Simulating complex ion channel kinetics with IonChannelLab

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Abbreviations: GUI, graphical user interface; \( V_m \), membrane potential

In-silico simulation based on Markov chains is a powerful way to describe and predict the activity of many transport proteins including ion channels. However, modeling and simulation using realistic models of voltage- or ligand-gated ion channels exposed to a wide range of experimental conditions require building complex kinetic schemes and solving complicated differential equations. To circumvent these problems, we developed IonChannelLab a software tool that includes a user-friendly Graphical User Interface and a simulation library. This program supports channels with Ohmic or Goldman-Hodgkin-Katz behavior and can simulate the time-course of ionic and gating currents, single channel behavior and steady-state conditions. The program allows the simulation of experiments where voltage, ligand and ionic concentration are varied independently or simultaneously.

Introduction

Ion channels are membrane-spanning proteins that control the flow of ions across the membranes of living cells. These proteins are essential to the generation of electrical signals in excitable cells and, therefore, are of fundamental importance for the functions of the brain, heart and muscle. Gating is the process that controls the opening and closing of the ion conducting pore. During gating, an ion channel may visit many conformational states while undergoing transitions controlled by transmembrane voltage or ligand binding. Discrete conformational states and the transitions that connect them are commonly represented by Markov chain models, which help understand the molecular conformational changes that occur during ion channel gating. General-purpose software packages, such as MATLAB and Mathematica, can be used to simulate gating kinetics of ion channels. Also, more specialized simulation programs, such as QUB Express, ChannelLab, IChSim, StateEditor and PulseSim, are available to perform this task. The main advantage of general-purpose programs is their flexibility; the user can perform almost any kind of simulation thanks to a large number of algorithms available in these packages. The main disadvantage is that implementation of the model and stimulus is in general time consuming and sometimes requires a high-level of programming experience. In contrast, specialized programs for kinetic simulation of ion channels are more popular because researchers can place more attention on the experimental work and analysis rather than model implementation. Because we consider that a successful program for kinetic simulation needs to be user-friendly and flexible enough for general applications across different fields we developed IonChannelLab. This program includes a Graphical User Interface (GUI), intuitive model-building dialogues and tools and a simulation library. IonChannelLab can be used to build kinetic models, stimulus protocols and perform simulations over a large range of experimental conditions.

Implementation

IonChannelLab uses Markov chain models to simulate the dynamic behavior of voltage- and ligand-gated ion channels. Accordingly, a kinetic model has states that represent discrete conformations and/or ligand occupancies of the channel and rate constants that govern transitions between states. Figure 1 provides a schematic overview of the IonChannelLab architecture. As mentioned before, the software includes a GUI and a simulation library. The GUI can be used to build kinetic models and experiments with voltage and ligand dependences. The simulation library includes algorithms to simulate time-courses, steady-state conditions and time constants. The time-course and steady-state simulations include: ionic current, gating current, conductance and custom functions of state probabilities. The

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simulations can be obtained using numerical integration, spectral decomposition of the transition rate matrix (Q-Matrix) and Monte Carlo.

**Ion Currents**

Voltage-clamp experiments record either macroscopic currents resulting from an ensemble of ion channels or unitary currents through a single ion channel. Even though single channel current provides more detailed kinetic information, macroscopic current recordings are widely performed and used to build conceptual kinetic models. Thus, we describe a framework to study macroscopic recording in terms of kinetic models.

If the current through an ion channel in the jth state is represented by \( i_j \). The current through \( N_c \) ion channels can be written as:

\[
I(t) = N_c \sum_{j=1}^{N_c} i_j \cdot p_j(t) \tag{1}
\]

where \( N_c \) is the number of conformational states and \( p_j \) is the probability of occupation of the j state. The single-channel current \( i_j \) is commonly described using Ohm’s law. For ion channels obeying this law, the single channel current is given by:

\[
i = \sum_{S=1}^{M} g_S \left( V - \frac{RT}{z_S F} \ln \left[ \frac{[S]}{[S_i]} \right] \right) \tag{2}
\]

where \( M \) is the number of permeant ions, \( V \) is the voltage, \( g_S \) is the unitary conductance for the ion \( S \), \( R \) is the gas constant, \( z_S \) is the ion valence, \( F \) is the Faraday constant, \( T \) is the temperature (in K) and \([S]\) and \([S_i]\) are the extracellular and intracellular molar concentrations of permeant ions, respectively. However, for some open channels the current-voltage relationship is not linear, particularly when ion gradients are imposed, and therefore it is not well described by Equation 2. To take into account ionic gradients the Goldman-Hodgkin-Katz equation (GHK) is used to describe the open channel I-V curve:

\[
I = \sum_{S=1}^{M} P_S z_S F \left[ [S] - [S_i] \right] \frac{1}{z_S F} \exp\left(-z_S F V R T\right) \tag{3}
\]

where \( P_S \) is the permeability of ion \( S \).

The kinetic behavior of an ion channel with \( N_S \) states can be obtained by calculating the probability of visiting these states. This probability is determined by solving a system of differentials equations. If the probability of occupying the \( i \)th state is represented by \( p_i \), then \( p_i \) is given by:

\[
dp_i(t) \over dt = \sum_{j=1}^{N} k_{ij} \cdot p_j(t) - \sum_{j=0}^{N} k_{ji} \cdot p_i(t) \tag{4}
\]

where \( k_{ij} \) and \( k_{ji} \) are rate constants that describe the transition from state \( i \) to state \( j \) and vice versa. The set of differential equations for the probabilities can be expressed in terms of matrix notation as:

\[
dp(t) \over dt = Q' \cdot p(t) \tag{5}
\]

where \( p(t) \) is the probability vector and \( Q \) is the transition rate matrix, the elements of the matrix \( Q \) are specified by

\[
q_{ij} = \begin{cases} k_{ij} & \text{if } i \neq j \\ \sum_{j=0}^{N} k_{ji} & \text{if } i = j \end{cases}
\]

When voltage or ligand concentrations are changed in a stepwise manner (the most common protocol in voltage- and concentration-clamp experiments), the transition rate matrix is time-independent. Then, the probability of occupying the \( i \)th state can be written as:

\[
p_i(t) = p(\infty) + \sum_{j=2}^{N} a_j \exp(-t \tau_j) \tag{6}
\]

where the time constant \( \tau_j = 1/\lambda_j \); \( \lambda_j \) is an Eigen value of the matrix \(-Q^t\).

**Gating Currents**

Gating current results from displacing charged parts of a voltage-gated ion channel within the membrane electric field but without transporting these charges across the membrane. If \( q_{G,i} \) is the charge associated with the state \( i \) and \( q_G \) is a vector with elements \( q_{G,i} \), then the total charge \( Q_G \) can be written as:

\[
Q_G(t) = \sum_{i=1}^{N} p_i(t) \cdot q_{G,i} \tag{7}
\]

The gating current is \( I_g(t) = dQ_g/dt \). Thus, by taking the derivative of Equation 7 and using Equation 5, the gating currents can be written as:

\[
I_g(t) = (Q^t \cdot p(t)) \cdot q_G \tag{8}
\]

**Results and Discussion**

Implementation of a kinetic model using ionchannellab. Figure 2A displays a hypothetical Ca\(^{2+}\)-dependent ion channel in three different states: closed (C), closed with Ca\(^{2+}\) bound (C\(_{Ca}^2\)) and open with Ca\(^{2+}\) bound (O\(_{Ca}^2\)). A plausible kinetic model for this channel can be represented by the three-state model shown in Figure 2B. Because the Ca\(^{2+}\) binding site (green sphere) is located internally in the channel and the transition from the state C\(_{Ca}^2\) to O\(_{Ca}^2\) involves a movement of Ca\(^{2+}\), then the rate constants \( k(V) \), \( k_+(V) \), \( a(V) \) and \( b(V) \) are assumed to depend exponentially on
membrane potential. A screenshot of the kinetic model editor of IonChannelLab that illustrates implementation of such kinetic model is shown in Figure 3A. In this editor, the rate constants for the transitions between states C and C_{Ca} are represented by \( k_1 \) and \( k_2 \). As illustrated in Figure 2, this transition is triggered by \( Ca^{2+} \), therefore, \( k_1 = k_+ \cdot [Ca^{2+}] \). The rate constant \( k_1 \) is defined in the rate constant editor (Fig. 3C). This editor includes a set of predefined voltage and ligand-dependent functions that can be modified to generate custom rate constants.

Since \( k_1 \) is \( Ca^{2+} \)- and \( V \)-dependent, the rate constant \( k_1 \) is represented by the voltage and ligand-dependent function \( F = P_0 \cdot X \cdot e^{-P_1 \cdot V \cdot T^R} \), where the ligand concentration is represented for \( X \). The generic function \( F \) is then adapted to the rate constant \( k_1 \) by selecting internal \( Ca^{2+} \) as the ligand and specifying the values for \( P_0 \) and \( P_1 \). The permeation model, permeant ion(s) and the single channel conductance are implemented using the permeation editor (Fig. 3D). In this example, the permeant ion is \( Cl^- \) and the single-channel conductance is 1 pS. The implementation of complex kinetic models using IonChannelLab is illustrated in Figure 3B.

The variable editor is also used with the example shown in Figure 2, where the open probability of the channel is modulated by both voltage and the internal concentration of \( Ca^{2+} \), and the current in the open state is dependent on the internal and external concentration of \( Cl^- \). In IonChannelLab these experimental variables can be controlled using an unlimited number of steps and/or ramps. For example, Figure 4A and B display the voltage and \([Ca^{2+}]\) protocols used to simulate the currents shown in the Figure 5A. In each case the number and duration of sweeps can be indicated. Complex
Simulations using IonChannelLab. Another feature of IonChannelLab is the simulation editor that allows the user to define the type of experiment to be simulated (time course of macroscopic ionic current, gating currents, conductance, time constants, open probability or single channel current) as well as to execute the simulation and obtain a modeled response. This editor permits the creation of an unlimited number of experiments in the same project. In the experiment part the user can define the simulation method, number of channels, sampling rate, number of points to simulate each current trace, number of sweeps, temperature, noise, etc. Figure 5A displays the ionic current simulated according to the kinetic model showed in Figure 2B, when the voltage and [Ca\(^{2+}\)] are changed as indicated in the stimulus part (Fig. 4A and B). Figure 5B shows the waveforms of different amplitude and duration for voltage and ion concentrations can be constructed in a similar fashion as constructing voltage clamp protocols when using the software pClamp. As detailed in Figure 4A, the voltage is changed from -100 mV to 100 mV using square 500 ms pulses from a holding voltage of -100 mV, then the voltage is returned to -100 mV during 400 ms and finally changed to +100 mV for the remainder of the time (1,000 ms). Figure 4B illustrates a basal [Ca], of 50 nM during the first 1,000 ms of the experiment, then the concentration changes from 0 to 100 nM during 500 ms and then it is clamped at 0 nM during the last 500 ms. The holding values used for voltage and [Ca\(^{2+}\)], were -100 mV and 50 nM, respectively, whereas the internal and external Cl\(^{-}\) concentration were held at 100 mM.
single channel current simulated using the voltage square pulse (from -100 to +100 and back to +50 mV during 2.9s) shown above the simulated current. Single channel current is easily seen due to long open time duration even though the current amplitude is only 0.1 pA. Figure 5C (upper left part) displays the steady-state conductance as a function of voltage at different [Ca^{2+}]. As [Ca^{2+}] increases the sigmoidal curves are shifted to the left on the voltage axis since at a single V_m the conductance increased as the [Ca^{2+}] augmented. Our hypothetical channel (Fig. 2A) binds Ca^{2+} in a V_m-dependent manner. This property is illustrated in Figure 5C (upper right part) where the steady-state whole cell conductance is plotted as a function of [Ca^{2+}] (0 to 200 nM) at various voltage values ranging from -100 mV (small conductance) to +100 (larger conductance) mV in 20 mV steps. At the same [Ca^{2+}], positive V_m facilitated channel opening, which is manifested as an increase in conductance. Figure 5C also display the time constants (Eqn. 6) as a function of voltage when the [Ca^{2+}] = 100 nM (lower left part), or as a function of [Ca^{2+}] (lower right part) when the voltage is +100 mV. Finally, an important feature of IonChannelLab is the capability to visualize the dynamics of state occupancy. This is particularly important for teaching purposes since the user can visually judge how the channel visits each state during the transition from the closed to the open conformation. Figure 5D shows the dynamics of state occupancy (for the model in Fig. 2B) both numerically and schematically—color-coded—when a 300 ms depolarization to +100 mV is applied from a holding voltage of -100 mV.

**Figure 4. Variables Editor.** (A) Screenshot of voltage changes used to simulate the ionic current displayed in Figure 5A. In the holding values section, the experimentalist indicates the holding V_m and ion concentrations. In the variable section, we can indicate the variable that is changing, in this case V_m. We can also define the number of sweeps, duration of each steps and V_m value. In the illustrated example, the voltage was changed from -100 mV to 100 mV in 10 mV increments during 500 ms. (B) Screenshot of the internal Ca^{2+} concentration used during the simulation of the ionic current displayed in Figure 5A. The value of the [Ca^{2+}] was changed between 0 and 100 nM.

**Numerical Methods**

In IonChannelLab, the numerical calculations are based on the numerical library DotNumerics (www.dotnumerics.com). The solutions of Equation 5 can be calculated in IonChannelLab by numerical integration or by a spectral decomposition of the transition rate matrix Q. IonChannelLab includes two methods of numerical integration: the implicit Runge-Kutta method of order 5 with step size control and the Gear method or Backward Differentiation Formula.

**Conclusions**

IonChannelLab is a flexible and user-friendly software for ion channel simulation that can be readily applied to research and education. It includes intuitive GUI that allows building simple or complex kinetic schemes and easy manipulation of model parameters and experimental conditions. These kinetic schemes are readily adapted to study either ligand- or voltage-gated ion channels both at the single and macroscopic levels, as well as gating currents for voltage-gated ion channels. In the research laboratory, IonChannelLab could be used to suggest plausible gating mechanisms after an easy evaluation of many kinetic models. In addition, this software can be used to design experiments to test the suggested gating mechanism. IonChannelLab can be easily implemented in the classroom to teach basic principles of kinetic theory, advanced topics of kinetic modeling and the fundamentals of ion channel gating. The classical Hodgkin-Huxley
Channels

and contemporary kinetic models for Na⁺ and K⁺ channels are already built into this software and can be used to demonstrate the mechanisms of voltage-dependent activation and inactivation, processes that are vital for the initiation and propagation of action potentials in nerve cells.

Availability and Requirements

- Availability: The software is freely downloadable from: www.jadesantiago.com/Electrophysiology/IonChannelLab/
- Demo videos: http://jadesantiago.com/Electrophysiology/IonChannelLab/Videos/
- Operating system: Windows XP SP2, Windows Vista and Windows 7.

- Dependencies: Microsoft .NET Framework 2.0 (or higher).
- Programming Language: The core algorithm and the GUI were written in C#.

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