j | x^p_j, v^p_j) = p(\tilde{x}^p_j | x^p_j). \]  
(3.25)

This approximation makes the force \( \bar{f}^p \) in equation (3.23) dependent only on the location of the ion, and not on its velocity,
\[ \bar{f}^p(x) = \int f_{jp}^p(\tilde{x}) p(\tilde{x}^p_j | x^p_j = x) \, d\tilde{x}^p_j, \]  
(3.26)
where $p(\tilde{x}_j^p | x_j^p = x)$ is the stationary marginal (integrated) conditional density of the locations of all ions, excluding the $j$-th positive ion, given it is located at $x$.

With this approximation, the stationary pdf of the phase space coordinates of a single positive ion, $p(x, v)$, satisfies the FPE (3.24) with a forcing function that depends only on the location of the ion. In this case – in the limit of large friction – the marginal pdf of the ions’ location $p(x)$ satisfies the Smoluchowski equation [54]

$$0 = -\nabla \cdot J(x) = -\nabla \cdot \left( \frac{\tilde{f}_p^p(x)}{m^p \gamma^p(x)} p(x) - \frac{k_B T}{m^p \gamma^p(x)} \nabla p(x) \right),$$

(3.27)

while the full pdf $p(x, v)$ has the form

$$p(x, v) = \left( \frac{2\pi k_B T}{m^p} \right)^{-3/2} \exp \left( -\frac{m^p v^2}{2k_B T} \right) \left[ p(x) + \frac{m^p}{k_B T} J(x) \cdot v + O \left( \frac{1}{\Gamma^2} \right) \right],$$

where $J$ is the flux defined by the Smoluchowski equation (3.27) and $\Gamma$ is a measure of the friction.

Relation (3.10) converts equation (3.27) for the pdf of a single particle into a Nernst-Planck equation for the macroscopic positive charge density, $c_p(x)$, depending on the yet undetermined average force $\tilde{f}^p$,

$$0 = -\nabla \cdot \left( \frac{\tilde{f}_p^p(x)}{m^p \gamma^p(x)} c_p(x) - \frac{k_B T}{m^p \gamma^p(x)} \nabla c_p(x) \right).$$

(3.28)

Equation (3.28) depends on the approximation (3.25)-(3.26) but is otherwise exact. Obviously, a similar equation holds for the negative charge density $c_n(x)$, albeit with a different force $\tilde{f}^n(x)$, and the respective friction and mass coefficients, $\gamma^n(x)$ and $m^n$.

### 3.3.5 The averaged force

The Nernst-Planck equation (3.28) for the stationary charge density of the positive ions contains a yet undetermined average force, $\tilde{f}^p(x)$, given by equation (3.26). This force can be simplified considerably by noting that the force term $f_{j}^p(\tilde{x})$ can be represented as a sum of two terms, $f_D(x_j^p)$ and $f_{j}^p(\tilde{x}, P)$, according to (3.5). The latter term can be decomposed even further as follows,

$$f_{j}^p(x) = f_D(x_j^p) + f_{j}^p(\tilde{x}, P)$$

$$= f_D(x_j^p) - e \nabla_x \left( \phi_{perm}(x_j^p) + \sum_{i \neq j} \phi_D(x, x_j^p) - \sum_k \phi_D(x, x_k^n) \right) \bigg|_{x=x_j^p}.$$
where \( f_D(x^p_j) \) is the self induced force of the ion, given by (3.8), \( \phi_{\text{perm}}(x) \) is the potential at \( x \) created by the permanent charge and the applied voltage, and \( \phi_D(x, y) \) is the potential at \( x \) created by a positive ion located at \( y \), with no applied voltage. The potential \( \phi_D(x, y) \) satisfies Poisson’s equation (3.9).

We now insert decomposition (3.29) of \( f^p \), into (3.26). The first two terms in (3.29) depend only on the location of the \( j \)-th positive ion and are thus constant with respect to the integration variables. Furthermore, each one of the remaining terms in the two sums is a function of only one of the integration variables. Thus, integration with respect to the other variables can be performed. This integration reduces the conditional pdf \( p(\tilde{x}^p | x^p_j = x) \) to the marginal (integrated) conditional pdf \( p(x^c_i | x^p_j = x) \) of the variable not integrated so far

\[
p(x^c_i | x^p_j = x) = \int p(\tilde{x}^p_i | x^p_j = x) \prod_{x_k \neq x^p_j, x^c_i} dx_k.
\]

Performing these integrations in (3.26) yields

\[
\bar{f}^p(x) = f_D(x) - e \nabla_y \phi_{\text{perm}}(y) \bigg|_{y=x} - e \nabla_y \left\{ \sum_{i \neq j} \int_\Omega \phi_D(y, x^p_i)p(x^p_i | x^p_j = x) dx^p_i \right\} \bigg|_{y=x} + e \nabla_y \left\{ \sum_k \int_\Omega \phi_D(y, x^p_k)p(x^p_k | x^p_j = x) dx^p_k \right\} \bigg|_{y=x},
\]

(3.30)

where \( \nabla_y \) denotes gradient with respect to \( y \). In the steady state all positive ions of the same species are indistinguishable and therefore interchangeable, so the conditional pdf’s of the different positive ions, given the position of the \( j \)-th positive ion, are all equal. The same property holds of course for the negative ions as well. Thus, we conclude that all terms in the first sum in equation (3.30) are equal to each other, and so are the terms in the second sum.

We denote by \( c_p(y | x) \) and \( c_n(y | x) \) the positive and negative conditional charge densities at \( y \), given that a positive ion is located at \( x \). Arguments similar to those in Section 3.1 show that

\[
c_p(y | x) = (N-1)p(x^p_i = y | x^p_j = x),
\]

\[
c_n(y | x) = Np(x^p_k = y | x^p_j = x).
\]

(3.31)

In terms of these quantities, the total force can be written as

\[
\bar{f}^p(x) = f_D(x) - e \nabla_x \phi_{\text{perm}}(x)
\]

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\[- e \nabla z \left\{ \int_{\Omega} \phi_D(z, y) \left[ c_p(y | x) - c_n(y | x) \right] \, dy \right\} \bigg|_{z=x} \cdot (3.32) \]

We define a potential \( \bar{\phi}^p(z | x) \) by

\[
\bar{\phi}^p(z | x) = \phi_{\text{perm}}(z) + \int_{\Omega} \phi_D(z, y) \left[ c_p(y | x) - c_n(y | x) \right] \, dy.
\]

Since \( \phi_D(y, x) \) satisfies equation (3.9), it follows that \( \bar{\phi}^p(z | x) \) satisfies the Poisson equation

\[
\nabla y \cdot \left[ \varepsilon(y) \nabla y \bar{\phi}^p(y | x) \right] = -\frac{e}{\varepsilon_0} \left[ \rho_{\text{perm}}(y) + c_p(y | x) - c_n(y | x) \right], \quad (3.33)
\]

with the applied voltage conditions on the electrodes. Obviously, the average force \( \bar{f}^n(x) \) appearing in the NP equation for the negative charge densities can be written as the sum of the self force and the gradient of an analogous electric potential \( \bar{\phi}^n(y | x) \).

To conclude, the averaging procedure described in Sections 3.1-3.4 show that the macroscopic charge densities \( c_p(x) \) and \( c_n(x) \), satisfy the following Nernst-Planck equations,

\[
0 = \nabla \cdot \left[ \frac{1}{m^p \gamma^p(x)} c_p(x) \left( e \nabla y \bar{\phi}^p(y | x) \bigg|_{y=x} - f_D(x) \right) + \frac{k_B T}{m^p \gamma^p(x)} \nabla c_p(x) \right]
\]

\[
0 = \nabla \cdot \left[ \frac{1}{m^n \gamma^n(x)} c_n(x) \left( -e \nabla y \bar{\phi}^n(y | x) \bigg|_{y=x} - f_D(x) \right) + \frac{k_B T}{m^n \gamma^n(x)} \nabla c_n(x) \right], \quad (3.34)
\]

with averaged mean field potentials that satisfy Poisson equations with conditional charge densities, and with the additional self-force term \( f_D(x) \). The charge densities in the Poisson equation are different from the unconditional charge densities in the NP equations. We denote the resulting system (3.33)-(3.34) as the CPNP system. This system differs from the standard PNP system, because: (i) It contains conditional potentials satisfying Poisson equations with conditional charge densities; (ii) it contains the self induced force.

Note that there are different conditional Poisson equations for the positive and negative species. For a mixed solution, we would have a separate NP equation and a separate conditional Poisson equation for each ionic species.

Finally, we note that calculations with conditional charge densities play a central role in statistical mechanics of liquids [71],[21]. Our work shows that conditional densities arise inevitably in a stochastic analysis of averaged macroscopic charge densities.
3.3.6 Conditional and unconditional charge densities

To solve eqs. (3.34) for the unconditional densities $c_p(x)$ and $c_n(x)$, it is necessary to solve Poisson’s equation (3.33) for $\bar{\phi}^p$ and a similar equation for $\bar{\phi}^n$. However, these equations contain the conditional charge densities $c_p(y|x)$ and $c_n(y|x)$ so that the CPNP system (3.33)-(3.34) is not closed. These conditional charge densities at location $y$, given a positive (or negative) ion at location $x$, are in general different from the unconditional charge densities at the same location, $c_p(y)$ and $c_n(y)$. Therefore, to close the CPNP system, it is necessary to either derive an additional set of equations for the conditional charge densities, or to determine closure relations between the conditional and unconditional charge densities.

At this point, we note that according to (3.31), the conditional charge densities are related to the well studied pair correlation functions [21], which are the conditional pdf, $p(y|x)$, of a positive or negative ion at $y$, given a positive or negative ion at $x$. There are several theories for computing the pair correlation functions under various assumptions [10, 21]. The outcome of each of these theories is a different, and often rather complicated, closure relation between the conditional and the unconditional charge densities. Applying either one of these existing theories to the CPNP system yields a closed system of Poisson and Nernst-Planck equations which can be solved simultaneously, at least in principle. In this way, our work can relate to the substantial literature of statistical mechanics of ionic solutions and to their recent applications to channels [23],[116].

The pair correlation functions, or equivalently, the conditional charge densities present in the Poisson equations contain within them excluded volume effects. The simplest and most crude approximation, however, is to neglect these effects and replace the conditional charge densities in eq. (3.33) by the unconditional charge densities. This approximation leads to the standard Poisson equation,

$$\nabla_x \cdot \varepsilon(x) \nabla_x \bar{\phi}(x) = -\frac{e}{\varepsilon_0} \left[ \rho_{\text{perm}}(x) + c_p(x) - c_n(x) \right]. \quad (3.35)$$

Equations (3.34)-(3.35), with $\bar{\phi}^c(y|x)$ replaced by $\bar{\phi}(y)$, are the standard Poisson-Nernst-Planck model [51], but with an additional self induced force term, $f_D(x)$.

More refined theories attempt to compensate for the error in the evaluation of the net force introduced in the above approximation. We consider this compensation in a typical bath-channel-membrane setup. First, we consider the net force on an ion located at $x$ in
the bulk solution far away (many Debye lengths [21]) from the membrane and channel. We assume that this region of the solution is approximately in equilibrium, and thus approximate the conditional charge densities $c(y \mid x)$ by the simple Debye-Hückel theory [21].

This leads to an isotropic displacement of the ionic atmosphere, i.e. the charge cloud around the ion. According to the well known sum rule, the total charge of the cloud equals the opposite charge of the fixed ion at $x$ [71]. Since this displacement of the charge of the ionic atmosphere is isotropic, the direct Coulomb force on the ion due to this cloud is zero. The charge displacement of the ionic atmosphere, however, acts on the ion not only through the direct Coulomb force, but also through the surface charges induced at dielectric interfaces by the charge displacement. Since the spherically symmetric cloud centered at the ion has an equal but opposite charge as that of the ion, the spherically symmetric cloud induces equal and opposite surface charges at the far away dielectric interfaces. Therefore, the effect of the displacement cloud, or equivalently, of the reaction field computed from the conditional charge densities, is a cancelation of the self induced force on the ion, due to the charge induced by the ion at dielectric boundaries. That is, the displacement cloud screens the self induced force of the ion (see figure 3.2(a)).

The replacement of the conditional charge densities with unconditional densities in Poisson’s equation (3.33) changes the distribution of positive and negative charges around the ion located at $x$. While the conditional densities form a cloud around the ion as described above, the unconditional densities do not. Thus, in a bath with an ion at $x$ and unconditional densities around it, the surface charge induced by the ion at dielectric boundaries is not compensated by a surface charge induced by a displacement cloud. This means that there is a difference in the net force acting on the ion between a bath with conditional densities, and a bath with unconditional densities. Specifically, in a bath with conditional densities, the self induced force due to the induced surface charges is screened and decays exponentially fast with distance from dielectric boundaries. In contrast, in a bath with an ion at $x$ and unconditional densities the induced force is not screened and is therefore long range, decaying as the inverse square of the distance to the dielectric boundary.

Thus, for ions located far away from dielectric interfaces, the (long range) self induced force $f_D(x)$ in equation (3.34) has to be eliminated to compensate for the replacement of the conditional charge densities with the unconditional ones. Related phenomena involving screening in ionic solutions is seen in both Modified Poisson Boltzmann theories and
As the ion approaches the dielectric interface, the isotropy of the displacement cloud is broken, so that the direct Coulombic force of the cloud on the ion no longer vanishes, and the induced surface charges of the screening cloud do not cancel the induced surface charges of the ion (see figure 3.2(b)). These complexities also affect ions approaching the mouth of the pore, where the flux may not be considered negligible, so the ionic cloud may not be considered spherically symmetric [22].

The situation inside the channel is opposite to the situation in bulk solution. Inside the channel there is usually only one mobile ion. Therefore, we expect that there will be almost no screening of the self induced force on this ion by the conditional charge densities in the bath [108]. In replacing conditional charge densities by the unconditional ones, the self force must be retained inside the channel.

To conclude, replacement of conditional charge densities by unconditional ones has an effect on the net force acting on the ion. This effect can be compensated by replacing the self induced force \( f_D(x) \) by an effective induced force \( f_{\text{eff}}^D(x) \), so that the net force in the NP equations for the positive and negative ions (of valence one) is given by

\[
\tilde{f}^p(x) = -e \nabla \tilde{\phi}(x) + f_{\text{eff}}^p_D(x)
\]

\[
\tilde{f}^n(x) = +e \nabla \tilde{\phi}(x) + f_{\text{eff}}^n_D(x).
\]

Note that if the positive and negative ions have same diameter and valence then the effective forces are equal.

Outside the channel, there is perfect screening of the self induced force by the conditional densities, so their replacement with unconditional ones makes the effective induced force zero. Inside the channel there is almost no screening so the effective induced force approximately equals the self induced force. In the intermediate region, between the membrane and the bulk solution (several Debye lengths away from the membrane), the situation is more complicated and the compensation for the replacement has to be calculated from a more detailed theory.

3.4 Quasi-ions and simulation of permeation

A different approach to the study of the permeation process through the channel uses Langevin simulations instead of closure relations. In Section 3.3 we have shown that the
stationary probability density function, $p(x, v)$, for the phase space coordinates of a single positive ion is governed by an FPE type equation, (3.24). This FPE corresponds to the Langevin equation

$$\ddot{x} + \gamma^p(x) \dot{x} = \frac{\bar{f}^p(x)}{m^p} + \sqrt{2\gamma^p(x)k_B T \over m^p} \dot{w}$$

(3.36)

with the additional approximation (3.25)-(3.26) so that the force $\bar{f}^p(x)$ depends only on $x$. Note that this Langevin equation does not define the trajectories of a real positive ion, but rather defines trajectories of a quasi-ion, that is driven by the averaged force $\bar{f}^p(x)$, and not by the real fluctuating force that drives a real ion. Yet, according to our analysis, the average flux computed from the trajectories of this quasi-particle equals the averaged flux of real ions. Note also that the boundary behavior of the trajectories of this quasi-ion are the same as those of the real ions – reflection at hard walls and immediate recycling at the opposite electrode of ions reaching a given electrode. The idea of a quasi-ion (called a permion) has been mentioned before in the permeation literature [55].

The permeation properties of a channel can thus be studied by simulating trajectories of quasi-ions, according to equation (3.36), once the force $\bar{f}^p(x)$ has been evaluated. One possible procedure for approximating the force $\bar{f}^p(x)$ is to fix the quasi-ion at various locations $x$, and for every such location compute the average force exerted on it, either by some theory or by a simulation in which the force is approximated by an ensemble average. In the latter method, for every system within the ensemble, the quasi-ion is kept fixed at its location, and the other ions are allowed to relax to a random realization of their stationary distribution. This procedure is only an approximation of the force, because keeping the quasi-ion fixed is not strictly consistent with the flux of the quasi-ion. However, the mean velocity of the quasi-ion associated with this flux is small compared to its thermal velocity, so this procedure is a reasonable first step [54]. Once the force is known, a simulation of equation (3.36) can be carried out.

Now consider the case of a channel that can contain more than one ion at a time. In this case, it might be necessary to simultaneously simulate two or more ionic trajectories at a time. In analogy to the case of a single simulated quasi-ion, a simulation of the motion of two ions leads to forces of the form $\bar{f}(x_1, x_2)$ which depend on the locations of the two simulated quasi-ions, and on the conditional charge densities, but conditioned now on the locations of both ions. In order to proceed in this direction, a theory must be derived or a simulation carried out to compute these forces.
There are, however, quite a few issues to be resolved before a practical simulation of a quasi-ion can be carried out. The first issue involves approximating the full phase space coordinates \((x, v)\) with only the spatial coordinate \(x\). This approximation enables a simpler and faster simulation of the quasi-ion but is problematic because in simulating only the location of the ion and not its velocity there is no way to distinguish between incoming and outgoing trajectories at the electrodes [54]. Thus, the recycling mechanism of ions at the electrodes has to be revised in this limit. Another issue is the size of the domain. In our formalism the domain \(\Omega\) in which the solution was confined is assumed large enough so that density fluctuations are neglected and the instantaneous recycling mechanism at the electrodes is assumed. However, running a simulation in such a large domain may prove to be too slow. Thus, ideally one would like to simulate a quasi-ion in a much smaller domain surrounding the protein channel. However, in this domain the density fluctuations in the original formulation of the problem may not necessarily be negligible, and thus automatic reinjection of quasi-ions at the electrodes needs to be reconsidered. These issues require further investigation not covered in this chapter.

3.5 Discussion

In this chapter, an averaging procedure of a Langevin model for the coupled motion of many interacting ions in an electrolyte solution is described, that results in an approximate description of the solution with averaged charge densities and mean electric fields. The result of the averaging procedure is a CPNP system (3.33)-(3.34) containing a set of conditional Nernst-Planck and conditional Poisson equations. The average charge density of each ionic species in the solution is described by a separate Nernst-Planck equation coupled to a separate Poisson equation for its conditional electrostatic potential. The force in each NP equation is the sum of two terms; one is the self induced force on an ion of that species, and the other is the gradient of the corresponding conditional electrostatic potential. This potential is described by a Poisson equation that (1) depends on the conditional charge densities of all the ionic species, conditioned on the location of an ion of that species; (2) depends on the permanent charge and (3) depends on the applied voltage boundary conditions. In a bi-ionic solution the CPNP system consists of a total of four equations: two NP equations for the charge densities of the positive and negative ions, and two Poisson equations for the conditional
potentials, each corresponding to one of the NP equations.

In equilibrium, the conditional charge densities appearing in the Poisson equations are closely related to the pair correlation functions in the theory of fluids [21]. The conditional densities are *different* from the (unconditional) densities in the NP equations, and their difference is a measure of inter-ionic repulsion and attraction forces, and in particular excluded volume effects. This difference renders the resulting CPNP system incomplete. As in the theory of fluids, a *closure relation* between the conditional and unconditional charge densities and between the conditional and unconditional potentials is needed to complete the system. With a closure relation, all the equations in the CPNP system are coupled to each other. One of the simplest closure relations is the MSA-type closure which expresses the excluded volume of ions of finite size. MSA-type closure relations are quite successful in describing properties of free solutions (with no dielectric interfaces and no narrow channels) [21]. These closure relations have recently been applied to ionic permeation in protein channels [23, 116, 117].

In this chapter, we examine a specific closure relation, that replaces the conditional charge densities in the Poisson equations by the unconditional ones, and also replaces the self induced force in the NP equations by an effective induced force $f_{D}^{eff}$. The exact form of the effective induced force in the access region needs to be resolved by a higher resolution theory; but far away from channel and membrane the effective induced force is approximately zero, as we have discussed, and near dielectric interfaces—in particular inside the channel—there is hardly any screening so the effective induced force approximately equals the self induced force. We note that the proposed closure relation is not based on the Boltzmann distribution, and therefore may be applied also in non-equilibrium systems.

The replacement of conditional densities by unconditional ones leads to the PNP system, with the additional effective induced force term $f_{D}^{eff}$. This replacement represents the finite sized ions as point charges. Thus, excluded volume effects are *lost* in this description, and all the related phenomena of channels, such as single filing and flux saturation, cannot be recovered by the PNP system.

A different approach to include the finite sizes of the ions into an averaged PNP description introduces Lennard-Jones force terms between the individual ions in the Langevin system (3.1). Then, in the averaging procedure, an additional averaged conditional Lennard-Jones force term appears in the resulting Nernst-Planck equations of the CPNP system. The
specific closure relation that replaces conditional densities by unconditional ones must also evaluate this averaged Lennard-Jones force, thus leading to a PNP description with excluded volume effects. Note, however, that when short range Lennard-Jones forces are present, in particular inside a multiply occupied channel, the assumption of independent noise terms in the Langevin equations of different ions should be reexamined. Last but not least, other closure relations, not necessarily leading to a PNP description of the system, are also possible, and should be examined.

In the derivation presented in this chapter, the CPNP system (3.33)-(3.34) can be considered as the result of ensemble averaging over many independent realizations of the stochastic system (3.1)-(3.2). Obviously, for a rigid channel, in which the permanent charge does not move at all, the permanent charge density \( \rho_{\text{perm}}(x) \), the dielectric constant \( \varepsilon(x) \), and the boundary conditions remain unchanged in the averaging process. If, however, the permanent charge fluctuates around a fixed position, then the dynamics of the permanent charge need to be described and coupled to the full Langevin system (3.1). The averaging procedure will result in a conditional permanent charge distribution appearing in the conditional Poisson equations.

In this way, the geometric and electrostatic properties of a channel carry over to the averaged system. However, the fluctuating state of the system with the (random) locations and velocities \( \tilde{x}(t) \) and \( \tilde{v}(t) \) of all the ions is replaced in the averaged system by the non-fluctuating averaged charge densities \( c_p(x) \) and \( c_n(x) \). Thus, potential fluctuations are lost in the PNP system. In steady state these charge densities can be viewed as averages over many snapshots (samples) of a single system, taken at different times, sufficiently far apart for all correlations to vanish. In this view, the charge densities appearing in the NP equations are time averages of discrete ionic concentrations, over times much larger than the relaxation time of the system. This view defines a continuum description of the discrete contents of an ionic channel. This continuum description results from averaging over times much longer than the passage time of a single ion through a channel so that all unidirectional fluxes meld into a single averaged net flux and lose their individual identity.

Finally, note that ion specific excess chemical potentials included in the Langevin model are preserved by the averaging process, and appear in the effective NP equations. Such excess chemical potentials might arise from a more detailed atomic model, describing ‘chemical’ interactions of an ion (in the bulk phase) with its hydration shell, and of an ion (in the
channel) with its solvation shell (of atoms of the protein as well as channel water).

Appendix: The Self Induced Force

Consider the three-dimensional space $\mathbb{R}^3$ decomposed into an arbitrary number of dielectric regions of arbitrary shapes $\Omega_i$. We assume that in each region $\Omega_i$ the dielectric coefficient is constant, with respective value $\varepsilon_i$, and jumps abruptly only at the boundaries $\partial \Omega_i$ separating regions of different dielectric coefficients. Consider a point charge of strength $q$ located at location $r_0 \in \Omega_1$, a region with dielectric coefficient $\varepsilon_1$. In this section we consider only the self induced force on this point charge, due to the presence of dielectric boundaries, so we assume no other fixed or mobile charges are present in the system. As explained in the text, the interaction forces of this point charge with the other charges (and their induced surface charges) is treated separately (see eq.(3.5)). The presence of the charge at $r_0$ induces surface charges at all the boundaries $\partial \Omega_i$, separating regions of different dielectric media. These surface charges, in turn, exert a force on this charge, which we denote by $F_{\text{ind}}(r_0)$. The following lemma states the connection between this force and the electric potential, $\Phi(r)$, in the entire space.

**Lemma:** Let $\Phi(r)$ denote the electric potential at $r$, created by a point charge at $r_0$, and let $F_{\text{ind}}(r_0)$ denote the resulting net force on that point charge. Then

$$F_{\text{ind}}(r_0) = -q \nabla_r \left( \Phi(r) - \frac{q}{4\pi \varepsilon_0 \varepsilon_1 |r - r_0|} \right) \bigg|_{r = r_0}.$$

In other words, the force acting on the charge can be computed by subtracting from the total electric potential the singular Coulombic term produced by the charge, and then computing the gradient at the charge’s location.

**Proof:** The potential $\Phi(r)$ satisfies Poisson’s equation

$$\nabla \cdot \varepsilon(r) \nabla \Phi(r) = -\frac{q}{\varepsilon_0} \delta(r - r_0), \quad (3.37)$$

with the standard jump conditions of the normal field at dielectric interfaces

$$[\varepsilon(r) \nabla \Phi(r) \cdot n] \bigg|_{\partial \Omega_i} = 0, \quad (3.38)$$

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where \( \mathbf{n} \) is a unit vector in the outer normal direction to a surface element on \( \partial \Omega_i \), and the square brackets denote the difference in the variable enclosed within them, between the value outside the region \( \Omega_i \) and inside it. Note that \( \Phi(\mathbf{r}) \) also vanishes as \( |\mathbf{r}| \to \infty \).

The presence of the point charge at \( \mathbf{r}_0 \) induces surface charges, \( \sigma_{\text{ind}} \), at the dielectric boundaries \( \partial \Omega_i \), given by

\[
\sigma_{\text{ind}} \bigg|_{\partial \Omega_i} = [\nabla \Phi \cdot \mathbf{n}] \bigg|_{\partial \Omega_i}.
\] (3.39)

These induced surface charges create a force on the point charge. To compute this force, it is necessary to compute the electric potential formed by the surface charges, denoted \( \Phi_{\text{ind}}(\mathbf{r}) \). Then, the force on the ion is simply the gradient of this potential, computed at the ion’s location,

\[
F_{\text{ind}}(\mathbf{r}_0) = -q \nabla \Phi_{\text{ind}}(\mathbf{r}) \bigg|_{\mathbf{r} = \mathbf{r}_0}.
\] (3.40)

We now consider the equation that the potential \( \Phi_{\text{ind}}(\mathbf{r}) \) satisfies. Since the polarization of the different regions \( \Omega_i \) has already been taken into account in the computation of the induced surface charges via equations (3.37)-(3.39), the potential \( \Phi_{\text{ind}}(\mathbf{r}) \) satisfies Laplace’s equation \textit{without} a dielectric coefficient, i.e., with \( \varepsilon(\mathbf{r}) = 1 \) throughout whole space,

\[
\Delta \Phi_{\text{ind}}(\mathbf{r}) = 0.
\] (3.41)

In addition, as with \( \Phi(\mathbf{r}) \), the potential \( \Phi_{\text{ind}}(\mathbf{r}) \) also vanishes as \( |\mathbf{r}| \to \infty \). No point charge is present at \( \mathbf{r}_0 \) in equation (3.41), since the potential \( \Phi_{\text{ind}} \) is produced only by the surface charges induced on the dielectric interfaces.

While there are no dielectric boundaries for the potential \( \Phi_{\text{ind}}(\mathbf{r}) \) (\( \varepsilon(\mathbf{r}) = 1 \) everywhere), there are surface charges, given by (3.39), located at these boundaries. This amounts to the following jump conditions,

\[
[\nabla \Phi_{\text{ind}} \cdot \mathbf{n}] \bigg|_{\partial \Omega_i} = \sigma_{\text{ind}} \bigg|_{\partial \Omega_i}.
\] (3.42)

The key point in the proof of the lemma is the observation that the potential \( \Phi(\mathbf{r}) \) created by the point charge at \( \mathbf{r}_0 \), that satisfies equations (3.37)-(3.38), \textit{equivalently} satisfies Poisson’s equation with a constant dielectric coefficient, \( \varepsilon(\mathbf{r}) = \varepsilon_1 \), throughout space,

\[
\Delta \Phi(\mathbf{r}) = -\frac{q}{\varepsilon_1 \varepsilon_0} \delta(\mathbf{r} - \mathbf{r}_0),
\] (3.43)

but with jump conditions across (now non-existent) dielectric boundaries that depend on the induced surface charges found there,

\[
[\nabla \Phi \cdot \mathbf{n}] \bigg|_{\partial \Omega_i} = \sigma_{\text{ind}} \bigg|_{\partial \Omega_i}.
\] (3.44)
This equivalent representation of $\Phi(r)$ follows from the textbook pillbox treatment of Gauss’ law at dielectric boundaries [81]. Note also that these jump conditions are exactly the same as those that $\Phi_{ind}$ satisfies.

To prove the lemma, we subtract the two potentials $\Phi_{ind}$ and $\Phi$. Both potentials satisfy the same jump conditions, eqs.(3.42) and (3.44). Then, according to (3.41) and (3.43), their difference satisfies the following Poisson equation throughout space

$$\Delta \left( \Phi_{ind}(r) - \Phi(r) \right) = + \frac{q}{\varepsilon_0 \varepsilon_1} \delta(r - r_0)$$

without any jump conditions at the boundaries $\partial \Omega_i$. The solution to this equation, with a plus sign in the right hand side, is of course the free space potential created by a point charge of strength $-q/\varepsilon_1$ at $r_0$,

$$\Phi_{ind}(r) - \Phi(r) = - \frac{1}{4\pi \varepsilon_0 \varepsilon_1} \frac{q}{|r - r_0|}. \quad (3.45)$$

Combining (3.45) with (3.40) concludes the proof of the lemma.

Note that the potential $\Phi(r)$ is proportional to the charge $q$ at $r_0$. Thus according to (3.39) and (3.41)-(3.42) both the induced surface charges and the potential $\Phi_{ind}$ are also proportional to $q$. This renders the self induced force, given by (3.40), proportional to $q^2$. Thus, the force vector has the same direction and magnitude regardless of the sign of the charge at $r_0$.

Note also that for the same problem in a finite domain $\Omega$, with homogeneous boundary conditions on $\partial \Omega$ (grounded metal electrodes at the boundary), the lemma does not hold. This is because the solution of (3.45) in a finite domain with zero boundary conditions is not exactly the Coulomb term, but rather contains boundary effects as well. However, in terms of the force on the ion, these effects decay as the square distance from the outer boundary [61], so for all practical purposes for an ion located far away from the outer boundaries of the domain the lemma is still valid.
Figure 3.1: A typical experimental setup. The region Ω consists of two baths separated by an impermeable membrane, with a possible channel embedded in it. The electrodes immersed in the two baths are connected to an external feedback mechanism (not shown in figure) that maintains a constant voltage difference between the electrodes, and constant concentrations in the baths.

Figure 3.2: (a) The displacement cloud around an ion located many Debye lengths from a membrane, and (b) around an ion located near a membrane.
Chapter 4

1D-PNP with Large Permanent Charge

The Poisson-Nernst-Planck (PNP) equations are a self consistent continuum model for the flow of charged particles in an electric field. These set of equations, also known as the drift diffusion equations, have been used for a long time to model semiconductor devices [84, 101, 149]. In the past few years the same set of equations have also been applied to describe ionic flow through single protein channels. In the simplest one dimensional form, researchers have been able to fit experimental current voltage curves of ionic flow through single channels under a wide range of conditions, with only a few fit parameters [31, 32, 33]. Recently, more general 3-D PNP models have been successfully applied to ionic permeation through channels with known three dimensional structure [27, 77, 93].

In this chapter we analyze mathematically the one dimensional system of Poisson-Nernst-Planck equations, under the assumption of large permanent charge density inside the channel. In our analysis, we assume Donnan built-in potential boundary conditions for both the potential and the concentrations, as used in [31, 32, 33]. A derivation of these boundary conditions under the assumption of equilibrium and charge neutrality at the edge of the channel is presented in section 4.8. Recently these boundary conditions have been shown to be the leading order expression also for the non-equilibrium case [62]. The main result of our analysis is that to leading order, the current flowing through the channel is a linear function of the applied potential, thus rederiving Ohm’s law for ion flow through highly charged channels. This result explains the success of the one dimensional PNP theory in fitting experimental I-V curves of various channels, which are mostly linear in nature.
Another key result of this chapter is that for a uniformly charged channel, the net flux flowing through a highly charged channel is linearly proportional to the permanent charge of the channel. Thus, the higher the permanent charge is, the larger the current flowing through the channel. An explanation of this phenomenon from a molecular point of view still awaits a thorough treatment.

4.1 Problem Setup

Consider a single cylindrically symmetric channel of length \( L \) and radius \( R \), immersed in a membrane between two neutral baths. We choose the coordinate system such that the channel axis extends in the \( \tilde{z} \) direction, from 0 to \( L \), while the channel radius extends in the \( \tilde{r} \) coordinate, from 0 to \( R \).

Following Chen & al. [31], we assume that the flow of ions through this channel is described by the three dimensional Poisson Nernst Planck system of equations. Under the assumption of a long and narrow channel, \( L \gg R \), Barcilon & al [7, 8] have shown that the leading order set of equations are the reduced one dimensional version of PNP, with an additional term in the Poisson equation.

Thus, the corrected leading order one dimensional Poisson equation for the electric potential \( \Psi(\tilde{z}) \) reads

\[
-\varepsilon_ch\varepsilon_0 \frac{d^2\Psi(\tilde{z})}{d\tilde{z}^2} = \frac{2\varepsilon_m\varepsilon_0}{R^2 \ln(R/L)} \left[ (1 - \frac{\tilde{z}}{L}) \Delta - \Psi(\tilde{z}) + \Psi(L) \right] + \sum_j e\tilde{z}_j\tilde{c}_j(\tilde{z}) + e\rho_{ch}(\tilde{z}). \quad (4.1)
\]

The Nernst-Planck equation for the ionic species \( j \) is

\[
\frac{d\tilde{J}_j}{d\tilde{z}} = -\frac{d}{d\tilde{z}} \left( D_j \left[ \frac{d\tilde{c}_j(\tilde{z})}{d\tilde{z}} + \tilde{c}_j(\tilde{z}) \frac{e\tilde{z}_j}{k_B T} \frac{d\Psi(\tilde{z})}{d\tilde{z}} \right] \right) = 0, \quad (4.2)
\]

where \( \tilde{J}_j \) is the flux of the \( j \)-th ionic species (number of particles per unit area per unit time).

The boundary conditions for the potential are

\[
\Psi(0) = \Phi_{bi}(0) + V_{applied}, \quad \Psi(L) = \Phi_{bi}(L), \quad (4.3)
\]

and for the concentrations,

\[
\tilde{c}_j(0) = c_{L,j} e^{-e\tilde{z}_j\Phi_{bi}(0)/k_B T},
\]

\[
\tilde{c}_j(L) = c_{R,j} e^{-e\tilde{z}_j\Phi_{bi}(L)/k_B T}. \quad (4.4)
\]
The set of equations (4.1)-(4.4) are all written in dimensional form, where \(L\) is the length of the channel, \(R\) its radius, \(\tilde{z}\) is the coordinate along the channel axis, \(\rho_{ch}(\tilde{z})\) is the permanent charge of the channel, \(\varepsilon_{ch}\) is the dielectric constant inside the pore of the channel, \(\varepsilon_m\) is the dielectric constant of the membrane, \(k_B\) is Boltzmann’s constant, \(T\) is temperature and \(\Delta = V_{applied} + \Phi_{bi}(0) - \Phi_{bi}(L)\) is the Donnan potential. The quantities \(\Phi_{bi}(0)\) and \(\Phi_{bi}(L)\) are the values of the built in potential, given by the following expressions (see section 4.8 for the derivation),

\[
\Phi_{bi}(0) = -\frac{k_B T}{e} \ln \left( -\frac{\rho_{ch}(0)}{2c_L} + \sqrt{\rho_{ch}^2(0) + 4c_L^2} \right),
\]

\[
\Phi_{bi}(L) = -\frac{k_B T}{e} \ln \left( -\frac{\rho_{ch}(L)}{2c_R} + \sqrt{\rho_{ch}^2(L) + 4c_R^2} \right),
\]

(4.5)

with \(c_L\) and \(c_R\) denoting the bulk concentrations in the left and right baths respectively.

Note the additional first term in the right hand side of the Poisson equation (4.1), which is the result of the restriction of the three dimensional problem into a one dimensional leading order approximation.

The quantities of interest are the total current flowing through the channel,

\[
I = \pi R^2 \sum_j ez_j \tilde{J}_j,
\]

the reversal potential \(V_r\) at which the total current through the channel is zero, and the conductance of the channel

\[
g(C) = \frac{dI}{dV},
\]

computed at symmetric bath concentrations, \(c_L = c_R = c\).

The set of equations (4.1)-(4.5) is the exact system with which fits to experimental data have been done. In the following sections, we find the asymptotic expansion of the solution of this system with the above boundary conditions, in the limit of large permanent charge. For simplicity, we present the calculations for a 1:1 bi-ionic solution, such as Na\(^+\)Cl\(^-\), but the method can be generalized to any number of ionic species. An explicit expression for the current as a function of applied voltage and concentrations is calculated, as well as expressions for the reversal potential at which the net current is zero, and limiting slopes of the IV curve. As stated above, the main result of this paper is that to leading order, the IV curves are linear, thus rederiving Ohm’s Law for ionic flow through channels.
Special attention is given to the case of large and uniform permanent charge, which according to [32] is indeed a good fit for the experimental data of at least one type of channel, the calcium release channel of cardiac muscle.

4.2 Non-Dimensional Equations

The first step towards the mathematical analysis of the above system is the non dimensionalization of all physical variables in the equations. Distance is scaled according to the length of the channel, \( z = \tilde{z}/L \). All electrostatic potentials are scaled with the factor \( k_B T/e \),

\[
V = \frac{e}{k_B T} V_{\text{applied}}, \quad \psi(z) = \frac{e}{k_B T} \Psi(\tilde{z}), \quad \phi_{bi}(z) = \frac{e}{k_B T} \Phi_{bi}(\tilde{z}).
\]

Densities are scaled with a characteristic density \( C \) for the bulk bath concentrations,

\[
\tilde{c}_j(\tilde{z}) = C \cdot c_j(z), \quad \rho_{ch}(\tilde{z}) = C \cdot P_{ch}(z).
\]

and fluxes are nondimensionalized as follows, \( J_j = L\tilde{J}_j/D_jC \). After these scalings, the non dimensional form of Poisson’s equation (4.1) is

\[
-\frac{d^2\psi(z)}{dz^2} = \lambda^2 \left[ P_{ch}(z) + \sum_j z_j c_j(z) \right] + \lambda^2 \epsilon^2 \left[ \psi(0) + z(\psi(1) - \psi(0)) - \psi(z) \right],
\]

and the non-dimensional form of the Nernst-Planck equation is

\[
\frac{dJ_j}{dz} = -\frac{d}{dz} \left[ \frac{d c_j(z)}{dz} + z_j c_j(z) \frac{d \psi(z)}{dz} \right] = 0.
\]

The non-dimensional boundary conditions for the electric potential are

\[
\psi(0) = \phi_{bi}(0) + V, \quad \psi(1) = \phi_{bi}(1),
\]

and for the concentrations are

\[
c_j(0) = \frac{c_{L,j}}{C} \exp (-z_j \phi_{bi}(0)), \quad c_j(1) = \frac{c_{R,j}}{C} \exp (-z_j \phi_{bi}(1)).
\]

The non-dimensional Poisson equation (4.7) contains two non-dimensional parameters,

\[
\lambda^2 = \frac{e^2 L^2 C}{\varepsilon_{ch} \varepsilon_0 k_B T}, \quad \epsilon^2 = \frac{2 \varepsilon_m \varepsilon_0 k_B T}{e^2 C R^2 \ln(R/L)}.
\]
For a typical channel of length $L = 20\,\text{Å}$ and radius $R = 2\,\text{Å}$, with a dielectric constant of $\varepsilon_{ch} = 20$ embedded in a membrane with dielectric constant $\varepsilon_m = 2$, and a typical bath concentration of $C = 0.1M$, the values of the non dimensional parameters are $\lambda^2 \approx 8$ and $\epsilon^2 \approx 1$.

However, one unit of charge uniformly spread over inside this channel leads to a charge concentration of $\rho_{ch} = 4M \gg C$. Therefore, in a typical situation $\lambda^2 = O(1)$, $\epsilon^2 = O(1)$, but the scaled concentration, given by (4.6), $P_{ch}(z) \gg 1$. In the following sections, we shall perform the asymptotics of the equations for large permanent charge based on these assumptions.

### 4.3 Exact Solution For Symmetric Bath Concentrations and Uniform Permanent Charge

First we consider the solution of the PNP equations with a constant though not necessarily large permanent charge in the case of equal symmetric ionic concentrations on both sides of the channel. In this special case, the values of the built-in potential at both edges of the channel are equal, as are the boundary conditions for each ionic species. Thus, it is easy to verify that the exact solution of equations (4.7) - (4.10) is a linear profile for the potential, and constant profiles for each of the ionic concentrations,

$$\psi(z) = \phi_{bi}(0) + (1 - z) \cdot V,$$

$$c_j(z) = c_j(0) = c_j(1) = \exp(-z_j \phi_{bi}(0)), \quad \text{for all } j.$$

The total current is given by

$$I = V \cdot \frac{e \pi R^2}{L} \sum_j D_j z_j^2 \exp(-z_j \phi_{bi}(0)).$$

(4.11)

In the simplest case of a bi-ionic 1:1 solution with bath concentration $c$, and a uniformly charged channel with charge density $\rho_{ch}$, inserting (4.5) into (4.12) yields

$$I = -V \cdot \frac{e \pi R^2}{L} \left[ D_p \frac{\rho_{ch} + \sqrt{\rho_{ch}^2 + 4c^2}}{2} + D_n \frac{2c^2}{\rho_{ch} + \sqrt{\rho_{ch}^2 + 4c^2}} \right],$$

(4.13)
where $D_p$ and $D_n$ denote the diffusion coefficients of the positive and negative ions respectively. For $\rho_{ch} \gg c$ equation (4.13) can be further simplified

$$I = -V \rho_{ch} \frac{e \pi R^2}{L} \left[ D_p + \frac{c^2}{\rho_{ch}^2} (D_n + D_p) + O \left( \frac{c}{\rho_{ch}} \right)^4 \right].$$

(4.14)

The conductance in this case is therefore given by

$$g = \frac{dI}{dV} = \rho_{ch} \frac{e \pi R^2}{L} \left[ D_p + \frac{c^2}{\rho_{ch}^2} \right].$$

(4.15)

As seen from equation (4.14), the current is linear with the applied voltage, and with the permanent charge density. To leading order, because the channel is negatively charged, the contribution to the current comes only from permeation of the positive ions. Strangely, note that to leading order the current does not depend on the bath concentrations. This of course, cannot be true for all bath concentrations, and is merely a manifestation of the use of the built-in potential boundary conditions at the edges of the channel. These boundary conditions might not be valid for all bath concentrations. Specifically, these boundary conditions are not valid for very low concentrations, since then the current flowing through the channel has a large effect on the potential and concentration profile in the bath, and the assumptions made in the derivation of the built-in potential are not satisfied.

From equation (4.15) we note that the conductance of the channel is almost constant, with a slight hyperlinear increase as a function of $c^2/\rho_{ch}^2$, the square of bath concentration divided by the channel’s permanent charge.

As we shall see in the next section, in the general case of unequal bath concentrations, and a general profile of the permanent charge inside the channel, the resulting I-V relationship is still linear to first order, with a shift of the potential by $\ln(c_R/c_L)$.

### 4.4 Asymptotic Solution of PNP equations

Consider the asymptotic solution of the PNP equations (4.7)-(4.10) in the limit of a large permanent charge. For simplicity, we consider only the case of a bi-ionic solution with two ionic species of valence $\pm 1$, positive and negative. We denote the concentrations of the positive and negative species by $p(z)$ and $n(z)$ respectively.

We also denote by $P$ a characteristic non-dimensional value for the permanent charge inside the channel, measured in units of the characteristic bulk concentration $C$. Under our
assumptions, $P \gg 1$, and we scale the negative permanent charge profile inside the channel with this parameter $P$,

$$P_{ch}(z) = -P f(z),$$

so $f(z) = O(1)$ is a positive function bounded away from zero.

### 4.4.1 Asymptotics of the boundary conditions

In the limit of large negative permanent charge the asymptotic expansion of the built in boundary conditions (4.5), written in non-dimensional form is

\[ \phi_{bi}(0) = \ln \left( \frac{c_L}{|P_{ch}(0)|} \right) - \left( \frac{c_L}{P_{ch}(0)} \right)^2 + O \left( \frac{c_L}{|P_{ch}(0)|} \right)^4, \]

\[ \phi_{bi}(1) = \ln \left( \frac{c_R}{|P_{ch}(1)|} \right) - \left( \frac{c_R}{P_{ch}(1)} \right)^2 + O \left( \frac{c_R}{|P_{ch}(1)|} \right)^4. \]

Therefore, the potential difference between the two edges of the channel is

\[ \Psi(0) - \Psi(1) = V - \ln \left( \frac{c_R f(0)}{c_L f(1)} \right) + \frac{1}{P^2} \left( \frac{c_R^2}{f(1)^2} - \frac{c_L^2}{f(0)^2} \right) + O \left( \frac{1}{P^4} \right). \]

We now consider the asymptotic expansion of the concentration boundary conditions. According to (4.10) and (4.16), the expansion for the positive species is

\[ p(0) = |P_{ch}(0)| \left( 1 + \left( \frac{c_L}{P_{ch}(0)} \right)^2 + O \left( \frac{c_L}{P_{ch}(0)} \right)^4 \right), \]

\[ p(1) = |P_{ch}(1)| \left( 1 + \left( \frac{c_R}{P_{ch}(1)} \right)^2 + O \left( \frac{c_R}{P_{ch}(1)} \right)^4 \right), \]

while for the negative species it is

\[ n(0) = c_L \left( \left( \frac{c_L}{|P_{ch}(0)|} \right) + O \left( \frac{c_L}{|P_{ch}(0)|} \right)^3 \right), \]

\[ n(1) = c_R \left( \left( \frac{c_R}{|P_{ch}(1)|} \right) + O \left( \frac{c_R}{|P_{ch}(1)|} \right)^3 \right). \]
4.4.2 Asymptotics of the solution

Based on the expressions (4.16)-(4.19) for the asymptotics of the boundary conditions and the exact solution (4.11) for the case of equal bath concentrations, we consider the following asymptotic expansion for the PNP system,

\[
\psi(z) = \psi(1) + V - \ln \left( \frac{c_R f(0)}{c_L f(1)} \right) (1 - z) + \psi_0(z) + \frac{1}{P} \psi_{-1}(z) + O(1/P^2),
\]

\[
p(z) = P p_1(z) + p_0(z) + \frac{1}{P} p_{-1}(z) + O(1/P^2),
\]

\[
n(z) = P n_1(z) + n_0(z) + \frac{1}{P} n_{-1}(z) + O(1/P^2).
\]

(4.20)

Note that in this formulation, according to (4.17), \(\psi_0(z)\) satisfies homogeneous boundary conditions. For simplicity, we define a shifted potential \(V_S\) as

\[
V_S = V - \ln \left( \frac{c_R f(0)}{c_L f(1)} \right).
\]

Inserting (4.20) into the Poisson equation (4.7) gives

\[
-1/ \lambda^2 \psi''_0 = P \left[ -f(z) + p_1(z) - n_1(z) \right] + (p_0(z) - n_0(z)) + \epsilon^2 (V_S (1 - z) - \psi_0(z)) + O \left( \frac{1}{P} \right).
\]

4.4.3 Leading Order Equations

The leading order Poisson equation reads

\[
-f(z) + p_1(z) - n_1(z) = 0.
\]

(4.21)

Insertion of (4.20) into the Nernst-Planck equations (4.8) gives to leading order

\[
\frac{d}{dz} \left[ \frac{dp_1(z)}{dz} + p_1(z) \left( -V_S + \frac{d \psi_0}{dz} \right) \right] = 0,
\]

\[
\frac{d}{dz} \left[ \frac{dn_1(z)}{dz} - n_1(z) \left( -V_S + \frac{d \psi_0}{dz} \right) \right] = 0.
\]

(4.22)

The solution to these equations can be found via two integrations. One integration of (4.22a) gives

\[
\frac{d}{dz} \left( e^{V_S (1-z) + \psi_0(z)} p_1(z) \right) = -e^{V_S (1-z) + \psi_0(z)} J_p^{(1)}(z),
\]

where \(J_p^{(1)}\) is a yet undetermined coefficient. Another integration gives

\[
p_1(z) = p_1(0) e^{V_S z - \psi_0(z)} - J_p^{(1)} e^{V_S (1-z) - \psi_0(z)} \int_0^z e^{V_S (1-y) + \psi_0(y)} dy.
\]

(4.23)
The coefficient \( J_p^{(1)} \) is found from the boundary condition (4.18) at \( z = 1 \),

\[
J_p^{(1)} = -\frac{p_1(1) - p_1(0)e^{V_S}}{\int_0^1 e^{V_S(1-y) + \psi_0(y)} dy}, \tag{4.24}
\]

A similar equation holds for \( J_n^{(1)} \). However, according to (4.19) \( n_1(0) = n_1(1) = 0 \), so \( J_n^{(1)} = 0 \) and \( n_1(z) \equiv 0 \).

The yet undetermined potential \( \psi_0(z) \) is found from the leading order Poisson equation (4.21), which now reads

\[
p_1(z) = f(z), \tag{4.25}
\]

Insertion of (4.23) into (4.25) gives

\[
p_1(0)e^{V_Sz - \psi_0(z)} - J_p^{(1)}e^{-V_S(1-z) - \psi_0(z)} \int_0^z e^{V_S(1-y) + \psi_0(y)} dy = f(z). \tag{4.26}
\]

This is an integral equation that the potential \( \psi_0(z) \) must satisfy. The equation is more complicated than it looks, because \( J_p^{(1)} \) depends on the unknown potential \( \psi_0(z) \) as well, through (4.24). To solve this equation we define a new function, \( h(z) \)

\[
h(z) = e^{-V_Sz + \psi_0(z)}, \tag{4.27}
\]

and denote by \( h_1 \) the value of its integral along the channel,

\[
h_1 = \int_0^1 g(y)dy. \tag{4.28}
\]

In terms of \( h(z) \) and \( h_1 \), the integral equation (4.26) reads

\[
f(0) + \frac{f(1)e^{-V_S} - f(0)}{h_1} \int_0^z g(y)dy = f(z)h(z).
\]

Differentiation of this equation with respect to \( z \) gives

\[
\frac{f(1)e^{-V_S} - f(0)}{h_1} h(z) = f'(z)h(z) + f(z)h'(z).
\]

Dividing by \( h(z) \) and \( f(z) \), and integrating once we obtain that

\[
h(z) = \frac{f(0)}{f(z)} \exp \left( \frac{f(1)e^{-V_S} - f(0)}{h_1} \int_0^z \frac{1}{f(y)} dy \right).
\]

The function \( h(z) \) contains a yet undetermined coefficient \( h_1 \), which should be determined by the consistency condition (4.28). This gives after some algebra the following equation

\[
h_1 = \frac{f(1)e^{-V_S} - f(0)}{-V_S + \ln \left( \frac{f(1)}{f(0)} \right)} \int_0^1 \frac{1}{f(y)} dy.
\]
Inserting this expression into the previous formula gives the final explicit formula for $h(z)$,

$$h(z) = \frac{f(0)}{f(z)} \exp \left( -V_S + \ln \left( \frac{f(1)}{f(0)} \right) \int_0^z \frac{1}{f(y)} dy \right).$$  

(4.29)

An explicit expression for $\psi_0(z)$ can now be written by combining (4.29) and (4.27).

4.4.4 The Case of Uniform Permanent Charge

In the case of uniform permanent charge $f(z) \equiv 1$ so equation (4.29) simply reads

$$h(z) = \exp(-V_S z),$$

which implies that $\psi_0(z) = 0$. Therefore, to leading order the potential profile is a linear function. For this particular case, this is actually the exact solution, as derived above in equation (4.11).

4.5 The Current Flowing through the Channel

To leading order, the contribution to the current flowing through the negatively charged channel comes, not surprisingly, only from the positive species (recall that $n_1(z) \equiv 0$). The leading order term in the non-dimensional flux is given by formula (4.24), which simplifies to

$$J_p^{(1)} = \frac{V - \ln \left( \frac{c_R}{c_L} \right)}{\int_0^1 \frac{1}{f(y)} dy}.$$

Therefore, returning to dimensional variables, to leading order the net current is

$$I = -\pi R^2 e D_p \frac{eV_{app}}{k_B T} - \ln \left( \frac{c_R}{c_L} \right) \int_0^L \frac{1}{\rho_{ch}(\tilde{z})} d\tilde{z}.$$

(4.30)

From this equation we can learn a few things about the properties of this PNP model: (i) the dependence of the net current flowing through the channel on the permanent charge is encapsulated into a single constant, the integral $\int_0^L 1/\rho_{ch}(y)dy$; (ii) The net current flowing
through the channel is linear with respect to the applied potential, $V_{\text{app}}$, but this potential is shifted by $\ln(c_R/c_L)$; (iii) to leading order, the current flowing through the channel is independent of the dielectric constant of the solvent inside the channel $\varepsilon_{\text{ch}}$, and of the dielectric constant of the membrane $\varepsilon_m$. (iv) for large concentrations $c$, the current in this model grows sublinearly as $\ln(c)$.

### 4.6 The Reversal Potential

The reversal potential is obtained to leading order from the condition $I = 0$, or equivalently from the condition $J_p^{(1)} = 0$. Inspection of (4.30) for the net current yields

$$V_{\text{rev}} = \frac{k_B T}{e} \left( \ln \left( \frac{c_R}{c_L} \right) + O \left( \frac{1}{P} \right) \right). \tag{4.31}$$

Thus, to leading order, the reversal potential is the same as that predicted by the Nernst-Planck theory, $V_{\text{rev}} = \ln(c_R/c_L)$ [72], and it is independent of the exact shape of the permanent charge inside the channel.

### 4.7 Comparison of Asymptotics vs. Numerical Results

We have solved numerically the system (4.1)-(4.4) and compared our results to the analytic asymptotic expressions derived above. For the numerical solution, the following values were chosen: $R = 2\,\text{Å}$, $L = 20\,\text{Å}$, $\varepsilon_{\text{ch}} = 20$, $\varepsilon_m = 2$, $T = 300^0\text{K}$.

In figure 4.1, a graph of the current as a function of right bath concentration $c_R$ is shown for fixed applied potential and fixed left bath concentration $c_L$. This graph corresponds to a uniformly negatively charged channel with density $\rho_{\text{ch}} = 5 M$. As seen from the graph, the asymptotic formula (4.30) fits perfectly the numerical results.

In figure 4.2, a graph of the current as a function of the applied voltage is shown, for constant bath concentrations, $c_L = 0.2 M$, $c_R = 0.1 M$. The upper curve corresponds to a linear profile of permanent charge inside the channel, $\rho_{\text{ch}}(z) = -(3 + 2z)$ Molar, while the lower curve corresponds to a uniform charge of strength $\rho_{\text{ch}}(z) = -1$ Molar. Note that in both cases, although the permanent charge profiles and strengths inside the channel are different, still the reversal potential, at which the current is zero, is the same for both permanent charge profiles, as predicted by equation (4.31).

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Figure 4.1: The current as a function of concentration. Comparison of numerical results with the analytical asymptotic formula (4.30).

Figure 4.2: The current as a function of applied voltage. Comparison of numerical results with the analytical asymptotic formula (4.30).

4.8 Derivation of the built-in potential boundary conditions

The derivation of the built-in potential and concentration boundary conditions is based on the following assumptions.

- On both sides of the channel, at distant locations from the channel, the concentrations and voltages are kept constant by some external feedback mechanism in the experiment.
- Charge neutrality is conserved at the pore’s entrance.
- In the bath there is no flux, i.e. the flux through the channel is negligible.
• The electric potential and concentration profiles in the bath are described by the Poisson Nernst Planck system of equations.

For simplicity we shall compute the boundary conditions for a bath containing a monovalent bi-ionic solution, of positive and negative ions, with concentration profiles \( p(\tilde{z}) \) and \( n(\tilde{z}) \), respectively.

Denote by \( z_L \) a point far away from the left channel entrance, where the voltage and concentrations are kept constant (on average), then we have the following boundary conditions far away from the channel,

\[
\Psi(z_L) = V_L, \quad p(z_L) = n(z_L) = c_L. \tag{4.32}
\]

According to the fourth assumption, the concentration profiles are described by the three dimensional Nernst-Planck equations. As the membrane walls are impermeable to ionic flow, no flux boundary conditions are imposed there. Now, according to the third assumption, that the current flowing through the channel is negligible, no flux boundary conditions are imposed also at the entrance to the channel. This means that there is approximate symmetry in the \((x, y)\) plane in the baths, and the concentrations in the bath can be approximately described by a one dimensional the Nernst-Planck equation with no flux,

\[
\tilde{J}_p = -D_p \left[ \frac{dp}{d\tilde{z}} + \frac{ep(\tilde{z})}{k_B T} \cdot \frac{d\Psi}{d\tilde{z}} \right] = 0, \tag{4.33}
\]

\[
\tilde{J}_n = -D_n \left[ \frac{dn}{d\tilde{z}} - \frac{en(\tilde{z})}{k_B T} \cdot \frac{d\Psi}{d\tilde{z}} \right] = 0, \tag{4.34}
\]

which can also be written in the following form

\[
\frac{d}{d\tilde{z}} \left[ \exp\left( \frac{e\Psi}{k_B T} \right) \cdot p(\tilde{z}) \right] = 0,
\]

\[
\frac{d}{d\tilde{z}} \left[ \exp\left( -\frac{e\Psi}{k_B T} \right) \cdot n(\tilde{z}) \right] = 0.
\]

One integration from \( z_L \) to \( \tilde{z} \) of these equations, and use of the boundary conditions (4.32) yields

\[
p(\tilde{z}) \exp\left( \frac{e\Psi(\tilde{z})}{k_B T} \right) = c_L \exp\left( \frac{eV_L}{k_B T} \right), \tag{4.35}
\]

\[
n(\tilde{z}) \exp\left( -\frac{e\Psi(\tilde{z})}{k_B T} \right) = c_L \exp\left( -\frac{eV_L}{k_B T} \right).
\]
The value of the electric potential, $\Psi(0)$, at the channel entrance at $\tilde{z} = 0$, is found by the condition that at $\tilde{z} = 0$ the sum of all concentrations, including the permanent charge, is zero. Denoting by $\rho_P$ the value of the permanent charge at $\tilde{z} = 0$, then the equation for $\Psi(0)$ is

$$p(0) - n(0) + \rho_P = 0. \quad (4.36)$$

Inserting the expressions for $p(0)$ and $n(0)$ from (4.35), we obtain the quadratic equation

$$c_L \cdot \left( y - \frac{1}{y} \right) + \rho_P = 0,$$

where $y = \exp\left( e(V_L - \Psi(0))/k_BT \right)$. The solution for $\Psi(0)$ is

$$\Psi(0) = V_L + \frac{k_BT}{e} \cdot \ln \left( \frac{-\rho_P + \sqrt{\rho_P^2 + 4 \cdot c_L^2}}{2c_L} \right).$$

The built-in potential is defined as the difference $\Psi(0) - V_L$,

$$\phi_{bi}(0) = -\ln \left( \frac{-\rho_P + \sqrt{\rho_P^2 + 4 \cdot c_L^2}}{2c_L} \right). \quad (4.37)$$

This expression together with (4.35), yield the corresponding boundary conditions for the concentrations,

$$p(0) = \frac{-\rho_P + \sqrt{\rho_P^2 + 4c_L^2}}{2}, \quad n(0) = \frac{2c_L}{-\rho_P + \sqrt{\rho_P^2 + 4c_L^2}}. \quad (4.38)$$

The last two equations are known as the built-in potential boundary conditions.
Chapter 5

Description of Ionic Permeation with Markov Chains

For the past 20 years, rate models have been the most common approach for the description of ionic permeation through protein channels [72, 73]. Rate models, also known as barrier models, are based on the assumptions that inside the channel there are well defined affinity sites for mobile ions, surrounded by high barriers, and that mobile ions "hop" from one site to the other at exponential times described by effective rates.

These simplified assumptions, however, are also at the center of some of the criticisms against rate models [38, 42, 50, 148]. The exponentially distributed times of transitions between sites is derived from the results of Eyring transition state theory in the presence of high barriers [94]. However, most rate models do not use the standard form of Eyring state theory with the pre-exponential factor of $k_B T/h$, but rather let this factor be a fit parameter of the model. The resulting value for this pre-exponential factor is usually orders of magnitude larger than $k_B T/h$. Besides this inconsistency, the use of Eyring theory is problematic in another two aspects. The first concerns the assumption of high potential barriers inside the channel, which is rather unlikely, given the large currents that flow through channels. Various other arguments also indicate that in general the potential barriers that mobile ions face inside the channel are low [38, 60]. The second concerns the underlying ballistic motion assumption, used in the derivation of the Eyring theory. As seen in molecular dynamics simulations, the motion of the mobile ions inside the channel is very complicated, coupled to the surrounding water molecules, and thus inconsistent with this assumption, that the ion undergoes no collisions during the crossing of the barrier. In addition, even the presence of well defined affinity sites inside the channel is questionable. Last but not
least, barrier models with multiple occupancy (more that one ion inside the channel) rarely consider the possibility of coupled motion of the mobile ions inside the channel.

In this chapter, we aim to resolve some of these problems, present in rate models. We consider a generalization of rate models to occupancy state models with a \textit{general} distribution of lifetimes of the different states. In contrast to rate models, this renders the permeation process not Markovian. However, by applying tools from renewal theory, we describe the permeation process with a Markov chain, and develop methods to compute all the relevant macroscopic quantities, such as the unidirectional and net currents.

Another novel result is the application of Markov chains to coupled motion type models of multiple occupancy, and their comparison to the analogous rate type models. While some of the transition diagrams for coupled type models are different than those of previously analyzed rate models, there are many qualitative similarities. For example, as shown in section 5.5, for a channel that can contain at most $k$ ions, but at least $k - 1$ ions, all performing coupled motion, the flux ratio exponent is exactly $k$. A similar result holds for analogous (non coupled) rate type models [136].

Our analysis shows that, as expected, the net and unidirectional currents depend only on the average residence times of the different states and on their transition probabilities, and not on any other properties of the general lifetime distributions of the different states. However, as shown in Section 5.6, the unidirectional currents depend only on the transition probabilities. This result seems to be new, to the best of the author’s knowledge.

The importance of the generalization of rate models to Markov chains is that it provides a method to bridge between molecular dynamics simulations and the actual computation of macroscopic quantities such as the net current. This is because the inputs needed for a Markov chain model are the average lifetimes of the different occupancy states of the channel and the transition probabilities between the different states. While at the present time, molecular dynamics simulations are unable to compute the current flowing through a channel, these quantities are certainly within the reach of such simulations. This opens the possibility for a long awaited hierarchy of theories, where one detailed theory provides effective parameters for a coarse grained one, from which macroscopic quantities can be computed.
5.1 Introduction

The first descriptions of ionic permeation through biological membranes used the formalism of bulk electrodiffusion or the conductance approximation (the HH Theory) [72]. These theories assumed the principle of independence, i.e., independence of the unidirectional flows across the membrane. However, as early as 1955, the work with tracer fluxes of Hodgkin and Keynes [76], revealed deviations from the principle of independence for ionic flow across membranes. The experimental results showed that for certain membranes ions diffuse in a single-file coupled motion. In order to quantitatively measure the amount of single-file coupling, Hodgkin and Keynes introduced the flux ratio exponent $n'$, also known as the Ussing exponent. This exponent is defined according to the following equation

$$\frac{I_{LR}}{I_{RL}} = \exp\left(n'\frac{z\varepsilon(V - V_N)}{k_BT}\right), \quad (5.1)$$

where $V_N = (k_BT/z\varepsilon)\ln(C_R/C_L)$ is the Nernst potential associated with the permeating ion of valence $z$, and $V$ is the applied potential, so $z\varepsilon(V - V_N)$ is the electrochemical energy difference. For ions moving independently across the membrane, the Nernst-Planck theory predicts a value of $n' = 1$ for the flux exponent. However, experiments reveal that while for certain channels the flux exponent is roughly one, other channels have flux exponents larger than one, ranging from 1 to even 3.5 [26, 56, 145]. Moreover, in certain channels the flux ratio exponent depends on the bulk concentrations [13].

These findings initiated the formulation and application of rate type models to permeation of ions through ionic channels (see [72], chapter 14, and references therein). These models assume the existence of affinity sites inside the channel, with ions ”hopping” from one site to another at exponential rates. With these models, some of the microscopic effects of channel permeation, such as single filing, are captured. For some channels it is even possible to recover experimental data by fit of parameters [53, 153, 158].

The simplest rate type model is that of a single ion channel, that is a channel that can be occupied by at most one ion at a time [72]. This model assumes that an ion inside the channel is found in a deep well with two barriers on each side of the channel. In the literature, the standard nomenclature for this model is 2B1S, that is two barriers and one site. A simple analysis shows that the Ussing exponent for this model is exactly one.

Since many channels exhibit a flux ratio exponent larger than one, this has lead to the formulation of more complicated rate models for multi ion channels, that can hold more
than one ion at a time. These models predict an exponent $n' \geq 1$, with various voltage and concentration dependencies for different models. The most common of these models is the 3B2S model for a two ion channel, assuming three barriers and two ion sites inside the channel [60, 73], for which the flux ratio exponent ranges between one and two. Based on the predictions of multi ion rate models, an experimentally measured flux ratio exponent $n' > 1$ is considered a definite criterion for the presence of multiple ions in a channel [72, 136].

Almost all rate type models rely on the following assumptions:

1. At any given time, the channel with the permeating ions inside it can be found in only one state from a small finite set of channel occupancy states.

2. There are pre-assigned transition probabilities between states, and each state has a pre-assigned exponentially distributed lifetime, as customarily used in Eyring rate theory [57, 72].

Under these assumptions, it is possible to formulate and solve the resulting system of rate equations, either analytically or numerically, to obtain the macroscopic observables, such as the total current, the current noise, and the unidirectional flux ratio. In the past twenty years, numerous rate type models have been formulated, and their parameters fitted to reproduce experimental results. As mentioned above, for some channels it has been possible to fit all the experimental data, while for others an attempt to fit some of the measurements failed in reproducing others.

As describe above, there are many criticisms against rate type models. The main two issues are 1) rate models are *ad-hoc*, without underlying physics, and their parameters are set in order to fit experiments, and not as an outcome of an underlying more detailed physical model. 2) Almost all rate theories consider *vacancy* models with predefined affinity sites for the ions inside the channel, and do not consider the possible *coupled* motion of the ions inside the channel, not necessarily confined to special affinity sites.

In this chapter we present a generalization of rate models to occupancy state models with a general distribution of lifetimes of the different states, This renders the permeation process not Markovian, in contrast to rate models. We also consider coupled motion type models and compare them to the analogous vacancy rate models.
We stress that the aim of the chapter is not to calculate fit parameters to match experimental data, but rather to obtain general qualitative insights into conduction mechanisms based on the predictions of Markov chain models, and compare them to the predictions of similar rate type models. In our view, all parameters of such models, such as residence times and transition probabilities can and should be calculated as output of more detailed and self consistent molecular physical models. In this way, the semi-Markov chain models presented in this chapter can provide a method to bridge between computationally intensive molecular dynamics simulations or other physical microscopic theories of ionic motion inside the channel, and the actual computation of macroscopic quantities such as the net and unidirectional currents through the channel.

The rest of the chapter is organized as follows: We start in section 5.2 by analyzing the simplest model of a channel that can contain only one ion of a given species. In this case, the results of the Markov chain approach are identical to those of the rate type model 2B1S. Then, in section 5.3 we consider a Markov chain model for a two-ion channel with coupled motion. In this case. In section 5.4 we analyze a Markov chain model for a channel that can contain any number between 0 and 2 ions. The results for the Ussing flux exponent of this model differ from those of the corresponding 3B2S rate model, due to the coupled motion of the ions. The connection between the Ussing flux exponent and the number of ions inside the channel is explored in section 5.5. Finally in section 5.6 we show how to calculate the current for a general Markov chain state model with a general distribution of residence times.

5.2 A 3-State Model for a Single Ion Channel

The simplest state model for permeation through a channel is that of a single-ion channel, that is a channel that can occupy at most one ion at a time. For simplicity, we consider a single-ion channel embedded in an impermeable membrane, separating between two simple bi-ionic electrolytic solutions. Furthermore, we assume that the channel admits only positively charged ions.

For this system, there can be only two states: Either the channel is empty or either it is occupied by one positive ion. We can further subdivide the occupied state according to the origin of the ion inside the channel, that is, from which side did this ion enter the channel. This leads to the definition of three different states for the channel: \{0, L, R\}, where
0 denotes an empty channel, and \( L \) and \( R \) denote a channel occupied by an ion that entered from the left bath or from the right bath, respectively. The possible transitions between the different states are denoted in the following figure,

\[
\begin{array}{c}
\text{0} \\
\text{L} \\
\text{R}
\end{array}
\]

Notice the implicit assumption that as a left bath ion exits the channel into the right bath, it is immediately absorbed by the bath, and forgets its origin. This assumption is justified by considering the Born self energy barrier of a few \( k_B T \) that any ion entering the channel must overcome. Thus, as an ion exits the channel, its probability of immediate re-enterance into the channel is low, and neglected in this model. While more complex models also include a site at the edge of the channel, or just outside it, this assumption, that exiting ions are instantaneously absorbed by the baths, is also found in rate theory models, in some form or another. We shall follow this assumption throughout all the Markov chain models presented in this chapter.

The permeation of ions through the channel in this simple model depends only on four parameters. The first two parameters concern the motion of the ion inside the channel. They are the mean residence time of an ion inside the channel until its exit, denoted by \( \tau_1 \), and the probability that the mobile ion inside the channel will exit into the right bath, denoted by \( p \). Notice the assumption that these two parameters, \( \tau_1 \) and \( p \) are equal for both the \( R \) and \( L \) states. This is consistent with the assumptions of the model, that the channel has one occupied state indistinguishable of the origin of the channel ion, and the sub-division of this state according to the ion’s origin is for the purpose of ionic flux calculations. The other two parameters concern the arrival process into an empty channel. They are the mean arrival time of an ion from either bath into an empty channel, denoted by \( \tau_0 \), and the probability that the origin of the arriving ion is from the left bath, denoted by \( a \). In other words, \( \tau_0 \) is the average time until the first entrance of an ion (from either bath) into an empty channel, and \( a \) is the probability that a left bath ion will enter the channel before a right bath ion.

Our first aim is to compute the unidirectional currents from left to right and from right to left, denoted \( I_{LR} \) and \( I_{RL} \) respectively. Note that in contrast to rate type models, our system is not memoryless. Since the lifetimes of the different states are not necessarily exponentially distributed, the probability of a transition at a given time interval \( (t, t + \Delta t) \) does not equal
\[ \lambda \Delta t, \] where \( \lambda \) is a rate constant, but rather depends on the time already spent in that state. Therefore, it is not possible to directly apply the standard tools of rate theory and chemical kinetics to compute the currents in our model.

To compute the unidirectional currents, we define the time \( \tau_L \) as the mean first passage time from the \( L \) state to the \( L \) state. That is, \( \tau_L \) is the average time till the first return to the \( L \) state, when starting from the \( L \) state. Similarly, we define the time \( \tau_R \) as the average time to return to the \( R \) state, from the \( R \) state. In terms of these times, the unidirectional currents are given by

\[ I_{LR} = \frac{ze \rho}{\tau_L}, \quad I_{RL} = \frac{ze(1-p)}{\tau_R}, \] (5.3)

where \( e \) is the charge of an electron, and \( z \) the valence of the mobile ion.

The time \( \tau_L \) can be easily computed from the following set of linear recursion relations,

\[ \begin{align*}
\tau_L &= \tau_1 + \tau_{0,L}, \\
\tau_{0,L} &= \tau_0 + (1-a)(\tau_1 + \tau_{0,L}).
\end{align*} \] (5.4)

The reasoning behind these equations is as follows: Consider first the equation for \( \tau_L \). Starting from the \( L \) state, according to the state diagram (5.2), the only possible transition is to the empty channel state, which takes an average time \( \tau_1 \). Thus, the total average time to return to the state \( L \) is the sum of \( \tau_1 \) and the average time to reach the \( L \) state from the \( 0 \) state, denoted \( \tau_{0,L} \). This time is described by the second equation in (5.4). The first term in this equation is the mean residence time at the empty state, \( \tau_0 \). Then, from the empty state, either a transition is made to the required \( L \) state with probability \( a \), or a transition to the \( R \) state is made with probability \( (1-a) \). In the latter case, it takes an average time \( \tau_1 \) to return to the empty state, and from there an additional average time \( \tau_{0,L} \) to reach the \( L \) state. Thus, the second equation is a linear recursive equation for \( \tau_{0,L} \). The system (5.4) of two linear equations for the two unknowns, \( \tau_L \) and \( \tau_{0,L} \) is readily solved, and the solution for \( \tau_L \) is given by

\[ \tau_L = \frac{\tau_0 + \tau_1}{a}. \]

A similar system of equations can be formulated for \( \tau_R \), the average time to return to the \( R \) state, when starting from the \( R \) state. However, this need not be done since from symmetry considerations \( \tau_R \) is given by

\[ \tau_R = \frac{\tau_0 + \tau_1}{1-a}. \]
Combining the last two equations with (5.4), we obtain that the unidirectional flux ratio is given by

\[ \frac{I_{LR}}{I_{RL}} = \frac{p}{1-p} \cdot \frac{a}{1-a}. \]

Note that the flux ratio is independent of the residence times \( \tau_0 \) and \( \tau_1 \) of the empty and occupied states, and depends only on the transition probabilities \( a \) and \( p \). Now recall that the parameter \( p \) denoted the probability that an ion in an occupied channel exits on the right. This parameter depends strongly on the structure and fixed charge of the channel, and on the applied potential, but rather weakly on the bath concentrations. The other parameter, \( a \), denoted the probability of entrance of a left bath ion into an empty channel, before the entrance of a right bath ion. For an uncharged symmetric channel with no applied potential, \( a \) is equal to \( C_L / (C_L + C_R) \), where \( C_L \) and \( C_R \) denote the left and right bath concentrations, respectively. For other cases, such as a non-symmetric charged channel with an applied potential, a common practice is to assume that this probability is given by

\[ a = \frac{C_L k_L \exp(V_L)}{C_L k_L \exp(V_L) + C_R k_R \exp(V_R)}, \tag{5.5} \]

where \( V_L \) and \( V_R \) are the values of the electric potential at the edges of the channel, and \( k_L \) and \( k_R \) are rate constants. Thus, assuming the above functional relation of the probability \( a \) on the bath concentrations, we obtain that the Ussing flux ratio is approximately given by

\[ \frac{I_{LR}}{I_{RL}} = \text{Constant} \times \left( \frac{C_L}{C_R} \right). \tag{5.6} \]

Therefore, according to definition (5.1), the flux ratio exponent for this model is exactly one. This result is essentially equivalent to that predicted by the 2B1S rate type model. The main difference is that result (5.6) was derived under a general distribution of residence times, without the assumption that these times are exponentially distributed.

This concludes our analysis of this simple single ion channel. A detailed study of the noise characteristics of this non-Markovian model of a singly occupied channel appears in [9].

### 5.3 A 5-State Two Ion Model

We now consider a more complicated model, of a two ion channel. As above, for simplicity we consider only simple bi-ionic surrounding electrolytic solutions, and assume that only
the single species of positive ions can enter the channel. In addition, we make the following assumptions:

- **At Least One Ion:** We assume that for the bath concentrations under consideration, the mean lifetime of an ion inside the channel is much larger than the average entrance time of a bath ion into the channel. Therefore, at any given time there is at least one ion inside the channel.

- **Two Ion Channel:** There is a stable state for two positive ions inside the channel. However, there is no stable state for three positive ions inside the channel, and the probability of a third ion entering the channel is negligible.

- **Single Filing and Coupled Motion:** We assume that the channel is narrow so that two ions inside the channel cannot cross each other. Furthermore we also assume that water molecules cannot cross ions inside the channel, and that the two ions inside the channel perform coupled motion, similar to a *quasi particle*.

The assumptions of this model set it apart from the traditional and well analyzed 3B2S rate model of a channel with three barriers and two sites, which can also accommodate two ions inside it. More specifically, in our model it is implicitly assumed that two ions inside the channel perform *correlated motion*. As one of the two channel ions moves inside the channel, the other ion moves as well in a correlated fashion, with their distance essentially unchanged. Once one of the two ions exits the channel, the remaining ion almost immediately settles in its steady state inside the channel, before the entrance of another ion into the channel.

Under these assumptions, the channel is almost always occupied by either one or two ions. This leads to the definition of the following five possible states for the channel, \( \{L, R, LL, LR, RR\} \), with the following possible transitions between the different states

\[
\begin{array}{ccc}
L & & R \\
LL & \rightarrow & \downarrow & LR & \rightarrow & \downarrow & RR \\
\end{array}
\]  

(5.7)

As in the single ion model, also here we have only four parameters governing the permeation process. The first set of parameters concerns the motion of ions inside the channel. These are \( p \), the probability of exit on the right from a 2-ion occupied state of the channel, and \( \tau_2 \), the average residence time of the 2-ion occupied state, till the exit of one of the ions.
The other two parameters characterize the entrance process of bath ions into the 1-ion occupied state. These parameters are \( a \), the entrance probability of a left bath ion into a 1-ion occupied state of the channel, before the entrance of a right bath ion, and \( \tau_1 \), the average time until the entrance of a bath ion (from either side) into the 1-ion occupied state.

We now compute the unidirectional currents as a function of these four parameters. There are only two transitions that contribute to the current. The transition \( LL \rightarrow L \) contributes a current of \((+1)\) units of electric charge, only if the rightmost ion left the channel into the right bath. This event has probability \( p \). The other transition contributing to the current is \( RR \rightarrow R \), contributing a current of \((-1)\), only if the leftmost ion of the \( RR \) state exited the channel into the left bath, with probability \( 1 - p \).

Following the analysis of the single-ion channel model, we denote by \( \tau_{LL} \) the average time to return to the state \( LL \), when starting from the \( LL \) state. An analogous definition holds for the time \( \tau_{RR} \). With these definitions, the unidirectional currents are given by

\[
I_{LR} = \frac{zep}{\tau_{LL}}, \quad I_{RL} = \frac{ze(1-p)}{\tau_{RR}}.
\] (5.8)

The time \( \tau_{LL} \) can be computed from the following set of linear recursion equations

\[
\tau_{LL} = \tau_2 + \tau_{L,LL}, \\
\tau_{L,LL} = \tau_1 + (1-a)[\tau_{LR,L} + \tau_{L,LL}], \\
\tau_{LR,L} = \tau_2 + (1-p)[\tau_{R,LR} + \tau_{LR,L}], \\
\tau_{R,LR} = \tau_1 + (1-a)[\tau_2 + \tau_{R,LR}].
\]

The reasoning of these equations follows the same principles as in the 3-state model, and in this context, \( \tau_{i,j} \) denotes the average time to reach the \( j \) state from the \( i \) state. The solution for \( \tau_{LL} \) is

\[
\tau_{LL} = \frac{1}{a^2p}(\tau_1 + \tau_2)(1 + 2ap - a - p).
\] (5.9)

By symmetry considerations, the expression for \( \tau_{RR} \) can be found by replacing \( a \) by \( 1 - a \) and \( p \) by \( 1 - p \) in (5.9), which gives

\[
\tau_{RR} = \frac{1}{(1-a)^2(1-p)}(\tau_1 + \tau_2)(1 + 2ap - a - p).
\] (5.10)

Combining (5.8)-(5.10), the unidirectional flux ratio is given by

\[
\frac{I_{LR}}{I_{RL}} = \frac{p}{\tau_{LL}} \frac{\tau_{RR}}{1-p} = \left( \frac{p}{1-p} \right)^2 \left( \frac{a}{1-a} \right)^2.
\] (5.11)
Note that as in the 3-state model, the unidirectional flux ratio depends only on the transition probabilities between states, and is independent of the residence times of the different states. This is a general feature of Markov chain models, as proved in Section 5.6.

We now consider the flux ratio exponent for this model. According to eq. (5.11), the exponent depends only on the two parameters \( p \) and \( a \). Recall that \( p \) denotes the exit probability on the right of an ion from the 2-ion occupied state. This quantity thus depends strongly on the channel characteristics, its permanent charge, radius and length, as well as on the applied potential, but only weakly on the bath concentrations. Further recall that \( a \) denotes the probability that the first ion entering a channel with an ion already inside it, will be from the left bath (and not from the right bath). Assuming equation (5.5) for the functional dependence of the probability \( a \) on the bath concentrations, we obtain that the Ussing flux ratio is given by

\[
\frac{I_{LR}}{I_{RL}} = \text{Constant} \times \left( \frac{C_L}{C_R} \right)^2.
\]

That is, the Ussing flux exponent \( n' \) is exactly 2. Note that \( n' \) is equal to the number of ions inside the channel in our model, needed to transfer current. This is not a coincidence, but merely a private case of a general feature for this kind of model. As proven in Section 5.5, for a channel that can contain at most \( k \) ions but at least \( k - 1 \) ions, the flux ratio exponent is exactly \( k \). Finally we note that the flux ratio exponent for this model is very different than that of the 3B2S model, in which at high concentrations, the exponent decreases back to a value of 1 [73].

### 5.4 A 6 State Model for One/Two Ion Channel

We now combine the two models of a single ion channel and a two ion channel. We assume that the channel can be in either one of three states: empty, occupied by one ion, or occupied by two ions. The figure of all possible states and transitions is the combination of the former two transition diagrams (5.2) and (5.7)

![Diagram of a 6 state model](image-url)
This model has eight parameters: $\tau_0$, $\tau_1$, $\tau_2$ are the residence times of the empty, 1-ion and 2-ion channel occupancy states respectively. We denote by $q$ the probability of entrance of a second ion into the channel before the exit of the ion already present in the channel. We denote by $p_1$ and $p_2$ the probability of exit on the right of an ion inside the channel, from the 1-ion and 2-ion occupancy states, respectively. Finally we denote by $a_0$ the probability of entrance of a left bath ion into an empty channel, and we denote by $a_1$ the probability of entrance of a left bath ion into a 1-ion occupied channel, given that a second ion entered the channel before the exit of the ion already there.

In this model there are two types of transitions that contribute to the unidirectional current from left to right. One is the transition $L \rightarrow 0$, which contributes $(+1)$ to the current, but only with probability $p_1$, and the other is the transition $LL \rightarrow L$, which also contributes $(+1)$ to the current, but with probability $p_2$. One method to calculate the current is as was done previously. Compute the average return time to these states, $\tau_L$ and $\tau_{LL}$. Then the current is given by

$$I_{LR} = \frac{ze p_1}{\tau_L} + \frac{ze p_2}{\tau_{LL}}.$$ 

However, there is a different method to calculate the current, which resembles the computations made in rate models. The basic idea is to consider the different states of the channel as a Markov chain, compute the steady state of this system, disregarding the different residence times of the different states. Then apply renewal theory methods to account for the different residence times of the different states, in order to compute the resulting current. The exact method and a formula for the current of a general Markov chain is outlined in Section 5.6.

For our case, labeling the states in the following order, $\{0, L, R, LL, LR, RR\}$, the $6 \times 6$ transition matrix is given by

$$M = \begin{pmatrix} 
0 & 1 - q & 1 - q & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & p_2 & 0 \\
1 - a_0 & 0 & 0 & 0 & 1 - p_2 & 1 \\
0 & qa_1 & 0 & 0 & 0 & 0 \\
0 & q(1 - a_1) & qa_1 & 0 & 0 & 0 \\
0 & 0 & q(1 - a_1) & 0 & 0 & 0 
\end{pmatrix}$$
The steady state vector \( \mathbf{p} \), satisfying the equation \( M\mathbf{p} = \mathbf{p} \), is given by

\[
\mathbf{p} = \frac{1}{2(1 + 2qp_2a_1 - qp - qa_1)} \begin{pmatrix}
(1 - q)(1 + 2qp_2a_1 - qa_1 - qp)

a_0(1 - q) + qp_2a_1

a_0q - a_0 - qa_1 - p_2q + p_2qa_1 + 1

qa_1[a_0(1 - q) + qp_2a_1]

q(a_0 - a_0q - 2a_1a_0 + 2a_1a_0q - qa_1^2 + a_1)

q(1 - a_1)(a_0q - a_0 - qa_1 - p_2q + p_2qa_1 + 1)
\end{pmatrix}.
\]

As shown in section 5.6, in terms of the steady state vector \( \mathbf{p} \) and the lifetimes of the different states, the undirectional currents are given by

\[
I_{LR} = \sum_k p_k \frac{1}{p_1} \left[ p_L(1 - q)p_1 + p_{LL}p_2 \right],
\]

\[
I_{RL} = \sum_k p_k \frac{1}{p_1} \left[ p_R(1 - q)(1 - p_1) + p_{RR}(1 - p_2) \right],
\]

and their ratio is

\[
\frac{I_{LR}}{I_{RL}} = \frac{p_L(1 - q)p_1 + p_{LL}p_2}{p_R(1 - q)(1 - p_1) + p_{RR}(1 - p_2)}. \tag{5.13}
\]

Consider for example a symmetric channel with no applied voltage. In this case, from symmetry considerations

\[
p_1 = p_2 = \frac{1}{2} \quad \text{and} \quad a_0 = a_1 = \frac{C_L}{C_L + C_R}.
\]

Assume further that the residence time \( \tau_1 \) of a single ion inside the channel is exponentially distributed. Since the entrance time of another ion into the channel is also exponentially distributed, it follows that \( q \) admits the functional form

\[
q = \Pr\{\tau_1 < \tau_1\} = \frac{C_L + C_R}{C_L + C_R + \kappa/ < \tau_1 >},
\]

for some value of \( \kappa \).

For this case, as shown in fig. 5.1, a graph of the Ussing flux exponent \( n \) as a function of \( C_L \) starts at \( n = 1 \) for low concentrations and goes up to a value of \( n = 2 \) for high concentrations. As discussed above, this is a different behavior of the flux exponent than the one exhibited by the well studied 3B2S model of rate theory.
5.5 The Ussing Flux Ratio and the Number of Ions in the Channel

An interesting question concerns the connection between the Ussing flux ratio, and the number of ions in the channel. As we have already seen above, for a single ion channel, the flux ratio exponent is exactly one, eq. (5.6), and for a channel that can contain at most 2 ions but at least one, the Ussing flux ratio is exactly 2, eq. (5.12).

We hence consider the general case of a channel that can contain at most $k$ ions, but at least $k-1$ ions. This channel has obviously only two occupancy states, either with $k$ ions or with $k-1$ ions. However, for the purpose of calculating currents, we subdivide each of these states according to the origin of the ions in the channel. Since ions inside the channel are not allowed to cross each other, there are only $k$ possible states for the $k-1$ occupancy state of the channel. We denote these states as \{\text{LL...LL}, \text{LL...LR}, \text{LL...RR}, ..., \text{RR...RR}\}. Similarly there are only $k+1$ different states for a channel with $k$ ions, and in total $2k+1$ states. For example, for $k = 3$ we have a total of $2k+1 = 7$ possible states, with the following transition diagram,

\[
\begin{array}{c}
\text{LLL} \\
\text{LLR} \\
\text{LRR} \\
\text{LLL} \\
\text{LR} \\
\text{RR} \\
\text{RR} \\
\end{array}
\]

Numbering the states from left to right, that is \{LLL, LL, LLR, LR, LRR, RR, RRR\} for

Figure 5.1: The unidirectional current ratio (left), and the corresponding Ussing flux exponent $n$ (right), as a function of concentration on the left bath $C_L$. 
example for $k = 3$, gives the following $(2k + 1) \times (2k + 1)$ transition matrix,

$$M = \begin{pmatrix}
0 & a & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & p & 0 & 0 & 0 & 0 & 0 \\
0 & 1 - a & 0 & a & 0 & 0 & 0 & 0 \\
0 & 0 & \ddots & \ddots & \ddots & 0 & 0 & 0 \\
0 & 0 & 0 & \ddots & \ddots & \ddots & 0 & 0 \\
0 & 0 & 0 & 0 & 1 - p & 0 & p & 0 \\
0 & 0 & 0 & 0 & 0 & 1 - a & 0 & a \\
0 & 0 & 0 & 0 & 0 & 0 & 1 - p & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 - a & 0
\end{pmatrix} \tag{5.14}$$

With these definitions, we are ready to prove the following lemma,

**Lemma 5.1:** Consider a channel that can contain at most $k$ ions but at least $k - 1$ ions. Let $p$ denote the probability of exit on the right of an ion, from the $k$-occupied state, and let $a$ denote the probability of entrance on the left of an ion into the $(k - 1)$-occupied state. Under the assumption that $p$ is independent of the bulk concentrations, and that the functional dependence of $a$ on the bulk concentrations is of the following form

$$a = \frac{k_L C_L \exp(V_L)}{k_L C_L \exp(V_L) + k_R C_R \exp(V_R)} + k_R C_R \exp(V_R), \tag{5.15}$$

then the Ussing flux exponent is exactly $k$. That is, the unidirectional flux ratio admits the following form

$$\frac{I_{LR}}{I_{RL}} = A \times \left(\frac{C_L}{C_R}\right)^k,$$

where the constant $A$ depends on the applied potential, and on the structure of the channel, but not on bulk concentrations.

**Proof:** Let $\mathbf{p}$ denote the vector of steady state residence probabilities of the states defined by the matrix $M$, defined above in eq.(5.14). It is the normalized eigenvector corresponding to the eigenvalue $\lambda = 1$ of the matrix $M$. Simple algebraic manipulations show that up to a normalization factor, this vector is given by

$$\mathbf{p} = \left( a, 1, \alpha, \alpha \beta, \alpha^2 \beta, \ldots, \alpha^{k-1} \beta^{k-1}, \alpha^{k-1} \beta^k (1 - a) \right) \tag{5.16}$$

where

$$\alpha = \frac{1 - a}{p}, \quad \beta = \frac{1 - p}{a}.$$
Therefore, according to lemma 5.2 the unidirectional currents ratio is given by

\[
\frac{I_{LR}}{I_{RL}} = \frac{p_1}{p_{2k+1}} \frac{p}{1-p} = \left(\frac{p}{1-p}\right)^k \left(\frac{a}{1-a}\right)^k.
\]

Under the assumptions mentioned above, that the probability \(p\) is independent of bulk concentrations, and the parameter \(a\) admits the functional dependence of eq. (5.15), we obtain that

\[
\frac{I_{LR}}{I_{RL}} = \text{Constant} \times \left(\frac{C_L}{C_R}\right)^k.
\]

### 5.6 Computation of the current for a Markov chain

Consider the following problem: Let \(M\) denote an irreducible Markov chain with \(N\) states, denoted simply by \(\{i\}_{i=1}^N\), with transition probabilities \(p_{i,j}\) and mean residence times \(\tau_i\). Assume furthermore that there is a score function associated with this chain, such that for every transition from state \(i\) to state \(j\), there is an associated score, a real number denoted by \(\text{Score}(i,j)\).

Each path or trajectory on this chain, is a function of time, \(f(t)\), admitting the values \(\{i\}_{i=1}^N\), with jumps at the random transition times \(\{t_j\}_{j=1}^\infty\). For each trajectory \(f(t)\) let \(A(f,T)\) denote its total score up to time \(T\). This total score is the sum of the scores of all the transitions by time \(T\), multiplied by the respective weights \(\text{Score}(i,j)\). Our aim is to compute the average score per unit time, or current of this stochastic process, which is defined as the limit \(A(t)/t\) as \(t \to \infty\).

**Lemma 5.2** The average score is independent of the trajectory \(f(t)\). That is, there exists a constant \(J\), independent of \(f(t)\), such that

\[
J = \lim_{T \to \infty} \frac{A(f,T)}{T} \quad \text{w.p. 1.} \tag{5.17}
\]

We shall not give a proof of this lemma here. The interested reader is referred to [41, 88].

**Lemma 5.3** The current of the process \(A(t)\) is given by

\[
\lim_{t \to \infty} \frac{A(t)}{t} = \frac{1}{\tau_{\text{renewal}}} \sum_i p_i \sum_j p_{i,j} \text{Score}(i,j),
\]
where \( \tau_{\text{renewal}} \) is the average renewal time associated with this chain, defined as

\[
\tau_{\text{renewal}} = \sum_k p_k \tau_k.
\]

Note that \( \tau_{\text{renewal}} \) is the average time between transitions, also known as the renewal time of the Markov chain.

**Proof:** Since the Markov chain is irreducible with a finite number of states, there is a single steady state for the Markov chain of states, denoted by \( p = \{p_i\} \). It satisfies the equation

\[
M'p = p,
\]

where \( M \) is the \( N \times N \) matrix of transition probabilities \( p_{i,j} \).

Computing the above limit is the same as considering a system already found in steady state, and computing the limit [41]

\[
\lim_{\Delta t \to 0} E \left\{ \frac{A(t + \Delta t) - A(t)}{\Delta t} \right\}.
\]

Consider thus a system found in steady state, which we sample at time \( t \). Had the residence times of all different states been equal, then the probability of finding the system at state \( i \) would be of course \( p_i \). However, since different states have different residence times, the probability of finding the system in state \( i \) is given by

\[
\Pr\{\text{system in state } i\} = \frac{p_i \tau_i}{\sum_k p_k \tau_k}.
\]

Now, for a system found in state \( i \), the probability of transition by time \( \Delta t \), is a residual time that depends on the distribution \( a_i(t) \) of the residence time at state \( i \). It is given by [59]

\[
\Pr\{\text{Transition from state } i \text{ at time interval } [s, s + \Delta t]\} = \frac{A_i^c(s) \Delta t}{\tau_i} \left[ 1 + o(\Delta t) \right],
\]

where \( A_i^c(s) \) is the complementary cumulative distribution function of the density \( a_i(s) \). For small \( \Delta t \), since \( A^c(0) = 1 \), we have that

\[
\Pr\{\text{Transition from state } i \text{ by time } \Delta t\} = \frac{\Delta t}{\tau_i} \left( 1 + O(\Delta t) \right).
\]

Combining all of the above, yields the required formula and proves the Lemma. \( \blacksquare \)
Corollary: The unidirectional flux ratio does not depend on the residence times of the different states, but only on the transition probabilities.

Proof: Let $S_{LR}(i,j)$ and $S_{RL}(i,j)$ denote the contributions to the unidirectional currents from left to right and from right to left, of the transition from state $i$ to state $j$. According to the lemma, the unidirectional currents are given by

$$I_{LR} = \frac{1}{\tau_{\text{renewal}}} \sum_i p_i \sum_j p_{i,j} S_{LR}(i,j),$$

$$I_{RL} = \frac{1}{\tau_{\text{renewal}}} \sum_i p_i \sum_j p_{i,j} S_{RL}(i,j).$$

Dividing one unidirectional current by the other, the only time dependent quantity $\tau_{\text{renewal}}$ cancels out, and the unidirectional flux ratio depends only on the transition probabilities between the different states.
Chapter 6

Langevin Trajectories Between Fixed Concentrations

In this chapter we consider Langevin trajectories in a region between fixed concentrations. The continuum description of such a system is based on the Nernst-Planck equation with fixed concentrations at the boundaries. The underlying motion of particles is Brownian diffusion in a field of force, usually described by the Langevin equation. The boundary conditions for the Nernst-Planck equation, however, do not describe the behavior of the Langevin trajectories at the boundaries. In this chapter we describe the Langevin trajectories of particles diffusing between fixed concentrations at the boundaries. On the microscopic level the boundary concentrations fluctuate and are maintained only on the average, either by an external feedback mechanism or by connection to effectively infinite reservoirs. The feedback mechanism or the infinite reservoirs serve therefore as both sources for ingoing trajectories and absorbers of outgoing trajectories. We calculate the strengths of the sources needed to maintain the given average concentrations. This description is applied to the design of a Langevin simulation of the system, both for interacting and non-interacting particles. In particular, we propose to apply our method to the simulation of ionic diffusion through protein channels across biological membranes separating salt solutions of different concentrations.

6.1 Introduction

We consider particles that diffuse between two regions where fixed, but different concentrations are maintained. The fixed concentrations can be maintained by several different
mechanisms. For example, in a chemical reactor a feedback mechanism senses the concentrations and removes or injects particles accordingly. Another example is the diffusion of ions through a protein channel of a biological membrane that separates two salt solutions of different fixed concentrations [72]. In this case the diffusion current through the channel is so small that effectively the ambient concentrations of the two baths are fixed. Thus, the fixed concentrations are maintained by connection to practically infinite reservoirs.

Continuum theories of such diffusive systems describe the concentration field by the Nernst-Planck equation with fixed boundary concentrations [21, 50, 72, 114, 138]. In these theories there is no time dependence of the macroscopic boundary concentrations. The force field does not fluctuate and is usually calculated from a Poisson equation coupled to the Nernst-Planck equations. The huge voltage fluctuations in the salt solution and in the channel are averaged out in these theories.

On the other hand, the underlying microscopic theory of diffusion describes the motion of the diffusing particles by Langevin’s equations [21, 54, 92, 137]. This means that on a microscopic scale there are fluctuations in the concentrations at the boundaries. The question of the boundary behavior of the Langevin trajectories, corresponding to fixed boundary concentrations, arises both in theory and in the practice of molecular simulations of diffusive motion [16, 19, 25, 79, 106, 107, 110]. Thus, in the case of fixed boundary concentrations maintained by an external feedback mechanism at the boundaries, ingoing trajectories are physically generated and outgoing trajectories are terminated there. In this case the strengths of the sources of new trajectories are chosen by the feedback mechanism so that the given concentrations are maintained at the boundaries. However, if the concentrations are maintained by connection to infinite reservoirs, there are no physical sources and absorbers of trajectories at any definite location in the reservoir or the system. The boundaries in this setup can be chosen anywhere in the reservoirs where the average concentrations are effectively fixed. Thus, limiting the system to a finite region replaces the baths, in effect, by sources and absorbers, as in the former case. However, the generation and absorption of Langevin trajectories in this case has to be identical to the exchange of trajectories between the confined system and the surrounding reservoirs. Not only the strengths of the sources have to be chosen to maintain the average concentration, but the time course of generating new trajectories has to be identical to the time course of the exchange of trajectories between the confined system and the reservoirs, as done in [106, 107, 110] for a similar setup. The
problem of the time course is not addressed in this chapter.

The boundary behavior of diffusing particles has been studied in various cases, including absorbing, reflecting, sticky boundaries, and many other modes of boundary behavior [88, 102]. It seems, however, that the boundary behavior of particles diffusing between fixed concentrations has not been described mathematically in an adequate way. In Langevin and other simulations of this type of diffusion ad hoc methods are usually adopted for maintaining fixed concentrations. It is the purpose of this chapter to analyze the boundary behavior of Langevin trajectories between fixed concentrations and to apply the result to the design of simulations.

In this chapter we show that real or imaginary boundaries with fixed concentrations can be described as sources of ingoing Langevin trajectories and absorbers of outgoing trajectories. This description accounts for outgoing trajectories that recross the boundary and return to the given region. In this chapter we do not consider the exact time course of the injection process, but only calculate the strengths of the sources needed to maintain the fixed concentrations. The time course of the injection process, which takes into account trajectories that recross the boundary many times, has been studied in [106, 107, 110] for a problem with equal boundary concentrations for a simulation with a buffer zone. The method of [106, 107, 110] can be adapted for the determination of the time course in the problem at hand.

We apply our result to channel simulations by proposing an algorithm that maintains fixed average concentrations at the boundaries, but allows for concentration fluctuations in the system. This boundary behavior of Langevin trajectories is consistent with the corresponding Nernst-Planck equation for the density of particles, with fixed concentrations imposed at the boundaries.

The mathematical issue at hand is as follows. While a concentration boundary condition on the NPE does not express in an obvious way a condition on the underlying Langevin trajectories, a flux condition on the boundary does. A given flux on the boundary represents a density and intensity of sources for incoming Langevin trajectories and termination of outgoing trajectories [54].

In Section 2 we find the relationship between the boundary fluxes and boundary concentrations and in Section 3 we discuss the implication of this relation on Langevin simulations.
First, we consider Langevin trajectories in a mean field and derive the relationship between the strength of the sources and the imposed average boundary concentrations. Then we argue that this relationship is valid for Langevin trajectories of interacting particles and apply it to Langevin simulations with long and short range forces. The long range forces that the reservoir particles exert on the simulated ions are assumed known. Their computation requires a separate theory (see, e.g. [110]).

### 6.2 Trajectories, fluxes, and boundary concentrations

Consider a bath connecting two finite, but large reservoirs of fixed concentrations. The fixed concentrations in the reservoirs are maintained by some external mechanism that is not a part of the model. Particles move between the two reservoirs through the connecting bath by diffusion in a field of force. The force on each particle may be due to interactions with the other particles and with an external field. To facilitate the analysis, we consider only interactions with a mean field, which can be an approximate representation of inter-particle forces, e.g., as described in [138]. Of particular interest is the case of a bath of salt solutions of different concentrations separated by an impermeable lipid membrane containing a narrow protein channel [50, 72].

We work in non-dimensional variables, and denote the 6-dimensional coordinate of a particle by \((x, v)\), where \(x = (x, y, z)\) and \(v = (v_x, v_y, v_z)\). The electric potential of the mean field at location \(x\) is denoted \(\Phi(x)\) and the concentration there is \(c(x)\). We denote by \(\Omega\) the finite bath, which occupies a bounded piecewise smooth domain containing parts of the planes \(x = 0\) and \(x = 1\). The given concentrations are maintained at these portions of the boundary. If they are fixed in the infinite reservoirs on the left and on the right at values \(C_L\) and \(C_R\), respectively, we have the boundary conditions

\[
c(0, y, z) = C_L, \quad c(1, y, z) = C_R.
\]  

We assume that there is no flux across the other parts of the boundary of \(\Omega\).

We denote by \(N\) the total number of particles in \(\Omega\) and in the reservoirs. Note that the number of particles in \(\Omega\) is not fixed, but rather fluctuates in time, due to diffusion of particles from the reservoirs to \(\Omega\) and vice versa. Since the ions interact only with a mean field, their motions in the bath and in the reservoirs are described by the uncoupled Langevin
equations
\[ \ddot{x}_i + \gamma(x_i) \dot{x}_i + \nabla_x \Phi(x_i) = \sqrt{2 \gamma(x_i) \varepsilon} \dot{w}_i, \quad (i = 1, \ldots, N), \tag{6.2} \]
where \( \gamma(x) \) is the (state-dependent) friction per unit mass, \( \varepsilon \) is a thermal factor, and \( \dot{w}_i \) are standard independent Gaussian white noises [137].

We denote by \( p_i(x, v) \) the stationary probability density function of the \( i \)-th Langevin trajectory in phase space \((x, v)\), where \( v = \dot{x} \). It is the probability density of finding the trajectory of the \( i \)-th diffusing particle at location \( x \) with velocity \( v \). It satisfies the stationary Fokker-Planck equation (FPE) in the bath and in the reservoirs,
\[ -\nabla_x \cdot \nabla_x p_i + \gamma(x) \varepsilon \Delta v p_i + \nabla \cdot [\gamma(x)v + \nabla_x \Phi(x)] p_i = 0, \tag{6.3} \]
where \( \nabla_x \) and \( \Delta_x \) denote the gradient and Laplacian operators with respect to the variable \( x \).

We now consider the concentration of particles (trajectories), denoted \( c(x, v) \), defined as the number of particles per phase space unit volume, centered at \((x, v)\). By definition,
\[ c(x, v) = \sum_{i=1}^{N} p_i(x, v). \tag{6.4} \]
Obviously, \( c(x, v) \) also satisfies the FPE (6.3),
\[ -\nabla_x \cdot \nabla_x c + \gamma(x) \varepsilon \Delta v c + \nabla \cdot [\gamma(x)v + \nabla_x \Phi(x)] c = 0. \tag{6.5} \]
The concentration of particles per unit (spatial) volume, denoted \( c(x) \), is the marginal concentration
\[ c(x) = \int_{R^3} c(x, v) dv. \tag{6.6} \]
The total phase space particle flux density vector in the \( x \) direction is given by
\[ J(x) = (J_x(x), J_y(x), J_z(x)) \equiv \int_{R^3} vc(x, v) dv. \tag{6.7} \]
We denote by \( J_x(y, z) \) the particle flux density in the \( x \)-direction on any cross section of \( \Omega \) by the plane \( x = \text{const} \). At the boundaries \( x = 0 \) and \( x = 1 \) it can be decomposed into incoming and outgoing fluxes according to the particle’s velocity. Thus, the incoming flux on the left is given by
\[ J_{\text{in}}(0, y, z) = \int_{R^2} \int_{0}^{\infty} v_x c(0, y, z, v) dv_x \]
\[ J_{\text{in}}(0, y, z) = \int_{R^2} \int_{0}^{\infty} v_x c(0, y, z, v) dv_x \tag{6.8} \]
and the outgoing flux on the left is given by

\[
\mathcal{J}_{\text{out}}(0, y, z) = -\int_{\mathbb{R}^2} \int dv_y dv_z \int_{-\infty}^{0} v_x c(0, y, z, v) \, dv_x,
\]

where \(c(x, v)\) is the particle density in phase space. Similarly, the incoming flux on the right is given by

\[
\mathcal{J}_{\text{in}}(1, y, z) = -\int_{\mathbb{R}^2} \int dv_y dv_z \int_{-\infty}^{0} v_x c(1, y, z, v) \, dv_x
\]

and the outgoing flux on the right is given by

\[
\mathcal{J}_{\text{out}}(1, y, z) = \int_{\mathbb{R}^2} \int dv_y dv_z \int_{0}^{\infty} v_x c(1, y, z, v) \, dv_x.
\]

The net flux densities at location \((y, z)\) on the boundaries \(x = 0\) and \(x = 1\) are given by

\[
\mathcal{J}(0, y, z) = \mathcal{J}_{\text{in}}(0, y, z) - \mathcal{J}_{\text{out}}(0, y, z)
\]

\[
\mathcal{J}(1, y, z) = \mathcal{J}_{\text{out}}(1, y, z) - \mathcal{J}_{\text{in}}(1, y, z).
\]

In the Smoluchowski limit of large friction the stationary solution of the FPE (6.5) is given by [54]

\[
c(x, v) = \frac{e^{-\frac{|v|^2}{2\varepsilon}}}{(2\pi\varepsilon)^{3/2}} \left\{ c(x) + \frac{\mathbf{J}(x) \cdot \mathbf{v}}{\varepsilon} + O\left(\frac{1}{\gamma^2}\right) \right\}
\]

(6.10) and the flux density is given by

\[
\mathbf{J}(x) = -\frac{1}{\gamma(x)} \left\{ \varepsilon \nabla c(x) + \nabla \Phi(x)c(x) \right\} + O\left(\frac{1}{\gamma^2}\right),
\]

(6.11)

where \(c(x)\) is the solution of the Smoluchowski-Nernst-Planck equation (SNPE)

\[
\nabla \cdot \frac{1}{\gamma(x)} \left\{ \varepsilon \nabla c(x) + \nabla \Phi(x)c(x) \right\} = 0.
\]

(6.12)

Assuming that the stationary flux density \(\mathbf{J}(x)\) is curl free, we find that \(\mathbf{J}(x)\) and the normalization conditions

\[
\int_{\text{bath and reservoirs}} c(x) \, dx = N,
\]

determine the solution \(c(x)\) of the SNPE (6.12) uniquely.

Inserting (6.10) into (6.8) gives the following expression for the incoming flux at \(x = 0\)

\[
\mathcal{J}_{\text{in}}(0, y, z) = \sqrt{\frac{\varepsilon}{2\pi}} c(0, y, z) + \frac{\mathcal{J}(0, y, z)}{2} + O\left(\frac{1}{\gamma^2}\right),
\]

(6.13)
and for the outgoing flux on the left

\[ J_{\text{out}}(0, y, z) = \sqrt{\varepsilon/2\pi} c(0, y, z) - \frac{\mathcal{J}(0, y, z)}{2} + O\left(\frac{1}{\gamma^2}\right). \] (6.14)

Similarly, the incoming flux on the right is given by

\[ J_{\text{in}}(1, y, z) = \sqrt{\varepsilon/2\pi} c(1, y, z) - \frac{\mathcal{J}(1, y, z)}{2} + O\left(\frac{1}{\gamma^2}\right) \] (6.15)

and the outgoing flux on the right is given by

\[ J_{\text{out}}(1, y, z) = \sqrt{\varepsilon/2\pi} c(1, y, z) + \frac{\mathcal{J}(1, y, z)}{2} + O\left(\frac{1}{\gamma^2}\right). \] (6.16)

Next, we relate the boundary fluxes to the concentrations at \( x = 0 \) and \( x = 1 \). Placing sources of incoming ions at \( x = 0 \) and \( x = 1 \), with strengths \( J_{\text{in}}(0, y, z) \) on the left and \( J_{\text{in}}(1, y, z) \) on the right, and removing exiting ions, we obtain from eqs.(6.13) and (6.15) that to leading order in large \( \gamma \)

\[ J_{\text{in}}(0, y, z) = \sqrt{\varepsilon/2\pi} c(0, y, z) + \frac{\mathcal{J}(0, y, z)}{2} \] (6.17)

\[ J_{\text{in}}(1, y, z) = -\sqrt{\varepsilon/2\pi} c(1, y, z) + \frac{\mathcal{J}(1, y, z)}{2}. \]

These are identities that relate boundary uni-directional and net flux densities to the boundary densities. In terms of Langevin trajectories they represent the relationship between boundary densities and intensities of sources of Langevin trajectories that enter \( \Omega \), the net flux density on the boundary, and the boundary concentrations. This is the solution to the problem of description of Langevin trajectories of particles diffusing between two given concentrations.

If the boundary concentrations are constant, as in eq.(6.1), a coarser theory can be derived. The total particle flux on any cross section of \( \Omega \) by the plane \( x = \text{const.} \), denoted \( \Omega_x \), is a constant \( \mathcal{J} \). It is given by

\[ \mathcal{J} = \int_{\Omega_x} \int J_x(x) \, dy \, dz, \]

for any \( 0 < x < 1 \), where \( J_x(x) \) is defined in (6.7). The total ingoing and outgoing fluxes on the left are given by

\[ J_{\text{in}}(0) = \int_{\Omega_0} \mathcal{J}(0, y, z) \, dy \, dz \] (6.18)

\[ J_{\text{out}}(0) = -\int_{\Omega_0} \mathcal{J}(0, y, z) \, dy \, dz \]
In an analogous manner, similar expressions hold for the incoming and outgoing fluxes on the right. Equations (6.9) reduce to

\[ J = J_{\text{in}}(0) - J_{\text{out}}(0) = J_{\text{out}}(1) - J_{\text{in}}(1). \]

If the boundary conditions (6.1) are given, equations (6.13)-(6.16) integrate to

\[ J_{\text{in}}(0) = \sqrt{\frac{\varepsilon}{2\pi}}|\Omega_0|C_L + \frac{J}{2}, \]

\[ J_{\text{out}}(0) = \sqrt{\frac{\varepsilon}{2\pi}}|\Omega_0|C_L - \frac{J}{2}, \]

\[ J_{\text{in}}(1) = \sqrt{\frac{\varepsilon}{2\pi}}|\Omega_1|C_R - \frac{J}{2}, \]

\[ J_{\text{out}}(1) = \sqrt{\frac{\varepsilon}{2\pi}}|\Omega_1|C_R + \frac{J}{2}. \]

where \(|\Omega_0|\) is the area of \(\Omega_0\), and equations (6.17) reduce to

\[ J_{\text{in}}(0) = \sqrt{\frac{\varepsilon}{2\pi}}|\Omega_0|C_L + \frac{J}{2}, \]

\[ J_{\text{in}}(1) = -\sqrt{\frac{\varepsilon}{2\pi}}|\Omega_1|C_R + \frac{J}{2}. \]

Equations (6.20) relate the fixed boundary concentrations at \(x = 0\) and \(x = 1\) to the total flux across \(\Omega\) and to the total ingoing fluxes there, without imposing any restrictions on the behavior of the Langevin trajectories at the boundaries. The trajectories may enter and exit \(\Omega\) any number of times. The concentrations remain unchanged if outgoing trajectories are terminated at the boundaries and ingoing trajectories are generated there at rates that are determined by eqs.(6.20). This procedure represents a feedback mechanism that maintains boundary concentrations by injecting and removing particles there.

For the analysis of the diffusion of interacting particles the averaged potentials \(\Phi(x_i)\) in the Langevin equations (6.2) have to be replaced with the full potential of all interactions, \(\Phi(x_1, \ldots, x_N)\). The Fokker-Planck equation for the joint probability density function of all \(3N\) trajectories in phase space is \(6N\)-dimensional (see [138]) and so is the phase space probability flux density vector. Generalizing the 3-dimensional analysis of this section to
3N dimensions and summing the 3N fluxes at the boundaries recovers eq.(6.20). Thus the results of this section apply not only to Langevin equations of particles diffusing in a mean field, but also to the diffusion of interacting particles and to their Langevin simulations. For a simulation of interacting particles it is necessary to calculate the contribution to the field of the reservoirs beyond the boundaries of the simulation. A partial step in this direction was made in [110]. In this case the strengths of the boundary sources for positive and negative particles fluctuate as charged particles enter or leave the simulation. We do not consider this problem here.

The effect of short range forces, such as Lennard-Jones interactions, can be distorted by terminating trajectories as they leave the simulation. However, these distortions do not affect the structure of the solution inside the simulation volume, away from the boundaries.

### 6.3 Application to channel simulation

Permeation of ions from one electrolytic solution to another through a protein channel is a biological process of considerable importance [50, 72]. Permeation occurs on a time scale of micro- to milliseconds, far longer than the femtosecond time scales of atomic motion. Direct simulations of atomic dynamics are not yet possible for such long time scales, so that coarse-grained Langevin simulations are called for. Protein channels are small natural nano-devices of length in the range of 20 – 100Å, and 5-20Å diameter. A computer simulation of a channel involves the simulation of motion of the mobile ions both inside the channel and around it, in a volume comparable to the channel size. Such a simulation must be connected to its surrounding continuum. This leads to a small simulation with large time dependent density and potential fluctuations.

We consider a Langevin simulation of bath and channel. The left reservoir occupies the slab 0 < x < L, the narrow channel occupies the interval [L, R] and has a given cross-section profile. The right bath occupies the slab R < x < 1 (all variables are dimensionless, see notation in [54]). The lateral walls of the bath are assumed impermeable to ions and the electrostatic field there has a vanishing normal component. The fixed time averaged concentrations \(C_L\) and \(C_R\) at \(x = 0\) and \(x = 1\), respectively, are maintained by a feedback mechanism or by connection to effectively infinite reservoirs, and are assumed known. The potential \(\Phi(x)\) of the ambient mean field is assumed known as well. The net time averaged
flux from left to right is denoted $J$ and is a measurable quantity.

The theoretical problem of channel modeling is to find the relation between the experimentally set variables $\Phi(0, y, z) = \Phi_L$, $\Phi(1, y, z) = \Phi_R$, $C_L$, $C_R$, and the resulting measured net flux $J$, given the structure of the channel (either the three-dimensional stereometry and charge distribution or the one-dimensional reduction of the above). This relation can be approximated with continuum coarse grained Poisson-Nernst-Planck (PNP) model. In the latest PNP models [138] the average potential $\Phi(x)$ is calculated from the permanent and mobile charge distribution, the hard sphere (or Lennard-Jones) inter-ionic repulsions, and the applied external voltage in the system. The boundary conditions for the stationary PNP system represent the feedback mechanism by imposing sources at both boundaries of the baths, that inject incoming ions and remove outgoing ions. The strengths of the sources are chosen so as to maintain the given concentrations at the boundaries. This way the feedback mechanism is approximated by the sources.

The part of the bath included in the simulation is microscopic and has to be connected to the macroscopic surrounding continuum, that is not part of the simulation. The connection problem arises in simulations of ionic permeation in protein channels of biological membranes. In these simulations the bath concentration is often imposed as a boundary condition at the end of the channel. However, the boundary densities cannot be assumed known there, because they are not measurable there. They are known, on the average, in the bath at a macroscopic distance from the channel. Therefore a channel simulation necessarily has to include a part of the surrounding baths on both sides of the membrane and the fluctuating concentrations at the boundaries of the simulation region have to be computed in the simulation, not imposed there.

In channel simulations that include portions of the surrounding baths, we assume that a cylindrical simulation region is sufficiently long to make left and right boundary concentrations fixed. If the cylinder is also sufficiently wide, the concentrations are fixed on the entire boundary of the cylinder, so that the flux density is parallel to the field. Since the field in a cylinder with a transversal uniform dielectric membrane is parallel to the cylinder’s axis, there is no flux across the lateral boundary of the cylinder. This implies that both concentration and flux density are constant at the left and right boundaries.

Equations (6.20) indicate how to connect the microscopic simulation region to the macroscopic baths so as to maintain the correct average concentrations at the interface between
the two. The net current $\mathcal{J}$ should be determined from the following shooting procedure. Begin with a guess at the value of $\mathcal{J}$ and compute the source strength at each end from eqs.(6.20). Choose an initial number of particles $N$, and inject new ions at the left end with an inward velocity distribution $v_x$ given by

$$p_L(v) \sim \frac{e^{-v^2/2\epsilon}}{\sqrt{2\pi\epsilon}} \left\{ \frac{1 + \mathcal{J}v}{\epsilon C_L} \right\} \frac{1}{2 + \mathcal{J}C_L \sqrt{2\pi\epsilon}}$$

for $v > 0$ (6.21)

and at the right end with an inward velocity distribution

$$p_R(v) \sim \frac{e^{-v^2/2\epsilon}}{\sqrt{2\pi\epsilon}} \left\{ \frac{1 - \mathcal{J}v}{\epsilon C_R} \right\} \frac{1}{2 + \mathcal{J}C_R \sqrt{2\pi\epsilon}}$$

for $v < 0$. (6.22)

The velocity components in the $y$ and $z$ directions of incoming particles follow the equilibrium distribution with no flux.

Since the average field in the bath is nearly zero and current is essentially diffusion-driven, the time course of the injection processes into the baths on either side of the membrane is essentially that described in [110] and [107]. In a simplified version ions can be injected on the left at exponential waiting times with average number $J_{\text{in}}(L)$ per unit time, and an analogous rate on the right.
Chapter 7

The Arrival Process to an Absorbing Boundary

In this chapter we present a mathematical analysis of the arrival process of infinitely many independent diffusers from a continuum to an absorbing boundary. We show that in steady state the arrival process is Poissonian. This result is used in the mathematical analysis of the time course of a simulation with a buffer region. The results of this chapter were also published in [106].

7.1 Introduction

The study of the arrival process of diffusing particles from a continuum to an absorbing boundary started at the earliest stages of the probabilistic theory of diffusion. In 1917 Smoluchowski [143] (see also [28], [157]) calculated the flux on an absorbing sphere immersed in an infinite bath of independent Brownian particles with fixed concentration at infinity, and applied his result to the theory of coagulation of colloids. The time to the arrival of the first Brownian particle from an equilibrated continuum bath to an absorbing sphere was shown by Bordewijk in 1975 [24] to be exponentially distributed with rate equal to the Smoluchowski flux. The result was applied to the theory of defect relaxation in dielectrics. Obviously, the existence of a stationary flux, which represents the average number of absorbed particles per unit time, does not in general imply that the first arrival time or any other inter-arrival times are exponentially distributed. The flux and the exponential rate coincide only for Poisson processes [59, 88]. The proof that all inter-arrival times of free Brownian particles to an absorbing sphere are identically exponentially distributed, thus rendering the absorption
process Poissonian with rate equal to the Smoluchowski flux, was given by Nadler in 1994 [105]. All of these results concern problems with spherical symmetry and rely on explicit known solutions of the relevant equations.

The probabilistic characterization of the arrival process of particles into an absorbing boundary has also important applications, apart from its mathematical interest and history. This problem arises, for example, in the simulation of discrete particles in a finite region of a continuum bath. A common approach in these simulations is to define a relatively small finite simulation region, start with an initial configuration of the required (average) number of particles inside the simulation region and move the particles according to their dynamics. The two most common treatments of the boundaries of the simulation region are to define them as reflecting or periodic boundaries [151]. In both treatments of the boundaries, the total number of particles inside the simulation region is fixed at all times. Thus, all fluctuations in the number of particles inside the simulation region are lost, and all physical phenomena related to these fluctuations may not be recovered correctly by the simulation. Of course, if the simulation region is large enough, then in a small sub-region of the simulation fluctuations in the number of particles do exist, but then the simulation itself might be infeasible in terms of computer resources due to the large number of simulated particles in the larger region. A different approach, as described in [105, 110], is to simulate only the motion of particles in a small region, but on the one hand to let the particles exit the simulation region and be absorbed by the continuum bath, and on the other hand let particles from the continuum bath enter the simulation region. In such a simulation scheme, the total number of particles inside the simulation region is not fixed, but rather fluctuates in time. The exact absorption and injection mechanisms between the continuum bath and the discrete simulation region should of course mimic as much as possible the situation in the real physical system and maintain the correct average number of particles in the simulation region. To carry out such a simulation it is necessary to compute the statistics of these mechanisms, and in particular the arrival times of particles from the continuum bath into the simulation region. We divide arrivals of particles into the simulation region into two types: (i) arrivals of “new” particles, which have not visited the simulation region so far, and (ii) arrivals of “returning” particles, which have already visited and exited the simulation region. In this chapter we study the arrival process (i). The more complicated process (ii) as well as the integration of the processes (i) and (ii) into a simulation of interacting ions in
solution appear in [107, 110].

In this chapter we generalize previous results on this problem, which were confined to independent Brownian particles arriving at an absorbing sphere. The present work extends these results to general diffusion processes, without any symmetries and without resorting to explicit expressions for solutions to the relevant equations. We consider an infinite three dimensional bath of independent non-interacting Brownian particles diffusing in the presence of a general force field and an absorbing boundary with general geometry. The main result of this chapter is that the steady state absorption stream at an absorbing boundary of particles diffusing independently in a force field is Poissonian with rate equal the total absorption flux on the absorbing boundary, as calculated from continuum theory of diffusion with transport.

The significance of this result for simulations of ions in solution is that the arrival process of new particles into the simulation is memoryless, when the assumptions of independent diffusion are satisfied outside the simulation region. This means that the inter-arrival times of new particles into the simulation region are independent identically distributed (i.i.d.) random variables, and can be easily generated without need to store the history of previous arrival times.

It is a common practice in physical chemistry to describe ionic solutions by an electrochemical potential. This means that the motions of ions in solution are assumed independent diffusion processes in a mean force field. To compensate for the lost inter-ionic interactions an activity factor is introduced [21]. Thus, our assumptions are satisfied in regions that can be described by independent diffusion (usually outside the simulation region).

The rest of the chapter is organized as follows. In Section 2 the flux in a one-dimensional problem is computed, and in Section 3 we show that the first arrival time is exponentially distributed with a rate that equals the calculated flux. The first arrival time in a general three-dimensional setting is formulated and solved in Section 4. The probability law of all subsequent inter arrival times is calculated in Section 5. Finally, a summary and discussion are given in Section 6.
7.2 The stationary flux at an absorbing boundary in one dimension

We begin with a simplified one-dimensional model of a continuum bath and an absorbing boundary. In this section we calculate the continuum flux into the absorbing boundary, and in the next section we show that the distribution of the first arrival time at the same boundary is exponential with a rate that equals the continuum flux.

Consider a continuum bath located on the $x$-axis at $x > 0$, with an absorbing boundary at $x = 0$. The bath is composed of an infinite number of independent non-interacting Brownian particles with average density $\rho$ as $x \to \infty$, diffusing in a potential field $U(x)$. We assume that $U'(x) \to a > 0$ as $x \to \infty$, so that the steady state average density of particles is uniform at infinity.

In this setting, the motion of each particle is governed by the one-dimensional Langevin equation

$$\dot{x} = -\frac{dU}{dx} + \sqrt{2}\dot{w},$$

where $w(t)$ is standard Brownian motion.

We assume the existence of a stationary concentration of bath particles, denoted $p(x)$. By assumption, the motions of different bath particles are independent, so the stationary concentration $p(x)$ satisfies the Nernst-Planck equation

$$\frac{d}{dx} \left[ \frac{dp}{dx} + p \frac{dU}{dx} \right] = 0,$$

with the boundary conditions

$$p(0) = 0, \quad \text{and} \quad p(x) \to \rho \quad \text{as} \quad x \to \infty.$$ (7.3)

First, we modify the problem to that of a finite bath in the region $[0, L]$, with the boundary conditions

$$p(0) = 0, \quad p(L) = \rho.$$ (7.4)

The solution of the modified problem, denoted $p_L(x)$, is given by

$$p_L(x) = \rho \frac{e^{-U(x)} \int_0^x e^{U(s)} ds}{e^{-U(L)} \int_0^L e^{U(s)} ds}.$$ (7.4)
The steady state density of the infinite system $p(x)$, whenever it exists, is given as the limit

$$p(x) = \lim_{L \to \infty} p_L(x). \quad (7.5)$$

Under the given assumptions concerning the existence of a steady state density, the following limit exists

$$0 < a^{-1} = \lim_{L \to \infty} \int_0^L e^{U(s)-U(L)} ds < \infty. \quad (7.6)$$

In this case, combining (7.6) with (7.5) and (7.4) gives

$$p(x) = \rho a e^{-U(x)} \int_0^x e^{U(s)} ds. \quad (7.7)$$

The continuum flux at the absorbing boundary is

$$J = -\frac{dp}{dx} \bigg|_{x=0} = -\rho a. \quad (7.8)$$

Note that the flux is negative as particles are flowing out of the bath, thus ”decreasing” the number of bath particles.

In the next section we prove that when a steady state exists, the first arrival time from the continuum to the absorbing boundary is exponentially distributed, with a rate equal to the above flux (in absolute value), $\lambda = \rho a = |J|$. The proof that all subsequent inter-arrival times are also exponentially distributed with the same rate is postponed to section 5.

### 7.3 The probability law of the first arrival time

Before computing arrival times from an infinite continuum bath, a definition of a steady state infinite bath with an infinite number of discrete particles is in order. We define an observation, or a measurement of the first arrival time from a steady state infinite bath as follows. Following [24], at the start of observation, at time $t = 0$, we consider only the finite number of particles initially distributed in the interval $[0, L]$ of the infinite bath and compute the first arrival time of these particles. The first arrival time from the infinite bath is defined as the limit of the above arrival time when we let the length of the interval and the number of particles tend to infinity.

We denote by $N(L)$ the number of particles initially located in the interval $[0, L]$ and denote by $\tau(L)$ the first arrival time from this system. By definition, the time $\tau(L)$ is the
minimum of the arrival times of all the $N(L)$ particles. Since the bath is in steady state and all diffusing particles are independent, the initial locations at time $t = 0$ of the $N(L)$ particles inside the finite region $[0, L]$, denoted $x_i(0)$, ($i = 1 \ldots N(L)$), are i.i.d. random variables, distributed according to the steady state density $p(x)$, eq. (7.7), but normalized to the region $[0, L]$,

$$
\Pr \left\{ x_i \in [x, x + dx] \right\} = \frac{p(x) \, dx}{\int_0^L p(s) \, ds}.
$$

By definition of the steady state particle concentration $p(x)$, it follows that the average number of particles inside the region $[0, L]$, denoted $E[N(L)]$, is given by

$$
E[N(L)] = \int_0^L p(x) \, dx.
$$

As is well known [59], $N(L)$, the total number of particles inside the region $[0, L]$, is a Poisson random variable whose average is $E[N(L)]$, as computed above in eq. (7.10), and its probability distribution function is

$$
\Pr \{ N(L) = k \} = \frac{E[N(L)]^k}{k!} e^{-E[N(L)]}.
$$

To summarize so far, in our approximation scheme we consider only the finite number of particles in the infinite bath, initially confined to the interval $[0, L]$. This number of particles $N(L)$ follows the Poisson distribution (7.11) and the initial locations of the particles are i.i.d. random variables, distributed according to (7.9).

Our purpose is to compute the probability law of the minimal arrival time to the origin of these $N(L)$ particles, sum over all possible values of $N(L)$ multiplied by their respective probabilities, and then take the limit $L \rightarrow \infty$. Since the motions of different particles are independent of each other, for a bath with $k$ particles

$$
\Pr \left\{ \tau(L) > t \mid N(L) = k \right\} = \left[ \Pr \{ \tau_1 > t \} \right]^k,
$$

where $\tau_1$ denotes the first passage time (FPT) to the origin of a single particle initially distributed inside the region $[0, L]$. Combining (7.12) and (7.11), the first passage time from the infinite bath, denoted $\tau$ is given by

$$
\Pr \{ \tau > t \} = \lim_{L \rightarrow \infty} \sum_{k=0}^{\infty} \Pr \{ \tau(L) > t \mid N(L) = k \} \Pr \{ N(L) = k \}
$$
\[
\begin{align*}
&= \lim_{L \to \infty} \sum_{k=0}^{\infty} \left[ \Pr \{ \tau_1 > t \} \right]^k E \left[ N(L) \right]^k E^{-E[N(L)]} \\
&= \lim_{L \to \infty} \exp \left\{ -E[N(L)] \left( 1 - \Pr \{ \tau_1 > t \} \right) \right\}.
\end{align*}
\] (7.13)

We denote by
\[
G(x, t) = \Pr \{ \tau_1 > t \mid x(0) = x \}
\]
the probability that a particle that starts out at \(x\) has not been absorbed by time \(t\). It satisfies the partial differential equation \[137\]
\[
\frac{\partial G}{\partial t} = \frac{\partial^2 G}{\partial x^2} - U'(x) \frac{\partial G}{\partial x}, \quad \text{for } x > 0,
\] (7.14)
with the boundary condition
\[
G(0, t) = 0,
\]
and the initial condition
\[
G(x, 0) = 1, \quad \text{for } x > 0.
\]
According to (7.9) and (7.10), the FPT of a single particle is given by
\[
\begin{align*}
\Pr \{ \tau_1 > t \} &= \int_0^L \Pr \{ x_i = x \} G(x, t) \, dx \\
&= \frac{\int_0^L p_L(x) G(x, t) \, dx}{E[N(L)]}.
\end{align*}
\] (7.15)

Inserting (7.15) and (7.10) into (7.13) gives
\[
\Pr \{ \tau > t \} = \exp \left\{ -\int_0^\infty p(x) [1 - G(x, t)] \, dx \right\}.
\] (7.16)

Using the abbreviation
\[
F(t) = \int_0^\infty p(x) \left[ 1 - G(x, t) \right] \, dx,
\]
we differentiate \(F(t)\) with respect to \(t\), interchange the order of integration (with respect to \(x\)) and differentiation, and use (7.14) to obtain
\[
\begin{align*}
\frac{dF}{dt} &= -\int_0^\infty p(x) \left[ -U'(x) \frac{\partial G}{\partial x} + \frac{\partial^2 G}{\partial x^2} \right] \, dx \\
&= -\int_0^\infty p(x) e^{U(x)} \frac{\partial}{\partial x} \left[ e^{-U(x)} \frac{\partial G}{\partial x} \right] \, dx.
\end{align*}
\] (7.17)
Two integrations by parts yield the identity

\[ \frac{dF}{dt} = -p(x) \frac{\partial G}{\partial x} \bigg|_0^\infty + Ge^{-U} \frac{d}{dx} \left( e^U p(x) \right) \bigg|_0^\infty - \int_0^\infty G(x) \frac{d}{dx} \left[ e^{-U} \frac{d}{dx} \left( e^U p(x) \right) \right] dx. \]

Note that the integral vanishes due to equation (7.2). In addition, all contributions from the lower limit \( x = 0 \) vanish as both \( G(0, t) = 0 \) and \( p(0) = 0 \). Thus, we are left only with the contributions from the upper limit \( x = \infty \),

\[ \frac{dF}{dt} = \lim_{L \to \infty} \left\{ -p(L) \frac{\partial G}{\partial x} \bigg|_{x=L} + G(L, t) \left[ U'(L)p(L) + \frac{dp}{dx} \bigg|_{x=L} \right] \right\}. \]  

(7.18)

We estimate separately each term on the right hand side of eq. (7.18), starting from the last term. It follows from (7.2) and (7.8) that for all values of \( x \),

\[ U'(x)p(x) + \frac{dp(x)}{dx} = -J. \]

Next, we consider the first term on the right hand side of (7.18). The boundary condition (7.3) implies that

\[ \lim_{L \to \infty} p(L) = \rho. \]

To compute the limit in equation (7.18) it remains to determine the asymptotic behavior of \( G(x, t) \) and its spatial derivative, as \( x \to \infty \). Obviously, by definition, for every fixed \( t \), \( G(x, t) \) is a monotone increasing function of \( x \) because it takes longer to reach the origin from a farther point. Similarly, it is obvious that the time to reach the origin from the point \( x = L \) increases to infinity with \( L \) so that for any finite \( t \), the probability of arriving at the origin from \( L \) after time \( t \) converges to 1 as \( L \to \infty \). That is,

\[ \lim_{L \to \infty} G(L, t) = 1. \]

Since \( G(x, t) \) is a smooth function of \( x \), it follows that

\[ \lim_{L \to \infty} \frac{\partial G}{\partial x} \bigg|_{x=L} = 0. \]

Thus, the first term on the right hand side of eq. (7.18) vanishes and we obtain the result

\[ \frac{dF}{dt} = -J. \]  

(7.19)

Since \( F(0) = 0 \), one integration of (7.19) gives \( F(t) = -Jt \). Inserting this result into (7.16) gives

\[ \Pr \{ \tau > t \} = \exp \{ Jt \}, \]  

(7.20)
which means that \( \tau \), the first arrival time from the continuum into the absorbing boundary, is exponentially distributed with rate \( \lambda = -J \). Note that the probability law of the first arrival time from a steady state bath into the absorbing boundary depends on the profile of the potential \( U(x) \) only through a single constant, the continuum flux \( J \).

### 7.4 The first arrival time for steady state 3-D diffusion

To obtain an analogous result in 3 dimensions, we consider independently diffusing particles outside an arbitrary bounded domain \( \Omega \) with a smooth boundary \( \partial \Omega \) (see fig. 7.1). The particles in this three dimensional bath are subjected to a potential field \( -\nabla U(r) \) such that a steady state density \( p(r) \) exists in the bath \( \mathbb{R}^3 - \Omega \). It satisfies the differential equation

\[
\mathcal{L}p(r) = \nabla \cdot \left( \nabla p(r) + p(r)\nabla U(r) \right) = -\nabla \cdot \mathcal{J}(r) = 0, \quad r \in \mathbb{R}^3 - \Omega,
\]

where \( \mathcal{J}(r) \) is the flux density vector.

We assume that \( \nabla U(r) \) vanishes fast enough as \( |r| \to \infty \) so that the stationary density is uniform at infinity (see Appendix A for details). We assume that a part of \( \partial \Omega \), denoted \( \partial \Omega_1 \), is absorbing, and the remaining part, \( \partial \Omega - \partial \Omega_1 \), is reflecting. Thus, the boundary conditions for the steady state density are an average density \( \rho \) at infinity,

\[
\lim_{|r| \to \infty} p(r) = \rho, \quad (7.22)
\]

absorption on \( \partial \Omega_1 \),

\[
p(r) \bigg|_{\partial \Omega_1} = 0,
\]
and no flux boundary conditions on the reflecting part of the boundary, \( \partial \Omega - \partial \Omega_1 \),
\[
J \cdot \nu \big|_{\partial \Omega - \partial \Omega_1} = -(\nabla p + p \nabla U) \cdot \nu \big|_{\partial \Omega - \partial \Omega_1} = 0,
\]
where \( \nu \) is the unit outer normal at the boundary.

As in the previous section, we start our analysis from a large ball of radius \( R \) centered at the origin, denoted \( \Omega_R \), such that \( \Omega \subset \Omega_R \) (see fig. 7.1). We place in \( \Omega_R - \Omega \) a finite number of particles \( N(R) \), initially located according to the steady state density \( p(r) \) normalized to the ball \( \Omega_R \),
\[
\Pr \{ r_i \in r + dr \} = \frac{p(r) dr}{\int_{\Omega_R - \Omega} p(y) dy}.
\]
As in the one dimensional case, the total number of particles \( N(R) \) is a Poisson distributed random variable with average
\[
E[N(R)] = \int_{\Omega_R - \Omega} p(y) dy.
\]
In analogy to eq.(7.13) for the one dimensional case, the first arrival time from the continuum bath into the absorbing boundary \( \partial \Omega_1 \) is given by
\[
\Pr \{ \tau > t \} = \lim_{R \to \infty} \exp \left\{ -E[N(R)] \left( 1 - \Pr \{ \tau_1 > t \} \right) \right\},
\]
where \( \tau_1 \) denotes the first arrival time of a single particle from the ball \( \Omega_R \) to the absorbing boundary.

We follow the same steps of computation as in the one dimensional case. We denote by
\[
G(r, t) = \Pr \{ \tau_1 > t | r(0) = r \}
\]
the probability of a diffusing particle to arrive at \( \partial \Omega_1 \) after time \( t \), starting from an initial position \( r \) at time \( t = 0 \). Then \( G(r, t) \) satisfies the evolution equation [137]
\[
\frac{\partial G(r, t)}{\partial t} = \mathcal{L}^* G(r, t) = \nabla \cdot \nabla G(r, t) - \nabla G(r, t) \cdot \nabla U,
\]
where \( \mathcal{L}^* \) is the backward operator (the formal adjoint to \( \mathcal{L} \) in eq.(7.21)). The boundary conditions for \( G(r, t) \) are
\[
G(r, t) \bigg|_{\partial \Omega_1} = 0,
\]
\[
\nabla G(r, t) \cdot \nu \bigg|_{\partial \Omega - \partial \Omega_1} = 0,
\]
and the initial condition is

\[ G(r, 0) = 1, \quad \text{for } r \in \mathbb{R}^3 - \Omega. \]

The quantity of interest, the probability law of \( \tau_1 \), is given by

\[
\Pr \{ \tau_1 > t \} = \frac{\int_{\Omega R - \Omega} p(r) G(r, t) \, dr}{\int_{\Omega R - \Omega} p(r) \, dr}.
\]  
(7.25)

Repeating the same steps as in the one dimensional computation leads to the formula

\[
\Pr \{ \tau > t \} = \lim_{R \to \infty} \exp \left\{ - \int_{\Omega R - \Omega} p(r) \left[ 1 - G(r, t) \right] \, dr \right\}
\]  
(7.26)

Differentiating \( F_R(t) \) with respect to \( t \) and using equation (7.24) gives

\[
\frac{dF_R}{dt} = \int_{\Omega R - \Omega} p(r) \mathcal{L}^* G(r, t) \, dr.
\]  
(7.27)

Since \( \mathcal{L}p(r) = 0 \), we can equivalently write

\[
\frac{dF_R}{dt} = \int_{\Omega R - \Omega} \left[ p \mathcal{L}^* G - G \mathcal{L} p \right] \, dr.
\]  
(7.27)

Inserting the expressions for the operators \( \mathcal{L} \) and \( \mathcal{L}^* \) from eqns. (7.21), (7.24), respectively, into eq.(7.27) yields

\[
\int_{\Omega R - \Omega} \left[ p \mathcal{L}^* G - G \mathcal{L} p \right] \, dr = \int_{\Omega R - \Omega} \left[ p \Delta G - G \Delta p \right] \, dr - \int_{\Omega R - \Omega} \nabla \cdot \left[ G p \nabla U \right] \, dr.
\]

Applying Green’s second identity to the first integral and the divergence theorem to the second integral, we obtain

\[
\frac{dF_R}{dt} = \int_{\partial [\Omega R - \Omega]} \left[ p \nabla G - G \nabla p - G p \nabla U \right] \cdot dS
\]  
(7.28)

where \( dS \) is a surface differential multiplied by a unit vector in the direction normal to the surface.

Consider first the contribution from the boundary \( \partial \Omega \). On the absorbing boundary \( \partial \Omega_1 \), both \( G(r, t) \) and \( p(r) \) vanish so there is no contribution to the surface integral from this
region. On the remaining part, \( \partial \Omega - \partial \Omega_1 \), the boundary conditions are \( \nabla G = 0 \) and \( \mathcal{J} = 0 \), so once again there is zero contribution to the surface integral. Thus, we retain only the contribution from the far boundary \( \partial \Omega_R \). On this boundary, at any finite time \( t \)

\[
\lim_{R \to \infty} G(r, t) \bigg|_{|r|=R} = 1, \quad \lim_{R \to \infty} \nabla G(r, t) \bigg|_{|r|=R} = 0,
\]

and the convergence is exponentially fast (see Appendix A). Thus, combining (7.29) with (7.28) the contribution from the boundary \( \partial \Omega_R \) can be approximated by

\[
\frac{dF_R}{dt} = \int_{\partial \Omega_R} \mathcal{J}(r) \cdot dS + o(1) \quad \text{for} \quad R \gg 1.
\]  

(7.30)

Recall from (7.21) that \( \nabla \cdot \mathcal{J}(r) = 0 \). Thus, using the divergence theorem

\[
0 = \int_{\Omega_R - \Omega} \nabla \cdot \mathcal{J}(r) \, dr = \oint_{\partial \Omega_R} \mathcal{J} \cdot dS - \oint_{\partial \Omega} \mathcal{J} \cdot dS,
\]

or equivalently,

\[
\oint_{\partial \Omega_R} \mathcal{J} \cdot dS = \oint_{\partial \Omega} \mathcal{J} \cdot dS = J,
\]

(7.31)

where \( J \) denotes the total continuum flux on the boundary \( \partial \Omega \). Combining equations (7.31) and (7.30) and integrating with respect to \( t \) gives

\[
\lim_{R \to \infty} F_R(t) = Jt.
\]

(7.32)

Equations (7.32) and (7.26) mean that the first arrival time from the bath to the absorbing boundary is exponentially distributed with rate that equals the continuum flux predicted from the steady state solution of the Nernst-Planck equation. Note that the total flux \( J \) is negative due to the fact that particles are exiting the bath and entering the absorbing boundary of \( \Omega \).

### 7.5 The next arrival times

So far, we have shown that the first arrival time of a particle from the continuum bath to the absorbing boundary is exponentially distributed. Now, we follow the analysis of [105] to show that all inter-arrival times are exponentially distributed, with the same rate.

As above, we first consider a bath with \( N(R) \) particles initially distributed in \( \Omega_R - \Omega \), calculate the pdf of the second arrival time, and then let \( R \to \infty \). We denote by \( t_1(R) \) and
According to equations (7.26) and (7.23) we have which further simplifies the right hand side of eq.(7.34) to

$$
\Pr \{ t_2 > t \mid t_1 = s \} = \lim_{R \to \infty} \Pr \{ t_2(R) > t \mid t_1(R) = s \}.
$$

For a bath with a finite number of particles, we have

$$
\Pr \{ t_2(R) > t \mid t_1(R) = s \} = \sum_{k=1}^{\infty} \Pr \{ t_2(R) > t \mid t_1(R) = s \mid N(R) = k \} \Pr \{ N(R) = k \}
$$

$$
= \sum_{k=1}^{\infty} \frac{\Pr \{ t_2(R) > t \cap t_1(R) = s \mid N(R) = k \}}{\Pr \{ t_1(R) = s \mid N(R) = k \}} \Pr \{ N(R) = k \}
$$

Since all diffusing particles are independent,

$$
\Pr \{ t_2(R) > t \cap t_1(R) = s \mid N(R) = k \} = \binom{k}{1} \Pr \{ \tau_1 = s \} \Pr \{ \tau_1 > t \}^{k-1},
$$

which means that there are \( k \) possibilities to choose the specific particle that arrives first, at time \( s \), and then all remaining \( k - 1 \) particles must arrive latter than time \( t \). Similarly,

$$
\Pr \{ t_1(R) = s \mid N(R) = k \} = \binom{k}{1} \Pr \{ \tau_1 = s \} \Pr \{ \tau_1 > s \}^{k-1}.
$$

Combining the last three equations gives

$$
\Pr \{ t_2(R) > t \mid t_1(R) = s \} = \sum_{k=1}^{\infty} \frac{\Pr \{ \tau_1 > t \}^{k-1}}{\Pr \{ \tau_1 > s \}^{k-1}} \Pr \{ N(R) = k \}.
$$

(7.33)

Inserting the Poisson distribution of \( N(R) \) into eq.(7.33) simplifies the right hand side to

$$
\Pr \{ t_2(R) > t \mid t_1(R) = s \} = \frac{\Pr \{ \tau_1 > s \}}{\Pr \{ \tau_1 > t \}} \sum_{k=1}^{\infty} \left( \frac{E[N(R)] \Pr \{ \tau_1 > t \}}{\Pr \{ \tau_1 > s \}} \right)^k \frac{e^{-E[N(R)]}}{k!}
$$

$$
= \frac{\Pr \{ \tau_1 > s \}}{\Pr \{ \tau_1 > t \}} e^{-E[N(R)]} \left( \exp \left\{ \frac{E[N(R)] \Pr \{ \tau_1 > t \}}{\Pr \{ \tau_1 > s \}} \right\} - 1 \right).
$$

(7.34)

According to equations (7.26) and (7.23) we have

$$
F_R(t) = E[N(R)] \Pr \{ \tau_1 < t \},
$$

which further simplifies the right hand side of eq.(7.34) to

$$
\frac{\Pr \{ \tau_1 > s \}}{\Pr \{ \tau_1 > t \}} \left\{ \exp \left( \frac{F_R(s) - F_R(t)}{\Pr \{ \tau_1 > s \}} \right) - \exp \left( -E[N(R)] \right) \right\}.
$$

(7.35)
We now take the limit $R \to \infty$. According to (7.25), the distribution of the first passage time of a single particle is

$$
\lim_{R \to \infty} \Pr \{ \tau_1 > t \} = \lim_{R \to \infty} \frac{\int_{\Omega_R - \Omega} p(r) G(r, t) \, dr}{\int_{\Omega_R - \Omega} p(r)}.
$$

According to (7.29), for any finite time $t$, as $|r| \to \infty$, $G(r, t) \to 1$. Also, according to (7.22), $p(r) \to \rho$ as $|r| \to \infty$. Therefore,

$$
\lim_{R \to \infty} \Pr \{ \tau_1 > t \} = 1.
$$

Obviously, for $R \to \infty$, $E[N(R)] \to \infty$, so the last term in (7.35) vanishes. Finally, according to (7.32), $F_R(t) \to Jt$ as $R \to \infty$. Therefore,

$$
\Pr \left\{ t_2 > t \mid t_1 = s \right\} = \exp \left( J(t - s) \right).
$$

That is, the inter-arrival time between the first and second particle depends only on the elapsed time $t - s$ since the first arrival at time $s$, and is independent of the first arrival time $s$. Moreover, this inter-arrival time is exponentially distributed with the same rate $\lambda = -J$, which is equal to the Smoluchowski flux.

In a similar manner, one can show that all inter-arrival times are exponentially distributed, rendering the arrivals a memoryless Poisson process.

### 7.6 Summary and discussion

Diffusion of independent (non-interacting) particles is a widely used approximation to describe the motion of ions in solution. Such an approximation represents a coarse-grained description of the effective motion of ions. It is used in physical chemistry of electrolytic solutions where solutions are described in terms of an electro-chemical potential and interactions are replaced by an activity coefficient. In this approximation ionic concentrations satisfy the Nernst-Planck equation, that is, Fickian diffusion and transport in a mean field. The underlying microscopic (Einsteinian) scenario for this description is Brownian motion of non-interacting individual particles that interact only with a non-fluctuating mean field.

In simulations of ions in solution [5, 14, 65, 68, 83, 91, 96, 113, 139, 146], especially in the context of molecular biophysics [87, 90, 141], a small scale simulation has to be connected
to the surrounding continuum. The interactions between the ions inside the simulation volume and their interactions with the far field of the surrounding continuum should be taken into account inside the simulation volume. Ions have to be introduced into the simulation volume and removed from it in a fashion consistent with their motion in the continuum. The exchange of ions between the discrete simulation volume and the surrounding continuum is a sorely missed link in the theory of ionic simulations. The exchange law is often assumed rather than computed from the assumed physical laws governing ions in solution [16, 19, 25, 79].

Two ionic species can be distinguished in a simulation of a discrete volume surrounded by a continuum: (i) the species of ions that have not been in the simulation so far, and (ii) the species of ions that have left the simulation into the continuum. The latter recirculate in and out of the simulation at random times. In this chapter, we have considered species (i), of ions that enter the simulation for the first time. They arrive at the simulation from a continuum, where they are described by physical chemistry as independent (non-interacting) Brownian particles that interact only with a mean field. Once they reach the simulation volume for the first time their species change to type (ii). This means that they are absorbed permanently in the boundary of the simulation volume (as ions of species (i)). When they re-emerge from the simulation volume they are no longer of species (i), but are rather permanently of species (ii). Thus the boundary of the simulation volume is absorbing for the diffusion process of species (i) in the continuum.

In this chapter, we have established that the absorption stream of species (i) is Poissonian with rate equal the absorption flux calculated from classical continuum diffusion theory. That is, the inter-arrival times of ions of species (i) to the absorbing boundary are i.i.d. exponential random variables. This result means that the process of introducing new ions into the simulation has no memory and thus no record of previous arrivals has to be kept. This property simplifies considerably the time course of the simulation.

Yet another application of our results is to ionic permeation through protein channels. In barrier models of ionic permeation through protein channels [72], transitions between the possible states of an ion inside and outside the channel are assumed Markovian. This implies that times between transitions have to be exponentially distributed. Our result shows that the transition times from the continuum into the channel are indeed exponentially distributed. The transitions times inside a channel are in general not exponential, because there are usually no high barriers for the diffusive motion of the ion to overcome.
Realistic simulations of ionic permeation in protein channels, be it molecular dynamics or Langevin dynamics, must connect the simulation volume to the surrounding continuum, because both ends of biological membrane channels are connected to salt water baths. The present computation, though not a full description of such a simulation, is the first step toward the development of these simulations.

Appendix A

We consider a smooth field $\nabla U (r)$ in $\mathbb{R}^3$ such that

$$|r||\nabla U (r)| = O (1), \quad \text{as} \quad |r| \to \infty. \quad (7.36)$$

We denote by $G (r, t)$ the solution of the initial boundary value problem

$$\frac{\partial G (r, t)}{\partial t} = \Delta G (r, t) - \nabla U (r) \cdot \nabla G (r, t)$$

in $\mathbb{R}^3 - \Omega$, where $\Omega$ is a smooth bounded domain in $\mathbb{R}^3$. We assume that $G (r, t)$ satisfies a homogeneous boundary condition on $\partial \Omega$, that is, an absorbing condition on a portion $\partial \Omega_1$ of $\partial \Omega$ and a reflecting condition on $\partial \Omega - \partial \Omega_1$. The initial condition for $G (r, t)$ is

$$G (r, 0) = 1.$$

We show that for each fixed $t$

$$G (r, t) \to 1 \quad \text{as} \quad |r| \to \infty,$$

with convergence at an exponential rate. First, we write

$$G (r, t) = \int_{\mathbb{R}^3 - \Omega} P (y, t | r) \, dy,$$

where $P (y, t | r)$ is the solution of the adjoint problem

$$\frac{\partial P (y, t | r)}{\partial t} = \Delta y P (y, t | r) + \nabla y \cdot [\nabla y U (y) P (y, t | r)] \quad (7.37)$$

in $\mathbb{R}^3 - \Omega$ with the mixed absorbing and no flux boundary conditions on $\partial \Omega_1$ and $\partial \Omega - \partial \Omega_1$, respectively. The initial condition for $P (y, t | r)$ is

$$P (y, 0 | r) = \delta (y - r).$$
For large $r$, we introduce the scaling $y = R\eta, r = R\xi, t = R^2\tau, U(y) = V(\eta)$, and $P(y,t|r) = Q(\eta,\tau|\xi)$. Eq.(7.37) takes the form
\[
\frac{\partial Q(\eta,\tau|\xi)}{\partial \tau} = \Delta_\eta Q(\eta,\tau|\xi) + \nabla_\eta \cdot [\nabla_\eta V(\eta) Q(\eta,\tau|\xi)]
\]
with the initial condition
\[
Q(\eta,0|\xi) = \frac{1}{R^3}\delta(\eta - \xi).
\]
For large $R$ the domain $\Omega$ shrinks to nearly a point at the origin. For fixed $t$ and large $R$ the scaled time $\tau$ becomes small. According to eq.(7.36), we have
\[
\nabla_\eta V(\eta) = O(1), \quad \text{as} \quad R \to \infty.
\]
It follows from [36] that for small $\tau$,
\[
Q(\eta,\tau|\xi) \sim \frac{1}{R^3 (4\pi \tau)^{3/2}} \exp \left\{ -\frac{|\eta - \xi|^2}{4\tau} \right\}
\]
Scaling back to the original variables, we find that for large $|r|
\[
P(y,t|r) \sim \frac{1}{(4\pi t)^{3/2}} \exp \left\{ -\frac{|y - r|^2}{4t} \right\}.
\]
Writing
\[
G(r,t) = \int_{\mathbb{R}^3 - \Omega} P(y,t|r) \, dy
\]
\[
\sim \frac{1}{(4\pi t)^{3/2}} \int_{\mathbb{R}^3} \exp \left\{ -\frac{|y - r|^2}{4t} \right\} \, dy - \frac{1}{(4\pi t)^{3/2}} \int_{\Omega} \exp \left\{ -\frac{|y - r|^2}{4t} \right\} \, dy
\]
\[
= 1 - \frac{1}{(4\pi t)^{3/2}} \int_{\Omega} \exp \left\{ -\frac{|y - r|^2}{4t} \right\} \, dy,
\]
we estimate
\[
\int_{\Omega} \exp \left\{ -\frac{|y - r|^2}{4t} \right\} \, dy \leq |\Omega| \exp \left\{ -\frac{|\text{dist}(\Omega, r)|^2}{4t} \right\},
\]
where $\text{dist}(\Omega, r)$ denotes the distance from the point $r$ to $\Omega$. This proves the assertion.
Chapter 8

Directions for Future Research

In the previous chapters we have presented some new results and ideas on mathematical models of ion flow through protein channels. We conclude this dissertation by presenting directions for future research.

First, consider the new set of continuum conditional Poisson-Nernst-Planck equations derived in chapter 3. These equations were derived from a molecular model of the coupled Langevin motion of many ions. As such, the resulting equations contain excluded volume effects, short range forces between ions and the dielectric self force on an ion. All of these terms, that were absent in previous treatments, may well be key players in the determination of channel function.

Excluded volume effects and short range forces are manifested in the resulting equations through the conditional densities. In equilibrium statistical mechanics, the analogous pair correlation functions play important roles in the determination of the energy, pressure, chemical potential and compressibility properties of electrolytes, to name just a few [21, 71, 125]. Non-equilibrium pair correlation functions also play an important role in the theoretical description of transport processes [10, 46, 49]. Some recent works show that inclusion of excluded volume effects into the standard PNP system leads to selectivity and other channel phenomena missed by the standard PNP system [116, 117]. The importance of excluded volume effects inside a narrow channel is also seen in Monte Carlo simulations [23]. In order to study these effects, there is a need to develop a mathematical and physical theory for closure relations for the non-equilibrium conditional densities, especially in confined geometries and near dielectric interfaces. In equilibrium statistical mechanics there exist many different closure relations for the equilibrium pair correlation functions. Some closure rela-
tions describe the pair function in terms of triplet functions and develop closure relations for the triplet correlation function [21, 71]. In a similar manner, Nernst-Planck equations for the non-equilibrium conditional densities can be written in terms of higher order conditional densities, conditioned on the presence of two or more ions at given locations. To complete the description of the system, not only is a non equilibrium closure relation needed, but also boundary conditions for the pair correlation function should be specified at the boundaries of the system.

Another research direction concerns the study of the self induced force. This force term, which is non negligible near dielectric interfaces, was absent in previous treatments, e.g., in the standard PNP system. Moreover, it is also absent in the equilibrium treatments of bath and membrane interfaces that use the Poisson-Boltzmann system. Both the Poisson-Boltzmann and the Poisson-Nernst-Planck systems of equations have been applied to the study of the membrane electrolyte interface as well as channels embedded in membranes [39, 50, 78]. Since the self induced force is not negligible near dielectric interfaces, it should be included into these systems and its effects should be studied.

In chapters 6 and 7 some results on the connection between a Langevin simulation and the surrounding non-simulated continuum have been described. There are still, however, many issues to be resolved for simulation of ionic motion inside a protein channel. One issue concerns the computation of the electrostatic field, and specifically its connection to the surrounding baths, which are described by continuum densities and not by discrete ions. Thus, while the electric field inside the simulation region fluctuates enormously, the field in the surrounding bath doesn’t and a connection scheme between the two needs to be developed. Another issue involves the validity of Brownian dynamics (BD) simulations inside the channel. A possible research direction is to describe the motions of ions inside the channel by detailed MD simulations (possibly including the water molecules as well), while outside the channel describe ionic motion by more coarse grained BD. In this case, there is a need to connect the MD simulation to the BD simulation, which in turn should be connected to the surrounding continuum.

The process of ionic permeation through a protein channel involves many different time and length scales. The vibrational and rotational motion of water molecules occurs at a time scale of $10^{-15}$ sec., and the diffusive motion of ions occurs at larger time scales of $10^{-12}$ sec. The permeation of a single ion through the channel occurs on a time scale of $10^{-8}$
sec., and measurement of macroscopic currents are on time scales of $10^{-3}$ seconds or higher. While the motion of single ions is described in units of Ångstroms, the length of protein channels are tens of Ångstroms, and the long range electric interactions span over much larger distances. Given the large gap in the time and length scales between the microscopic details of permeation and the macroscopic measurement, a hierarchy of models is called for. Such a hierarchy should include molecular dynamics simulations on the finer time and length scales, connected to a Brownian dynamics simulation on a coarser scale, possibly connected to a Markov chain or Poisson-Nernst-Planck model on an even coarser scale. In this hierarchy, the outputs of one model are effective parameters given as inputs for more coarse grained models, from which ultimately the macroscopic features can be computed. In Chapter 5 we have presented Markov chain models, which can be one of the models in such a hierarchy. Yet, the exact connection between models of different time and length scales still remains an open research problem.
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