Structure, function and regulation of ion channels

György Panyi

www.biophys.dote.hu
Artificial membranes are impermeable to ions.

- Gases: CO₂, N₂, O₂
- Small, polar molecules: urea, ethanol
- Water: H₂O
- Big, polar molecules: glucose
- Ions: K⁺, Mg²⁺, Ca²⁺, Cl⁻, HCO₃⁻, HPO₄²⁻
- Big, polar charged molecules: amino acids, ATP, G6P
Ion channels are integral (transmembrane) proteins.
Nobel Prize, Chemistry, 2003

Roderick MacKinnon for structural and mechanistic studies of ion channels

Crystal structure of the voltage gated potassium channel, S5-S6

Peter Agre, for the discovery of water channels
Ion transport across the cell membrane

- ATP-driven pump
  - closed
  - open
  - ion channel
  - transporter

- extracellular
- cytosolic

- ATP
- ADP + Pi

- $10^{0} - 10^{3}$ ions/s
- $10^{7} - 10^{8}$ ions/s
- $10^{2} - 10^{4}$ ion/s
Simple view of an ion channel:

driving force \ + \ open \ gate \ \rightarrow \ ionic \ current
Single channel currents are typically in the order of several pA ($10^{-12}$ A)
The magnitude and the direction of ion currents is determined by the electrochemical gradient and the conductance.

**At equilibrium → no net current**

\[ [K^+]_o = 5 \text{ mM} \]
\[ [K^+]_i = 140 \text{ mM} \]

\[ E_K = -\frac{RT}{zF} \ln \frac{[K^+]_i}{[K^+]_o} = E_m = -89 \text{ mV} \]

\[ T = 37 \, ^\circ\text{C}, \, z = 1 \]

\[ I = G(E_m - E_K) = 0 \]

**Away from equilibrium → net current**

\[ [K^+]_o = 5 \text{ mM} \]
\[ [K^+]_i = 140 \text{ mM} \]

\[ E_K = -\frac{RT}{zF} \ln \frac{[K^+]_i}{[K^+]_o} = -89 \text{ mV} \]

\[ E_m = -60 \text{ mV} \]

\[ I = G(E_m - E_K) \neq 0 \]
Fundamental properties of ion channels: basis for classification

Gating
appropriate trigger causes a conformational change in the protein resulting in the transition among different states (closed, open, inactivated) of the channels.

Selective permeability
the passage of one or some ion species is allowed through the pore (e.g. highly selective, mildly selective and non-selective channels).
Gating I. Classification based on the trigger causing the transition between non-conducting (closed) and conducting (open) states:

- **voltage-gated**
  - change in the membrane potential. (e.g. voltage gated K⁺ and Na⁺ channels of neurons)

- **ligand-gated**
  - binding of a specific extracellular ligand (e.g. nicotinic acetylcholine receptor in neuromuscular junction)

- **i.c. signal-gated**
  - binding of a specific intra-cellular molecule (e.g. Ca²⁺ activated K⁺ channel).

- **membrane stretch-gated channel**
  - change in membrane tension (e.g. volume activated Cl⁻ channels).
Structure of voltage-gated ion channels

- Extracellular
- Cytosolic
- Voltage sensor
- Pore forming S5-S6 loop
- Voltage sensor domain
- Pore domain
- Pore loops
- Selectivity filter
- S4-S5 linker
- Gate
- α-α-α
- K⁺
Gating II.

Gating states of ion channels:
- functional states (conducting, non-conducting)
- conformational states (e.g. closed, open, inactivated)
**Gating III.**
Identification of domains responsible for activation and inactivation.

**inactivation**

(a) Wild-type shaker $K^+$ channel

- Exterior
- Cytosol
- Inactivation domain (ball)
- Resting $\rightarrow$ Open $\rightarrow$ Inactivated

(b) Mutant shaker $K^+$ channel

- Inactivation domain (ball) missing
- Resting $\rightarrow$ Open $\rightarrow$ Open (No inactivation)

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**IM**

WT

Deletion mutant $\Delta$6-46

Deletion mutant $\Delta$6-46 + ball peptide

**Time (ms)**

0 20 40 60 80

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**50 µM**

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Gating IV.
Identification of domains responsible for voltage sensing

Voltage sensor contains 1 less positive charge

The diagram shows the structure of a membrane with voltage sensor domains labeled S1 through S6. The open probability curves for wild type (WT) and mutant R368Q are plotted against membrane potential, indicating a shift in the voltage sensitivity of the mutant compared to the wild type.
Classification of ion channels based on selectivity

Highly selective – four subunits:  
\[ K^+, Na^+, Ca^{2+}, Cl^- \]

Mildly selective, five subunits:  
Acetylcholine receptor, cation specific

Non-selective, six subunits:  
Gap junction channel
Mechanisms conferring selectivity to channels:

- **based on size:** acts as a molecular sieve
- **based on charge:** ions with appropriate charge may pass through the channel
- **based on specific interactions:** selection among ions with similar size and charge
Nicotinic acetylcholine receptor selects based on size and charge (ligand-gated channel, mildly selective)
Specific interactions between ions and the amino acids lining the pore I.

X-ray crystallographic picture of the pore
Specific interactions between ions and the amino acids lining the pore II. carbonyl oxygens in the selectivity filter act as surrogate water

- $K^+$ : 1.33 Å
- $H_{hyd} = -322$ kJ/mol
- $Na^+$ : 0.95 Å
- $H_{hyd} = -406$ kJ/mol
How do we study the channels?

By measuring ionic currents using the patch-clamp technique

![Diagram showing the process of studying ionic currents](chart.png)
cell attached patch

suction

pull

whole cell

outside out patch

K⁺ current (nA)

Time (ms)

0 500 1000

0 0.4 0.8 1.2

1 pA

100 ms
Patch pipette in contact with a cell and its electron micrograph after heat polishing

Major physiological functions of ion channels

Membrane potential

resting potential

\[
E_m = -\frac{RT}{Fz} \ln \frac{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o}{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_i}
\]
Major physiological functions of ion channels

Membrane potential
- resting potential
- action potential

Membrane potential (mV)
- Rising phase depolarization
- Threshold
- Declining phase repolarization
- Sub-threshold stimuli
- After hyperpolarization

Conductance ($\text{mmho/cm}^2$)
- Sodium permeability ($Na^+$ permeability)
- Potassium permeability ($K^+$ permeability)

Time (ms)
Major physiological functions of ion channels

Membrane potential
- resting potential
- action potential

Changes in intracellular ion concentrations
- $[Ca^{2+}]_i$ rise
Ion channels are drug targets I.

1, pore blockers

e.c.

block

i.c.

block
Ion channels are drug targets II.

2. gating modifiers

block