BE6003/Physiol 6003

Cellular Electrophysiology and Biophysics

Modeling of Ion Channels I

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Overview

- Motivation & Introduction
- Hodgkin-Huxley Modeling
  - Background
  - Approach
  - Examples
- Markovian Modeling
  - Background
  - Approach
  - Examples
Mathematical ion channel models allow for
- reconstruction
- quantitative description
- prediction
of
- electrical behavior
- molecular structure and dynamics
of single channels and channel populations

Ion channel models are frequently integrated into membrane, cell and tissue models to study complex interaction in biological systems

Further applications of models are in
- research: developing/testing hypotheses
- development: drug discovery, design and safety
General Approach of Modeling

1. Design or selection of initial model or set of models
2. Parameterization
3. Evaluation (Fit to measurements or models, computational efficiency)
4. Application

The process is iterative, with feedback loops allowing for refinement and improvement of the models.
Introduction: Types of Ion Channel Models

Markov Models
- Currents through a single channel and population of channels as well as gating currents
- Based on states and transitions

Hodgkin-Huxley Models
- Current through a population of channels and single channel as well as gating currents
- Based on gating variables and rate coefficients

Molecular Models
- Structure and dynamics
- Molecular interactions, drug binding, ion movement

Systems of ordinary differential equations (ODEs)

Partial differential equations,
... integration of motion in particle systems
Hodgkin-Huxley Ion Channel Model with Single Gating Variable

\[ I_{ion} = G_{ion,\text{max}} f(V_m - E_{ion}) \]

\[ \frac{df}{dt} = \alpha_f(1 - f) - \beta_f f \]

\[ \alpha_f \equiv \alpha_f(V_m) : \text{Rate coefficient} \]

\[ \beta_f \equiv \beta_f(V_m) : \text{Rate coefficient} \]

\[ f : \text{Gating variable} \]

\[ G_{ion,\text{max}} : \text{Maximal conductivity for ion} \]

\[ E_{ion} : \text{Nernst voltage} \]

\[ V_m : \text{Transmembrane voltage} \]
Molecular Structure of Phospholipid Bilayers

Nitrogen
Oxygen
Phosphor
Carbon
(Hydrogen not represented)

Hydrophilic
Hydrophobic


~ 6 nm
Phospholipid Bilayers

- Plasma membrane
- Membrane of organelle

Selective permeability

Transmembrane proteins responsible for transport:
- Ion Channels
- Pumps
- Exchangers

- Gases: CO₂, N₂, O₂ (Permeable)
- Small uncharged polar molecules: Ethanol, Urea, H₂O (Permeable)
- Large uncharged polar molecules: Glucose, fructose (Impermeable)
- Ions: K⁺, Mg²⁺, Ca²⁺, Cl⁻, HCO₃⁻, HPO₄²⁻ (Impermeable)
- Charged polar molecules: Amino acids, ATP, glucose 6-phosphate, proteins, nucleic acids (Impermeable)

(Lodish et al., Molecular Cell Biology, Fig. 7-1, 2004)
Modeling of Membrane: Nernst Equation

Region i
- Membrane
  - permeable for ion type k
  - homogeneous, planar, infinite

Region e

\[
\begin{align*}
[k]_i : & \text{ Concentration of k in region i} \\
\phi_i : & \text{ Potential in region i} \\
j_{D,k} : & \text{ Ionic current by diffusion} \\
[k]_e : & \text{ Concentration of k in region e} \\
\phi_e : & \text{ Potential in region e} \\
j_{E,k} : & \text{ Ionic current by electrical forces}
\end{align*}
\]
Modeling of Membrane: Nernst Potential

In Equilibrium

\[ j_{E,k} + j_{D,k} = 0 \]

\[ V_{m,k} = \phi_i - \phi_e = -\frac{RT}{z_k F} \ln \left( \frac{[k]_i}{[k]_e} \right) \]

- \( k \): Ion type
- \( V_{m,k} \): Nernst potential [V]
- \( R \): Gas constant [J/mol/K]
- \( T \): Absolute temperature [K]
- \( z_k \): Valence
- \( F \): Faraday’s constant [C/mol]
- \([k]_i\): intracellular concentration of ion type \( k \) [M]
- \([k]_e\): extracellular concentration of ion type \( k \) [M]
Nernst equation explains measured transmembrane voltage of animal and plant cells

For potassium (monovalent cation) at temperatures of 37ºC:

\[
V_{m,K} = -\frac{310K \cdot R}{F} \ln \left( \frac{[K]_i}{[K]_o} \right) = -61mV \log \left( \frac{[K]_i}{[K]_e} \right)
\]

For typical intra- and extracellular concentrations:

\[
[K]_i = 150 \text{ mM} \\
[K]_e = 5.5 \text{ mM}
\]

\[V_{m,K} = -88mV\]

Commonly, several types of ions are contributing to transmembrane voltage!
Modeling of Membrane: Resistor-Capacitor Circuit

\[ C_m = \frac{Q}{V_m} \]

- \( C_m \): membrane capacity \([\text{F}]\)
- \( Q \): electrical charge \([\text{As}]\)
- \( V_m = \phi_i - \phi_e \): voltage over membrane \([\text{V}]\)

\[ \frac{d}{dt} V_m = \frac{d}{dt} \frac{Q}{C_m} = \frac{I_m}{C_m} \]

- \( I_m \): Current through membrane \([\text{A}]\)

\[ R_m = -\frac{V_m}{I_m} \]

- \( R_m \): Resistance of membrane \([\Omega]\)
Hodgkin and Huxley: Measurements

Measurement and mathematical modeling of electrophysiological properties of cell membrane (published 1952, Nobel prize 1963)

“Giant” axon from squid with ~0.5 mm diameter

Techniques
• Space clamp
• Voltage clamp

Simplifications:

Extracellular space:
Salt solution

Semi permeable membrane

Intracellular space:
Axoplasm

Na: Sodium ions
K: Potassium ions
L: Other ions (primarily chlorine)
What are the important biophysical findings of Hodgkin and Huxley?

List 5 findings!
Hodgkin-Huxley: Clamp Techniques

• **Space Clamp**
  Electrophysiological properties are independent of \( x \)
  \[ I_m = I_i + C_m \frac{d}{dt} V_m \]
  \( I_i \): Injected current [A]
  \( I_m \): Current through membrane [A]
  \( C_m \): Membrane capacitor [F]
  \( V_m \): Membrane voltage [V]

• **Voltage Clamp**
  Voltage \( V_m \) is kept constant by injection of current \( I_i \): \( I_m = I_i \)
  Measurement of I-V relationship
  Reduction of capacitive effects
Hodgkin-Huxley: Voltage Clamping

Clamped voltage

Measured current

Membrane voltage [mV]

Clamped voltage

85 mV step (voltage clamp)

Membrane current [mA/cm²]

Capacitive current

Delayed outward current

Transient inward current

Time [ms]
Hodgkin-Huxley: Measurement Protocols

Protocols
Measurement of I-V relationship
Substitution of ions in intra- and extracellular space for separation of K and Na currents

Analysis
Based on extraction of measurement parameters, in particular:
• steady state currents
• time constants
Hodgkin-Huxley Model: Equivalent Circuit Diagram

\[ V_m = \Phi_i - \Phi_e \]

\[ I_m = C_m \frac{dV_m}{dt} + (V_m - V_{Na})G_{Na} + (V_m - V_{K})G_{K} + (V_m - V_{L})G_{L} \]

\[ G_{Na}, G_{K}, G_{L} \]
Membrane conductivity of Na, K and other ions [S/cm²]

\[ I_{Na}, I_{K}, I_{L} \]
Currents of Na, K and other ions [mA/cm²]

\[ V_{Na}, V_{K}, V_{L} \]
Nernst voltages of Na, K and other ions [mV]

\[ C_m, I_m, V_m \]
Membrane capacitor [F/cm²], current [mA/cm²] and voltage [mV]
Hodgkin-Huxley Model: Constants

Voltages are related to resting voltage $V_r$.
Conductivity and capacitance are related to membrane area.

<table>
<thead>
<tr>
<th>Relative Na voltage</th>
<th>$V_r - V_{Na}$</th>
<th>-115</th>
<th>mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative K voltage</td>
<td>$V_r - V_k$</td>
<td>12</td>
<td>mV</td>
</tr>
<tr>
<td>Relative voltage of other ions</td>
<td>$V_r - V_L$</td>
<td>-10.6</td>
<td>mV</td>
</tr>
<tr>
<td>Membrane capacitance</td>
<td>$C_m$</td>
<td>1</td>
<td>$\mu$F/cm$^2$</td>
</tr>
<tr>
<td>Maximal conductivity of Na</td>
<td>$G_{Na max}$</td>
<td>120</td>
<td>mS/cm$^2$</td>
</tr>
<tr>
<td>Maximal conductivity von K</td>
<td>$G_{K max}$</td>
<td>36</td>
<td>mS/cm$^2$</td>
</tr>
<tr>
<td>Conductivity for other ions</td>
<td>$G_L$</td>
<td>0.3</td>
<td>mS/cm$^2$</td>
</tr>
</tbody>
</table>

All ion channels open
Gating Variables Modulate Conductivities

\[ G_{Na} = G_{Na} \text{max} m^3 h \]
\[ \frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m \]
\[ \frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h \]
\[ G_K = G_{K\text{max}} n^4 \]
\[ \frac{dn}{dt} = \alpha_n (1 - n) - \beta_n n \]
\[ G_L = \text{const} \]
\[ \alpha_m = \frac{0.1(25 - V')}{e^{0.1(25-V')}-1} \frac{1}{\text{ms}} \]
\[ \beta_m = \frac{4}{e^{V'/18}} \frac{1}{\text{ms}} \]
\[ \alpha_n = \frac{0.07}{e^{V'/20}} \frac{1}{\text{ms}} \]
\[ \beta_n = \frac{1}{e^{0.1(30-V')}} + 1 \frac{1}{\text{ms}} \]
\[ \alpha_h = \frac{0.01(10 - V')}{e^{0.1(10-V')}-1} \frac{1}{\text{ms}} \]
\[ \beta_h = \frac{0.125}{e^{V'/80}} \frac{1}{\text{ms}} \]

Voltage-dependent

Sodium current

Potassium current

Current by other ions
Hodgkin-Huxley Model: Simulation of Voltage Clamp Measurements

http://genesis-sim.org
Hodgkin-Huxley Model: Simulation of Voltage Clamp Measurements

\[ G_{Na} = G_{Na,\text{max}} \ m^3 \ h \]
\[ G_{K} = G_{K,\text{max}} \ n^4 \]
Discuss limitations of the Hodgkin-Huxley models!

Which sub-models are missing?

Why is it nevertheless successfully reconstructing action potentials?
Markov Modeling of Ion Channels and Mutations

Markov models enable
- reconstruction of currents from single channels, population of channels and gating currents
- to be based upon thermodynamic principals
- assignment of physical meaning to states and transitions

Example: State diagram of cardiac sodium channel model
O: Open, I: Inactivated, C: Closed
(Irvine et al. Biophys J. 1999)

- Markov models consist of sets of 1st order ODEs
- Commonly, not all states are directly observable (hidden Markov model)
Molecular Structure of Ion Channels

Molecular structure of tetrameric $K^+$ channel (KcsA of bacterium streptomyces lividans)

Schematic Depiction of Voltage-Gated K\(^+\) Channel (Tetramer)

(Lodish et al., Molecular Cell Biology, Fig. 7-36a, 2004)
Schematic Depiction of Voltage-Gated Na\(^+\)/Ca\(^{2+}\) Channel (Monomer)

(Lodish et al., Molecular Cell Biology, Fig. 7-36b, 2004)
Experimental Studies: Patch Clamp Techniques

Measurement technique developed by Neher, Sakmann et al. (published 1976, Nobel prize 1991)

Micropipettes
- heat polished fluid filled glass pipette
- diameter of opening: 0.5–1 µm

Major configurations
- Cell attached recording
- Whole cell recording
- Outside-out patch
- Inside-out patch

Electrical measurements of
- population of channels
- single ion channels
- gating currents
Channel Characterization in Oocyte Expression Array

1. Microinject mRNA encoding channel protein of interest

2. Incubate 24–48 h for synthesis and movement of channel protein to plasma membrane

3. Measure channel-protein activity by patch-clamping technique

(Lodish et al., Molecular Cell Biology, Fig. 7-19, 2004)
Current traces of patch with single sodium channel

Average current per channel: 1.6 pA ~ 9900 ions/ms

Inside-out patch

(Lodish et al., Molecular Cell Biology, Fig. 7-18, 2004)
Current traces of patch with 2 potassium channels at different voltages

Transmembrane voltages determine
• open probability
• open time
• current amplitude

(Lodish et al., Molecular Cell Biology, Fig. 7-34, 2004)
2-State Markov Model

\[ \frac{dO}{dt} = \alpha \ C - \beta \ O \]
\[ \frac{dC}{dt} = \beta \ O - \alpha \ C \]

O : Probability that channel is in open state
C : Probability that channel is in closed state
\( \alpha, \beta \) : Rate coefficients.

Function of e.g. \( V_m \) and ion concentration
Rate Coefficient Functions

\[ \alpha = \alpha_0 \]

\[ \alpha = \alpha_0 V_m + a \]  
Linear

\[ \alpha = \alpha_0 e^{V_m/a} \]  
Exponential

\[ \alpha = \frac{\alpha_0}{e^{-\frac{(V_m - V_a)}{a}} + 1} \]  
Sigmoid

\[ \alpha = \alpha_0 \frac{V_m - V_a}{e^{-\frac{(V_m - V_a)}{a}} - 1} \]
Linear for extreme case

\( \alpha_0, V_a, a : \) Parameters

\( V_m : \) Membrane voltage
Voltage Dependent Rate Coefficient

\[ \alpha = \alpha_0 e^{\frac{zFV_m}{RT}} \]

\( \alpha_0 \): Rate coefficient at \( V_m = 0 \)
R, F: Gas and Faraday constant, resp.
z: Equivalent valence
T: Temperature
\( V_m \): Transmembrane voltage

Based on Boltzmann equation (Hille, Ion Channels of Excitable Membranes, chap. 1 and 10)
Matrix Formulation: Example

\[
\frac{dO}{dt} = \alpha \ C - \beta \ O \\
\frac{dC}{dt} = \beta \ O - \alpha \ C
\]

\[
\frac{dP}{dt} = QP
\]

with the states \( P = \begin{pmatrix} O \\ C \end{pmatrix} \)

and the matrix \( Q = \begin{pmatrix} \alpha & -\beta \\ \beta & -\alpha \end{pmatrix} \)

For larger models: parameters can be derived by submatrix selection and fit to macroscopic and single channel data.

(Colquhoun and Hawkes, chap. 19 and 20, Single-Channel Recording, eds. Sakmann and Neher)
Equivalence of Markov Models

Equivalent models with respect to steady-state observable probability distributions

Models are equivalent if

\[ Q_A = S^{-1} Q_B S \] with \[ S = \begin{pmatrix} S_{OO} & 0 \\ 0 & S_{CC} \end{pmatrix} \], \[ Q_A = \begin{pmatrix} Q_{A,OO} & Q_{A,OC} \\ Q_{A,CO} & Q_{A,CC} \end{pmatrix} \] and \[ Q_B = \begin{pmatrix} Q_{B,OO} & Q_{B,OC} \\ Q_{B,CO} & Q_{B,CC} \end{pmatrix} \]

\( Q_A \) and \( Q_B \) are partitioned into 4 sub-matrices related to O-O, O-C, C-O and C-C transitions

Channels can have
• several open states
• permeabilities/conductances for various ion types

\[ I_{\text{chan}} = N \times G \times O \left( V_m - E_{\text{ion}} \right) \]

Nernst approach

\[ I_{\text{chan}} = N \times O \times I_{\text{ion}} \]

Goldman-Hodgkin-Katz

\[ I_{\text{ion}} = P \times z^2 \frac{F^2 V_m [\text{ion}]_i - [\text{ion}]_o e^{-zFV_m/RT}}{RT} \left( 1 - e^{-zFV_m/RT} \right) \]

current equation

G: Conductance of single channel
O: Open probability of channels
N: Number of channels
V_m: Membrane voltage
P: Membrane permeability for ion
[ion]_i, [ion]_o: Concentration of ion in intra- and extracellular space
Markov model with gating charge movement associated to $A \leftrightarrow B$:

$$
\begin{array}{c}
\ldots \\
\xrightarrow{\alpha} \\
A \\
\xleftarrow{\beta} \\
B \\
\xrightarrow{\alpha} \\
\ldots
\end{array}
$$

Gating current:

$$I_{gAB} = z_{gAB} q_e (\alpha A - \beta B)$$

- $z_{gAB}$: Equivalent valence of moved charge
- $q_e$: Elementary charge, $1.60 \times 10^{-10}$ C

$$
\alpha = \alpha_0 e \frac{z_{gAB} q_e V_m}{kT}
$$

- $k$: Boltzmann's constant, $1.38 \times 10^{-23}$ V C/K

(Khalili-Araghi, Biophys J 2010)
Modeling of Gating Currents: Example

Three state model with gating charge movement associated to $A \leftrightarrow B$:

$$\begin{align*}
A & \xrightarrow{K_{AB}} B & K_{BA} & \xrightarrow{K_{BC}} C
\end{align*}$$

Commonly, more states are necessary to reconstruct gating currents, e.g. $\geq 6$ in Shaker K-channels.
Modeling of Gating Currents in Tetrameric Channels

(Jiang et al, Nature 2003)
Modeling of hERG Gating Currents

(Abbruzzese et al. J Gen Physiol 2010)
Group Work

What causes gating currents of ion channels?

Which other membrane proteins could produce similar currents?
Summary

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