A Multi-scale, Integrative Model of Cellular Electrophysiological Response Incorporating Intra/Extra-cellular Transport & Dynamics

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ABSTRACT

A novel hybrid computational approach to modeling integrated electrophysiological measurements (specifically action potentials) and biochemical endpoints (fluorescence/nanosensors) obtained while administering of compounds/drugs in a single cell environment is presented. To simulate the ion transport across the cell membrane and consequently to understand the electrophysiological response to external excitation (active potential or current), we propose a novel multi-scale approach (Figure 1) in which, high-fidelity spatial-temporal models describing intra and extra cellular fluid motion and ion transport are coupled with an equivalent electric circuit network model describing ion-channel activation. This approach will facilitate the data analysis and deconvolution of transport-related effects from the electrophysiology measurements, as well as design and development of microfluidics-based patch-clamp experimental platforms.

Keywords: ion channels, network models, continuum models, hybrid approach, voltage gating

1 INTRODUCTION

Ion-channel related research, especially modeling the ion permeation process, finds potential application in the modern drug discovery process [1,2] and nano devices [3]. Ion-channel are the proteins with a opening in the middle, exhibiting unique features such as sensitivity, specificity and efficiency in controlling ionic migration through them. The permeation process normally induces current, which is primarily controlled by the gradients in the voltage and concentration, as well as charge distribution along the walls of the pore. The pore diameter is extremely small (~1nm), compared to the size of the cell (~ few μm) and the density of the charge (mobile or fixed) is extremely high (~10 M). Besides, translocation of biomolecules can also significantly affect the current flowing through the channel.

With the advance in micro/nano fabrication technologies, and molecular biology, unprecedented opportunities are available to develop an integrated, hybrid, ion-channel based systems to convert biomolecular signals into electronic information (for example MOLDICE program from DARPA) [4]. These systems are expected to have a large number of pores distributed in a fashion that will facilitate the detection of biological events occurring at the cellular level. There is a need to develop predictive models that will simulate real-time (temporal) transduction of molecular events into an electrical signal. These call for an integrated computational tool that is capable of simulating the nanoscale biological events, coupled with macroscale fluidic phenomena to facilitate deconvolution transport effects from measure electrical signal. Such a tool will lead to a better understanding of the ion permeation, action potential and gating processes in response to mechanical, electrical or concentration stimulus. In addition, there is a significant amount quantitative information on the description of ion channel conformation for certain type of cell membrane available. This also catalysts simulation-based investigation ion permeation process.

Understanding transport phenomenon through ion channels and the associated regulatory mechanism are critical to understanding the transduction process. Modeling of the permeation process is extremely challenging. It occurs at femtosecond scale; while the ion-channel response (gating) is observed in milliseconds time scale. The data on complete molecular structure and charge distribution, which is important in simulation perspective, is known for a handful of channels. To summarize, the challenges are:

(a) Multi-scale effects (from individual ion channels at a molecular scale to carefully controlled spatial gradients at a device (micron) scale)
(b) Data analysis that requires de-convolution of pore electrokinetics, applied electric fields and transport effects

Current simulation approaches can be broadly classified in to two categories: molecular dynamics (MD) and continuum approach. MD methodology [5,6] has proved very successful in the simulating the behavior of the protein structure and conformation at the atomic scale, while continuum-based models [5,7,8] are successful in simulating the ionic transport at nano to microscales. MD simulations can be realistically performed over a short time scale, usually in the order of nanoseconds, thus restricting its ability to predict long time evolution of channel conduction and associated IV characteristics. On the other hand the continuum-based PNP formulation usually overestimates the current and is not amenable to include the effects of channel charges (discrete/surface) and protein confirmation. However, recently, progress has been made in simulating the permeation process as a transport of finite

sized particles to account for detailed dynamic and electrostatic interaction [9]. The bottom line is, both approaches are insufficient in understanding signal transduction in a device environment where multiple temporal and spatial scales are involved.

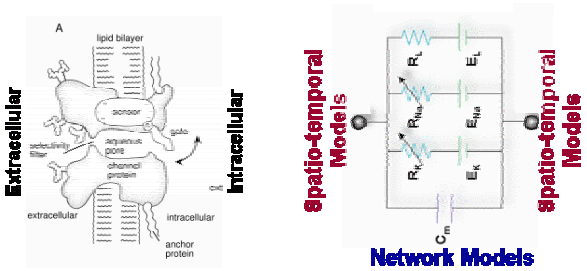

Figure 1: Hybrid Computational Approach

In this paper, we propose a hybrid computational strategy to simulate individual ion-channels and interactions among them. As shown in Figure 1, the analyte transport in both intra- and extra-cellular environments will be solved using continuum based PNP models, while the transport across the membrane will be solved via an equivalent electric circuit network model representing the membrane. The ionic flux and active potential are thus calculated for different loading conditions.

2 MODELING APPROACHES

2.1 Circuit network model for interacting ion-channels

The electric circuit representation of single ion channel using classic H-H type model for neural cell is very well established in literature [10]. The effective parameters used in these circuit model, such as capacity of the membrane, voltage/current dependent resistance and a battery accounting for pumping effect are sufficient to describe ion channel functions. Since the channel walls are bound with charged proteins and the surface charge will vary as ions permeate through the channel, a capacitor like component is added. This capacitor will mimic the effects of surface (protein) charges. A resistor is added to include the effects of the concentration gradient at the entrance and exit of the channel. However, the challenge is to extend these models to account for interactions of adjacent ion channels. In an integrated hybrid (*bio-silico*) ion channel system, surface currents can be generated facilitating the channel interactions. This effect is captured by the addition of a battery like component (along with resistors) when linking pores together (see Figure 2). The parameters for this modified Hodgkin-Huxley class models can be generated with the help of PNP/MD simulations. One way to accomplish model development is to perform a detailed simulation of few interacting ion channels by assuming a spatial distribution (such as doubly periodic). Once the detailed IV characteristics are generated for this configuration, the equivalent circuit parameters can be extracted and used for further validation of the network model.

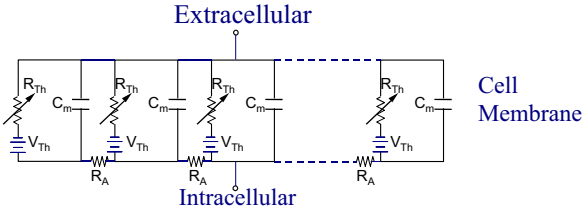

Figure 2: Equivalent network model for ion transport across the cell membrane. Each ion channel with adjacent membrane is represented by simple circuit model including a capacitor for membrane, a resistor and a battery for ion conduction through channel. Resistors connecting each ion channel component are introduced to account for current from one section of membrane to an adjacent region.

One of the unique functions of ion channel is its selectivity facilitated via gating effect for a specific type of ion. Simple two-state or multiple subunits model has been developed for K^+ channel, while more complex model for sodium channel has been reported [11]. The rate constants, which regulate the opening and closing status, can be determined from whole cell or single channel data. The Hodgkin-Huxley model, which relates the membrane potential and electric current for a Na^+ channel consists of three "m" gates and one "h" gate with either open or close status and four "n" gates. The model is expressed by coupled differential equations as [10]:

$$C_m \frac{dv}{dt} = -g_K(v - v_K) - g_{Na}(v - v_{Na}) - g_L(v - v_L) + I_{App}$$

$$\frac{dn}{dt} = 0.1(1-n)\Psi\left[\frac{-v+10}{10}\right] - 0.125ne^{\frac{-v}{80}}$$

$$\frac{dm}{dt} = (1-m)\Psi\left[\frac{-v+25}{10}\right] - 4me^{\frac{-v}{18}}$$

$$\frac{dh}{dt} = 0.07(1-h)e^{\frac{v}{20}} - \frac{h}{e^{\frac{30-v}{10}}+1}$$

Equations for Hodgkin-Huxley Model

The auxiliary functions is defined by

$$\Psi(x) = \frac{x}{e^x - 1}$$

Here v is the deviated potential from rest, measured in units of mV, current density in $\mu A/cm^2$, conductance in mS/cm^2 and capacitance in $\mu F/cm^2$. The remaining constants are

$$\overline{g}_K = 36, \overline{g}_{Na} = 120, \overline{g}_L = 0.3$$

$$v_K = -12, v_{Na} = 115, v_L = 10.6$$

The gating conductance for each ion is expressed as:

$$g_K = \overline{g}_K n^4, g_{Na} = \overline{g}_{Na} n^3 h, g_L = \overline{g}_L$$

2.2 Continuum model for spatial-temporal transport of species at device scale

In this model the extracellular and intracellular ion concentrations of every active ions (Na, K, Ca, Cl etc) are solved by transient Planck-Nernst-Poisson (PNP) equation, valid both inside and outside of the ion channel. PNP model automatically accounts for the I-V characteristics. The model is restricted by lack of information on cell geometry as well as ion channel distributions, and expensive computational time for realistic cell in biological environment. At the device level, the conventional diffusion, convection and drift process along with species concentration forms a well-posed partial differential equation that are solved by a finite volume method. The coupling at the pore level is determined by matching the ionic fluxes and usually involves iterative procedure. Since the network model is extremely simple to compute and the continuum model requires moderate amount of computational effort, it is feasible to model integrated, high-throughput bio-silico ion-channel devices in physiologically relevant environments and operation conditions, in a fast and efficient manner.

3 RESULTS AND DISCUSSIONS

H-H model is a set of highly nonlinear differential equations. To solve them in an integrated fashion with spatio-temporal models, user routines were written and integrated within CFD-ACE+ (multiphysics hifidelity software) framework to solve for action potential (AP) and gating function (n, m and h) under external excitation. An adaptive Runge-Kutta scheme is implemented to provide good accuracy and robustness. The results from the simulation are summarized in Figures 3 to 9. When a short electric pulse of 6.8 $\mu A/cm^2$ is applied for one millisecond period, we do not observe any response from the ion-channel (Figure 3). This phenomenon indicates that there is a critical value of current that is required to generate action potential. At this level of current, the opening and closing status for each ion, which are represented by gating functions in H-H model, do not change dramatically. However when the excitation current increases to 7 $\mu A/cm^2$ we observe that the membrane is excited with out any phase lag (Figure 4). The gating functions show significant change of channel status as the action potential is generated. Figure 5 and 6 show that AP can be generated by reverse current excitation for longer period of time when the magnitude exceeds the threshold value. The ion channel responds in a similar manner.

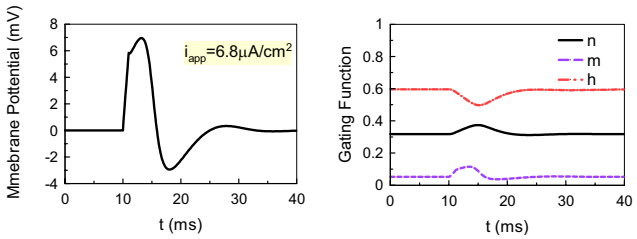

Figure 3: Threshold effect of Active potential. When pulse of applied current is 6.8$\mu A/cm^2$, no active potential is generated and gates for ions do not change significantly.

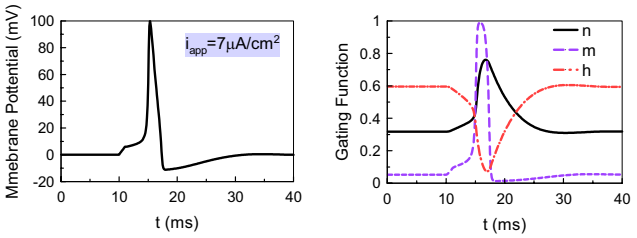

Figure 4 Action potential as produced by a short (1ms) but strong current pulse (7$\mu A/cm^2$) starting at t=10ms. Gating functions are also shown.

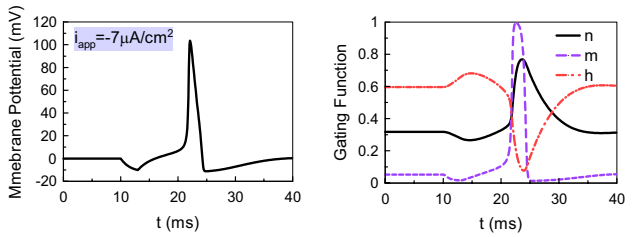

Figure 5 Active potential as produced by a short (3ms) but strong negative current pulse (-7$\mu A/cm^2$) starting at t=10ms. Gating functions are also shown.

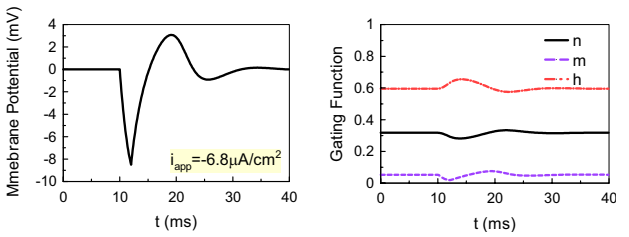

Figure 6: Threshold effect of Active potential. When pulse of applied current is -6.8$\mu A/cm^2$, no active potential is generated and gates for ions do not change significantly.

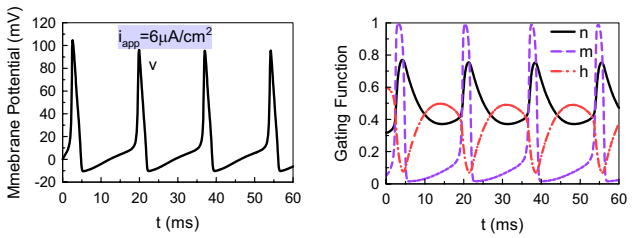

Figure 7: Spike Train of AC potential when a constant current is applied. Gating functions are also shown.

When a strong, continuous current is applied, it invokes a series of AP at constant time interval known as spike train of AC, as shown in Figure 7. Figure 8 presents the potential distribution to periodic current excitation for various magnitudes and time period. Results show a complex response of ion channel, where no AP, single spike of AP and spike train of AP can be generated, depending on the periodicity. In figure 9 we present the triggering of AP by a series of short impulse of current.

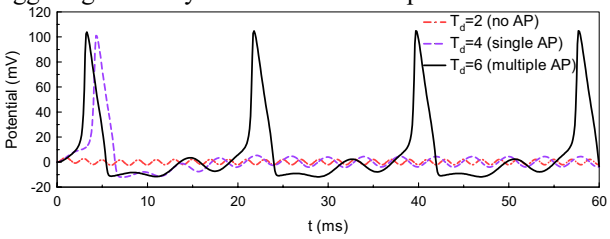

Figure 8: Potential response to time-dependent current $I_{app}=7\sin(2\pi t/T_d)$, where $T_d=2,4,6$.

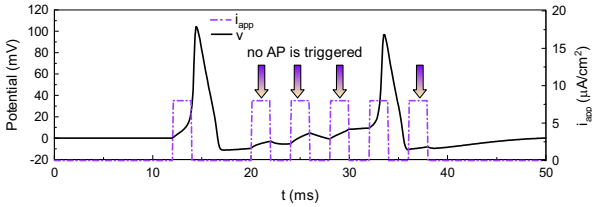

Figure 9: Repeated current after AP is invoked. The second through fourth signal are not strong enough to trigger another AP so a clear signature of membrane refractoriness is obtained. Yet the fifth signal triggers another AP.

4 CONCLISIONS

Ion-channel related research plays an increasing role in many areas of membrane electrophysiology and modern drug discovery research. With the advances in the technology, the quantitative descriptions of ion channel conformation for certain types of cell membrane are now available. Although current simulation methodologies have proved very successful in the study of single isolated ion-channel, the real challenge lies in understanding the ion-channel response in bio-silico devices, such as high throughput patch clamp. This motivates the development of multiscale, integrative framework for modeling cellular electrophysiology response to intra/extra-cellular transport & dynamics. In this paper, we have presented preliminary results using our modeling platform. Further work on detailed coupling of network model for ion-channel membrane and spatial-temporal model for extra/intra cellular matrix will follow.

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