BE6003/Physiol 6003

Cellular Electrophysiology and Biophysics

Modeling of Ion Channels I

CVRTI

Frank B. Sachse, University of Utah

Overview

- Motivation & Introduction

Group work

- Hodgkin-Huxley Modeling
    - Background
    - Approach
    - Examples

Group work

- Markovian Modeling
    - Background
    - Approach
    - Examples

Group work

Motivation

Mathematical ion channel models allow for
- reconstruction
- quantitative description
- prediction

of
- electrical behavior
- molecular structure and dynamics

of single channels and channel populations

Biophysics
Electrophysiology
Pharmacology
...

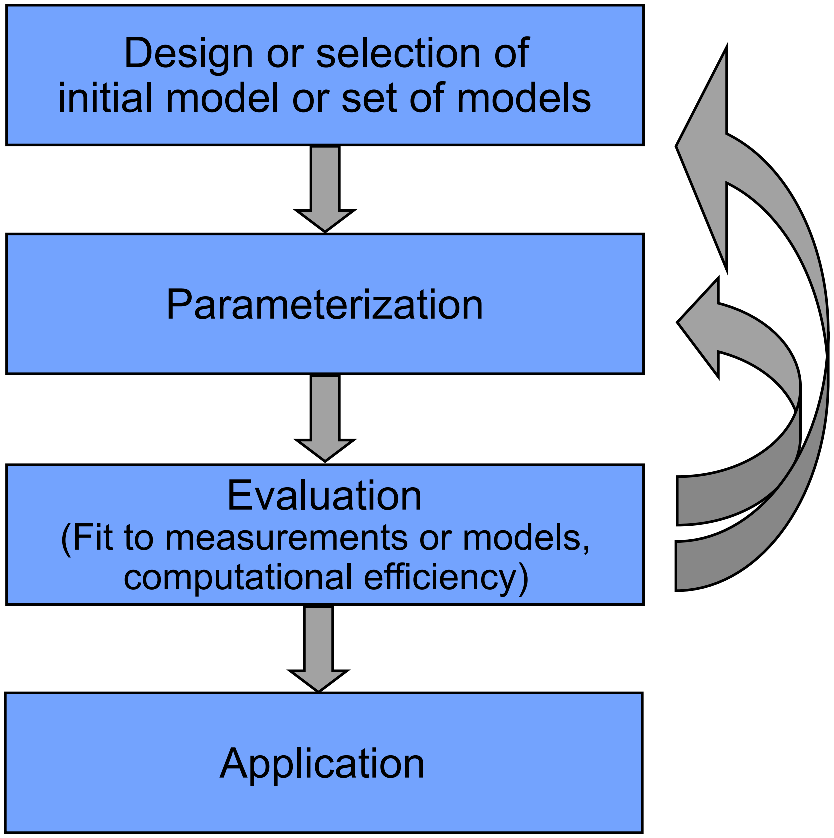
Ion channel models are frequently integrated into membrane, cell and tissue models to study complex interaction in biological systems

Systems biology

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

CVRTI

General Approach of Modeling



Design or selection of initial model or set of models

Parameterization

Evaluation
(Fit to measurements or models, computational efficiency)

Application

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

$$I_{ion} = G_{ion,max} f\left(V_m - E_{ion}\right)$$

$$\frac{df}{dt} = \alpha_f\left(1 - f\right) - \beta_f f$$

$$\text{“1-f”} \underset{\beta}{\overset{\alpha}{\rightleftarrows}} \text{“f”}$$

1st order ODE

$\alpha_f \equiv \alpha_f\left(V_m\right)$: Rate coefficient

$\beta_f \equiv \beta_f\left(V_m\right)$: Rate coefficient

f : Gating variable

$G_{ion,max}$: Maximal conductivity for ion

Channel Environment

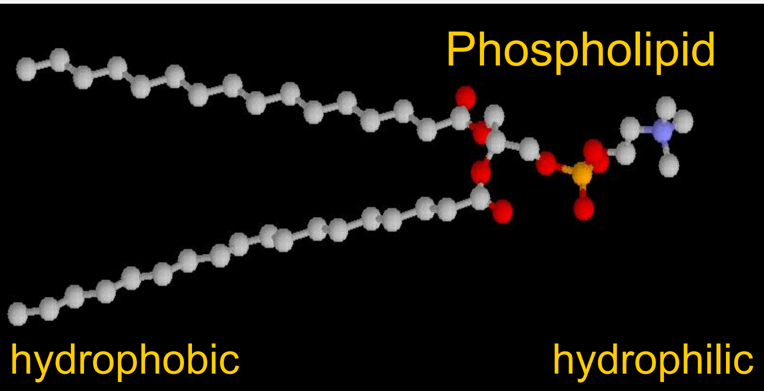
E_{ion} : Nernst voltage

V_m : Transmembrane voltage

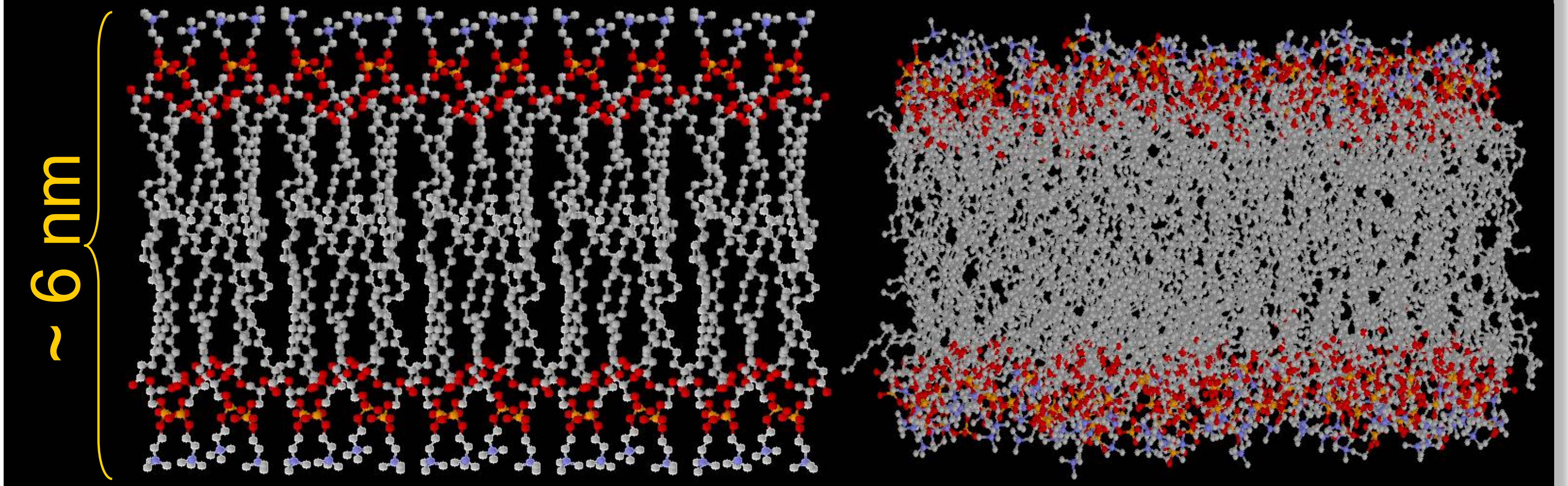
Membrane Environment

CVRTI

Molecular Structure of Phospholipid Bilayers



● Nitrogen
● Oxygen
● Phosphor
● Carbon
(Hydrogen not represented)



(Structure data from Heller et al, J. Phys. Chem., 1993)

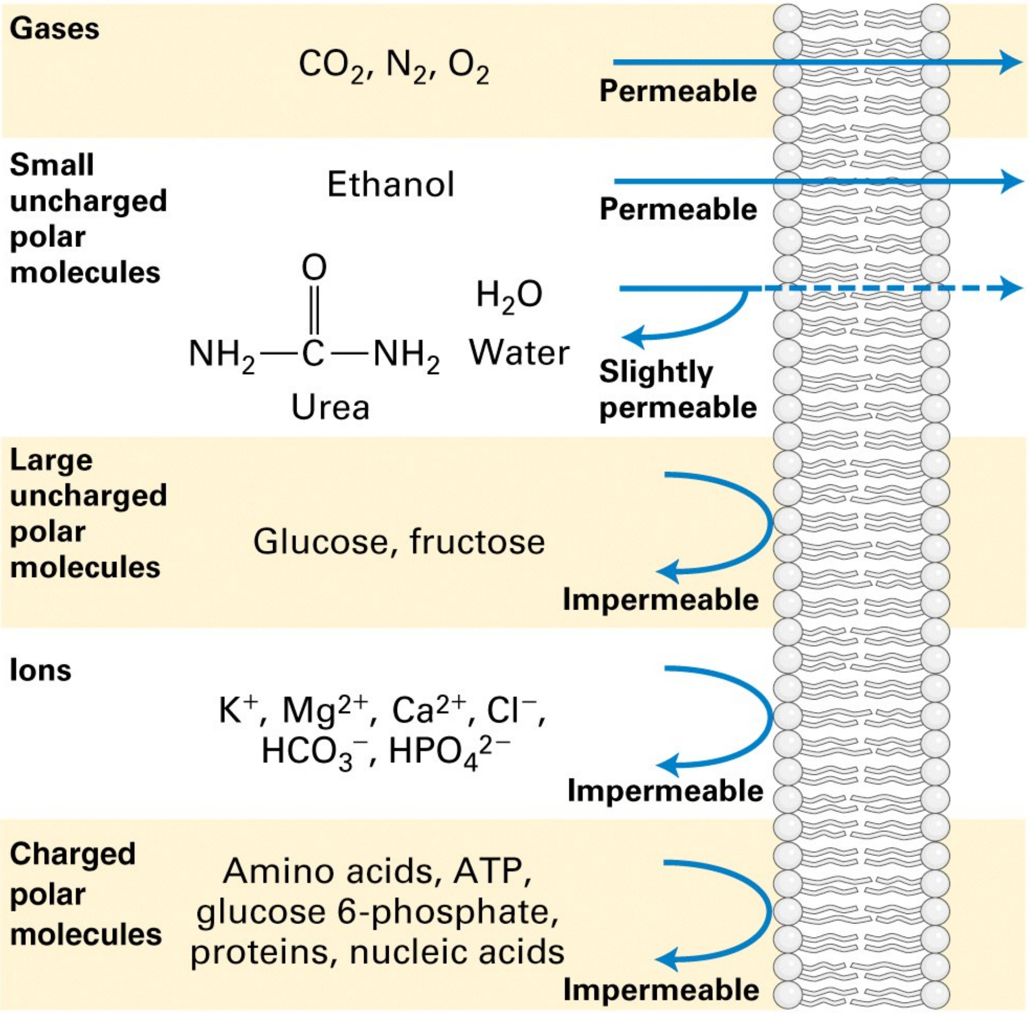
Phospholipid Bilayers

- Plasma membrane
- Membrane of organelle

Selective permeability

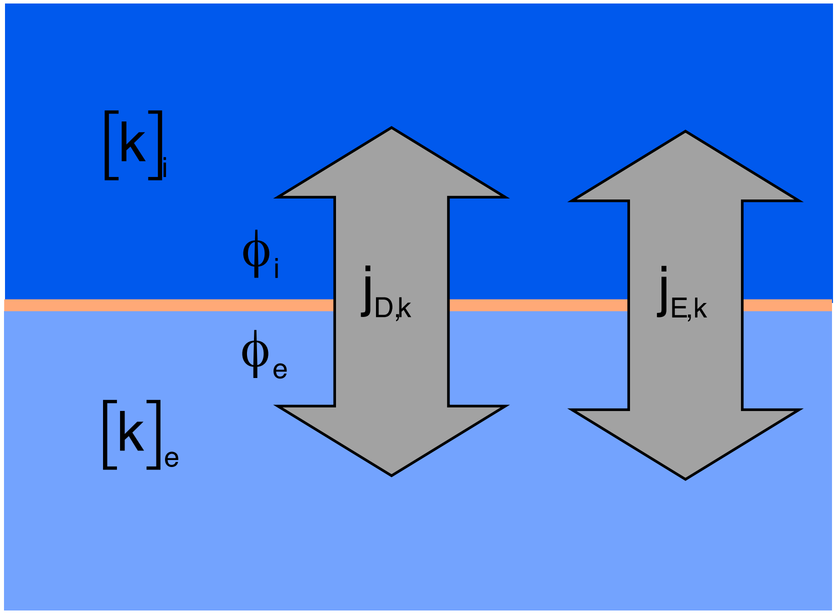
Transmembrane proteins responsible for transport:

- Ion Channels
- Pumps
- Exchangers



(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

$[k]_i$: Concentration of k in region i

ϕ_i : Potential in region i

$j_{D,k}$: Ionic current by diffusion

$[k]_e$: Concentration of k in region e

ϕ_e : Potential in region e

$j_{E,k}$: Ionic current by electrical forces

Modeling of Membrane: Nernst Potential

In Equilibrium

$$j_{E,k} + j_{D,k} = 0$$

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

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 $[M]$

Modeling of Membrane: Nernst Equation - Example

Nernst equation explains measured transmembrane voltage of animal and plant cells

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

$$V_{m,K} = -\frac{310K}{+1}\frac{R}{F}\ln\frac{[K]_i}{[K]_o} = -61mV\log\frac{[K]_i}{[K]_e}$$

For typical intra- and extracellular concentrations:

$[K]_i = 150$ mM

$[K]_e = 5.5$ 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 : membrance capacity $[F]$

Q : electrical charge $[As]$

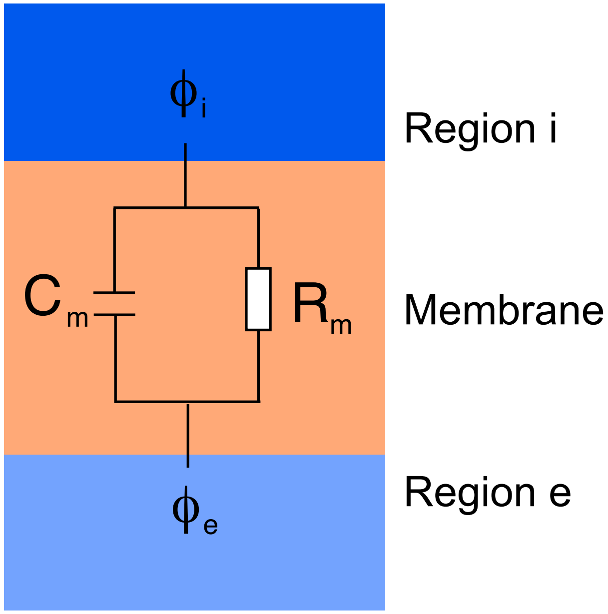
$V_m = \phi_i - \phi_e$: voltage over membrane $[V]$

$$\frac{d}{dt}V_m = \frac{d}{dt}\frac{Q}{C_m} = \frac{I_m}{C_m}$$

I_m : Current through membrane $[A]$

$$R_m = -\frac{V_m}{I_m}$$

R_m : Resistance of membrane $[\Omega]$



CVRTI

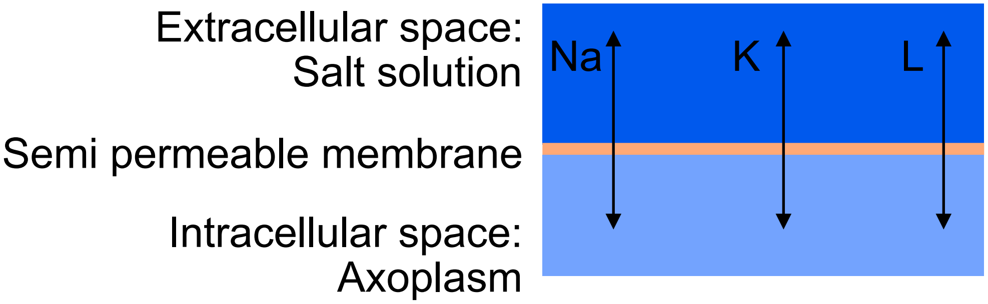
Hodgkin and Huxley: Measurements

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:



Na:	Sodium ions
K:	Potassium ions
L:	Other ions (primarily chlorine)

CVRTI

Group Work

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

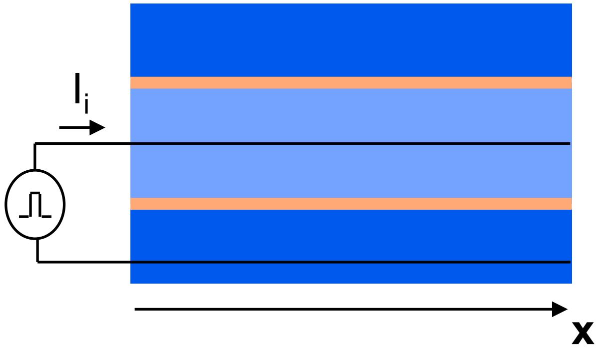
$$I_m = I_i + C_m \frac{d}{dt} V_m$$

I_i : Injected current $[A]$

I_m : Current through membrane $[A]$

C_m : Membrane capacitor $[F]$

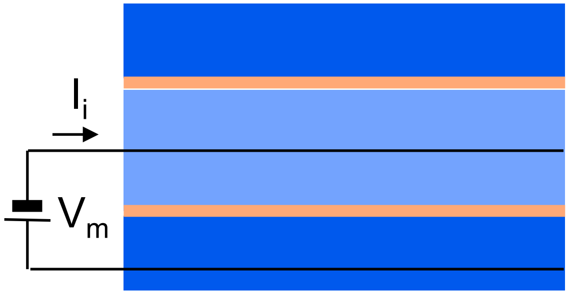
V_m : Membrane voltage $[V]$



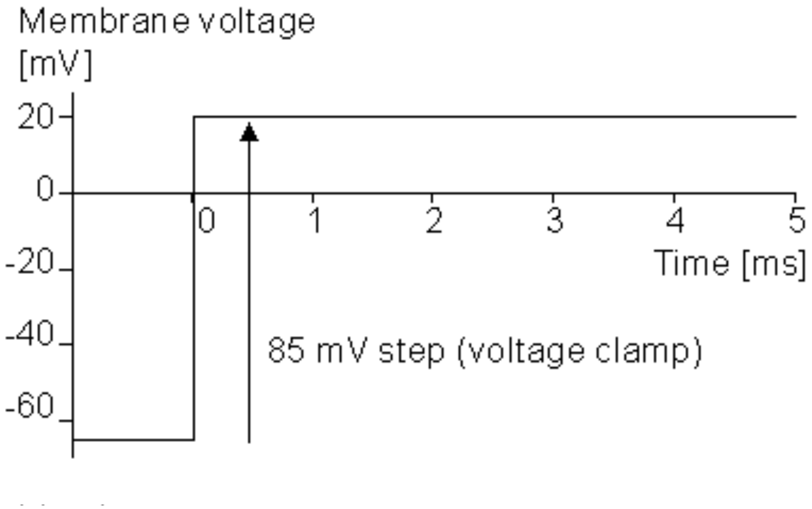
- **Voltage Clamp**
  Voltage V_m is kept constant by injection of current I_i: $I_m = I_i$

  Measurement of I-V relationship

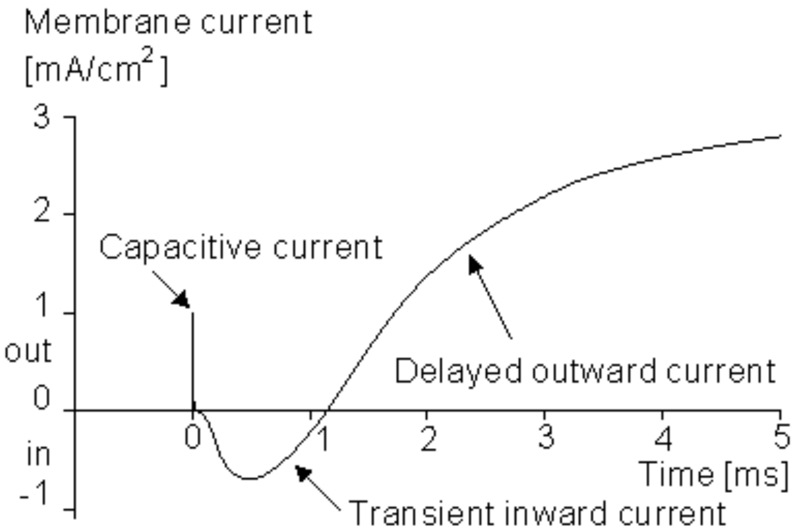
  Reduction of capacitive effects



Hodgkin-Huxley: Voltage Clamping

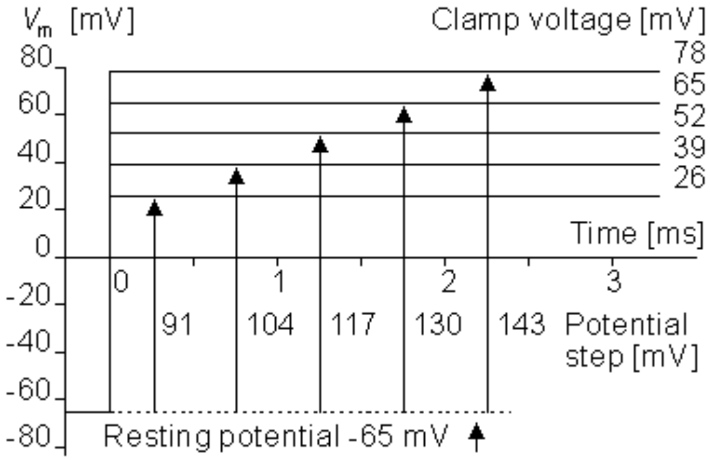


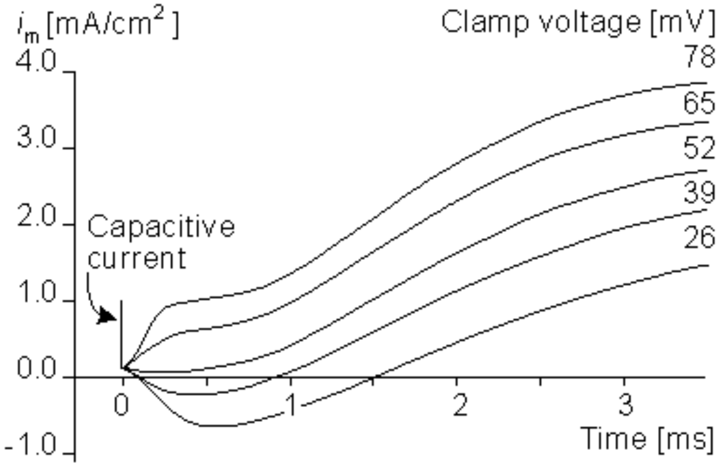
Clamped voltage



Measured current

Hodgkin-Huxley: Measurement Protocols





Protocols

Measurement of I-V relationship

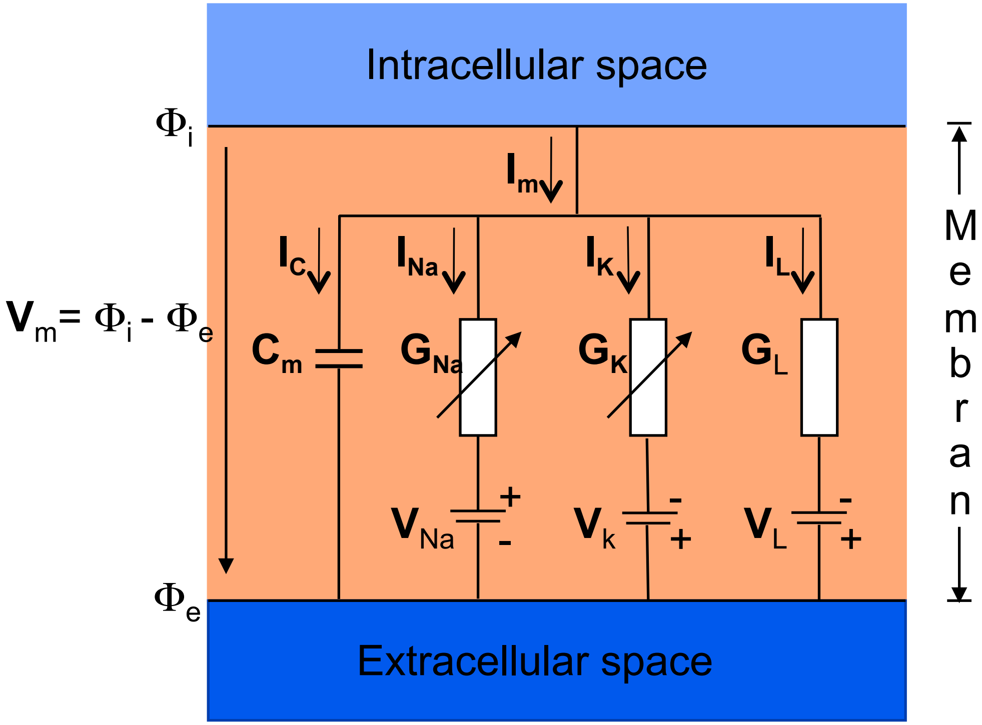
Substitution of ions in intra- and extracellular space for separation of K and Na currents

Analysis

Based on extraction of measurement parameters, in particular:

- steady state currents
- time constants

Hodgkin-Huxley Model: Equivalent Circuit Diagram



$$I_m = C_m \frac{dV_m}{dt} + (V_m - V_{Na})G_{Na} + (V_m - V_K)G_K + (V_m - V_L)G_L$$

G_{Na}, G_K, G_L
Membrane conductivity of Na, K and other ions [S/cm^2]

I_{Na}, I_K, I_L
Currents of Na, K and other ions [mA/cm^2]

V_{Na}, V_K, V_L
Nernst voltages of Na, K and other ions [mV]

C_m , I_m , V_m
Membrane capacitor [F/cm^2], current [mA/cm^2] and voltage [mV]

CVRTI

Hodgkin-Huxley Model: Constants

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

Relative Na voltage	$V_r - V_{Na}$	-115	mV	
Relative K voltage	$V_r - V_k$	12	mV	
Relative voltage of other ions	$V_r - V_L$	-10.6	mV	
Membrane capacitance	C_m	1	μF/cm^2	
Maximal conductivity of Na	$G_{Na\ max}$	120	mS/cm^2	} All ion channels open
Maximal conductivity von K	$G_{K\ max}$	36	mS/cm^2	
Conductivity for other ions	G_L	0.3	mS/cm^2	

Gating Variables Modulate Conductivities

$$G_{Na} = G_{Na\,max} m^3 h \qquad \frac{dm}{dt} = \alpha_m (1-m) - \beta_m m, \quad \frac{dh}{dt} = \alpha_h (1-h) - \beta_h h \quad \Bigg\} \text{ Sodium current}$$

$$G_K = G_{K\,max} n^4 \qquad \frac{dn}{dt} = \alpha_n (1-n) - \beta_n n \quad \Big\} \text{ Potassium current}$$

$$G_L = \text{const} \quad \Big\} \text{ Current by other ions}$$

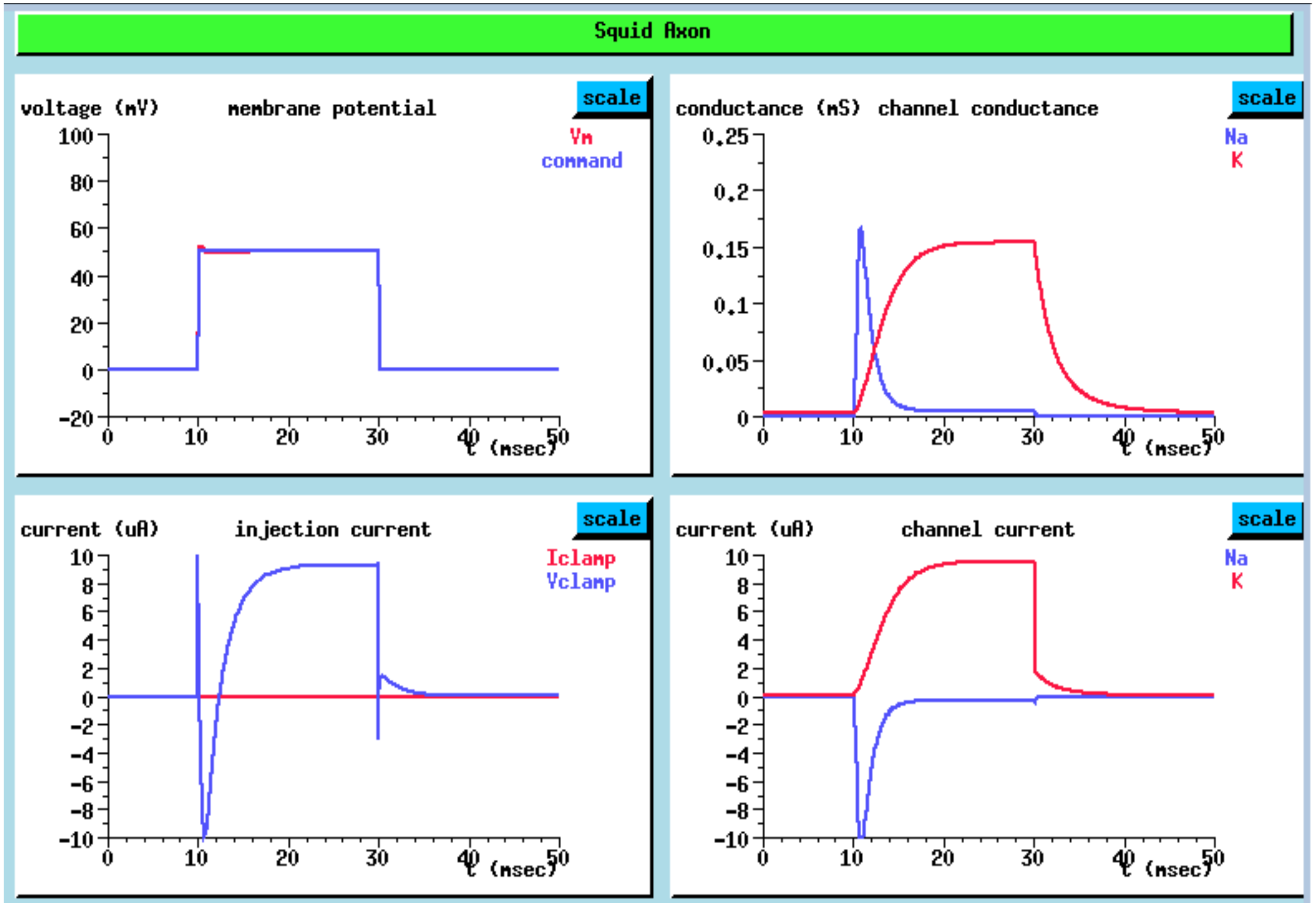
$$\alpha_m = \frac{0.1(25-V')}{e^{0.1(25-V')}-1} \frac{1}{ms} \qquad \beta_m = \frac{4}{e^{V'/18}} \frac{1}{ms}$$

$$\alpha_h = \frac{0.07}{e^{V'/20}} \frac{1}{ms} \qquad \beta_h = \frac{1}{e^{0.1(30-V')}+1} \frac{1}{ms}$$

$$\alpha_n = \frac{0.01(10-V')}{e^{0.1(10-V')}-1} \frac{1}{ms} \qquad \beta_n = \frac{0.125}{e^{V'/80}} \frac{1}{ms}$$

Voltage-dependent

Hodgkin-Huxley Model: Simulation of Voltage Clamp Measurements



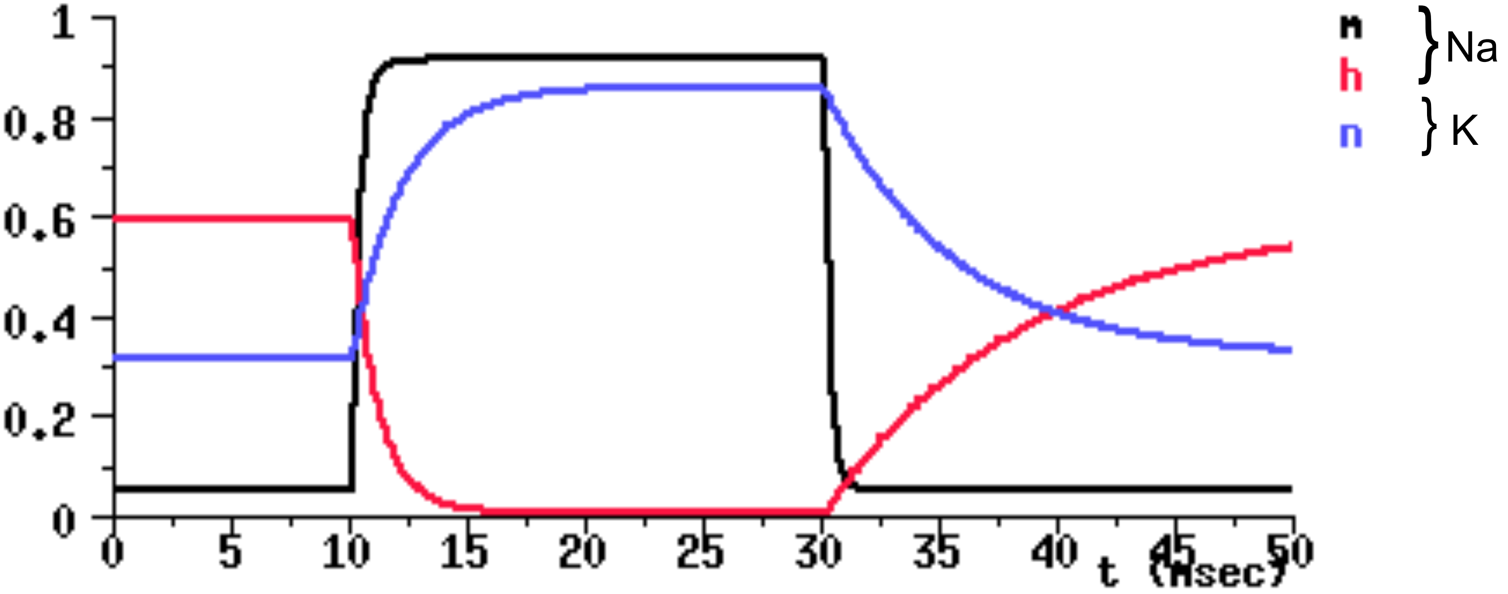
http://genesis-sim.org

CVRTI

Hodgkin-Huxley Model: Simulation of Voltage Clamp Measurements

$$G_{Na} = G_{Na,max}\ m^3\ h$$

$$G_K = G_{K,max}\ n^4$$



CVRTI

Group Work

Discuss limitations of the Hodgkin-Huxley models!

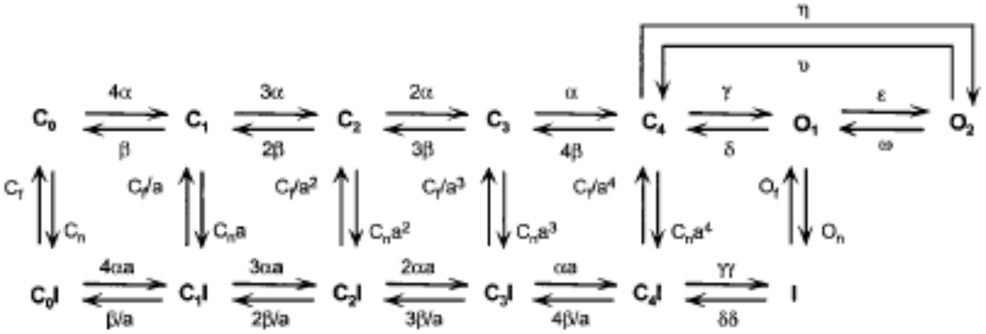
Which sub-models are missing?

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

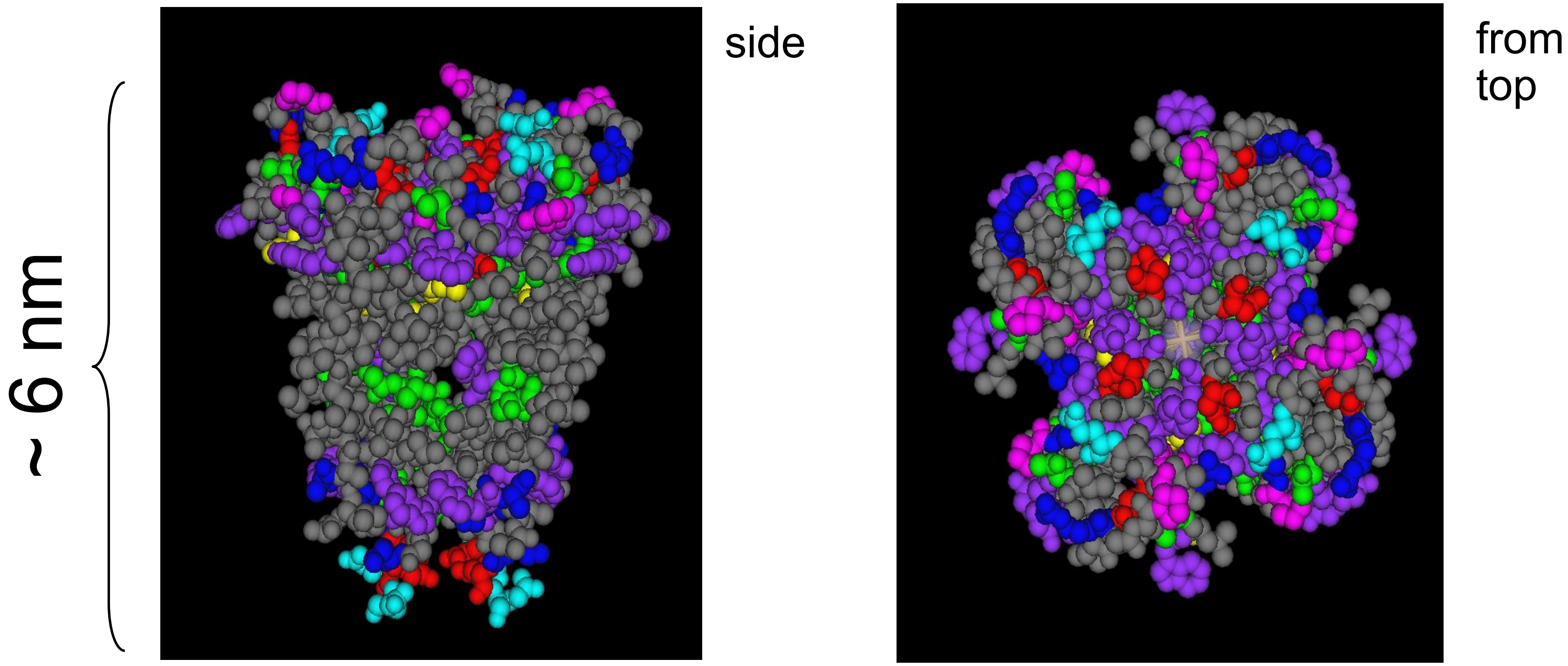
O: Open, I: Inactivated, C: Closed

(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)

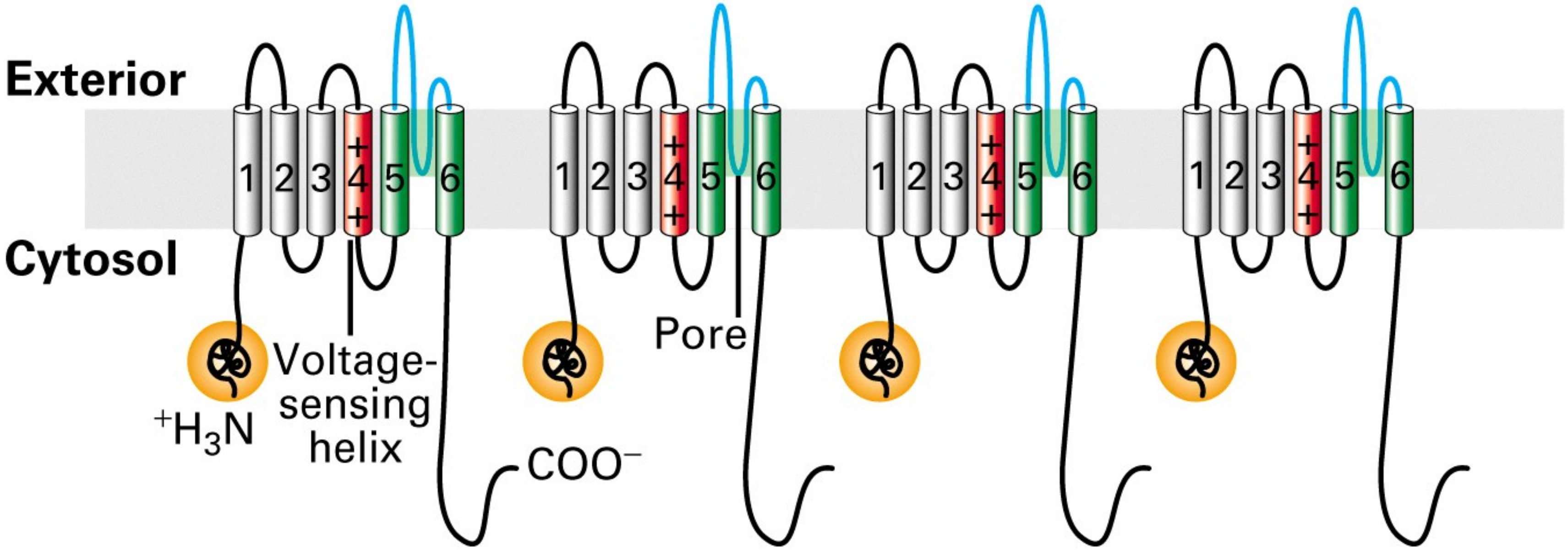
Molecular Structure of Ion Channels

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



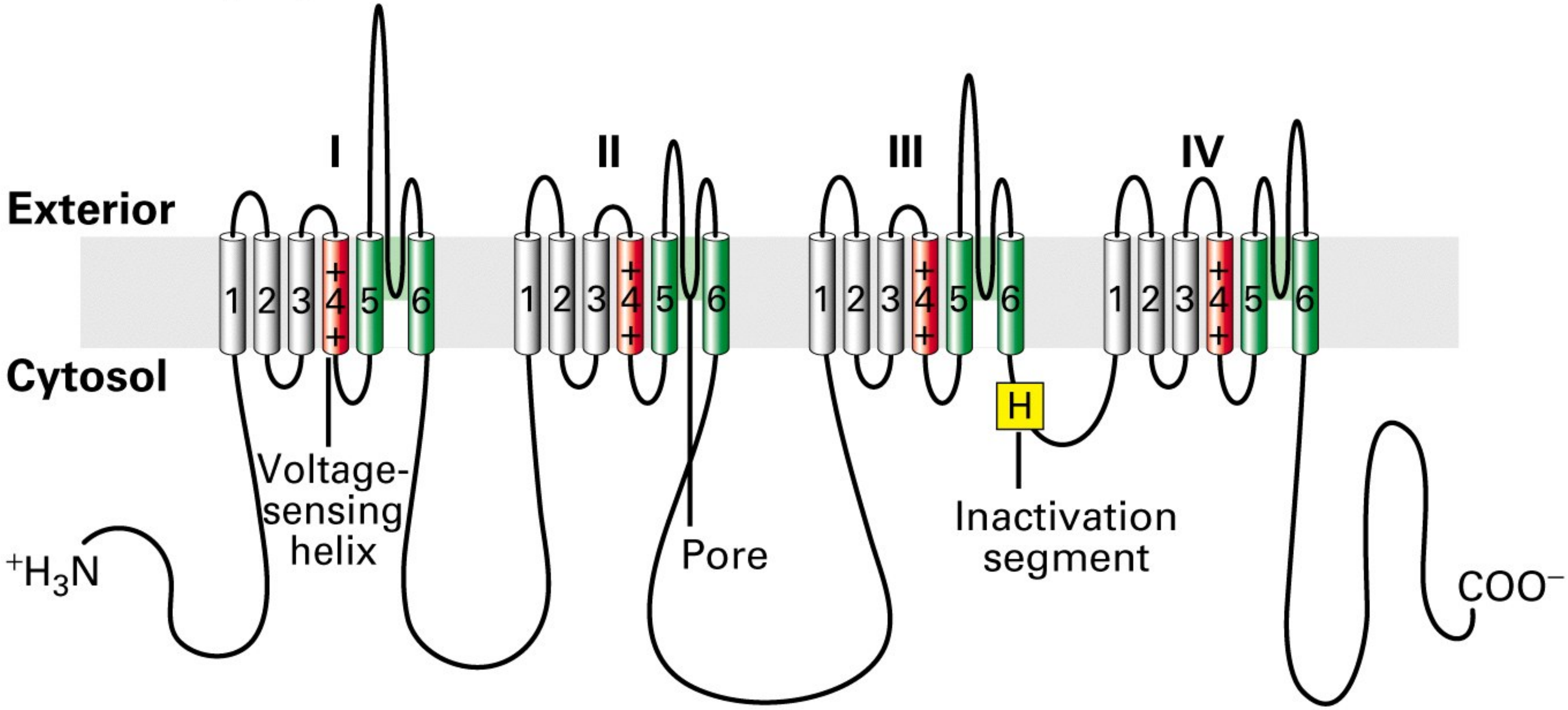
Structure data from NLM, NIH, USA, http://www.ncbi.nlm.nih.gov/sites/entrez

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)

CVRTI

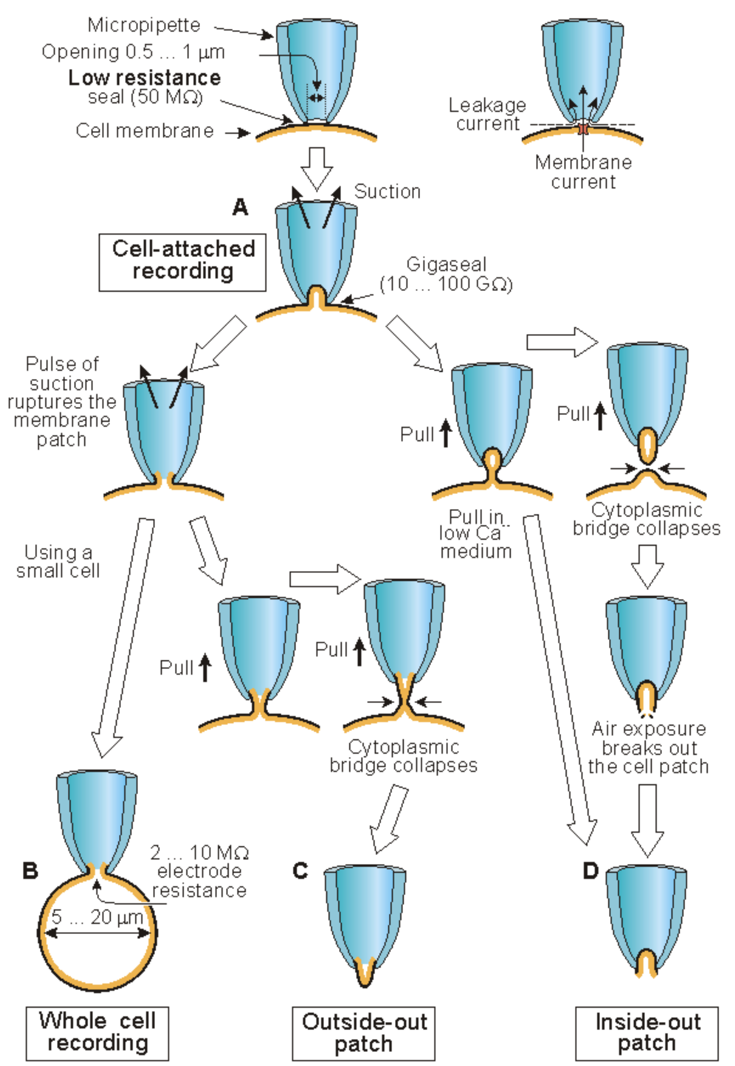
Experimental Studies: Patch Clamp Techniques

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

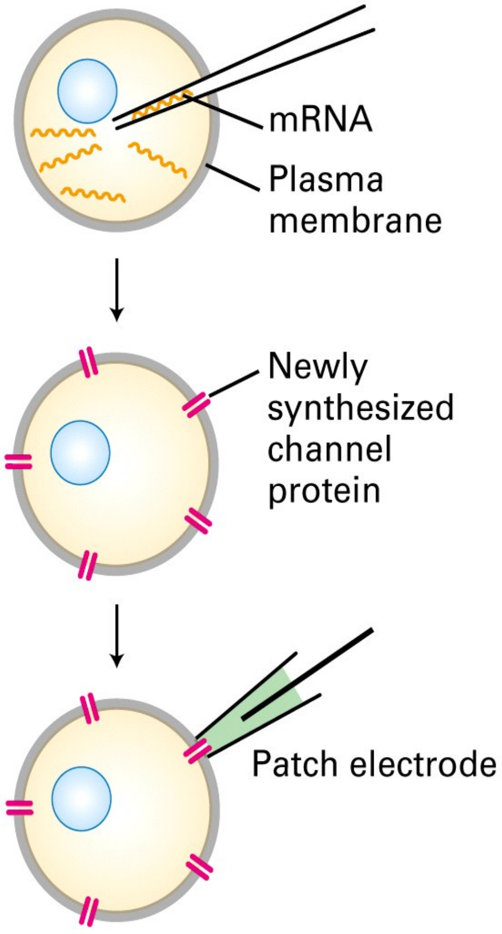
Micropipettes
- heat polished fluid filled glass pipette
- diameter of opening: 0.5-1 µm

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



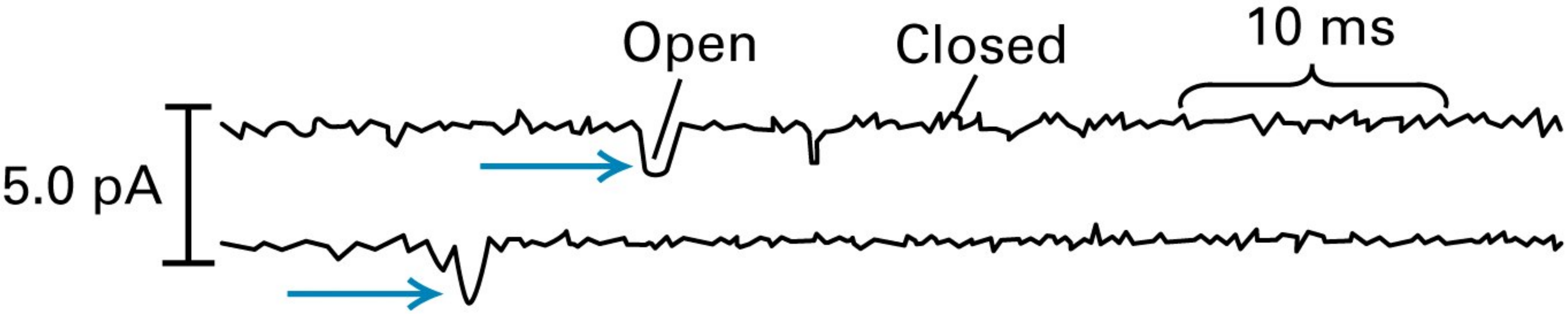
1 Microinject mRNA encoding channel protein of interest

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)

Currents Through Single Ion Channel



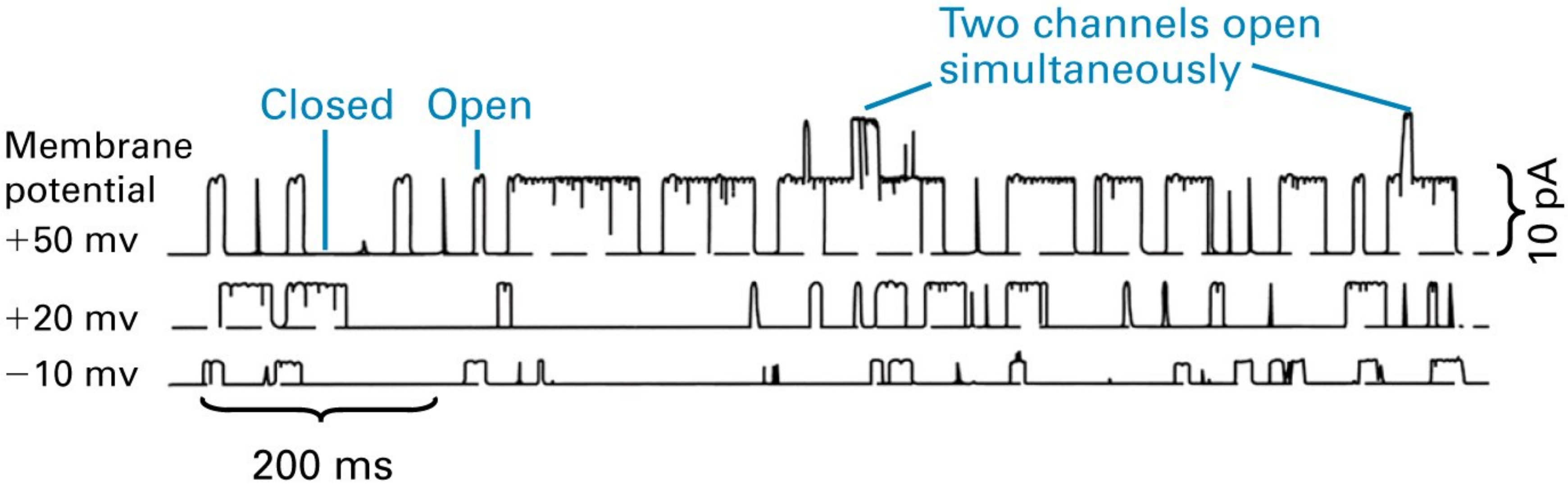
Current traces of patch with single sodium channel

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

Inside-out patch

(Lodish et al., Molecular Cell Biology, Fig. 7-18, 2004)

Currents Through Ion Channels



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

$$\frac{dO}{dt} = \alpha\, C - \beta\, O$$

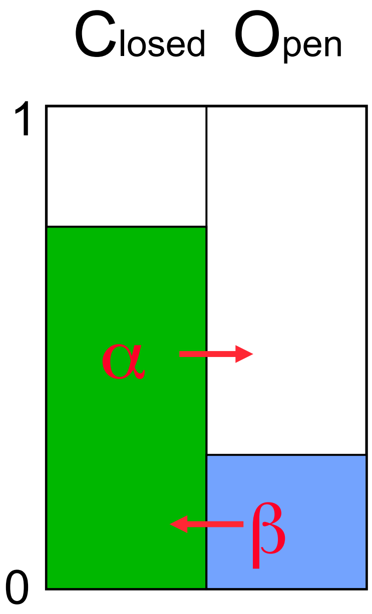
$$\frac{dC}{dt} = \beta\, O - \alpha\, C$$

O : Probability that channel is in open state

C : Probability that channel is in closed state

α, β : Rate coefficients.

Function of e.g. V_m and ion concentration



CVRTI

Rate Coefficient Functions

$\alpha = \alpha_0$	Constant
$\alpha = \alpha_0 \; V_m + a$	Linear
$\alpha = \alpha_0 e^{V_m/a}$	Exponential
$\alpha = \dfrac{\alpha_0}{e^{-(V_m - V_a)/a} + 1}$	Sigmoid
$\alpha = \alpha_0 \dfrac{V_m - V_a}{e^{-(V_m - V_a)/a} - 1}$	Linear for extreme case

α_0, V_a, a : Parameters

V_m : Membrane voltage

CVRTI

Voltage Dependent Rate Coefficient

$$\alpha = \alpha_0 e^{\frac{zFV_m}{RT}}$$

α_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

$$\frac{dO}{dt} = \alpha\, C - \beta\, O \qquad \frac{dC}{dt} = \beta\, O - \alpha\, C \quad \longleftrightarrow \quad \frac{d}{dt} P = QP$$

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

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

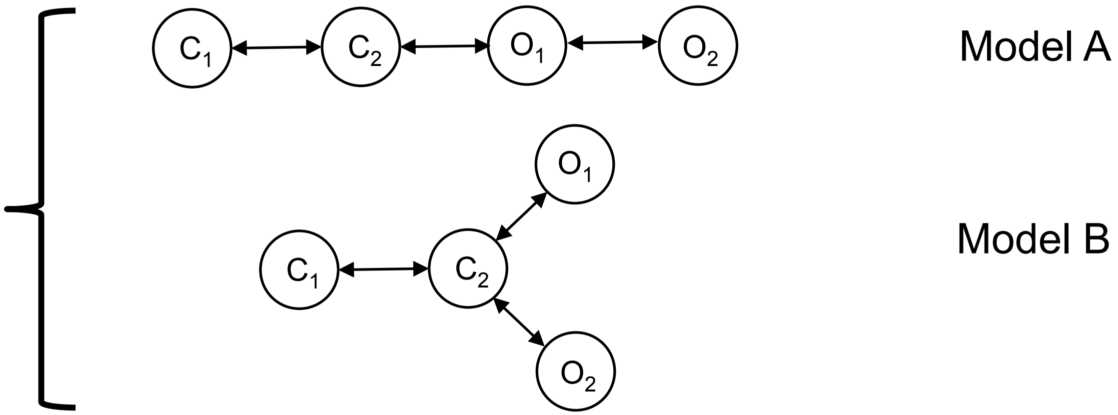
For larger models: parameters can be derived by submatrix selection and fit to macroscopic and single channel data.
(Colquhoun and Hawkes, chap. 19 and 20, Single-Channel Recording, eds. Sakmann and Neher)

CVRTI

Equivalence of Markov Models

Equivalent models

with respect to steady-state observable probability distributions



Models are equivalent if

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

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

(Kienker. Proc R Soc Lond B Biol Sci 1989)

Approaches for Modeling of Membrane Currents

$$I_{chan} = N\,G\,O\left(V_m - E_{ion}\right)$$

Nernst approach

$$I_{chan} = N\,O\,I_{ion}$$

$$I_{ion} = P\,z^2\,\frac{F^2 V_m}{RT}\,\frac{[ion]_i - [ion]_o e^{-zFV_m/RT}}{1 - e^{-zFV_m/RT}}$$

Goldman - Hodgkin - Katz 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

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

Markov Modeling of Gating Currents

Markov model with gating charge movement associated to A↔B:

$$\cdots \rightleftarrows A \underset{\beta}{\overset{\alpha}{\rightleftarrows}} B \rightleftarrows \cdots$$

Gating current:

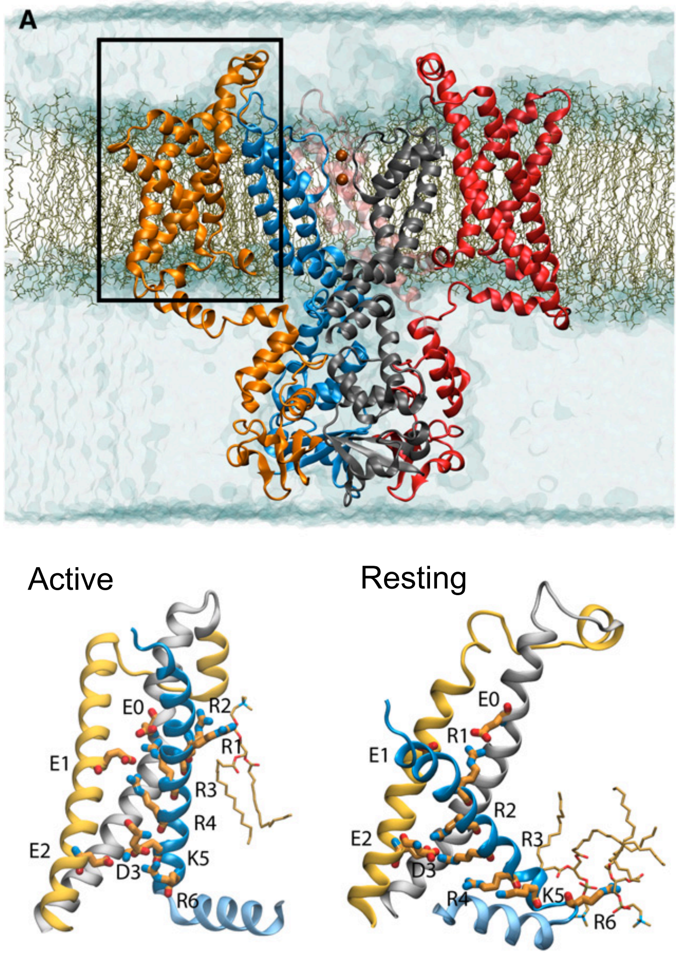
$$I_{gAB} = z_{gAB} q_e \left(\alpha A - \beta B \right)$$

z_{gAB} : Equivalent valence of moved charge

q_e : Elementary charge, 1.60×10^{-10} C

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

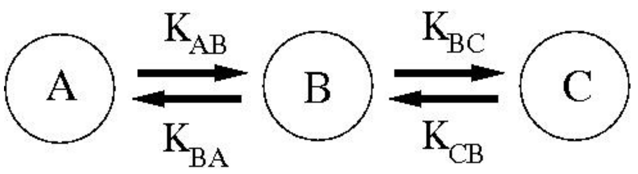
k : Boltzmann's constant, 1.38×10^{-23} V C/K

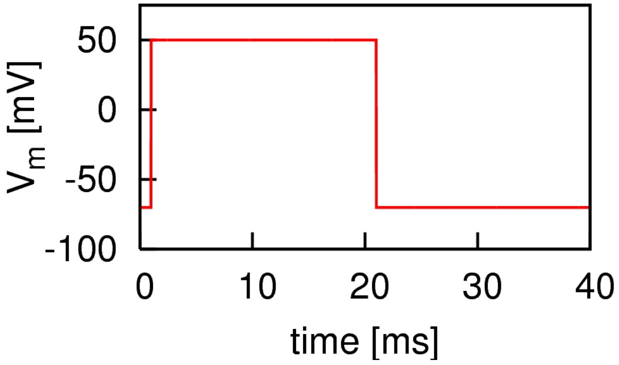


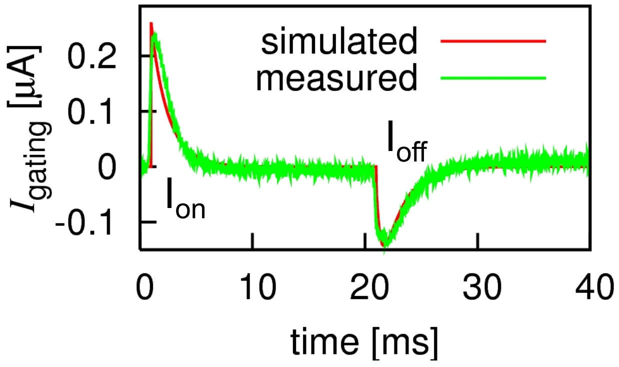
(Khalili-Araghi, Biophys J 2010)

Modeling of Gating Currents: Example

Three state model with gating charge movement associated to A↔B:

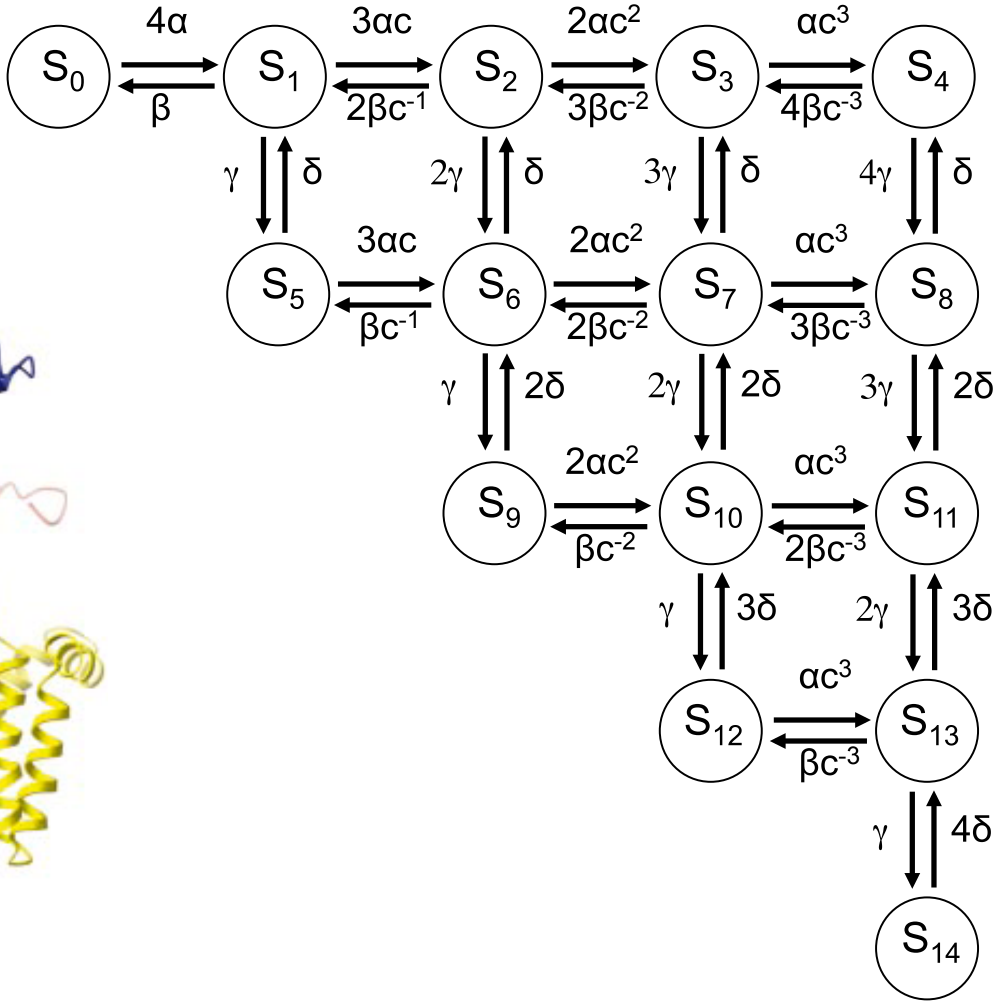


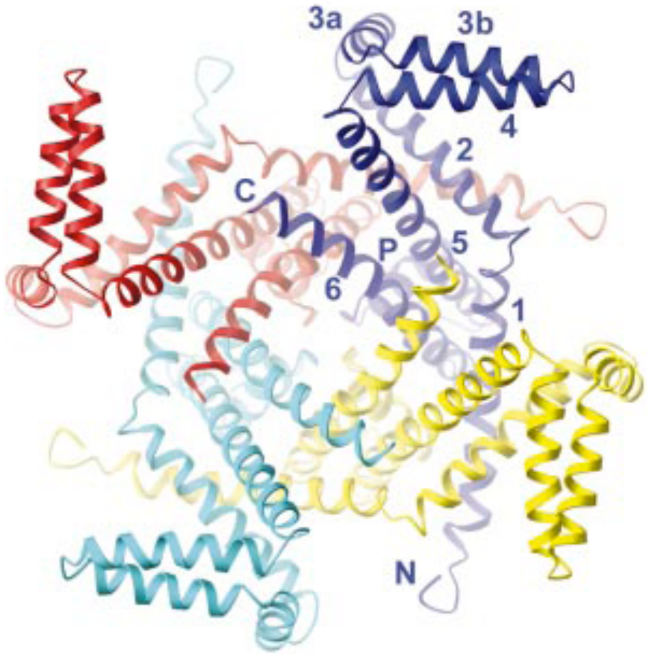




Commonly, more states are necessary to reconstruct gating currents, e.g. ≥6 in Shaker K-channels.

Modeling of Gating Currents in Tetrameric Channels

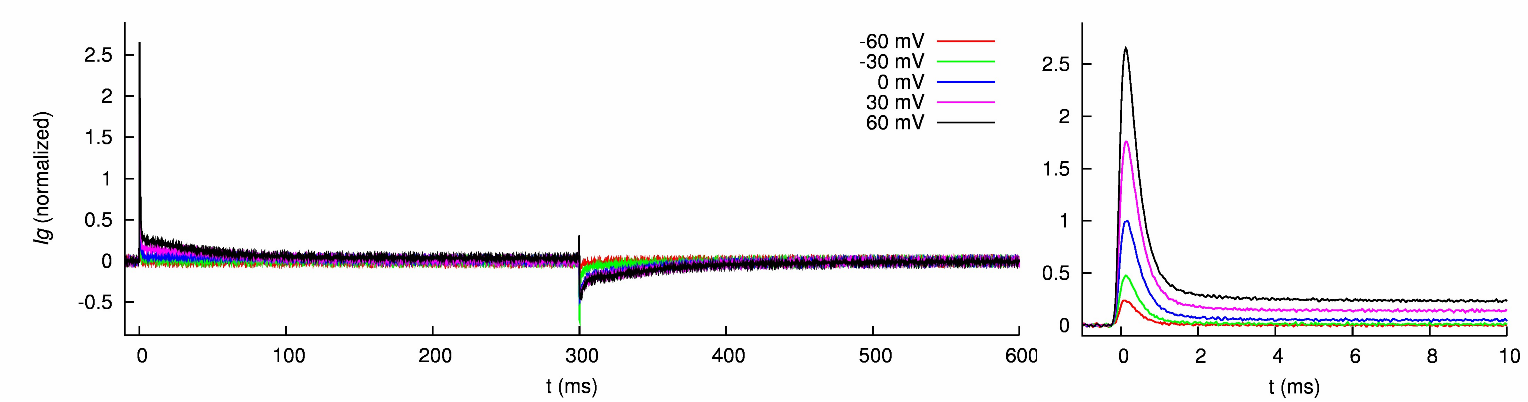




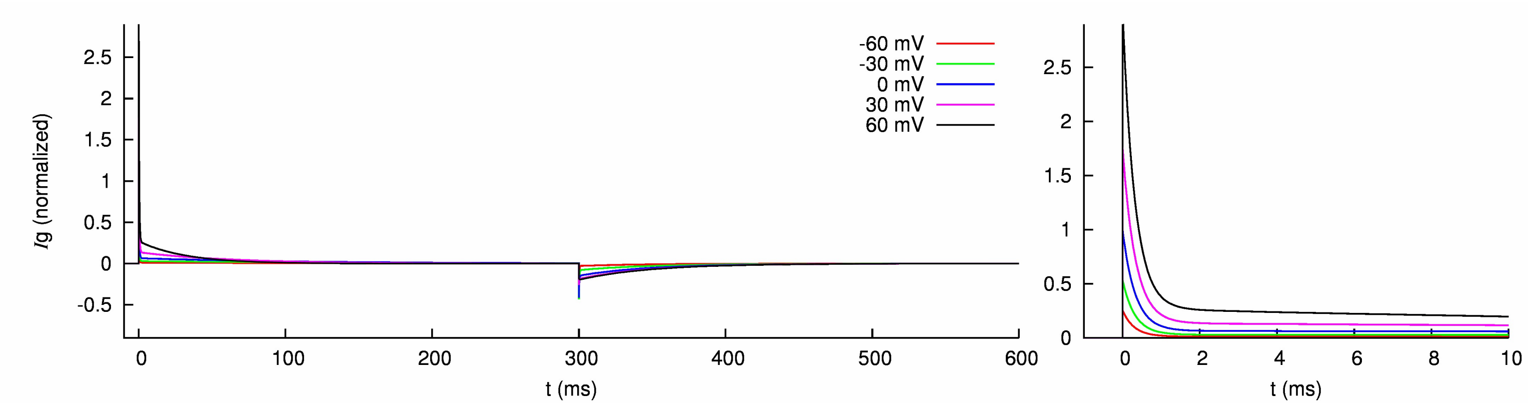
(Jiang et al, Nature 2003)

Modeling of hERG Gating Currents

Measured



Simulated



(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

- Motivation & Introduction
- Hodgkin-Huxley Modeling
  - Background
  - Approach
  - Examples
- Markovian Modeling
  - Background
  - Approach
  - Examples