CSE511 Brain & Memory Modeling

Lect08: Channels & Transporters
Chapter 4 of Purves et al., 4e

Larry Wittie
Computer Science, StonyBrook University

http://www.cs.sunysb.edu/~cse511 and ~lw
Delicate manipulations allow access to patches of single cell membrane.

1. **Cell-attached patch clamp recording method.**
   A glass pipette with a very small opening can make tight contact with a tiny area, or “patch”, of neuronal membrane. After a small amount of suction on the back of the pipette, the seal between the pipette and membrane becomes so tight that no ions can flow between them.

2. **Whole-cell recording.**
   A brief pulse of strong suction unites the pipette with the cell interior, allowing both electrical recording and injections into the cell cytoplasm.

3. **Inside-out patch recording.** With the pipette attached to the cell exterior, pulling the pipette into the air traps a bubble of membrane and cytoplasm, giving access to the inner surface of the cell membrane.

4. **Outside-out patch recording.** Pulling the pipette away during whole-cell recording brings the surrounding ring of membrane with it. The outermost edges of the ring fuse into a inverted bubble with the exterior of the cell membrane exposed.
Figure 4.1-1 Patch clamp measurements of ionic currents flowing through single Na\(^+\) channels in a squid giant axon. In these experiments, Cs\(^+\) was applied to the axon to block voltage-gated K\(^+\) channels. Depolarizing voltage pulses (A) applied to a patch of membrane containing a single Na\(^+\) channel result in brief currents (B, downward deflections) in the seven successive recordings of membrane current (I\(_{Na}\)). (B after Bezanilla and Correa, 1995.

A patch clamp is the voltage clamp method applied to a tiny patch of cell membrane within the minute tip opening of a thin, tapered pipette.
Figure 4.1-2

(C) The sum of many such current records shows that most channels open in the initial 1 - 2 ms following depolarization of the membrane, after which the probability of channel openings diminishes because of channel inactivation. (D) A macroscopic current measured from another axon shows the close correlation between the time courses of microscopic and macroscopic Na\(^+\) currents. (E) The probability of an Na\(^+\) channel opening depends on the membrane potential, increasing as the membrane is depolarized. (C after Bezanilla and Correa, 1995; D after Vandenburg and Bezanilla, 1991; E after Correa and Bezanilla, 1994.)
**Figure 4.2-1** Patch clamp measurements of ionic currents flowing through single K⁺ channels in a squid giant axon. In these experiments, tetrodotoxin was applied to the axon to block voltage-gated Na⁺ channels. Depolarizing voltage pulses (A) applied to a patch of membrane containing a single K⁺ channel results in brief currents (B, upward deflections) whenever the channel opens.

Note the opposite (up) polarity, delayed opening, and sustained activation for K⁺ channels versus the Na⁺ channels in Figure 4.1.
Figure 4.2-2 (C) The average of many current records shows that most channels open after a delay, but remain open for the duration of the depolarization.

Note the upward polarity, delayed opening, and sustained activation for K⁺ vs Na⁺.

(D) A macroscopic current measured from another axon shows the correlation between the time courses of microscopic and macroscopic K⁺ currents. (E) The probability of a K⁺ channel opening depends on the membrane potential, increasing as the membrane is depolarized. (C after Augustine and Bezanilla, in Hille 2001; D after Augustine and Bezanilla, 1990; E after Perozo et al., 1991.)
Figure 4.3  Functional states of voltage-gated Na\(^+\) and K\(^+\) channels

When the potential is depolarized, voltage sensors (indicated by +) allow the channel gates to open first the Na\(^+\) channels and then the K\(^+\) channels. Na\(^+\) channels also inactivate during prolonged depolarization, whereas many types of K\(^+\) channels do not.
Box 4B Expression of Ion Channels in *Xenopus* Oocytes

Making controlled amounts of foreign ion channels by mRNA injections into frog eggs.

(A) The clawed African frog, *Xenopus laevis*.

(B) Several oocytes from *Xenopus* highlighting the dark coloration of the animal pole and the lighter coloration of the vegetal pole. (Courtesy of P. Reinhart.)

The dark pole has most of the DNA and protein-producing organelles of the egg.

(C) Results of a voltage clamp experiment showing $K^+$ currents produced following injection of $K^+$ channel mRNA into an oocyte. (After Gundersen et al., 1984.)
Figure 4.4 Types of voltage-gated ion channels. Examples of voltage-gated channels include those selectively permeable to Na\(^+\) (A), Ca\(^{2+}\) (B), K\(^+\) (C), and Cl\(^-\) (D).

Ligand-gated ion channels include those activated by the extracellular presence of neurotransmitters, such as glutamate (E). Other ligand-gated channels are activated by intracellular second messengers, such as Ca\(^{2+}\) (F) or the cyclic nucleotides, cAMP and cGMP (G).
Figure 4.5 Diverse properties of K⁺ channels. Different types of K⁺ channels were expressed in *Xenopus* oocytes (see Box 4B) and the voltage clamp method was used to change the membrane potential (top) and measure the resulting currents flowing through each type of channel. These K⁺ channels vary markedly in their gating properties, as evident in their currents (left) and conductances (right). (A) Kᵥ2.1 channels show little inactivation and are closely related to the delayed rectifier K⁺ channels involved in action potential repolarization. (B) Kᵥ4.1 channels inactivate during a depolarization. (C) HERG channels inactivate so rapidly that current flows only when inactivation is rapidly removed at the end of a depolarization. (D) Inward rectifying K⁺ channels allow more K⁺ current to flow at hyperpolarized potentials than at depolarized potentials. (E) Ca²⁺-activated K⁺ channels open in response to intracellular Ca²⁺ ions and, in some cases, membrane depolarization. (F) K⁺ channels with two pores usually respond to chemical signals, such as pH, rather than changes in membrane potential.
Figure 4.5 Diverse properties of K⁺ channels (Part 1)

Figure 4.5-1 Diverse properties of K⁺ channels. Different types of K⁺ channels were expressed in *Xenopus* oocytes (see Box 4B) and the voltage clamp method was used to change the membrane potential (top) and measure the resulting currents flowing through each type of channel. These K⁺ channels vary markedly in their gating properties, as evident in their currents (left) and conductances (right).

(A) Kᵥ2.1 channels show little inactivation and are closely related to the delayed rectifier K⁺ channels involved in action potential repolarization. (B) Kᵥ4.1 channels inactivate during a depolarization.
Figure 4.5 Diverse properties of K⁺ channels (Part 2)

Figure 4.5-2 (C) HERG channels inactivate so rapidly that current flows only when inactivation is rapidly removed at the end of a depolarization. (D) Inward rectifying K⁺ channels allow more K⁺ current to flow at hyperpolarized potentials than at depolarized potentials.

(C) HERG human Ether-à-go-go-Related Gene => Kᵥ11.1 protein in heart, neuron & leukemic cells

(D) Inward rectifier
Figure 4.5-3  (E) Ca$^{2+}$-activated K$^+$ channels open in response to intracellular Ca$^{2+}$ ions and, in some cases, membrane depolarization. (F) K$^+$ channels with two pores usually respond to chemical signals, such as pH, rather than changes in membrane potential.
Figure 4.6 Topology of the principal subunits of voltage-gated Na⁺, Ca²⁺, K⁺, and Cl⁻ channels. Repeating motifs of Na⁺ (A) and Ca²⁺ (B) channels are labeled I, II, III, and IV; (C-F) K⁺ channels are more diverse. In all cases, four subunits combine to form a functional channel. (G) Chloride channels are structurally distinct from all other voltage-gated channels.
Box 4C  Toxins That Poison Ion Channels

(A) Effects of toxin treatment on frog axons. (1) $\alpha$-Toxin from the scorpion *Leiurus quinquestriatus* prolongs $\text{Na}^+$ currents recorded with the voltage clamp method.

(2) As a result of the increased $\text{Na}^+$ current, $\alpha$-toxin greatly prolongs the duration of the axonal action potential. Note the change in timescale ($2 \text{ ms vs } 8 \text{ s}$) after treatment with $\alpha$-toxin.

(B) Treatment of a frog axon with $\beta$-toxin from another scorpion, *Centruroides sculpturatus*, shifts the activation of $\text{Na}^+$ channels, so that $\text{Na}^+$ conductance begins to increase at potentials much more negative than usual. (A after Schmidt and Schmidt, 1972; B after Cahalan, 1975.)
**Figure 4.7** A charged voltage sensor permits voltage-dependent gating of ion channels. The process of voltage activation involves the movement of a positively-charged voltage sensor within the membrane.

The voltage-dependent sensor movement causes a change in the conformation of the channel pore, enabling the channel to conduct ions.
Figure 4.8-1 Structure of a simple bacterial $K^+$ channel determined by crystallography. (A) Structure of one subunit of the channel, which consists of two membrane-spanning domains and a pore loop that inserts into the membrane. (B) Three-dimensional arrangement of four subunits (each in a different color) to form a $K^+$ channel. The top view illustrates a $K^+$ ion (green) within the channel pore.
Figure 4.8-2  (C) The permeation pathway of the K⁺ channel consists of a large aqueous cavity connected to a narrow selectivity filter. Helical domains of the channel point negative charges (red) toward this cavity, allowing K⁺ ions (green) to become dehydrated and then move through the selectivity filter. (From Doyle et al., 1998.)
Figure 4.9 Structure of a mammalian voltage-gated $K^+$ channel. (A) The channel complex includes four subunits, each possessing a transmembrane domain and a T1 domain inside the cell. Below each T1 domain is a $\beta$ subunit. (B) When viewed from above, the transmembrane domain is seen to have separate domains for voltage-sensing and for forming the $K^+$ conduction pore. (C) Surface view of the complete channel reveals the presence of portals that allow $K^+$ to enter the channel from the cytoplasm and allow insertion of structures that inactivate the channel. Red and blue colors represent charged amino acids. (D) Model for voltage-dependent gating of the $K^+$ channel. Depolarization causes the voltage sensor to move toward the extracellular surface of the membrane, pulling on the S4-S5 linker (red) and opening the channel pore (blue). Conversely, hyperpolarization causes this linker to push down and shut the channel pore. (E) Paddle model for movement of the voltage sensor. Depolarization causes the paddle-like voltage sensor domain to move toward the extracellular surface of the membrane, while hyperpolarization causes it to move toward the intracellular surface. (A-C from Long et al., 2005a; D from Long et al., 2005b; E after Lee, 2006.)
Figure 4.9-1 Structure of a mammalian voltage-gated K\(^+\) channel. (A) The channel complex includes four subunits, each possessing a transmembrane domain and a T1 domain inside the cell. Below each T1 domain is a $\beta$ subunit.

(B) When viewed from above, the transmembrane domain is seen to have separate domains for voltage-sensing and for forming the K\(^+\) conduction pore. (A-B from Long et al., 2005a.)
Figure 4.9-2 (C) Surface view of the complete channel reveals the presence of portals that allow $\text{K}^+\,$ to enter the channel from the cytoplasm and allow insertion of structures that inactivate the channel. Red and blue colors represent charged amino acids.

(C from Long et al., 2005a.)
Figure 4.9-3 (D) Model for voltage-dependent gating of the K⁺ channel. Depolarization causes the voltage sensor to move toward the extracellular surface of the membrane, pulling on the S4-S5 linker (red) and opening the channel pore (blue). Conversely, hyperpolarization causes this linker to push down and shut the channel pore. (D) (D from Long et al., 2005b.)
Figure 4.9-3 (E) Paddle model for movement of the voltage sensor. Depolarization causes the paddle-like voltage sensor domain to move toward the extracellular surface of the membrane, while hyperpolarization causes it to move toward the intracellular surface. (E after Lee, 2006.)
Box 4D-B A defect in brain Na+ channels causes *generalized epilepsy with febrile seizures* (seizures brought on by a fever) (GEFS) that begins in infancy and usually continues through early puberty. Mutations within genes in chromosomes 2 and 19 encode for subunits of Na⁺ channels. That some mutations slow the rate of inactivation of Na⁺ currents may explain the neuronal hyperexcitability underlying GEFS. (After Barchi, 1995.)
Figure 4.10-1
Examples of ion transporters found in cell membranes.
(A,B) Some transporters are powered by the hydrolysis of ATP (ATPase pumps).

Adenosine-5'-triphosphate (ATP)

Adenosine diphosphate (ADP)
Figure 4.10-2 Examples of ion transporters found in cell membranes. (C–F) Other transporters use the electrochemical gradients of co-transported ions as a source of energy (ion exchangers).
Figure 4.11 Ionic movements due to the Na$^+$/K$^+$ pump.

(A) Measurement of radioactive Na$^+$ efflux from a squid giant axon. This efflux depends on external K$^+$ and intracellular ATP.

(B) A model for the movement of ions by the Na$^+$/K$^+$ pump. Uphill movements of Na$^+$ and K$^+$ are driven by ATP, which phosphorylates the pump. These fluxes are asymmetrical, with three Na$^+$ carried out for every two K$^+$ brought in. (A after Hodgkin and Keynes, 1955; B after Lingrel et al., 1994.)
Figure 4.11-1 Ionic movements due to the Na\(^+\)/K\(^+\) pump. (A) Measurement of radioactive Na\(^+\) efflux from a squid giant axon. This efflux depends on external K\(^+\) and intracellular ATP. (A after Hodgkin and Keynes, 1955.)
Figure 4.11-2 Ionic movements due to the Na⁺/K⁺ pump.

(B) A model for the movement of ions by the Na⁺/K⁺ pump. Uphill movements of Na⁺ and K⁺ are driven by ATP, which phosphorylates the pump. These fluxes are asymmetrical, with three Na⁺ carried out for every two K⁺ brought in. (B after Lingrel et al., 1994.)
The electrogenic transport of ions by the Na⁺/K⁺ pump can influence membrane potential. Measurements of the membrane potential of a small unmyelinated axon show that a train of action potentials is followed by a long-lasting hyperpolarization. This hyperpolarization is blocked by ouabain [wah-bah-in] (Somali: “arrow poison”), indicating that it results from the activity of the Na⁺/K⁺ pump. (After Rang and Ritchie, 1968.)
**Figure 4.13** Molecular organization of the Na\(^+\)/K\(^+\) pump. (A) General features of the pump. (B) The molecule spans the membrane 10 times. Amino acid residues thought to be important for binding of ATP, K\(^+\), and ouabain are highlighted. (After Lingrel et al., 1994.)
**Figure 4.13-1** Molecular structure of the Na\(^+\)/K\(^+\) pump. (A) General features of the pump. (After Lingrel et al., 1994.)
**Figure 4.13-2** Molecular structure of the Na\(^+\)/K\(^+\) pump. (B) The molecule spans the membrane 10 times. Amino acid residues thought to be important for binding of ATP, K\(^+\), and ouabain are highlighted. (After Lingrel et al., 1994.)
Cell-attached recording

Recording pipette

Mild suction

Tight contact between pipette and membrane
Whole-cell recording

Strong pulse of suction

Cytoplasm is continuous with pipette interior

Inside-out recording

Expose to air

Cytoplasmic domain accessible
Outside-out recording

Retract pipette

Ends of membrane anneal

Extracellular domain accessible
**Figure 4.1** Patch clamp measurements of ionic currents flowing through single Na\(^+\) channels in a squid giant axon. In these experiments, Cs\(^+\) was applied to the axon to block voltage-gated K\(^+\) channels. Depolarizing voltage pulses (A) applied to a patch of membrane containing a single Na\(^+\) channel result in brief currents (B, downward deflections) in the seven successive recordings of membrane current (I\(_Na\)). (C) The sum of many such current records shows that most channels open in the initial 1 - 2 ms following depolarization of the membrane, after which the probability of channel openings diminishes because of channel inactivation.

(D) A macroscopic current measured from another axon shows the close correlation between the time courses of microscopic and macroscopic Na\(^+\) currents. (E) The probability of an Na\(^+\) channel opening depends on the membrane potential, increasing as the membrane is depolarized.

(B,C after Bezanilla and Correa, 1995; D after Vandenburg and Bezanilla, 1991; E after Correa and Bezanilla, 1994.)
**Figure 4.2** Patch clamp measurements of ionic currents flowing through single K$^+$ channels in a squid giant axon. In these experiments, tetrodotoxin was applied to the axon to block voltage-gated Na$^+$ channels. Depolarizing voltage pulses (A) applied to a patch of membrane containing a single K$^+$ channel results in brief currents (B, upward deflections) whenever the channel opens. (C) The sum of such current records shows that most channels open after a delay, but remain open for the duration of the depolarization.

Note the opposite (up) polarity, delayed opening, and sustained activation for K$^+$ channels versus the Na$^+$ channels in Figure 4.1.

(D) A macroscopic current measured from another axon shows the correlation between the time courses of microscopic and macroscopic K$^+$ currents.

(E) The probability of a K$^+$ channel opening depends on the membrane potential, increasing as the membrane is depolarized.

(B and C after Augustine and Bezanilla, in Hille 2001; D after Augustine and Bezanilla, 1990; E after Perozo et al., 1991.)
Figure 4.4 Types of voltage-gated ion channels (Part 1)

VOLTAGE-GATED CHANNELS

(A) Na$^+$ channel
(B) Ca$^{2+}$ channel
(C) K$^+$ channel
(D) Cl$^-$ channel

$\text{Na}^+ \rightarrow \text{Ca}^{2+} \rightarrow \text{K}^+ \rightarrow \text{Cl}^-$

Voltage sensor

NEUROSCIENCE, Fourth Edition, Figure 4.4 (Part 1)
Figure 4.4 Types of voltage-gated ion channels (Part 2)

LIGAND-GATED CHANNELS

(E) Neurotransmitter receptor
(F) Ca^{2+}-activated K^{+} channel
(G) Cyclic nucleotide gated channel

Na^{+}  Glutamate  \(\text{Ca}^{2+}\)  \(\text{Na}^{+}\)  cAMP  cGMP  K^{+}  K^{+}  K^{+}
Figure 4.6  Topology of principal subunits of voltage-gated Na\(^+\), Ca\(^{2+}\), K\(^+\), and Cl\(^-\) channels (Part 1)
Figure 4.6  Topology of principal subunits of voltage-gated Na\(^+\), Ca\(^{2+}\), K\(^+\), and Cl\(^-\) channels (Part 2)
Figure 4.6  Topology of principal subunits of voltage-gated Na\(^+\), Ca\(^{2+}\), K\(^+\), and Cl\(^-\) channels (Part 3)

**K\(^+\) CHANNELS**

(C) \(K\textsubscript{v}\) and HERG  (D) Inward rectifier  (E) Ca\(^{2+}\)-activated  (F) 2-pore

\(\beta\) subunit
Figure 4.6 Topology of principal subunits of voltage-gated Na⁺, Ca²⁺, K⁺, and Cl⁻ channels (Part 4)
Figure 4.7 A charged voltage sensor permits voltage-dependent gating of ion channels.
Figure 4.8 Structure of a simple bacterial K⁺ channel

(A) Structure of one subunit of the channel, which consists of two membrane-spanning domains and a pore loop that inserts into the membrane.

(B) Three-dimensional arrangement of four subunits (each in a different color) to form a K⁺ channel. The top view illustrates a K⁺ ion (green) within the channel pore.

(C) The permeation pathway of the K⁺ channel consists of a large aqueous cavity connected to a narrow selectivity filter. Helical domains of the channel point negative charges (red) toward this cavity, allowing K⁺ ions (green) to become dehydrated and then move through the selectivity filter. (From Doyle et al., 1998.)
Box 4D(1) Diseases Caused by Altered Ion Channels

- **Ca²⁺ CHANNEL**
  - ETM
  - EA2
  - CSNB
  - Paralysis

- **Na⁺ CHANNEL**
  - β subunit
  - GEFS
  - Myotonia
  - Paralysis

- **K⁺ CHANNEL**
  - EAL
  - BFNC

- **Cl⁻ CHANNEL**
  - Myotonia
**Figure 4.10** Examples of ion transporters found in cell membranes. (A,B) Some transporters are powered by the hydrolysis of ATP (ATPase pumps). Others (C–F) use the electrochemical gradients of co-transported ions as a source of energy (ion exchangers).
Figure 4.14 Molecular structure of the Ca\(^{2+}\) pump. (A) Structure of the Ca\(^{2+}\) pump. Domains responsible for nucleotide binding (NB), phosphorylation (P), and ion translocation activity (TA) are indicated. Panel (A) and the first panel in (B) show the structure of the pump when bound to ADP; in this state, two Ca\(^{2+}\) are sequestered within the membrane-spanning regions of the pump. (B) Hypothetical sequence of structural changes associated with translocation of Ca\(^{2+}\) by the Ca\(^{2+}\) pump. Analogous to the sequence of events involved in the function of the Na\(^{+}/K\(^{+}\) pump (see Figure 4.11 B), the Ca\(^{2+}\) pump undergoes a cycle of phosphorylation and dephosphorylation that causes conformational changes (black arrows) that drive Ca\(^{2+}\) across the membrane. (After Toyoshima et al., 2004.)
Figure 4.14-1 Molecular structure of the Ca\textsuperscript{2+} pump. (A) Structure of the Ca\textsuperscript{2+} pump. Domains responsible for nucleotide binding (NB), phosphorylation (P), and ion translocation activity (TA) are indicated. Panel (A) and the first panel in (B) show the structure of the pump when bound to ADP; in this state, two Ca\textsuperscript{2+} (purple circles) are sequestered within the membrane-spanning regions of the pump. (After Toyoshima et al., 2004.)
**Figure 4.14-2** Molecular structure of the Ca\(^{2+}\) pump. (A) Structure of the Ca\(^{2+}\) pump. Domains responsible for nucleotide binding (NB), phosphorylation (P), and ion translocation activity (TA) are indicated. Panel (A) and the first panel in (B) show the structure of the pump when bound to ADP; in this state, two Ca\(^{2+}\) are sequestered within the membrane-spanning regions of the pump. (B) Hypothetical sequence of structural changes associated with translocation of Ca\(^{2+}\) by the Ca\(^{2+}\) pump. Analogous to the sequence of events involved in the function of the Na\(^{+}/K^{+}\) pump (see Figure 4.11 B), the Ca\(^{2+}\) pump undergoes a cycle of phosphorylation and dephosphorylation that causes conformational changes (black arrows) that drive Ca\(^{2+}\) across the membrane. (After Toyoshima et al., 2004.)