Ion channels
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Part I
Channel structure and gating

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Ion Channels - Structures and gating mechanisms

- Voltage-gated channels
- Idea of a gate - potassium channel example
- Voltage-sensors : how to open the gate
- Voltage-gated channels - inactivation
- Ligand-gated ion channels : a different sort of gate and gating mechanism

- Permeation - Chemical recognition at high speed
- Toxins and channels
- Engineering light driven channels

- Mechanosensation
- MALS
Ion channels are **FAST!**

Ion - ‘Greek for that which goes’ (Faraday 1834)

**Gating** – channel’s response to a stimulus, opening or closing – $100 \mu s$

**Selective permeability** – restricted passage of ion type – $25 \text{ ns ion}^{-1}$

Fastest enzyme (Carbonic anhydrase) $\sim 1 \mu s$
Helix-coil transitions 10 ns
Backbone motions 1 ns
Brief history of electrical signals in cellular systems

1881 – 1887 Sidney Ringer – perfused frog hearts require salts of sodium, potassium, and calcium in definite proportion for the heart to beat

1888 Walther Nernst – electrolyte diffusion theory inspires ideas about bioelectric potentials

1902, 1912 Julius Bernstein – Proposes correctly that excitable cells are surrounded by a membrane with selective potassium permeability and that during excitation this permeability changes for other ions

1930s-1950s Cole, Curtis, Hodgkin, Huxley – Squid giant axon, action potential theory

1960s-1970s – Pores or carriers?

1980s – First channels cloned

1998 – First high resolution crystal structure of a channel protein
Membrane bilayers – act as insulators and capacitors

Figure 3-4. Passive electrical properties of the plasma membrane. a: The plasma membrane can be depicted as a resistor ($R_m$) and a capacitor ($C_m$) connected in parallel. b: Voltage changes do not occur instantaneously.
Membrane potential and ion movements across membrane are governed by Nernst Equilibria Potentials.
Electrical signals in cellular systems
Action potential

Fast signals:
I’m ~170 cm tall. The fastest neurons can transmit signals at a rate of 100 m s⁻¹. That means that it would only take 17ms for a signal to go from my brain to the base of my foot.
Ways to measure ion channel activity

Two-electrode voltage clamp - *Xenopus* oocytes

Patch recording - any cell type

Lipid bilayer recording - purified proteins
Voltage-gated ion channels

Conceptual ideas about channel parts
Voltage-gated channels - a large family

143 members in humans

#3 in abundance

Only GPCRs and Kinases are more abundant

Voltage-gated channels show conservation of structural patterns based on primary sequence considerations.

For voltage-gated sodium and calcium channels, there are four homologous transmembrane repeats. The fourth transmembrane segment has a number of conserved positive charges.

Auxiliary subunits are important components of the native channel complex.

Voltage-gated potassium channels look like a single repeat from the voltage-gated sodium and calcium channels. For subunits are needed to make a channel. Auxiliary subunits are less well characterized.
Voltage-gated Na\textsuperscript{+} channels

Low resolution (19Å) electron microscopy pictures of voltage-gated sodium channels from *Electrophorus electricus* (electric eel) electric organ.

Lower left panel shows the raw images (top), average images (second row), surface views of the 3D reconstruction (third row), and projections of the 3D reconstruction (bottom row). The last row and the second row look similar suggesting that the 3D reconstruction reflects something close to the actual molecule.


Voltage-gating - how do charge movements in the channel open the pore?

Voltage drop across the membrane--most of the drop happens in the hydrophobic layer

Voltage-gated channels have intrinsic voltage sensors (charges/dipoles) in the membrane

The S4 segments with multiple basic residues correspond to the intrinsic voltage sensor
Voltage sensor positive charges are conserved across a wide range of voltage-sensitive channels.

Early models of voltage sensor motions postulated a helical-screw motion where the voltage-sensor moves towards the extracellular space upon depolarization.

Hille, B., Ion Channels of Excitable Membranes, 3rd edition, Sinauer (Sunderland, MA) 2001
Cysteine has a unique reactivity among the twenty natural amino acids, a thiol (-SH) group.

Experiments that measure changes in cysteine reactivity can reveal conformational changes.

There are many caveats.....
R/L/.. to C, which reacts with MTSET in solution

R, arginine; K, lysine, C, cysteine

MTSET from

Hille’s book
Voltage sensor positive charges are conserved across a wide range of voltage-sensitive channels.

N-terminal end of S4 carries most of the charge, but these types of experiments cannot distinguish if the mutations change mobility of other charges.

19.6 Contribution of Charged Residues to $z_g$. Measurements of total gating charge with wild type (WT) and mutated Shaker-IR channels. Mutations in all four subunits (1) neutralize individual positive charges (R or K), (2) neutralize individual negative (Neg.) charges (D or E), or (3) change R to K, preserving positive charge. For WT channels, $z_g$ is about 13 $q_e$. Several mutations reduce $z_g$ by 4 $q_e$ or more. The estimated values depend on the experimental method. [Data from Aggarwal and MacKinnon 1996 (circles) and Seoh et al. 1996 (triangles).]
Na channel S4 movement and $g(\text{Na})$ have similar V-dependence.

- MTSET

R1448C Reaction rate

$g(\text{Na})$ Peak

$$-140 \quad 20 \, \text{mV}$$

19.7 Voltage-Dependent Accessibility in S4 Voltage dependence of the rate of modification of mutated Na channels exposed to MTSET under voltage-clamp conditions. The natural R1448C mutation in this human muscle Na channel ($\text{Na}_v 1.4$) substitutes a cysteine for the outermost arginine of S4. Depolarization from $-140 \, \text{mV}$ speeds the modification. Open symbols show peak $g_{\text{Na}}$ during each pulse and the dashed line is the cube root of these values; in the HH theory, this would be the voltage dependence of each of the three voltage sensors. The maximum modification rate is equivalent to a second-order rate constant of $1.6 \times 10^3 \, \text{m}^{-1}\text{s}^{-1}$ (c.f. Figure 17.16). $T = 18^\circ \text{C}$. [From Yang and Horn 1995.]

(Hille’s book)
Model for opening of a voltage-gated channel. (Hille).

Closed channel

S4 responds to depolarization and moves ‘outward’, leading to channel opening

12-14 charges cross the field

Open channel

Model for opening of a voltage-gated channel. (Hille).
What moves when potassium channels open?

State-dependent changes in the modification rates of cysteines in Shaker S6

Liu, Y. et al., Neuron 19 175-184 (1997)
What types of gating motions might one expect?

A. Trap door motion

B. Collapsing motion

C. Pinching of selectivity filter

What sorts of physical changes might change a channel pore?

How do these types of motions couple to voltage sensor movement?

Liu, Y. et al., Neuron 19 175-184 (1997)
KcsA – Potassium channel from *Streptomyces lividans*

The world’s first view of an ion channel at high-resolution.

KcsA bears many of the hallmark features of potassium channels. Most importantly, it has the potassium channel conserved selectivity filter sequence ‘GYG’. This makes the part of the pore that has the most intimate contact with the permeant ions.

Top- Side view - four subunits

Bottom – Two subunits with key structural features noted

We still do not know why bacteria have ion channels….

MTSET modification rates of cysteine mutants in the S6 (pore lining) segment of Shaker (left).

Comparison of Shaker MTSET reaction rates with the KcsA bacterial potassium channel structure. Magenta shows the inferred positions of the residues that show strong state-dependent modification changes. Green shows those with little change.

White and yellow are residues that interact with external blockers.

Orange site interacts with tetrathylammonium ions within the cavity.

Liu, Y. et al., Neuron 19 175-184 (1997)
Comparison of transmembrane sequences shows conservation of key residues in the voltage-sensor, S4, residues that interact with the charges in the voltage sensor (S2 & S3), and in the potassium selectivity filter (all shown in red).

Voltage-gated channels
KvAP

Acts like a Kv channel

Blocked by spider venom

Purified VSTX1

Blocked by purified VSTX1

VSTX1 looks like known spider venom toxins that bind voltage sensors

CONSERVATION OF FUNCTION = CONSERVATION OF STRUCTURE?

Voltage-gated channels
KvAP

Looks like a channel??

View from the cytoplasmic side of the KvAP structure. Each subunit is colored differently. The green molecules are conformational specific monoclonal Fab’s that bind to the voltage sensor.

3.2 Å

Voltage-gated channels
KvAP

A) Bottom view, no Fab’s
B) Side view
C) Single subunit, side view
D) Single, subunit, side view, schematic

Voltage-gated channels
KvAP - The paddle model

An accessibility experiment to test the paddle model

Voltage-gated channels
KvAP - The paddle model

Red - External, Blue - Internal, Yellow - both
Voltage-gated channels
KvAP - The paddle model

Voltage-gated channels
KvAP Paddles vs. Pistons

A clear unified mode for voltage-sensor movement???

Voltage-gated channels
KvAP - The paddle model - further tests

Ruta, V., et al., Cell 123 463-475 (2005)
Voltage-gated channels
KvAP - The paddle model - further tests

Red - External, Blue - Internal
Yellow - both, Black - Neither
White - Zone of inaccessibility

Ruta, V., et al., Cell 123 463-475 (2005)
Voltage-gated channels
KvAP - The paddle model - further tests

Hold = -100 mV, Test = +100 mV

Ruta, V., et al., Cell 123 463-475 (2005)
Voltage-gated channels
KvAP - The paddle model - further tests S1/S2

Red- External, Blue - Internal
Yellow - both, Black - Neither
White - Zone of inaccessibility

Ruta, V., et al., Cell 123 463-475 (2005)
Voltage-gated channels

KvAP - The paddle model - further tests S3/S4

Red - External, Blue - Internal
Yellow - both, Black - Neither
White - Zone of inaccessibility

Monday, 11 February 13
Crystal structure of the $K_{V1.2}/K_{V\beta}$ complex

Crystal structure of the $K_v1.2/K_v\beta$ complex

Voltage sensors (S1-S4) are packed against the pore (S5-S6) of the adjacent subunit.
Electromechanical model for gating


Models of how voltage sensing works

Lone voltage sensor proteins

Voltage-sensor enzyme

Proton channel


TM field - $10^7$ V/m
$\Delta G$ opening - Shaker/Na$_v$ $\sim$14-16 kcal mol$^{-1}$
Lone voltage sensor proteins

Voltage-sensor enzyme (lipid phosphatase)
Lone voltage sensor proteins

Ci-VSP acts like a voltage-sensitive phosphatase
Lone voltage sensor proteins
New General Theme

Modular construction of channels membrane domains
Summary - Voltage gated channels

1) MODULAR construction (Pore, VSD, Assembly domains)
2) S4 moves in response to membrane voltage changes
3) Something must restrict ion flow in the closed state
4) Gating state transitions require rearrangements of the protein structure - level of conservation of mechanism is still unresolved
Ligand-gated ion channels

Acetylcholine receptor is a pentamer and is related to serotonin receptor (5HT₃R), glycine receptor and GABAₐ receptor.
Ligand-gated ion channels

Torpedo marmorata

Electric organ from *Torpedo* - rich source of nAChRs
Ligand-gated ion channels

Large family of neurotransmitter-gated ion channels including channels that gate in response to acetylcholine, GABA, glycine, 5HT3.

These channels are usually cation or anion permeable and show little selectivity besides charge.

General architecture

Medium resolution (4Å) electron microscopy pictures of voltage-gated sodium channels from *Torpedo marmorata* (electric ray) electric organ.

An experiment to mimic the synapse and trap the activated state

Synapse:

Experiment:

Analysis of 9 Å maps suggests a rotation of the subunits

Extracellular view

Level of Agonist Binding

Displacement following activation

Comparison of TM region

AChR pore-lining helices (M2) rotate to open pore

Density comparison at altitudes A, B, and C

Open pore - white
Closed pore - blue

Model for opening

Monday, 11 February 13
AChBP – high resolution structure of soluble snail protein reveals features relevant for the nAChR ligand binding pocket

AChBP – high resolution structure of soluble snail protein reveals features relevant for ligand binding

Nicotine

Carbamylcholine

Table 2. Thermodynamic Parameters of AChBP-Ligand Interaction

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_a$ (nM)</th>
<th>$\Delta H$ (kcal mol$^{-1}$)</th>
<th>$-T\Delta S$ (kcal mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Car$^a$</td>
<td>7575 ± 431</td>
<td>$-13.4 \pm 0.4$</td>
<td>6.46 ± 0.57</td>
</tr>
<tr>
<td>Ach$^a$</td>
<td>823 ± 40</td>
<td>$-12.5 \pm 0.1$</td>
<td>4.23 ± 0.32</td>
</tr>
<tr>
<td>Nic$^a$</td>
<td>45.2 ± 2.3</td>
<td>$-14.5 \pm 0.2$</td>
<td>4.52 ± 0.14</td>
</tr>
</tbody>
</table>

Isothermal titration calorimetry was performed in 100 mM NaCl and 25 mM sodium phosphate buffer (pH 8.0).

$^a$ Carbamylcholine.

$^a$ Acetylcholine.

$^a$ Nicotine.

AChBP – high resolution structure of soluble snail protein reveals features relevant for the nAChR ligand binding pocket

Same pocket/interface is targeted by a variety of plant toxins, venom peptides, and insecticides

4 Å Structure of nAChR

Features of a single subunit: each TM domain is a four helix bundle with M2 lining the pore
nAChR: A different kind of gate, no protein occlusion, instead, an energetic barrier.

Pore radius is < 3.5 Å over a ~ 8 Å long hydrophobic zone. The effective diameter of a hydrated Na⁺ or K⁺ is ~ 8 Å. This is too large to go through the hole.

nAChR: current model for gating

nAChR: model for gating - bacterial challenges

Microorganisms

Animals

Gloeobacter violaceus


Two bacterial LGICs

*Erwinia chrysanthemi*
ELIC - 3.3 Å
Ligand: ?


*Gloeobacter violaceus*
GLIC, 3.1 Å, 2.9 Å
Ligand: H⁺, ?

ELIC - Extracellular domain

ELIC (green)
AChBP (grey)
GLIC/ELIC - Ion binding site

GLIC - pore blockers?

Animal vs. Bacterial LGIC conflict or just different states?
Fluorescence-detection size exclusion chromatography (FSEC)

Express GFP-tagged target (E. coli, P. pastoris, mammalian cells)

Isolate membranes
Extract with detergent (1% DDM, 20 mM)

Apply 20 µl to FSEC column (1.5 mM DDM, 10 x CMC)

Can detect as little as 10 ng

Kawate and Gouaux 2006; Newstead et al. 2007
C. elegans LGIC Chloride channel

Required for crystals
Antibodies
Multiple deletions
Ligand
Lipids

3.3 Å

Shared site with
Ethanol
Anaesthetics
Other known modulators

Hibbs & Gouaux *Nature* **474**:54-60 (2011)
Ivermectin wedge
Ligand binding site
GluCl\textsubscript{cryst} - open channel