Ion channels – estimation and control at macroscopic and nano scales *

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SUMMARY: *All electrical activities in the nervous system, including communications between cells and the influence of hormones and drugs on cell function, are regulated by membrane ion channels. Therefore understanding their mechanisms at a molecular level is a fundamental problem in biology. This paper shows how ideas in Hidden Markov model signal processing, stochastic control and stochastic optimization can be used to understand the operation of ion channels at both a macroscopic and nano scales.*

1 INTRODUCTION

An ion channel is a hole or pore in a nerve cell membrane. In physical structure, an ion channel is a large protein molecule whose different configurations correspond to the ion channel being in a *closed* state or *open* state. The measurement of ionic currents flowing through single ion channels in cell membranes has been made possible by the giga-seal *patch-clamp* technique.[18,13] This was a major breakthrough for which the authors of[18] won the 1991 Nobel prize in Medicine. More recently, the 2003 Nobel prize in Chemistry was awarded to McKinnnon for determining the structure of several different types of ion channels from crystallographic analyses. Because all electrical activities in the nervous system, including communications between cells and the influence of hormones and drugs on cell function, are regulated by membrane ion channels, understanding their mechanisms at a molecular level is a fundamental problem in biology. Moreover, elucidation of how single ion channels work will ultimately help neurobiologists find the causes of, and possibly cures for, a number of neurological and muscular disorders.

This paper addresses two fundamental problems in ion channels from a estimation and control perspective: The *Gating Problem* and the *Permeation Problem*.

The *gating problem*[8,5,15] deals with understanding how ion channels undergo structural changes to regulate the flow of ions into and out of a cell. The ion channel currents are typically of the order of pico-amps (i.e. 10^{-12}, amps). The measured ion channel currents (obtained by sampling typically at 10 kHz, i.e, 0.1 milli-second time scale) are obfuscated by large amounts of thermal noise. In Sec.2 of this paper, we address the following issues related to the gating problem:

(i) we present a Hidden Markov model formulation of the observed ion channel current.

(ii) We present a discrete stochastic optimization algorithm for controlling a patch clamp experiment to determine the Nernst potential of the ion channel with minimal effort. This fits in the class of so called "experimental design" problems.

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(iii) We briefly discuss dynamic scheduling algorithms for activating multiple ion channels on a biological chip so as to extract maximal information from them.

The *permeation problem*[1, 19] seeks to explain the working of an ion channel at an Å (10^{-10} m) spatial scale by studying the propagation of individual ions through the ion channel at a femto (10^{-15}) second time scale. This setup is said to be at a mesoscopic scale since the individual ions (e.g., Na^+ ions) are of the order of a few Å in radius and are comparable in radius to the ion channel. At this mesoscopic level, point charge approximations and continuum electrostatics break down. The discrete finite nature of each ion needs to be taken into consideration. Also, failure of the mean field approximation in narrow channels implies that any theory that aspires to relate channel structure to its function must treat ions explicitly. In Sec.3 of this paper we show how Brownian dynamics simulation can be used to model the propagation of individual ions through an ion channel.

2 THE GATING PROBLEM

2.1 Hidden Markov Model Formulation

A typical trace of the ion channel current measurement from a patch clamp experiment (after suitable anti-aliasing filtering and sampling) shows that the channel current is piecewise constant discrete time signal that randomly jumps between two values - zero amperes which denotes the *closed stat*e of the channel, and $I(v)$ amperes which denotes the *open* state. $I(v)$ is called the *open-state* current level. Sometimes the current recorded from single ion channel dwells on one or more intermediate levels, known as conductance substates.

Chung et al.[6, 5] first introduced the powerful paradigm of Hidden Markov Models (HMMs) to characterize patch-clamp recordings of small ion channel currents contaminated by random and deterministic noise. By using sophisticated HMM signal processing methods, Chung and his colleagues[6, 5] demonstrated that the underlying parameters of the HMM could be obtained to a remarkable precision despite the extremely poor signal to noise ratio. These HMM parameter estimates yield important information into the dynamics of ion channels. Since the publication of[6, 5], several papers have appeared in the neuro-biological community that generalize the HMM signal models in[6, 5] in various ways to model measurements of ion channels, see[24] and the references therein. With these HMM techniques, it has now possible for neurobiologists to analyze not only large ion channel currents but also small conductance fluctuations occurring in noise.

Markov Model for Ion Channel Current: The idea of using Markov chains to model the piecewise constant finite state nature of ion channel currents was developed in detail in[7, 8]. Suppose a patch clamp experiment is conducted with a voltage v applied across the ion channel. Then, as described in[5, 24], the ion channel current $\{i_n(v)\}$, can be modelled as a three state homogeneous first order Markov chain. The state space of this Markov chain is $\{0g, 0b, I(v)\}$ corresponding to the physical states of *gap mode*, *burst-mode-closed* and *burst-mode-open*. For convenience, we will refer to the burst mode closed and burst-mode-open states as the open and closed states, respectively. In the gap mode and the closed state the ion channel current is zero. In the open state, the ion channel current has a value of $I(v)$.

The (3×3) transition probability matrix $A(v)$ of the Markov chain $\{i_n(v)\}$, which governs the probabilistic behaviour of the channel current, is given by

$A(v) =$

	0_g	0_b	$I(v)$
0_g	$a_{11}(v)$	$a_{12}(v)$	0
0_b	$a_{21}(v)$	$a_{22}(v)$	$a_{23}(v)$
$I(v)$	0	$a_{32}(v)$	$a_{33}(v)$

(1)

The elements of $A(v)$ are the transition probabilities $a_{ij}(v) = P(i_{n+1}(v) = j \mid i_n(v) = i)$ where $i, j \in \{0_{g,0_b}, I(v)\}$. The zero probabilities in the above matrix $A(v)$ reflect the fact that a ion channel current cannot directly jump from the gap mode to the open state, similarly an ion channel current cannot jump from the open state to the gap mode. Note that in general, the applied voltage affects both the transition probabilities and state levels of the ion channel current $\{i_n(v)\}$.

HMM Observations: Let $\{y_n(v)\}$ denote the measured noisy ion channel current at the electrode when conducting a patch clamp experiment:

$$y_n(v) = i_n(v) + w_n(v), \quad n = 1, 2, \ldots \tag{2}$$

Here $\{w_n(v)\}$ is thermal noise and is modelled as zero mean white Gaussian noise with variance $\sigma^2(v)$. Thus the observation process $\{y_n(v)\}$ is a Hidden Markov model sequence parameterized by the model

$$\lambda(v) = \{A(v), I(v), \sigma^2(v)\} \tag{3}$$

where v denotes the applied voltage. We remark here that the formulation trivially extends to observations models where the noise process $w_n(v)$ includes a time-varying deterministic component together with white noise - only the HMM parameter estimation algorithm needs to be modified as in[16].

HMM Parameter Estimation of Current Level $I(v)$: Given the HMM mode for the ion channel current above, estimating $I(v)$ for a fixed voltage v, involves processing the noisy observation $\{y_n(v)\}$ through a Hidden Markov Model maximum likelihood parameter estimator. The most popular way of computing the maximum likelihood estimate (MLE) $I(v)$ is via the Expectation Maximization (EM) algorithm (Baum Welch equations). The EM algorithm is an iterative algorithm for computing the MLE. It is now fairly standard in the signal processing and neuro-biology literature - see[10] for a recent exposition - or[5] which is aimed at neurobiologists. Alternatively a recursive EM algorithm can be used for online estimation of the parameters of the HMM - see[17] for details.

Let $\hat{I}_\Delta(v)$ denote MLE of $I(v)$ based on the Δ -point measured channel current sequence $(y_1(v),\ldots,y_\Delta(v))$. For sufficiently large batch size Δ of observations, due to the asymptotic normality of the MLE for a HMM,[4]

$$\sqrt{\Delta}\left(\hat{I}_\Delta(v) - I(v)\right) \quad N(0,\Sigma(v)) \tag{4}$$

where $\Sigma^{-1}(v)$ is the Fisher information matrix. Thus asymptotically $\hat{I}_\Delta(v)$ is an unbiased estimator of $I(v)$, i.e., $\mathbf{E}\{\hat{I}_\Delta(v)\} = I(v)$ where $\mathbf{E}\{\cdot\}$ denotes the mathematical expectation operator.

2.2 Nernst Potential and Discrete Stochastic Optimization

To record currents from single ion channels, the tip an electrode, with the diameter of about $1\,\mu$m, is pushed against the surface of a cell, and then a tight seal is formed between the rim of the electrode tip and the cell membrane. A patch of the membrane surrounded by the electrode tip usually contains one or more single ion channels. The current flowing from the inside of the cell to the tip of the electrode through a single ion channel is monitored. This is known as "cell-attached" configuration of patch clamp techniques for measuring ion channel currents through a single ion channel. Fig.1 shows the schematic setup of the cell in electrolyte and the electrode pushed against the surface of the cell.

In a living cell, there is a potential difference between its interior and the outside environment, known as the membrane potential. Typically, the cell interior is about 60 mV more negative with respect to outside. Also, the ionic concentrations (mainly Na^+, Cl^- and K^+) inside of a cell is very different from outside of the cell. In the cell-attached configuration, the ionic strength in the electrode is usually made same as that in the outside of the cell. Let E_i and ß E_o, respectively, denote the resting membrane potential and the potential applied to the electrode. If E_o is identical to the membrane potential, there will be no potential gradient across the membrane patch confined by the tip of the electrode. Let c_i denote the intra-cellular ionic concentration and c_o the ionic concentration in the electrode. Here the intra-cellular concentration c_i inside the cell is unknown as is the resting membrane potential E_i. c_o and E_o are set by the experimenter and are known.

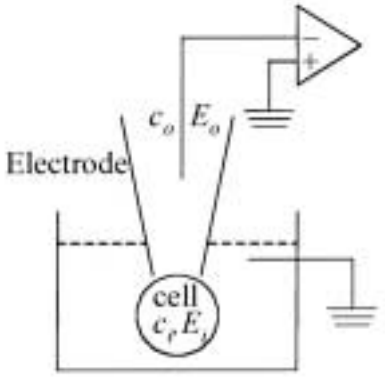

Figure 1: Cell-Attached Patch Experimental Setup

Let $v = E_o - E_i$ denote the potential gradient. Both the potential gradient v and concentration gradient $c_o - c_i$ drive ions across an ion channel resulting in an ion channel current. This ion channel current $\{i_n(v)\}$ is a piece-wise constant signal that jumps between the values of zero and $I(v)$, where $I(v)$ denotes the current when the ion channel is in the open state.

The potential E_o (and hence potential difference v) is adjusted experimentally until the current $I(v)$ goes to zero. This voltage v^* at which the current $I(v^*)$ vanishes is called the Nernst potential and satisfies the so called Nernst equation

$$v^* = -\frac{kT}{e}\ln\frac{c_o}{c_i} = -59\log_{10}\frac{c_o}{c_i}(\text{mV}), \tag{5}$$

where $e = 1.6\times10^{-19}$ C denotes the charge of an k electron, denotes Boltzmann's constant and T denotes the absolute temperature. The Nernst equation (5) gives the potential difference v required to maintain electro-chemical equilibrium when the concentrations are different on the two faces of the membrane.

Determining the Nernst potential v^* requires conducting experiments at different values of voltage v. In patch clamp experiments, the applied voltage v is usually chosen from a finite set. Let

$$v \in V = \{\theta(1),\ldots,\theta(M)\}$$

denote the finite set of possible voltage values that the experimenter can pick. For example, in typical experiments, if one needs to determine the Nernst potential to a resolution of 4 mV, then $M = 80$ and $\theta(i)$ are uniformly spaced in 4 mV steps from

$\theta(1) = -160$ mV and $\theta(M) = 160$ mV.

Note that the Nernst potential v^* (zero crossing point) does not necessarily belong to the discrete set V – instead we will find the point in V that is closest to v^* (with resolution $\theta(2) - \theta(1)$). With slight abuse of notation we will denote the element in V closest to the Nernst potential as v^*. Thus determining $v^* \in V$ can be formulated as a discrete optimization problem:

$$v^* = \arg\min_{v \in V} | I(v) |^2$$

Discrete Stochastic Approximation Algorithm Learning the Nernst Potential can be formulated as the following discrete stochastic optimization problem (we refer the reader to our recent paper[15] for details)

$$\text{Compute } v^* = \arg\min_{v \in V} [\mathbf{E}\{\hat{I}(v)\}]^2 \tag{6}$$

where $\hat{I}(v)$ is the MLE of the parameter $I(v)$ of the HMM. Since for a HMM, no closed form expression is available for $\Sigma^{-1}(v)$ in (4), the above expectation cannot be evaluated analytically. This motivates the need to develop a simulation based (stochastic approximation) algorithm.

The idea of discrete stochastic approximation[3] is to design a plan of experiments which provides more observations in areas where the Nernst potential is expected and less in other areas. More precisely what is needed is a dynamic resource allocation (control) algorithm that dynamically controls (schedules) the choice of voltage at which the HMM estimator operates in order to efficiently obtain the zero point and deduce how the current increases or decreases as the applied voltage deviates from the Nernst potential. We propose a discrete stochastic approximation algorithm that is both *consistent* and *attracted* to the Nernst potential. That is, the algorithm should spend more time gathering observations $\{y_n(v)\}$ at the Nernst potential $v = v^*$ and less time for other values of $v \in V$. Thus in discrete stochastic approximation the aim is to devise an *efficient*[20] adaptive search (sampling plan) which allows to find the minimizer v^* with as few samples as possible by not making unnecessary observations at non-promising values of v. Here we construct algorithms based on the random search procedures in[2, 3]. The basic idea is to generate a homogeneous Markov chain taking values in V which spends more time at the global optimum than at any other element of V. There are other classes of simulation-based discrete stochastic optimization algorithms such as nested partition methods[23] which combines partitioning, random sampling and backtracking to create a Markov chain that converges to the global optimum.

Let $n = 1,2,\ldots$ denote discrete time. The proposed algorithm is recursive and requires conducting experiments on batches of data. Since experiments will be conducted over batches of data, it is convenient to introduce the following notation. Group the discrete time into batches of length Δ - typically $\Delta = 10{,}000$ in experiments. We use the index $N = 1,2,\ldots$ to denote batch number. Thus batch N comprises of the Δ discrete time instants $n \in \{N\Delta, N\Delta + 1, \ldots, (N+1)\Delta - 1\}$.

Let $D_N = (D_N(1), \ldots, D_N(M))'$ denote the vector of duration times the algorithm spends at the M possible potential values in $m = 1, \ldots, M$.

Finally for notational convenience define the M dimensional unit vectors, e_m, as $m = 1, \ldots, M$

$$e_m = [0 \ \cdots \ 0 \ 1 \ 0 \ \cdots \ 0]', \tag{7}$$

with 1 in the m-th position and zeros elsewhere.

The discrete stochastic approximation algorithm of[2] is not directly applicable to the cost function (6) - since it applies to optimization problems of the $\min_{v \in V} \mathbf{E}\{C(v)\}$ form. However, (6) can easily be converted to this form as follows: Let $\hat{I}_1(v)$, $\hat{I}_2(v)$ be two statistically independent unbiased HMM estimates of $I(v)$. Then defining $\hat{C}(v) = \hat{I}_1(v)\hat{I}_2(v)$, it straightforwardly follows that

$$\mathbf{E}\{\hat{C}(v)\} = [\mathbf{E}\{\hat{I}(v)\}]^2 = | I(v) |^2 \tag{8}$$

The discrete stochastic approximation algorithm we propose is as follows:

Algorithm 1. [Algorithm for Learning Nernst Potential]

- Step 0: (Initialization.) At batch-time $N = 0$, select starting point $X_0 \in \{1, \ldots, M\}$ randomly. Set $D_0 = e_{X_0}$, Set initial solution estimate $\hat{v}_0 = \theta(X_0)$.
- Step 1: (Sampling.) At batch-time N, sample $\tilde{X}_N \in \{X_N - 1, X_N + 1\}$ with uniform distribution.
- Step 2: (Evaluation and Acceptance.) Apply $\tilde{v} = \theta(\tilde{X}_N)$ voltage to patch clamp experiment. Obtain two Δ length batches of HMM observations. Let $\hat{I}_N^{(1)}(\tilde{v})$ and $\hat{I}_N^{(2)}(\tilde{v})$ denote the HMM-MLE estimates for these two batches which are computed using the EM algorithm. Set $\hat{C}_N(\tilde{v}) = \hat{I}_N^{(1)}(\tilde{v})\hat{I}_N^{(2)}(\tilde{v})$.

  Then apply voltage $v = \theta(X_N)$. Compute the HMM-MLE estimates for these two

batches, denoted as $\hat{I}_N^{(1)}(v)$ and $\hat{I}_N^{(2)}(v)$. Set $\hat{C}_N(v)) = \hat{I}_N^{(1)}(v)\hat{I}_N^{(2)}(v)$.

If $\hat{C}_N(\tilde{v}) < \hat{C}_N(v)$, set $X_{N+1} = \tilde{X}_N$, else, set $X_{N+1} = X_N$.

- Step 3: (Update occupation probabilities of X_N.)

  $D_{N+1} = D_N + e_{X_{N+1}}$

- Step 4: (Update estimate of Nernst potential.)

  $\hat{v}_N^* = \theta(m^*)$ where

  $m^* = \text{argmax}_{m\in\{1,..,M\}} D_{N+1}(m)$, set

  $N \to N+1$, go to Step 1.

The proof of convergence of the algorithm is given in[15]. The main idea behind the above algorithm is that the sequence $\{X_N\}$ (or equivalently $\{\theta(X_N)\}$) generated by Steps 1 and 2 is a homogeneous Markov chain with state space $\{1,..., M\}$ (respectively, V) that is designed to spend more time at the global maximizer v^* than any other state. In the above algorithm, $\hat{v}_N^*$ denotes the estimate of the Nernst potential at batch N.

In[2], the following stochastic ordering assumption was used for convergence of the Algorithm 1.

(O) For any $m \in \{1,..., M-1\}$,

$$I^2(\theta(m+1)) > I^2(\theta(m)) \quad \Rightarrow$$
$$P(\hat{C}(\theta(m+1)) > \hat{C}(\theta(m))) > 0.5$$

$$I^2(\theta(m+1)) < I^2(\theta(m)) \quad \Rightarrow$$
$$P(\hat{C}(\theta(m+1)) > \hat{C}(\theta(m))) < 0.5$$

Theorem 1. *Under the condition (O) above, the sequence* $\{\theta(X_N)\}$ *generated by Algorithm 1 is a homogeneous, aperiodic, irreducible Markov chain with state space* V*. Furthermore, Algorithm 1 is attracted to the Nernst potential* v^**, i.e. , for sufficiently large* N*, the sequence* $\{\theta(X_N)\}$ *spends more time at* v^* *than an other state. (Equivalently, if* $\theta(m^*) = v^*$*, then* $D_N(m^*) > D_N(j)$ for $j \in \{1,..., M\} - \{m^*\}$.)

We also refer the reader to[26] for a weak convergence analysis of an adaptive version of the above algorithm which can be used to learn a time-varying Nernst potential.

2.2 Scheduling Multiple ion channels on a Chip

The patch clamping method described above has rapidly become the "gold standard"[11] for studying the dynamics of ion channel function by neuro-biologists. However, patch clamping is a laborious process requiring precision micro-manipulation under high power visual magnification, vibration damping and an experienced skillful experimenter. Because of this, high throughput studies required in proteomics and drug development have to rely on less valuable methods such as fluorescence-based measurement of intra-cellular ion concentrations.[25] There is thus significant interest in an automated version of the whole patch clamp principle, preferably one that has the potential to be used in parallel on a number of cells.

In 2002, Fertig, *et al*.[11] made a remarkable invention - the first successful demonstration of a patch clamp on a chip - a planar quartz based biological chip that consists of several hundred ion channels.[21] This *patch clamp chip* can be used for massively parallel screens for ion channel activity thereby providing a high-throughput screening tool for drug discovery efforts.

Typically, due to their expensive cost, most neuro-biological laboratories have only one patch clamp amplifier that can be connected to the patch clamp chip. As a result, only one ion channel in the patch clamp chip can be monitored at a given time. It is thus of significant interest to devise an adaptive scheduling strategy that dynamically decides which single ion channel to activate at each time instant in order to maximize the throughput (information) from the patch clamp experiment. Such a scheduling strategy will enable rapid evaluation and screening of drugs.

Here we make some brief comments on the problem of how to dynamically schedule the activation of individual ion channels using a laser beam to maximize the information obtained from the patch clamp chip for high throughout drug evaluation. The ion channel activation scheduling algorithm needs to dynamically plan and react to the presence of uncertain (random) dynamics of the individual ion channels in the chip. Moreover, excessive use of a single ion channel can make it de-sensitized. The aim is to answer the following question: *How should the ion channel activation scheduler dynamically decide which ion channel on the patch clamp chip to to activate at each time instant in order to minimize the overall de-sensitization of channels while simultaneously extracting maximum information from the channels?*

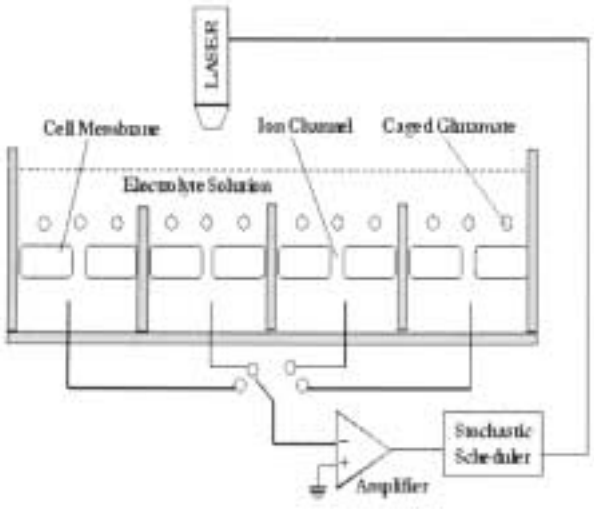

Figure 2: One dimensional section of planar biological chip

A schematic illustration of the ion channel scheduling problem for the patch clamp chip is given in Fig.2. The figure shows a cross section of the chip with 4 ion channels. The planar chip could for example consist of 50 rows each containing 4 ion channels. Each of the four wells contains a membrane patch with an ion channel. The external electrolyte solutions contain caged-ligands (such as caged-glutamate). When a beam of laser is directed at the well, the inert caged-ligands become active ligands that cause a channel to go from the closed conformation to an open conformation. Ions then flow across the open channel, and the current generated by the motion of charged particles is monitored with a patch-clamp amplifier. The amplifier is switched to the output of one well to another electronically. Typically, the magnitude of currents across each channel, when it is open, is about 1 pA (10^{-12} A).

The design of the ion channel activation scheduling algorithm needs to take into account the following sub-systems.

(i) *Heterogeneous Ion Channels (Macro-molecules) on Chip*: In a patch clamp chip, the dynamical behaviour of individual ion channels that are activated changes with time since they can become de-sensitized due to excessive use. De-activated ion channels behave quite differently to other ion channels. Their transition to the open state becomes less frequent when they are de-sensitized due to excessive use.

(ii) *Patch Clamp Amplifier and Heterogeneous Measurements*: The channel current of the activated ion channel is of the order of pico-amps and is measured in large amounts of thermal noise. Chung et al.,[6,5] used the powerful paradigm of Hidden Markov Models to characterize these noisy measurements of single ion channel currents. The added complexity in the patch clamp chip is that the signal to noise ratio is different at different parts of the chip - meaning that certain ion channels have higher SNR that other ion channels.

(iii) *Ion Channel Activation Scheduler*: The ion channel activation scheduler uses the noisy channel current observations of the activated ion channel in the patch clamp chip to decide which ion channel to activate at the next time instant to maximize a reward function that comprises of the information obtained from the experiment. It needs to avoid activating de-sensitized channels as they yield less information.

It can be shown that optimally scheduling between the different ion channels on the chip can be formulated as a partially observed stochastic multi-armed bandit problem. The optimal scheduling policy is to pick at each time the ion channel with the highest instantaneous Gittins index.[14]

3 THE PERMEATION PROBLEM

The permeation problem seeks to explain the working of an ion channel at an Å (10^{-10} m) spatial scale by studying the propagation of individual ions through the ion channel at a femto (10^{-15}) second time scale. This setup is said to be at a *mesoscopic scale* since the individual ions (e.g. Na^{+}, ions) are of the order of a few Å in radius and are comparable in radius to the ion channel. At this mesoscopic level, point charge approximations and continuum electrostatics break down. The discrete finite nature of each ion needs to be taken into consideration. Also, failure of the mean field approximation in narrow channels implies that any theory that aspires to relate channel structure to its function must treat ions explicitly.

For convenience we focus here on gramicidin A channels - which are one of the simplest channels. Gramicidin A is an antibiotic produced by *Bacillus brevis*. It was one of the first antibiotics to be isolated in the 1940s.[12] In sub-micromolar concentrations it can increase the conductance of a bacterial cell membrane (which is a planar lipid bilayer membrane) by more than seven orders of magnitude by the formation of cation selective channels. As a result the bacterial cell is flooded and dies. This property of dramatically increasing the conductance of a lipid bilayer membrane has recently been exploited by[9] to devise gramicidin A channel based biosensors with extremely high gains.

The aim of this section is to estimate the *potential mean force* (pmf) profile for a gramicidin A channel that optimizes the fit between the simulated current and the experimentally observed current. In the mesoscopic simulation of a gramicidin A channel, we propagate each individual ion using Brownian

dynamics (Langevin's equation) and the force experienced by each ion is a function of the pmf. As a result of the pmf and external applied potential to the ion channel there is a drift of ions from outside to inside the cell via the ion channel resulting in the simulated current.

Determining the pmf profile that optimizes the fit between the mescoscopic simulated current and observed current yields useful information and insight into how an ion channel works at a mesoscopic level. Determining the optimal pmf profile is important for several reasons: Firstly, it yields the effective charge density in the peptides that form the ion channel. This charge density yields insight into the crystal structure of the peptide. Secondly, for theoretical biophysicists, the pmf profile yields information about the permeation dynamics including information about where the ion is likely to be trapped called "*binding sites*", the mean velocity of propagation of ions through the channel and the average conductance of the ion channel.

3.1 Gramicidin Channel Model

Consider $2N$ ions, where N denotes a positive integer. Of these there are:

- N positive charged Na^+ ions each with charge $q^+ = 1.6\times10^{-19}$C, mass $m^+ = 3.8\times10^{-26}$ kg and frictional coefficient $m^+\gamma^+$ where from Einstein's relation

$$m^+\gamma^+ = \frac{kT}{D}, \quad D = 1.33\times10^{-9}\ \mathrm{m/s^2} \tag{9}$$

Na^+ ions have a radius of 0.95 Å .

- N negative charge Cl^- ions each with charge $q^- = -1.6\times10^{-19}$ C, mass $m^- = 5.9\times10^{-26}$ kg and frictional coefficient $m^-\gamma^- = \frac{kT}{D}$ where $D = 2.03\times10^{-9}$. Cl^- ions have a radius of 1.88 Å .

The setup comprises of 2 cylindrical reservoirs connected by the gramicidin ion channel as depicted in Fig.3. Each cylindrical reservoir is 30 Å in radius and N Å in height. Specifying the height of each reservoir to be N Å guarantees that the concentration of ions in each reservoir is at the physiological concentration of 150 mM. (Note: 1 Å (angstrom) = 10^{-10} m). The gramicidin A channel can be modelled as cylindrical nano-tube with diameter 4 Å length 25 Å[1] that connects the two reservoirs.

Let R_1 and R_2 denote the open sets comprised of the interior of the reservoirs, C the interior of the channel. So $R = R_1 \quad R_2 \quad C$ denotes the open

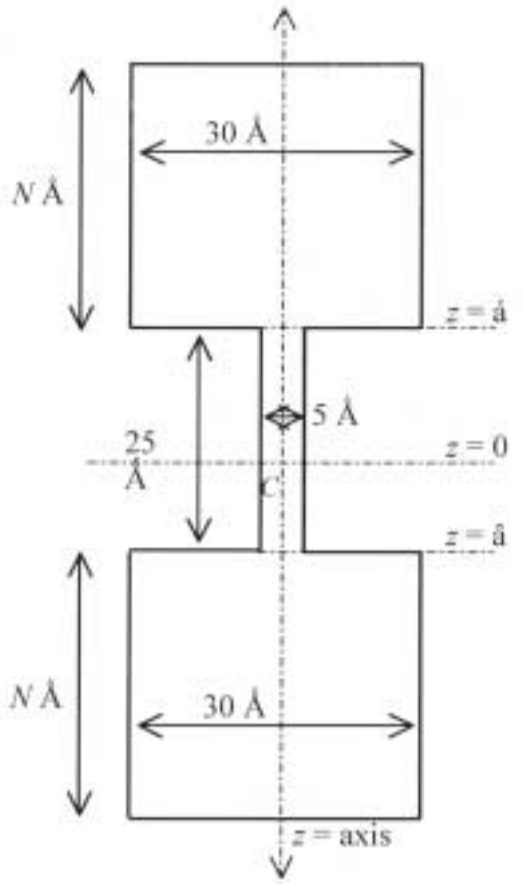

Figure 3: Gramicidin A Channel Model

set comprised of the interior of the reservoirs and channel. The corners of R_1, R_2 and their connection with C are smoothed out. so that the curve defining the boundary of R is differentiable.

Throught, we index the $2N$ ions by $i = 1,2,\ldots,2N$. Let N denote the index set of positive ions and the index set of negative ions, so that $P \quad N = \{1,2,\ldots,2N\}$.

3.2 Mesoscopic Brownian dynamics formulation

Let $t \geq 0$ denote continuous time. Each ion i, moves in 3 dimensional space over time. Let $x_t^{(i)} \in R^3$ and $v_t^{(i)} \in R^3$ denote the position and velocity of ion i and time t. The three components $x_t^{(i)}(1)$, $x_t^{(i)}(2)$ $x_t^{(i)}(3)$, of $x_t^{(i)} \in R^3$ are, respectively, the x, y and z position coordinates. Similarly, the three components of $v_t^{(i)}$ are the x, y. z velocity components.

At time $t = 0$, the position $x_0^{(i)}$ and velocity $v_0^{(i)}$ of each of the $2N$ ions indexed by $i = 1,2\ldots,2N$ are randomly initialized as follows: There are $N/2$ positive ions and $N/2$ negative ions in the upper reservoir, each with $x_0^{(i)} \quad U[R_1,\Delta]$. Similarly theare are $N/2$ positive ions and $N/2$ negative ions in

the lower reservoir, each with $x_0^{(i)} \quad U[R_2, \Delta]$. The velocities of the $2N$ particles are distributed according to a three dimensional Gaussian distribution with zero mean, and 3×3 diagonal positive definite covariance matrix.

Let $x_t = (x_t^{(1)}, x_t^{(2)}, \ldots, x_t^{(2N)})$ denote the positions of all the $2N$ particles. The position and velocity of each positive ions evolve according to the following continuous time stochastic dynamical system

$$x_t^{(i)} = x_0^{(i)} + \int_0^t v_s^{(i)} ds + Z_t^{(i),x}, \tag{10}$$

$$m^+ v_t^{(i)} = m^+ v_0^{(i)} - \int_0^t m^+ \gamma^+ v_s^{(i)} ds + \int_0^t F^{(i)}(x_s) ds + R^{1/2} w_t^{(i)} + Z_t^{(i),v}, \quad i \in N. \tag{11}$$

$$m^- v_t^{(i)} = m^- v_0^{(i)} - \int_0^t m^- \gamma^+ v_s^{(i)} ds + \int_0^t F^{(i)}(x_s) ds + R^{1/2} w_t^{(i)} + Z_t^{(i),v}, \quad i \in N. \tag{12}$$

Equation 10 merely says that velocity is the time derivative of the position. The *reflection* term $(Z_t^{(i),x}, Z_t^{(i),v})$ ensures that the position $x_t^{(i)}$ of each ion lies in R. In particular, $Z_t^{(i),x}$ and $Z_t^{(i),v} = 0$, if $x_t^{(i)} \in R^o$ where R^o denotes the interior of R. The term $Z_t^{(i),v}$ models elastic collisions at the boundary of R. Equations (11) and (12) are *reflected* versions of the well known *Langevin* equations.

$\{w_t^{(i)}\}$ denotes a 3 dimensional Brownian motion process, which is component wise independent. Similarly, $\{w_t^{(i)}\}$ and $\{w_t^{(j)}\}$, $j \neq i$ are mutually independent.

The systematic force $F^{(i)}(x_t) = \nabla_{x_t^{(i)}} V^{(i)}(x_t)$ where the scalar valued process $V^{(i)}(x_t)$ is the total electric potential experienced by ion i given the position $x_t = x_t^{(i)}$ of the $2N$ ions. It is convenient to break the potential $V^{(i)}(x_t)$ into the following four components:

$$V^{(i)}(x_t) = U(x_t^{(i)}, \theta) + \sum_{j \neq i} V^{C,(ij)}(x_t) + V^{IW,i}(x_t) + V^{SR,i}(x_t) \tag{13}$$

where

$$U(x_t^{(i)}, \theta) = V^{X,i}(x_t^{(i)}) + V^{SE,i}(x_t^{(i)}) + V^{P,i}(x_t^{(i)}) \tag{14}$$

$$V^{C,(ij)}(x_t) = \frac{1}{4\pi\varepsilon_0} \frac{q}{\varepsilon_w \| x_i - x_i \|}$$

(Coulomb potential) (15)

$$V^{IW,i}(x_t) = \frac{F_0}{9} \frac{(R_i + R_w)^{10}}{(R_c(x(3)) + R_w - a)^9}$$

(Ion - wall interaction - Lennard Jones potential) (16)

$$V^{SR,i}(x_t) = \quad \text{(Short range)} \tag{17}$$

$$V^{X,i}(x_t^{(i)}) =$$

(External potential due to applied field) (18)

$$V^{SE,i}(x_t^{(i)}) = \quad \text{(Surface effect)} \tag{19}$$

$$V^{P,i}(x_t^{(i)}) = \quad \text{(Protein ion interaction)} \tag{20}$$

3.3. Estimation of Potential Mean Force Profile

The electrolyte in the two reservoirs comprises of 55 M (moles) of H_2O, and 150 mM concetrations of Na^+ and Cl^- ions. An uniform electric field of 10^7 V/m is applied across the reservoirs and channel. Note that the length of the two reservoirs plus channel is approximately 100 Å. So the applied electric field results in approximately 100 mV across 100 Å which accurately matches the physiological potential.

As a result of the applied electrical field the ions drift from R_1 via the channel C to R_2 thus generating an ion channel current. Since experimental observations are made at the milli-second time scale, the BD simulation needs to be run for 10^{10} points at the slow time scale for the $2N-1$ ions in the reservoir and 10^{10} points at the fast time scale for the single ion in the gramicidin A channel.

In order to construct a mathematical expression for the current flowing from R_1 to R_2, we need to count the number of upcrossings of ions (i.e., the number of times ions cross from R_1 to R_2 across the region C) and **downcrossings of ions**. These are defined recursively by increasing sequences of random times $\{\sigma_n^{(i)}\}$, $\{\tau_n^{(i)}\}$ as follows:

$$\sigma_1^{(i)} = \inf\{t \geq 0; x_t^{(i)}(3) \leq \alpha\},$$
$$\tau_1^{(i)} = \inf\{t \geq \sigma_1^{(i)}; x_t^{(i)}(3) \geq \beta\} \quad (21)$$

and more generally for $n = 1,2,\ldots,$

$$\sigma_{n+1}^{(i)} = \inf\{t \geq \tau_n^{(i)}; x_t^{(i)}(3) \leq \alpha\},$$
$$\tau_{n+1}^{(i)} = \inf\{t \geq \sigma_{n+1}^{(i)}; x_t^{(i)}(3) \geq \beta\}. \quad (22)$$

Note that $\tau_n^{(i)}$ denotes the time of the n th upcrossing and $\sigma_n^{(i)}$ denotes the time of the n th downcrossing for ion i. In terms of these variables, over a time period $[0,T]$, denote the total number of upcrossings $U_T^{(i)}$ and downcrossings $D_T^{(i)}$ of particle i as

$$U_T^{(i)} = \max\{n : \sigma_n^{(i)} \leq T\}, \quad D_T^{(i)} = \max\{n : \tau_n^{(i)} \leq T\}.$$

Then the total current (random process) from R_1 to R_2 is

$$I(v) = \lim_{T\to\infty} \frac{1}{T} \sum_{i=1}^{2N} \left(U_T^{(i)} - D_T^{(i)} \right) \quad (23)$$

The expected current is the expected value of the above process:

$$\bar{I}(v) = \lim_{T\to\infty} \mathbf{E}\{\frac{1}{T} \sum_{i=1}^{2N} \left(U_T^{(i)} - D_T^{(i)} \right)\} \quad (24)$$

A stochastic gradient algorithm such as Smoothed Perturbation Stochastic Approximation (SPSA)[22] can be used to estimate the optimal profile θ that matches the above Brownian dynamics model to experimentally observed data.

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