Cellular and Molecular Biophysics

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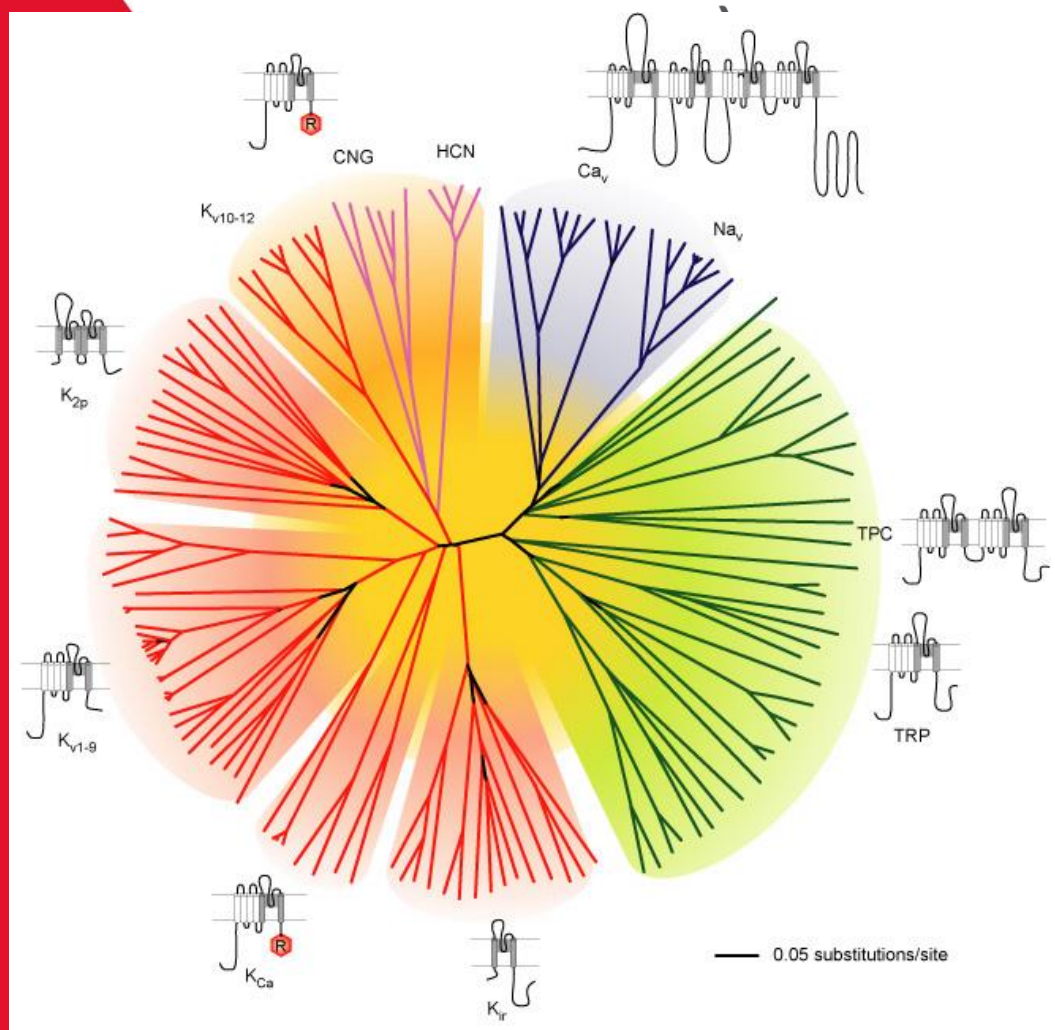
CFU 5 LM Biotecnologie Industriali- 6 LM Fisica - A.A. 2023/24

Corso di laurea in LM Biotecnologie Industriali- LM Fisica

Department of
Life Sciences
and Systems Biology

Ion Channels

STRUCTURE AND FUNCTION



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- Understand the molecular mechanism at the basis of voltage gated ion channels and correlation between structure and function
- Describe quantitatively the gating mechanism of activation and inactivation of voltage gated ion channels
- Describe quantitatively the gating mechanism of activation of ligand gated ion channels

Common properties of ION CHANNELS:

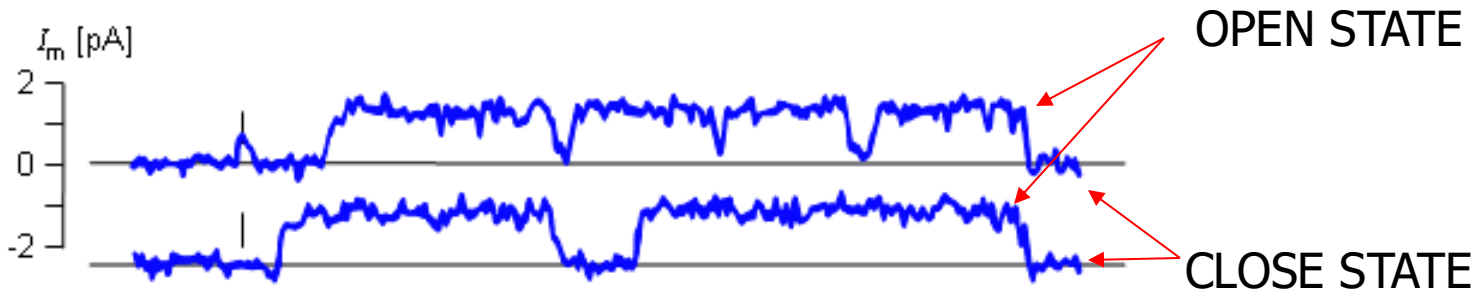
- **GATING**: mechanism that controls conformational transitions between open and closed state and therefore control OPENING and CLOSING of the channel

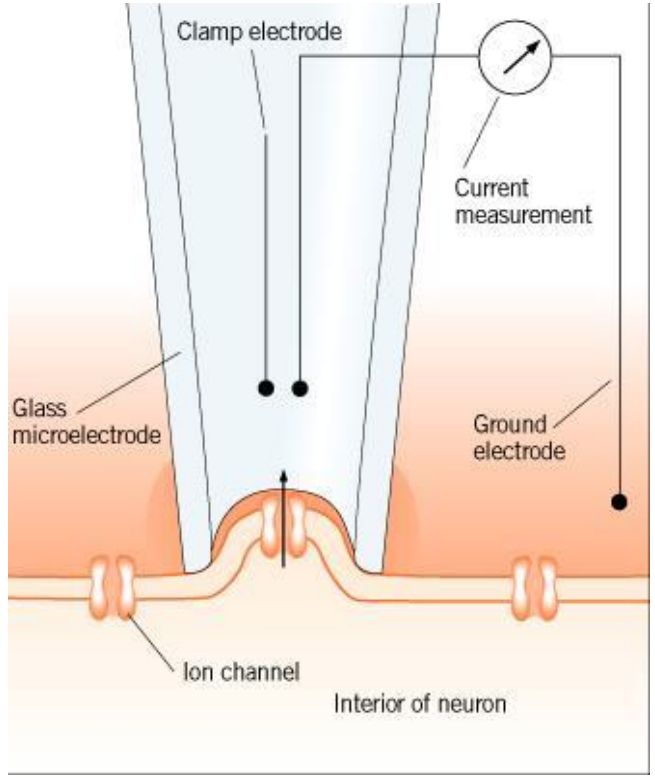
- **SELECTIVITY**: channel ability to select ion species that flows.

Channels can be therefore classified by the selectivity properties.

Gating mechanisms of ION CHANNELS:

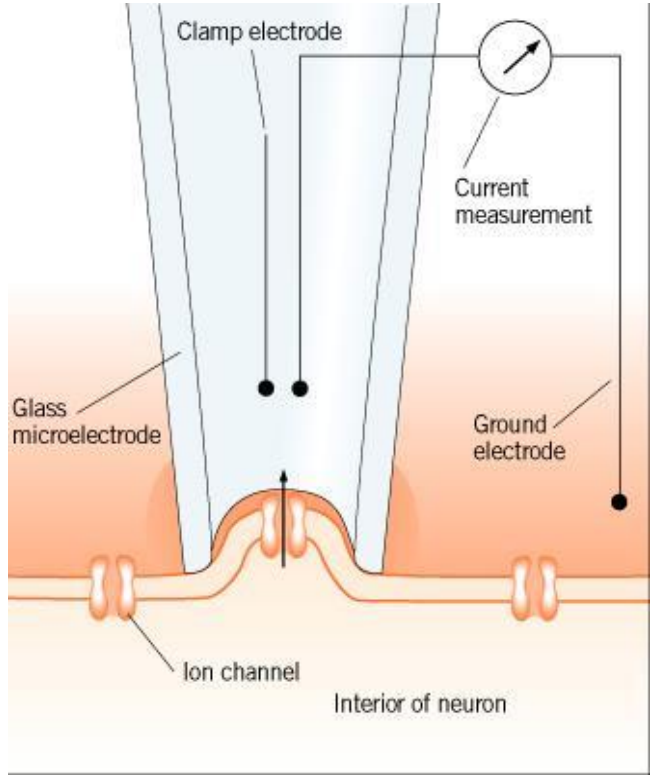
The most direct way to study ion channels properties is by measuring ion fluxes or more precisely the electric current that flows in the channel.
This is possible thanks to the patch-clamp technique introduced in the late 70's which allows to measure the current from single channel.

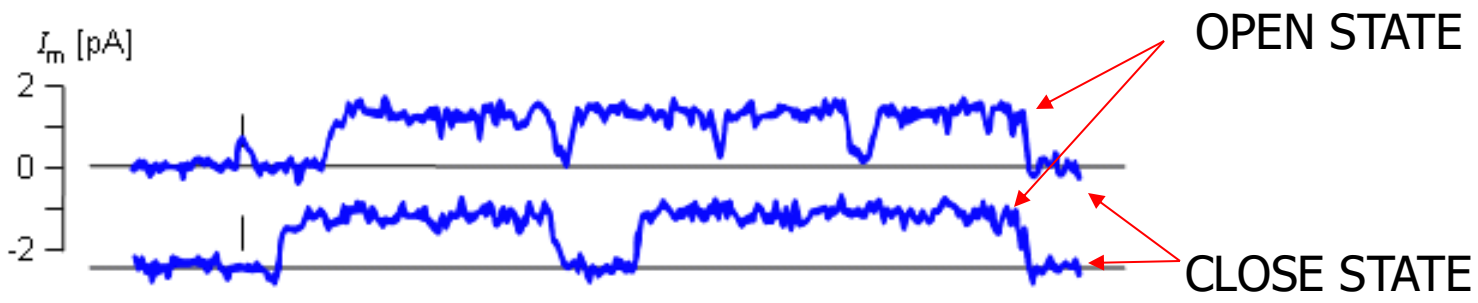




Gating mechanisms of ION CHANNELS:

From the figure is clear that even in absence of stimulation, the channels can shift between two different levels:
CLOSE STATE; OPEN STATE
This is a common behavior of ion channels indicating that at least two conformational states exist: OPEN and CLOSE. The channel continuously shift between these two states



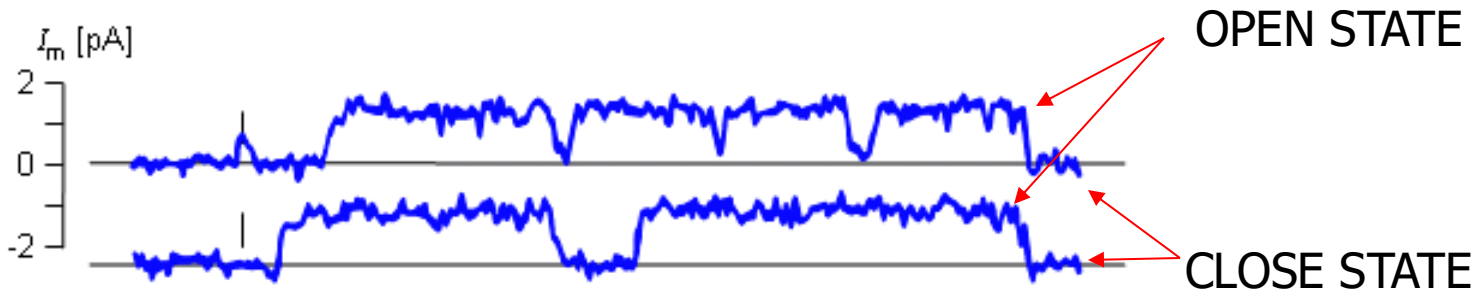


Gating mechanisms of ION CHANNELS:

The traces below also show a clear variability of open and close duration of the channel and therefore is not possible to predict how long a channel can stay in each of the functional state (open or close) or neither when the next transition will be

Stochastic events

The lows that describe these events are deducted from the probability distribution of several events number.



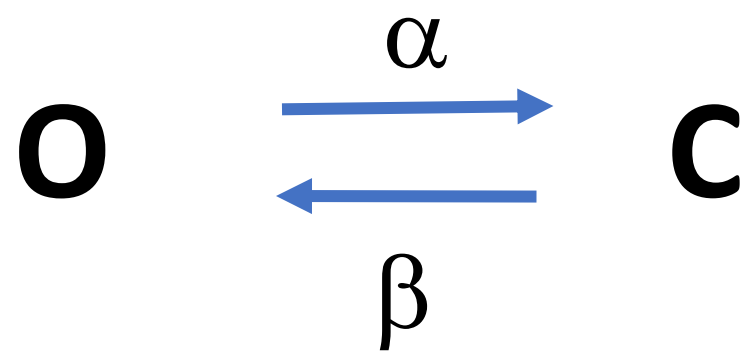
Gating mechanisms of ION CHANNELS:

The kinetic state (the transitions) of a single channel, the stochastic activity of one event and the exponential distribution of the “duration histograms” can be explained by the **TRANSITION STATE THEORY** **by Eyring which is premised on the somewhat tenuous assertion that “reactants (CHANNELS) rapidly thermalize with their surroundings until they reach the separatrix of the transition barrier, whereupon they inexorably turn to product.”**

In other words the channels undergo very rapid conformational changes which at the end determine shifts in the functional state (open or close states)

Gating mechanisms of ION CHANNELS:

Transitions from OPEN to CLOSE state can be described:

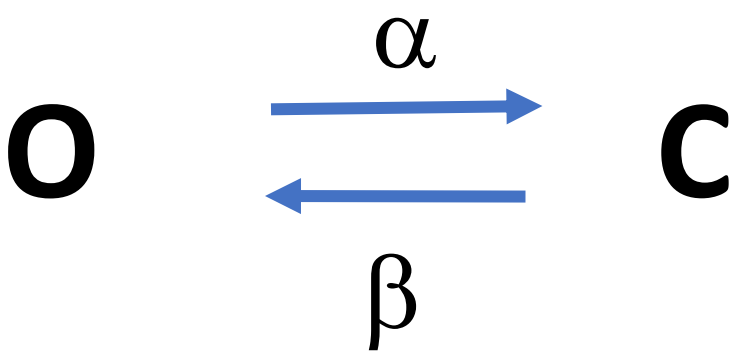


A and β are speed constant and represent the number of transitions in the time unit. **A and β can also be described in term of probability of transition in time frame t.**
This probability (that will not change in time if no conditions change) will be:

$\alpha\ dt$ $\beta\ dt$

Gating mechanisms of ION CHANNELS:

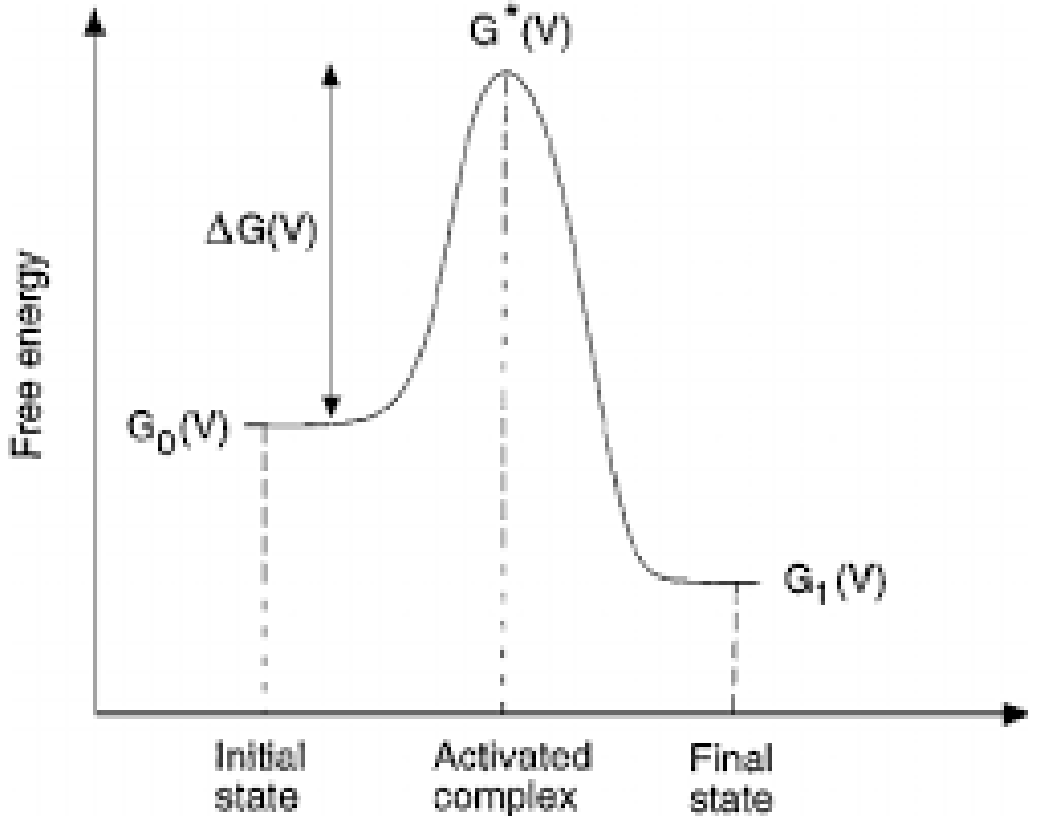
Transitions from OPEN to CLOSE state can be described:



We can talk therefore about Probability of transitions episodes

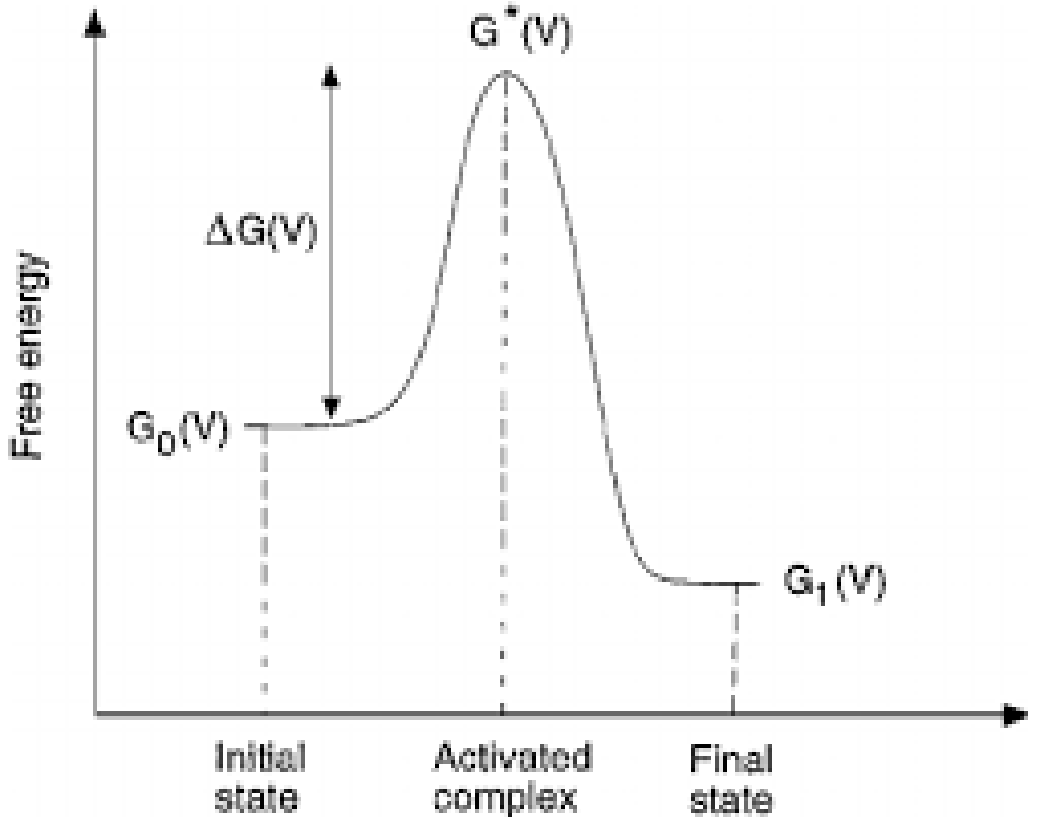
Gating mechanisms of ION CHANNELS:

In analogy with chemical reactions, the energetic profile of conformational changes is quite relevant.



Schematic representation of the free energy profile of conformational changes in ion channels. The diagram represents the free energy of different states involved in a transition: the initial state, activated complex, and final state. The equilibrium distribution between initial and final states depends on the relative value of their free energy (G 0 and G 1).

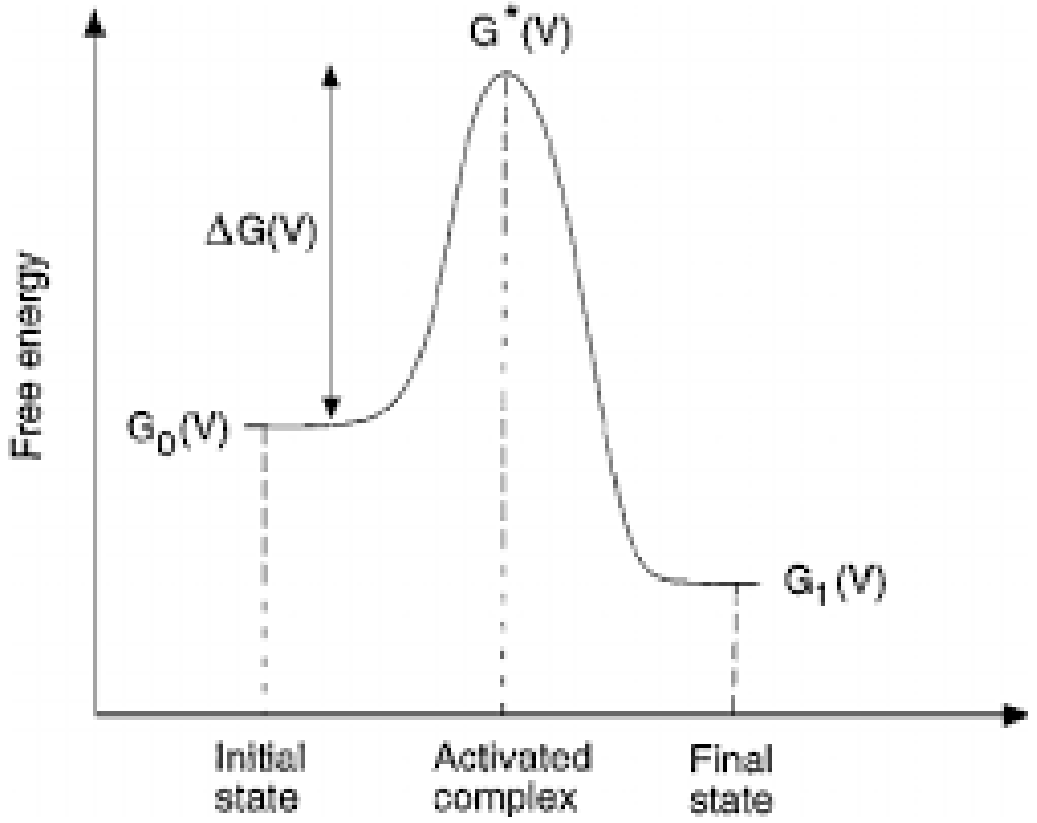
Gating mechanisms of ION CHANNELS:



During the transitions between states, the channels pass through a transition state which is not favorable from the energetic point of view = G*

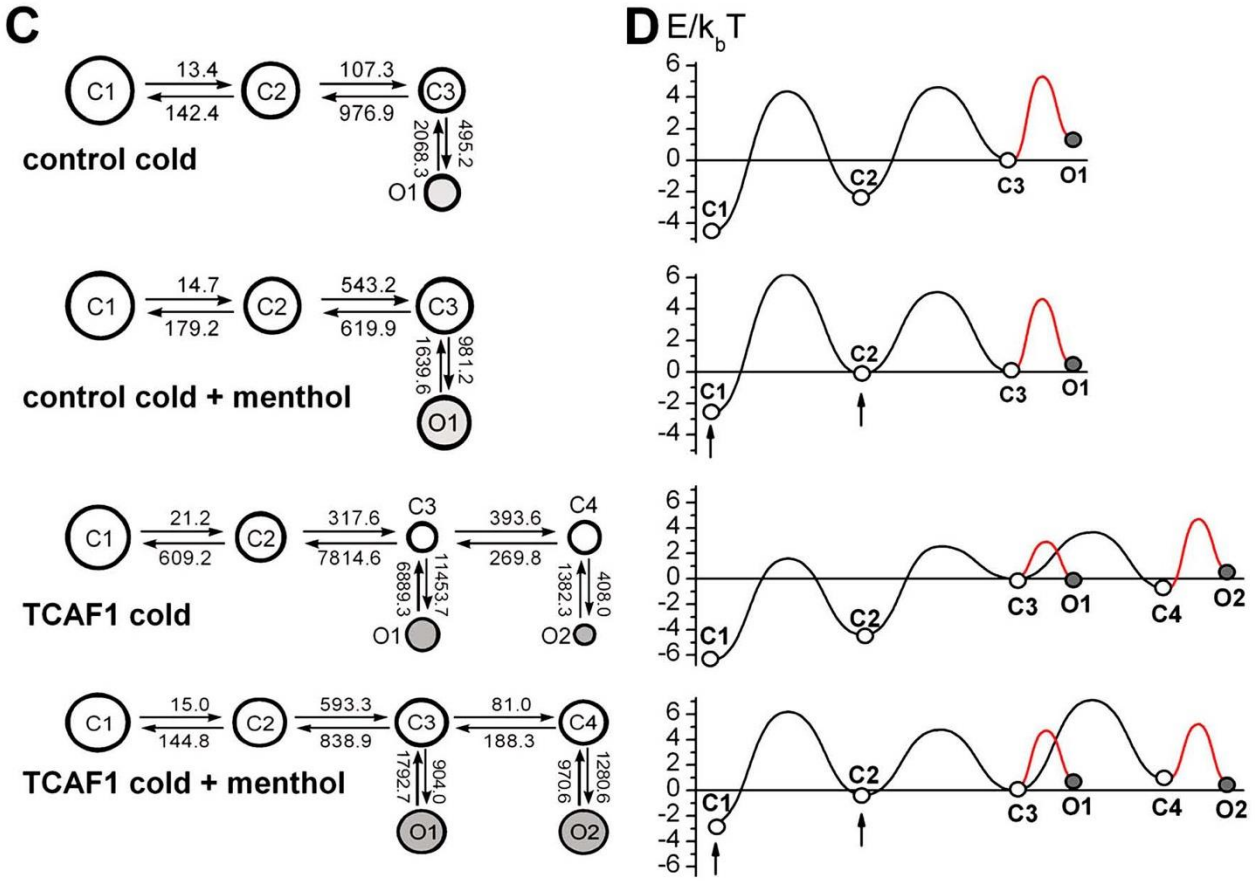
The bigger is ΔG, the smaller is the probability of transition from Initial to Final state and thus the smaller will be α

Gating mechanisms of ION CHANNELS:



This diagram represent the simplest case in which the channel present one initial and one final state.

Gating mechanisms of ION CHANNELS:



In general channels have more complex energetic states.

Gating mechanisms of ION CHANNELS: OPEN PROBABILITY

Open Probability (p_o) is a very useful parameter commonly used to to measure channels activity and therefore the amount of ions crossing the membrane

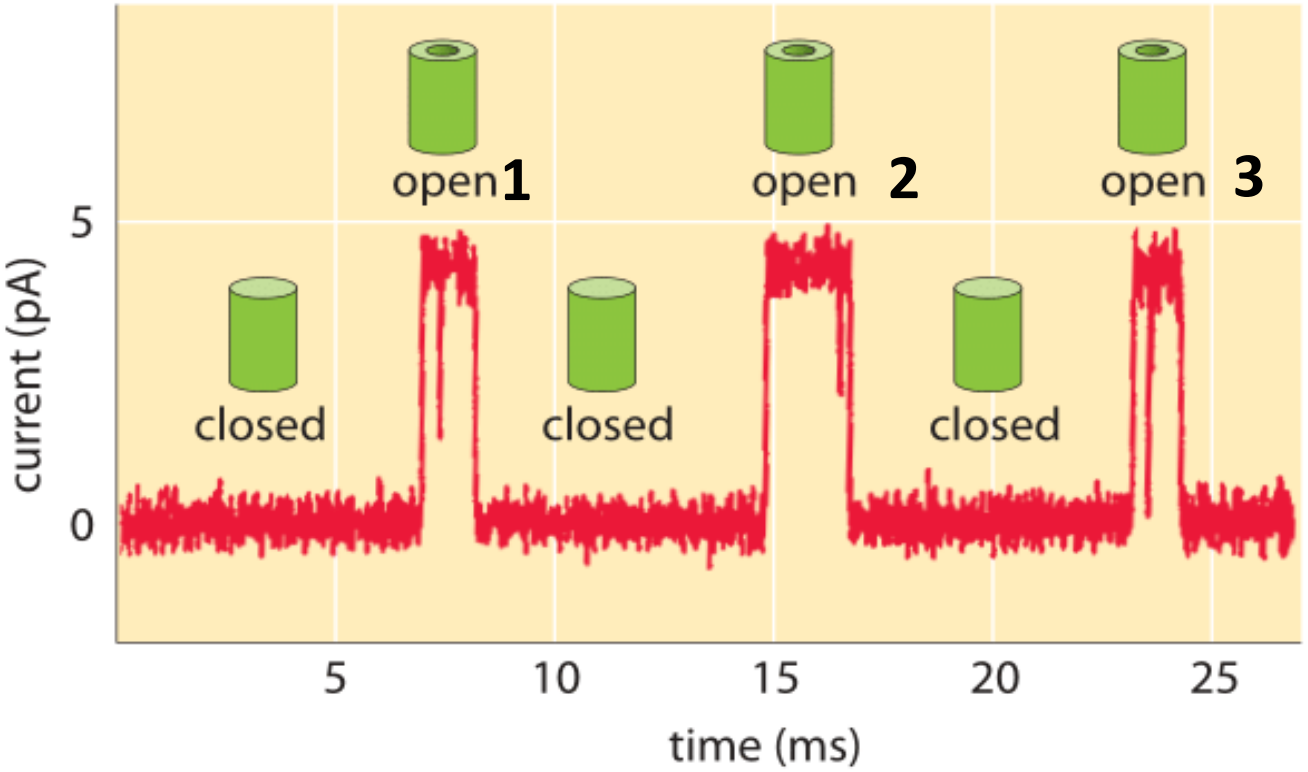
P_o describes the probability to find the channel open in a certain time fraction.

$$\mathbf{0 < p_o < 1}$$

Probability to find the channel close

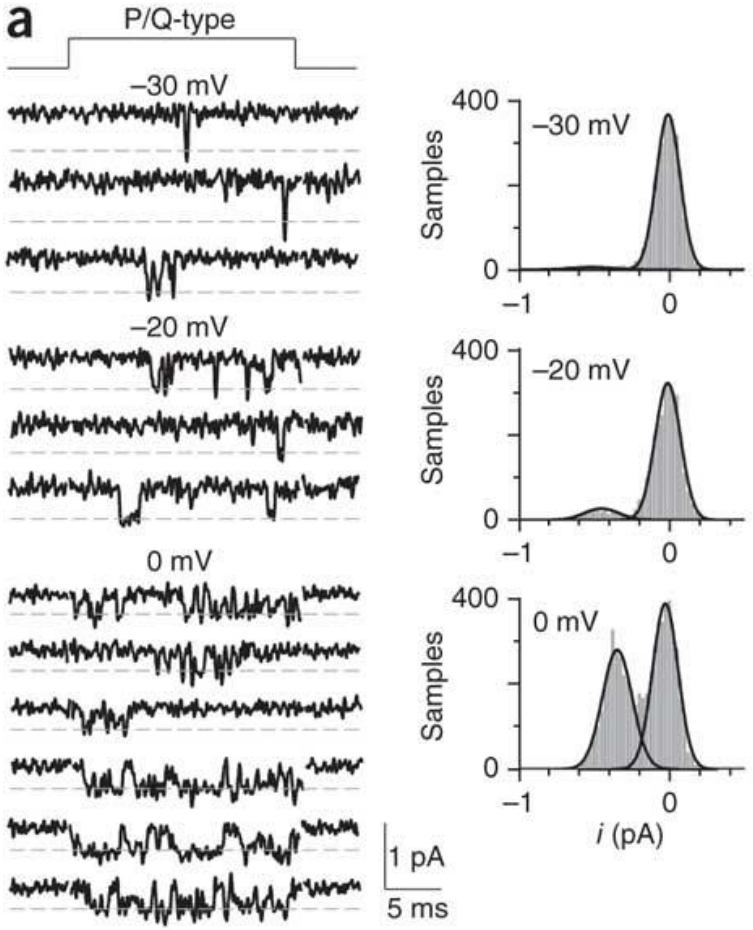
Probability to find the channel open

Gating mechanisms of ION CHANNELS: OPEN PROBABILITY



$$P_o = \frac{tO_1 + tO_2 + tO_3}{\text{Tot time}}$$

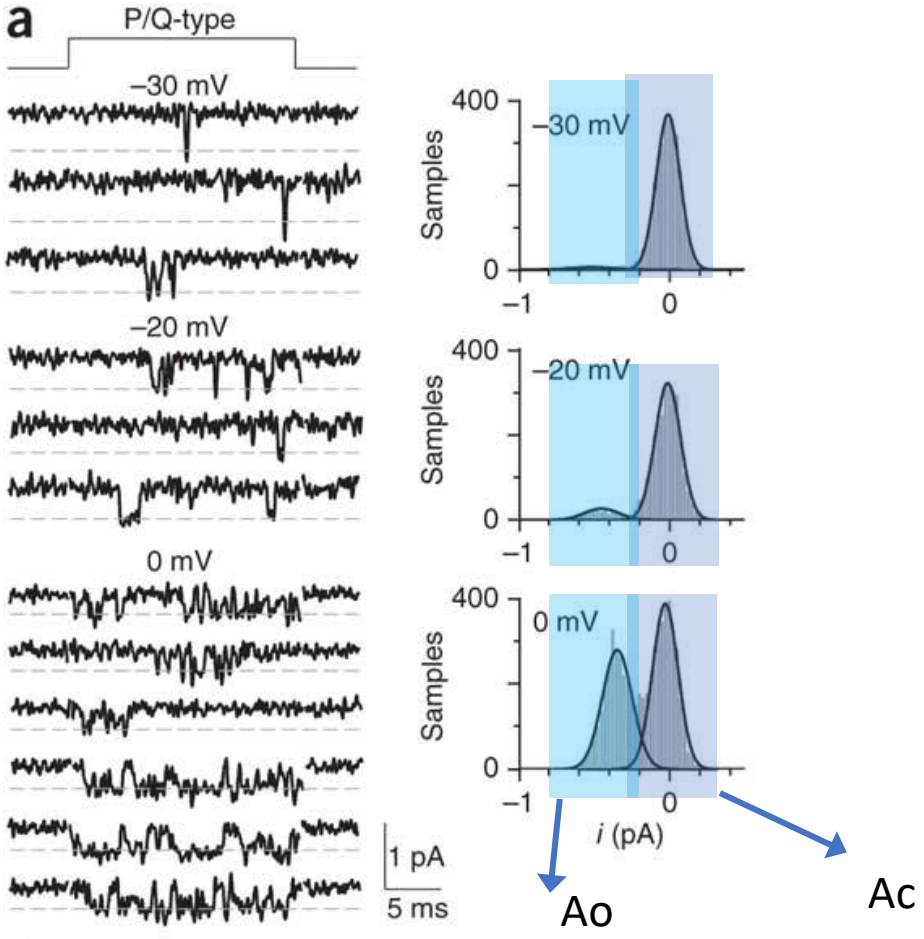
Gating mechanisms of ION CHANNELS: OPEN PROBABILITY



Another way to calculate po is by amplitude histograms

$$P_o = \frac{Ao}{Ao + Ac}$$

Gating mechanisms of ION CHANNELS: OPEN PROBABILITY

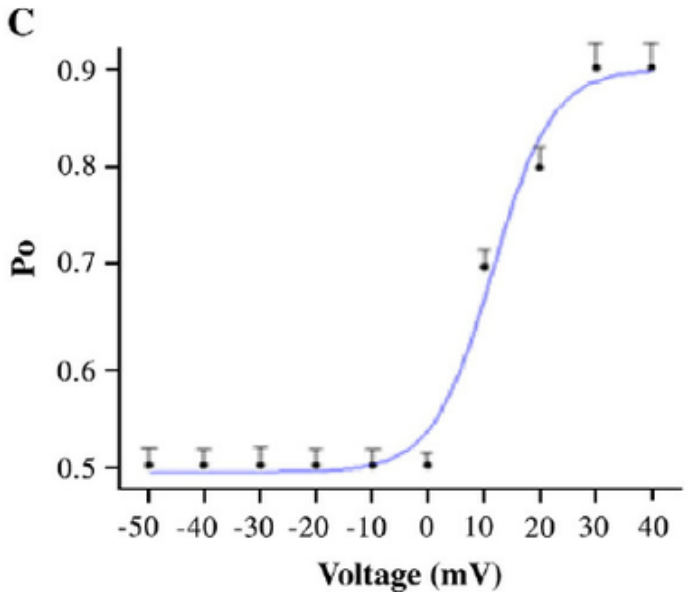


Another way to calculate po is by amplitude histograms

$$P_o = \frac{Ao}{Ao + Ac}$$

Gating mechanisms : Voltage-dependent gating

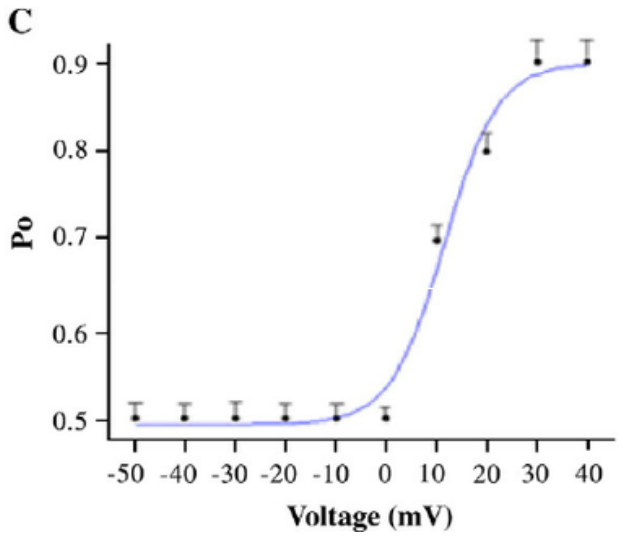
The channels continuously shift from O to C states.
If environmental condition (or experimental) change, the channels activity can change deeply.
Po differs at different V



Po can be plotted in a Po/V curve and described by Boltzman equation

$$Po = \frac{1}{1 + \exp - \frac{z_g\, e\, (V - V_{1/2})}{kT}}$$

Gating mechanisms : Voltage-dependent gating



Po can be plotted in a Po/V curve

$$Po = \frac{1}{1 + \exp\left(-\frac{z_g\, e\, (V - V_{1/2})}{kT}\right)}$$

e = elementary charge

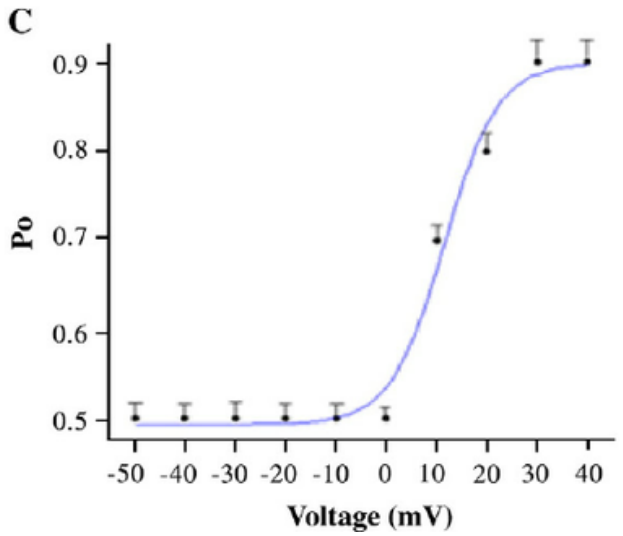
k = Boltzman k

T = Absolute T

z_g = gating charge

$V_{1/2}$ = half activation V

Gating mechanisms : Voltage-dependent gating



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$V_{1/2}$ = half activation V

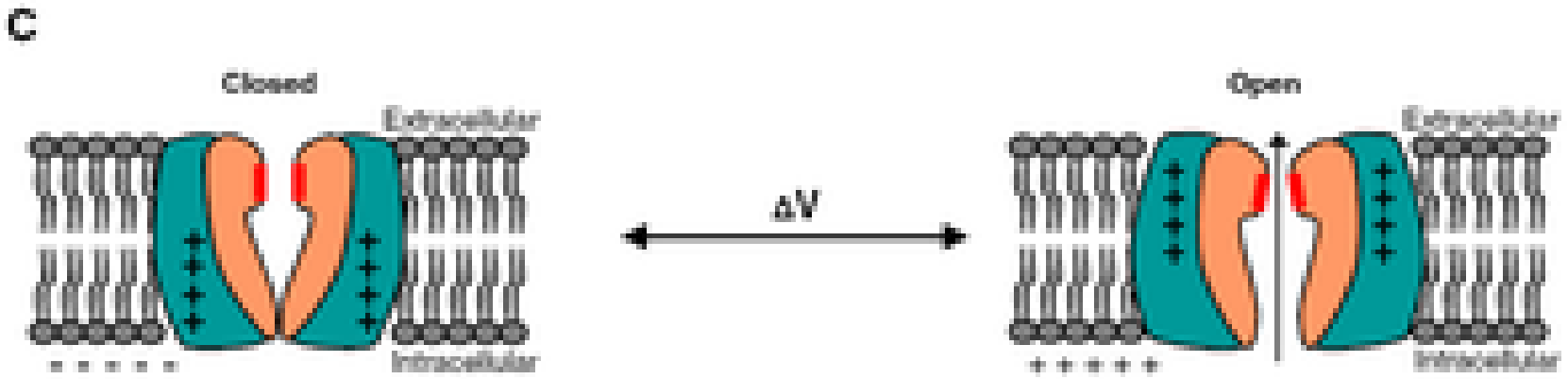
Depend on the properties of the different channels

Gating mechanisms : Voltage-dependent gating

The mechanism responsible of the voltage gating has been proposed initially by A.L. Hodgkin and A.F. Huxley.

They observed that the variation of membrane permeability to Na+ and K+ were dependent on V changes.

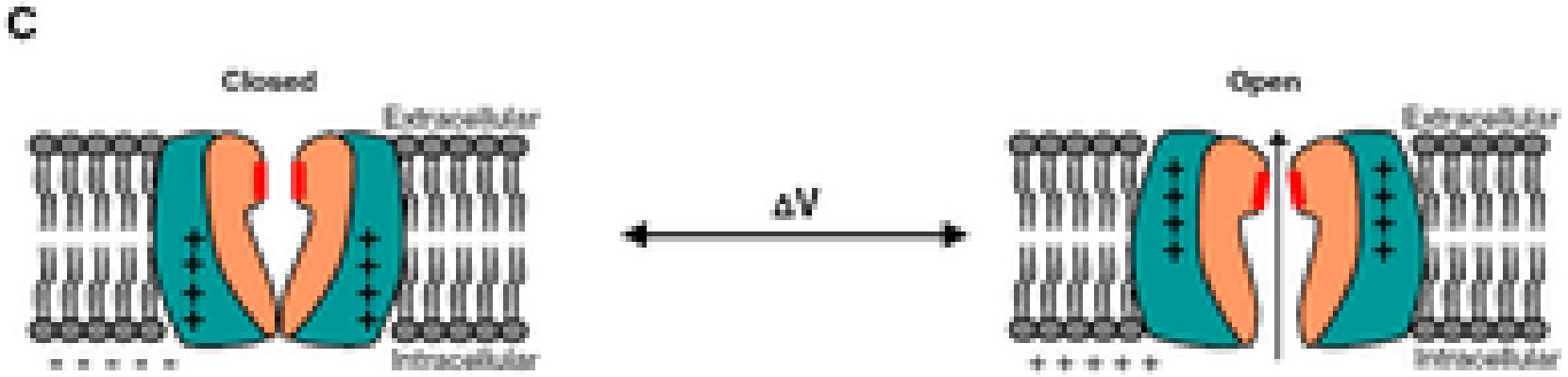
They hypothesized the **presence of V sensor in the form of charges** within the channel that are able to sense the voltage and move within the membrane thickness in response to V changes



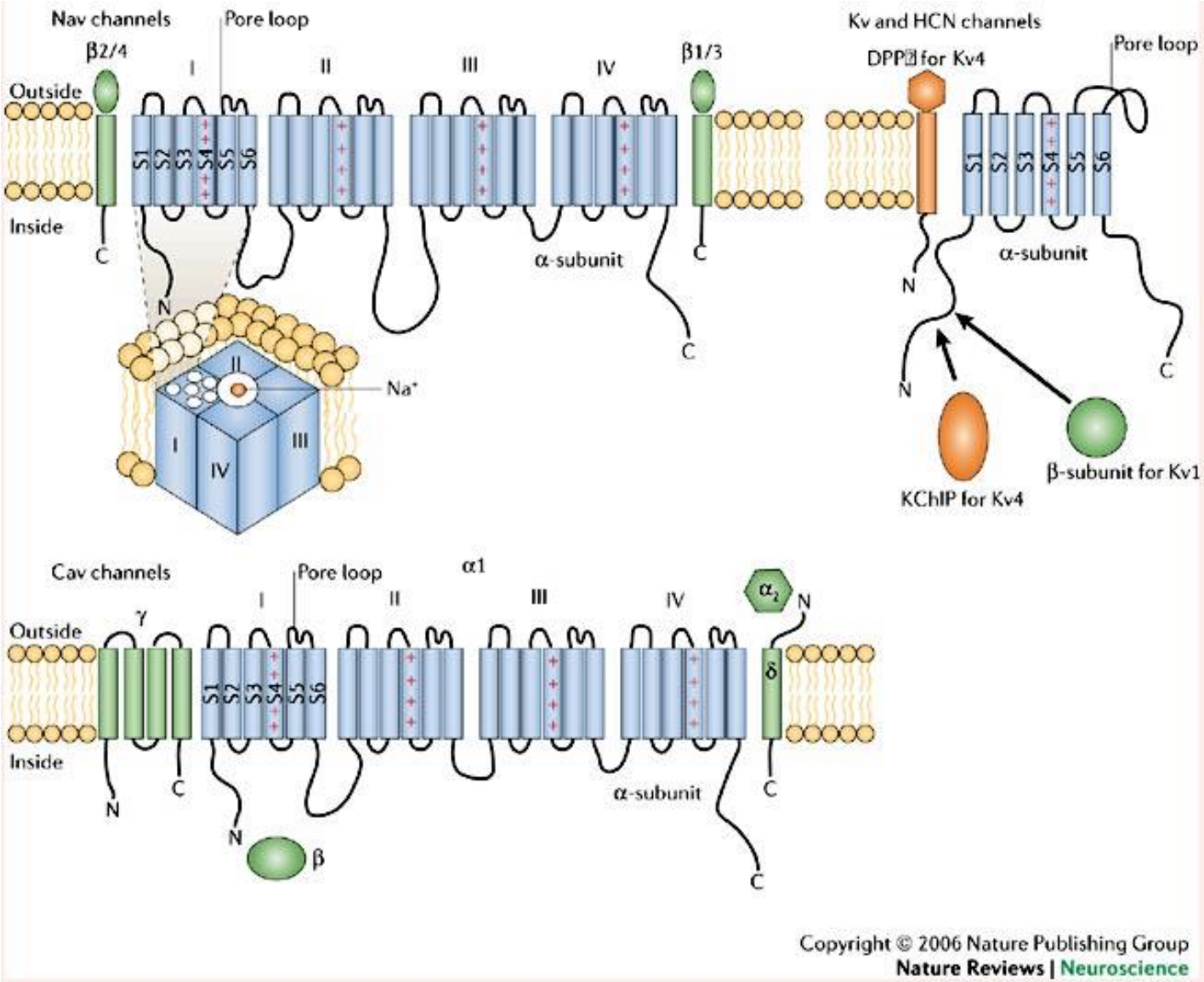
Gating mechanisms : Voltage-dependent gating

As a consequence the Gating charges (zg) that move along the electric field within the membrane should be able to generate **transient currents**.

These currents, although very small as compared with the ion currents through ion channels, **can be registered** strongly **supporting the hypothesis** of the voltage sensor



Molecular basis of Voltage sensor



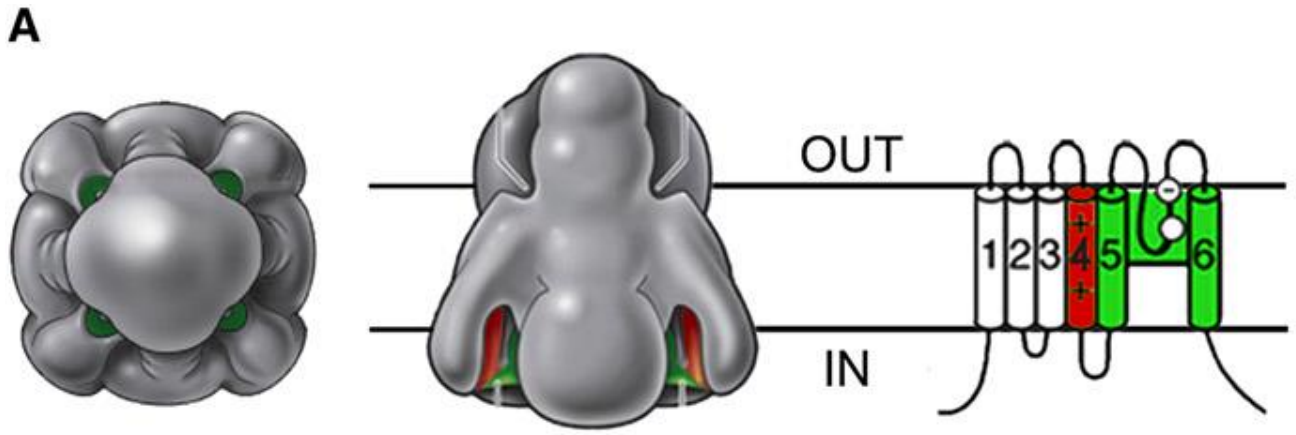
Voltage gated- ion channels present a close structural homology.

Each subunit of Kv or each of the 4 domains of the Na+ or Ca2+ V gated channels is formed by **6 TM α helix domains**.

S4 presents several basic aa: 4 to 7 repeated 3 positive residues followed by 2 hydrophobic aa residues

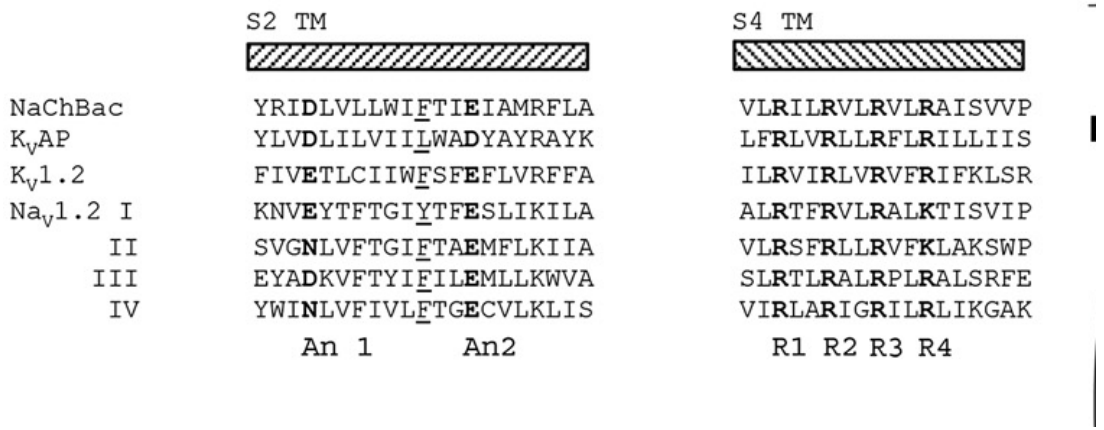
Molecular basis of Voltage sensor

These proposals presaged the idea that the **S1-S4 segments serve as the voltage-sensing** module while **the S5 and S6 segments serve as the pore-forming module** and eventually led to the now-familiar six-transmembrane-segment structural model for the domains of voltage-gated Na+ channels



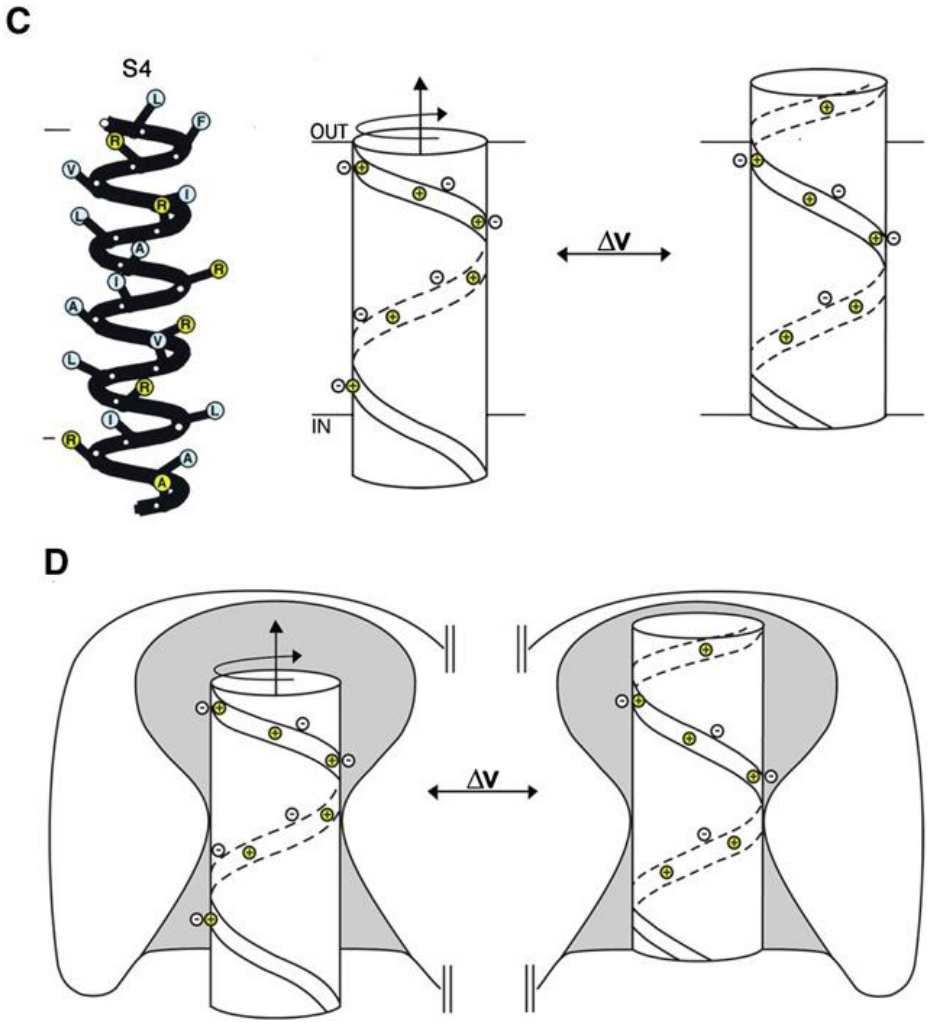
The Sliding Helix-Helical Screw Model for Voltage Sensor Function

How can an S4 segment containing four to seven positive charges (usually R) at three-residue intervals be stabilized in a transmembrane environment and move outward to translocate the gating charges across the membrane electric field?
Relying on thermodynamic and structural considerations, respectively, the ‘‘sliding helix’’ or ‘‘helical screw’’ models for voltage sensor function arrived at similar solutions to this conceptual problem.



The charged residues in the S4 segments were proposed to form ion pairs with negatively charged amino acid residues in the neighboring S1, S2, and/or S3 segments.

The Sliding Helix-Helical Screw Model for Voltage Sensor Function



In this configuration, the positively charged residues in the S4 segment are drawn inward by the electrostatic force of the negative internal resting membrane potential. Upon depolarization, this electrostatic force is relieved, and the S4 segments move outward along a spiral path such that each positively charged amino acid residue in the S4 segment makes a series of ion pairs with negative charges (Figure 1C).

See movie https://www.youtube.com/watch?v=hfXGsJCOC9A (minute 15:42)

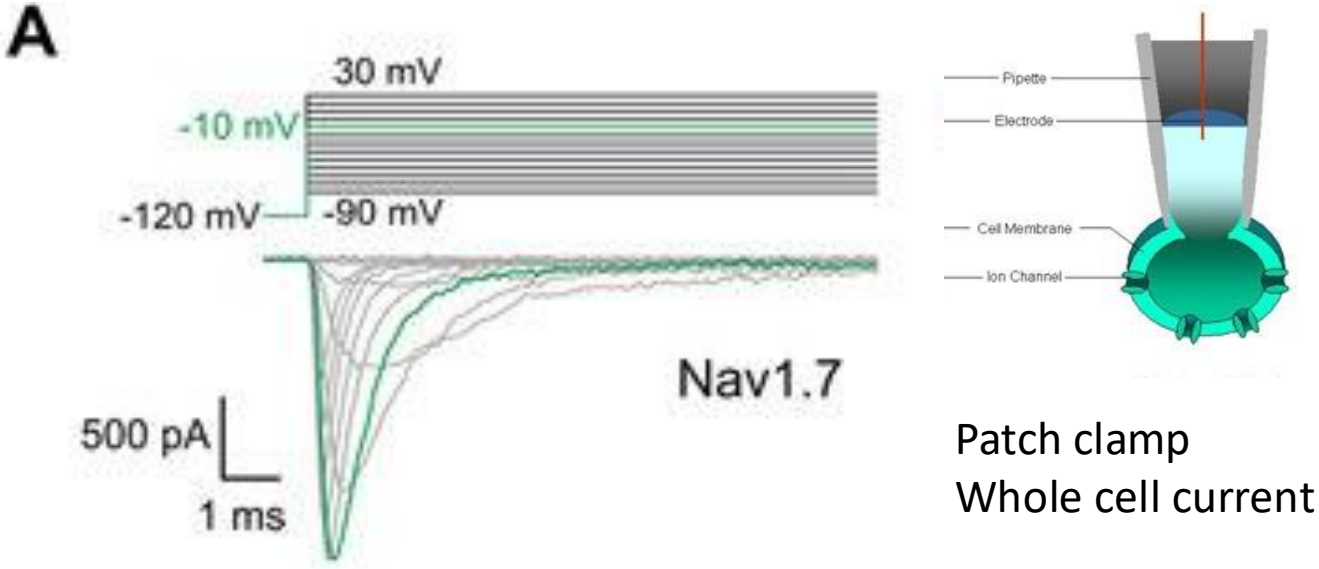
The Sliding Helix-Helical Screw Model for Voltage Sensor Function

This proposed model for gating charge movement, hereinafter termed the **sliding-helix model** for brevity, makes four testable predictions:

- the positively charged residues in S4 serve as the gating charges
- the S4 segment is in a transmembrane position in both resting and activated states
- the S4 segment moves outward and rotates during activation
- the positive charges in the S4 segment form ion pairs sequentially with negative charges in neighboring transmembrane segments

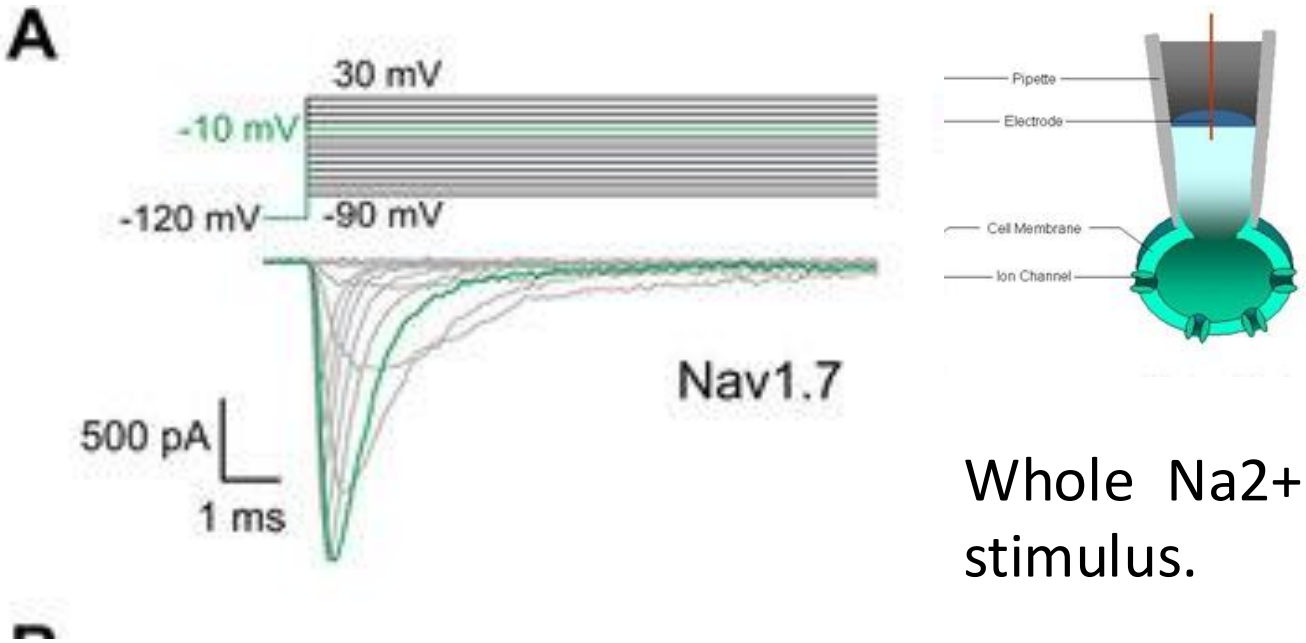
Inactivation of Voltage-gated channels

Some of the Voltage-gated channels under a depolarizing stimulus, remain open just for a short period of time and then go back to a non permeable state eve in the presence of continuous depolarization = INACTIVATION.
This is typical of Na^+ voltage-gated channels (Na^+_v) and some K^+_v.



Patch clamp
Whole cell current

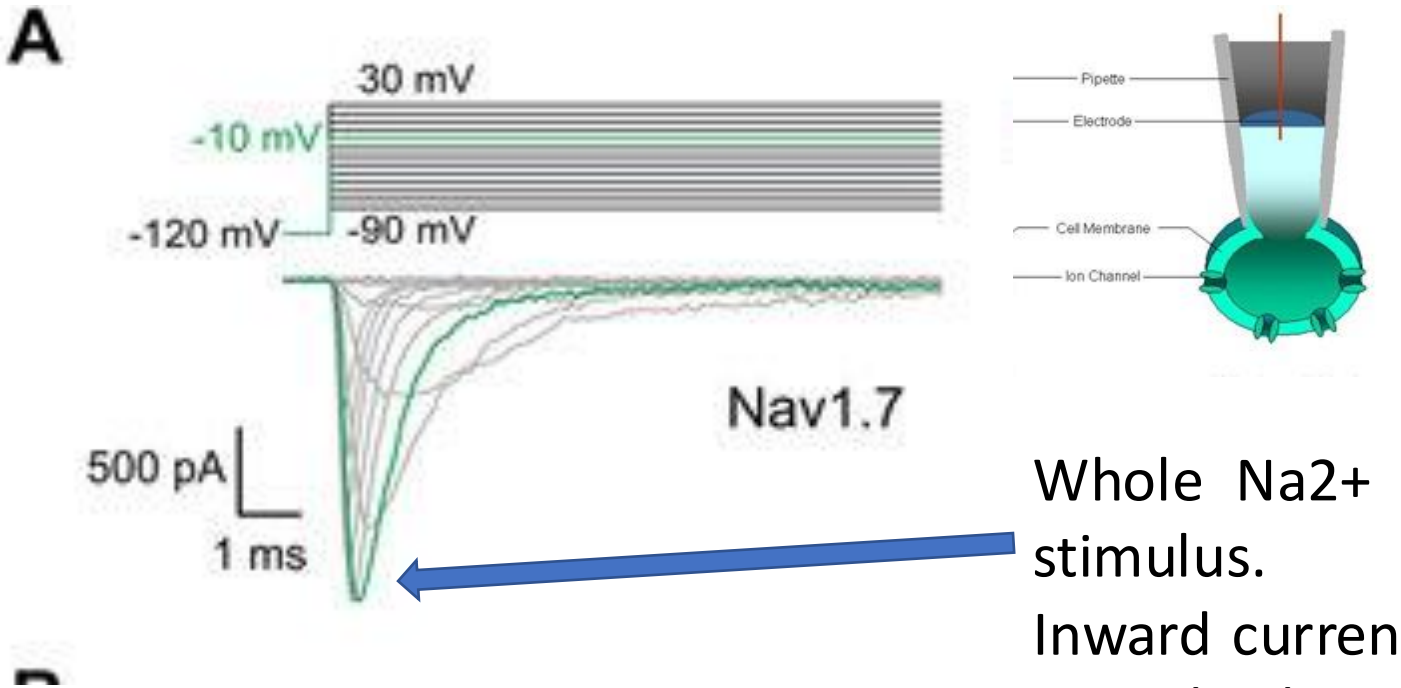
Inactivation of Voltage-gated channels



Patch clamp
Whole cell current

Whole Na2+ current in response to a depolarizing stimulus.

Inactivation of Voltage-gated channels



Patch clamp
Whole cell current

Whole Na2+ current in response to a depolarizing stimulus.

Inward current which last for few milliseconds before going back to 0.

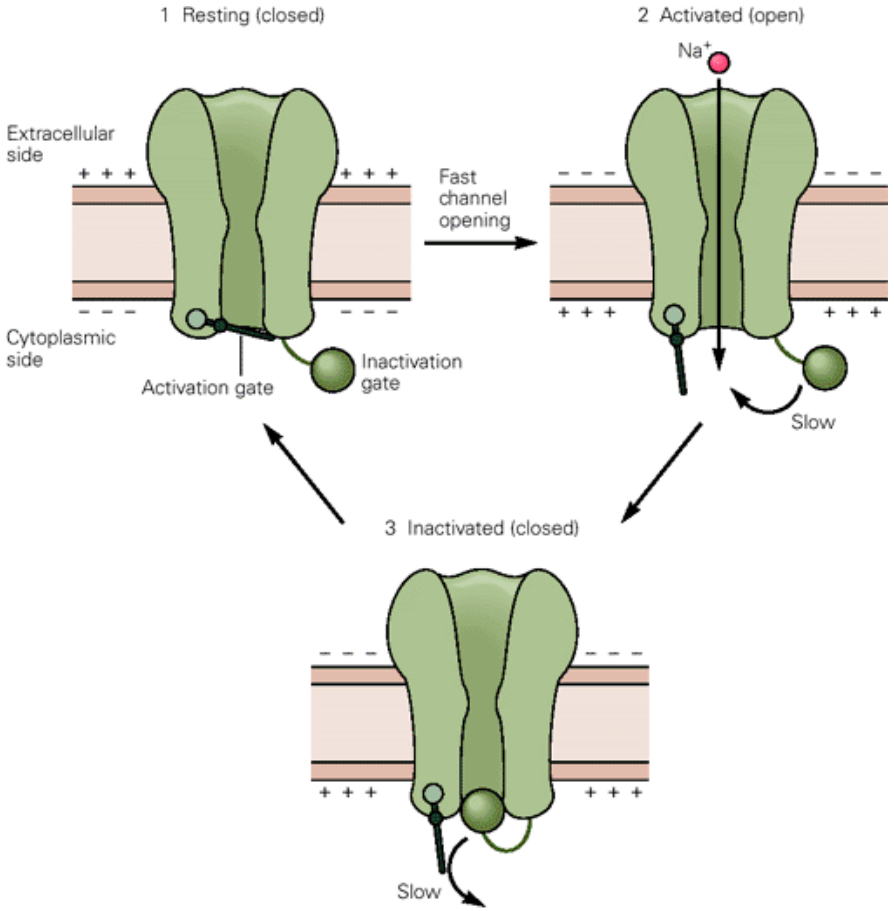
Inactivation of Voltage-gated channels

If a second impulse is evoked immediately after, the current is much smaller but if the membrane is kept for enough time to negative potential, than depolarization will evoke again inward current with the same amplitude as the first one

Recovery from INACTIVATION requires membrane repolarization during which the channels pass from the inactive to Close state

Inactivation of Voltage-gated channels

From the kinetic point of view we can therefore describe the channel with the scheme:



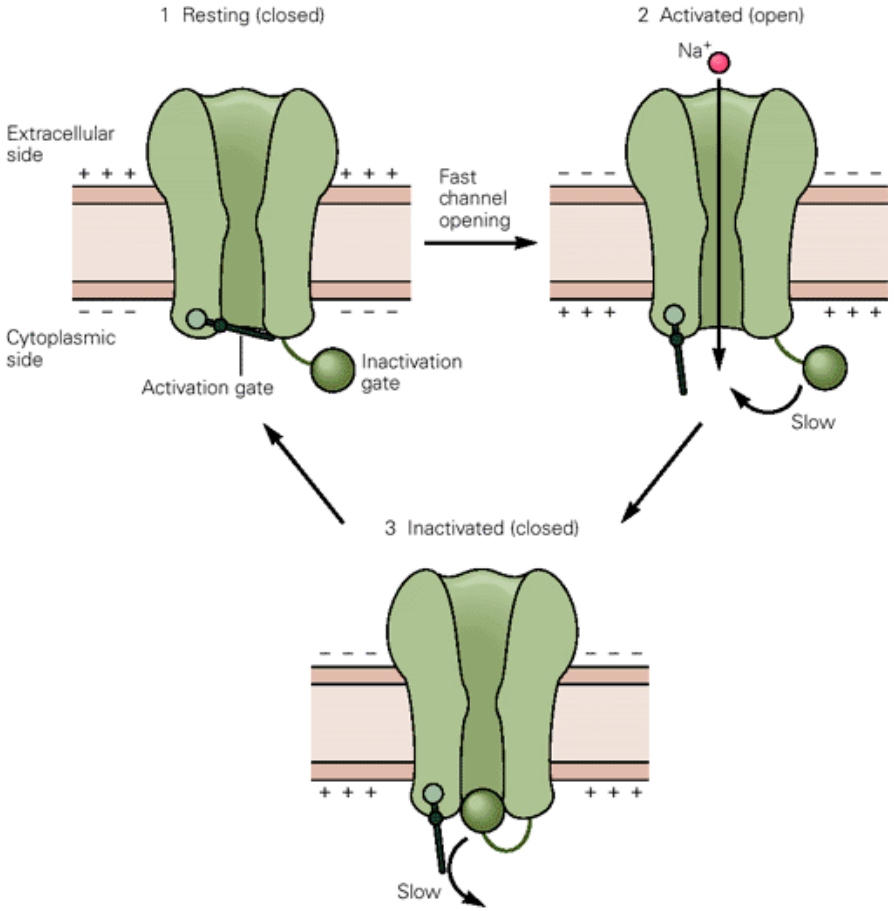
Beside OPEN and CLOSED states these channels have a INACTIVE state in which the channels are just after the OPEN state.

The INACTIVE state is undistinguishable from the CLOSE state form the functional point of view since in both cases no Current permeate through the channel

On the other hand huge molecular differences exists between the INACTIVE and CLOSE state.

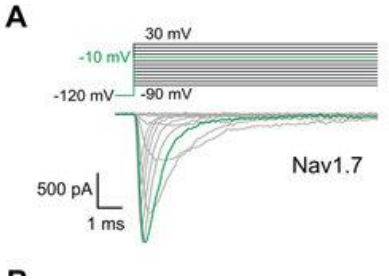
Inactivation of Voltage-gated channels

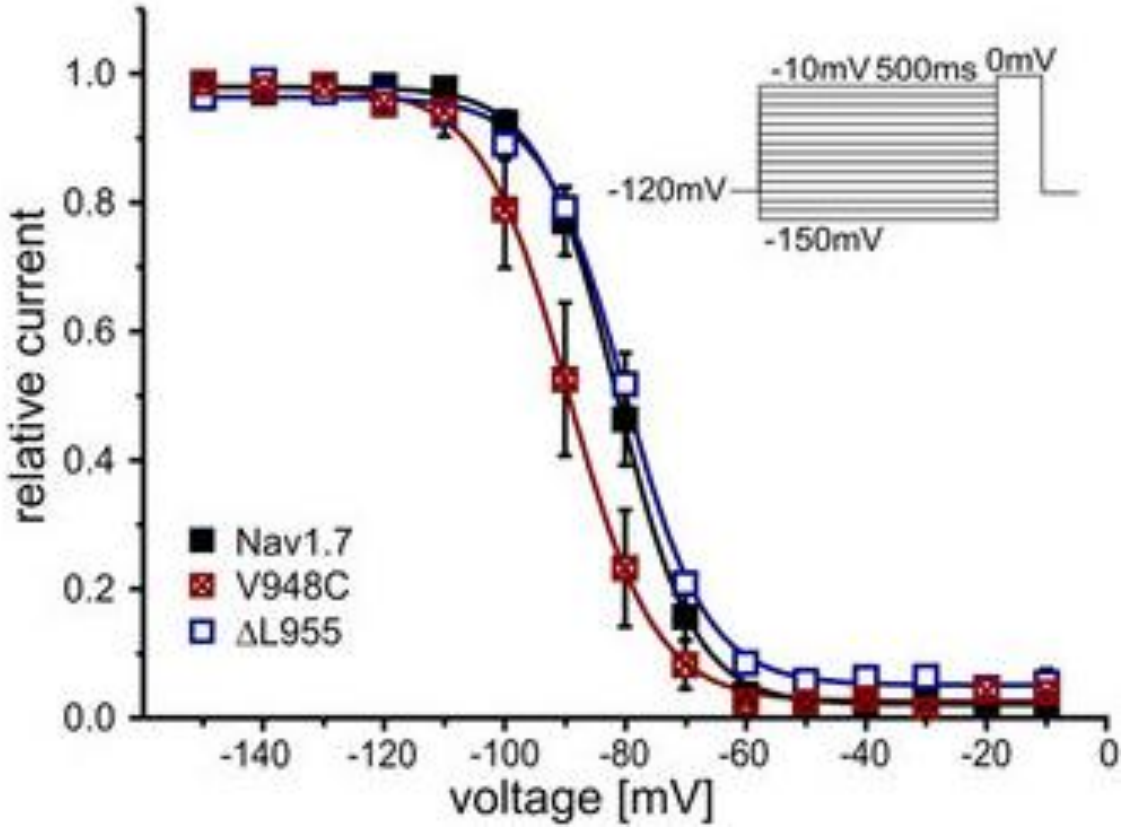
From the kinetic point of view we can therefore describe the channel with the scheme:



The passage to INACTIVE state at depolarizing potential is irreversible and the passage to CLOSE state requires hyperpolarizing condition of the plasma membrane.
Since the transition to INACTIVE state requires a depolarization step, we can state that it is a VOLTAGE DEPENDENT phenomenon such as the opening of the channel

Inactivation of Voltage-gated channels



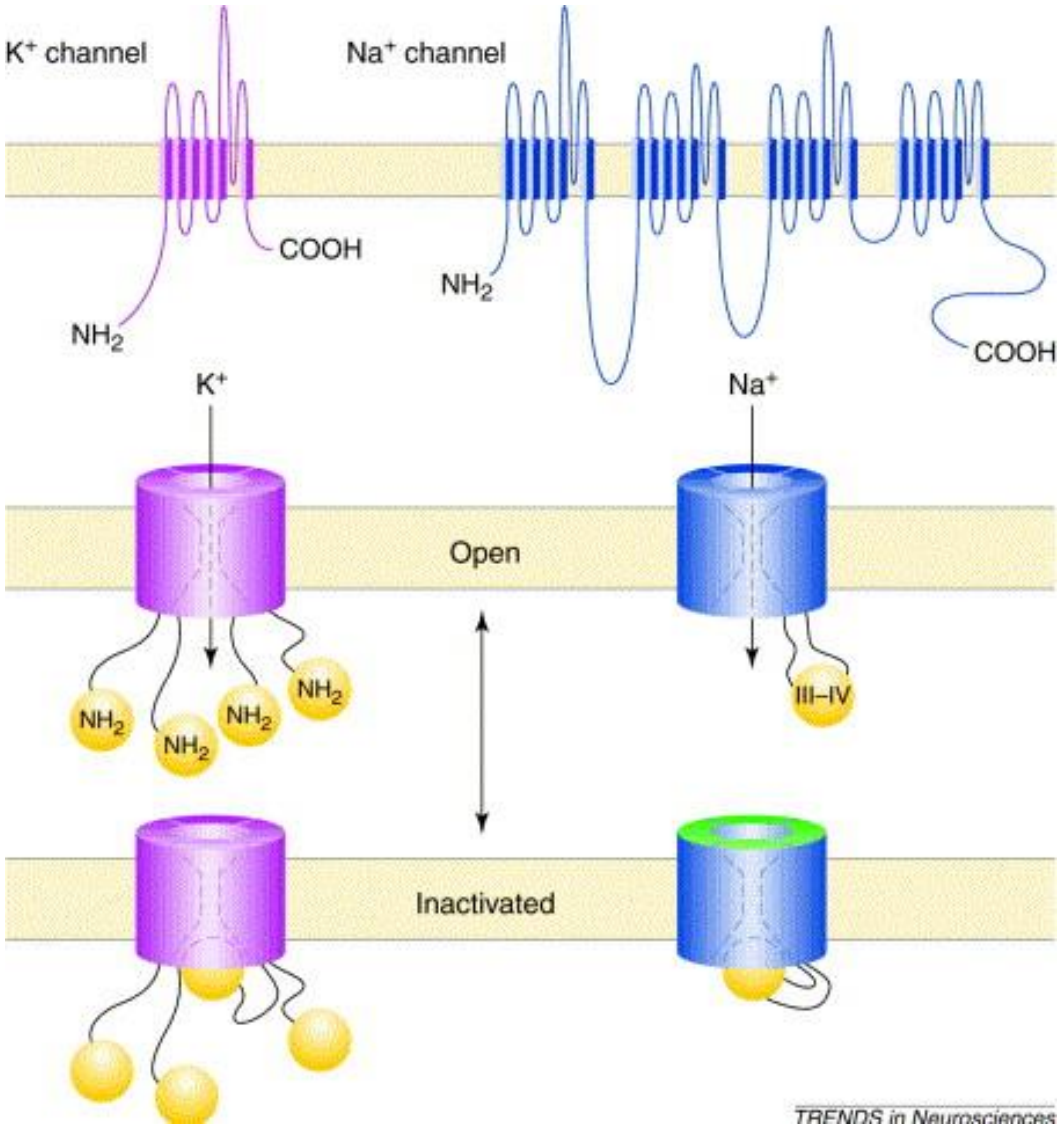


To study this voltage dependence we can plot the peak amplitude of the current as a function of the V impulse imposed. These graph normally well described by a Boltzmann relation similarly to the one describing the V-dependence of the activation

$$Po = \frac{1}{1 + \exp \frac{e\,(V - V_{1/2})}{kT}}$$

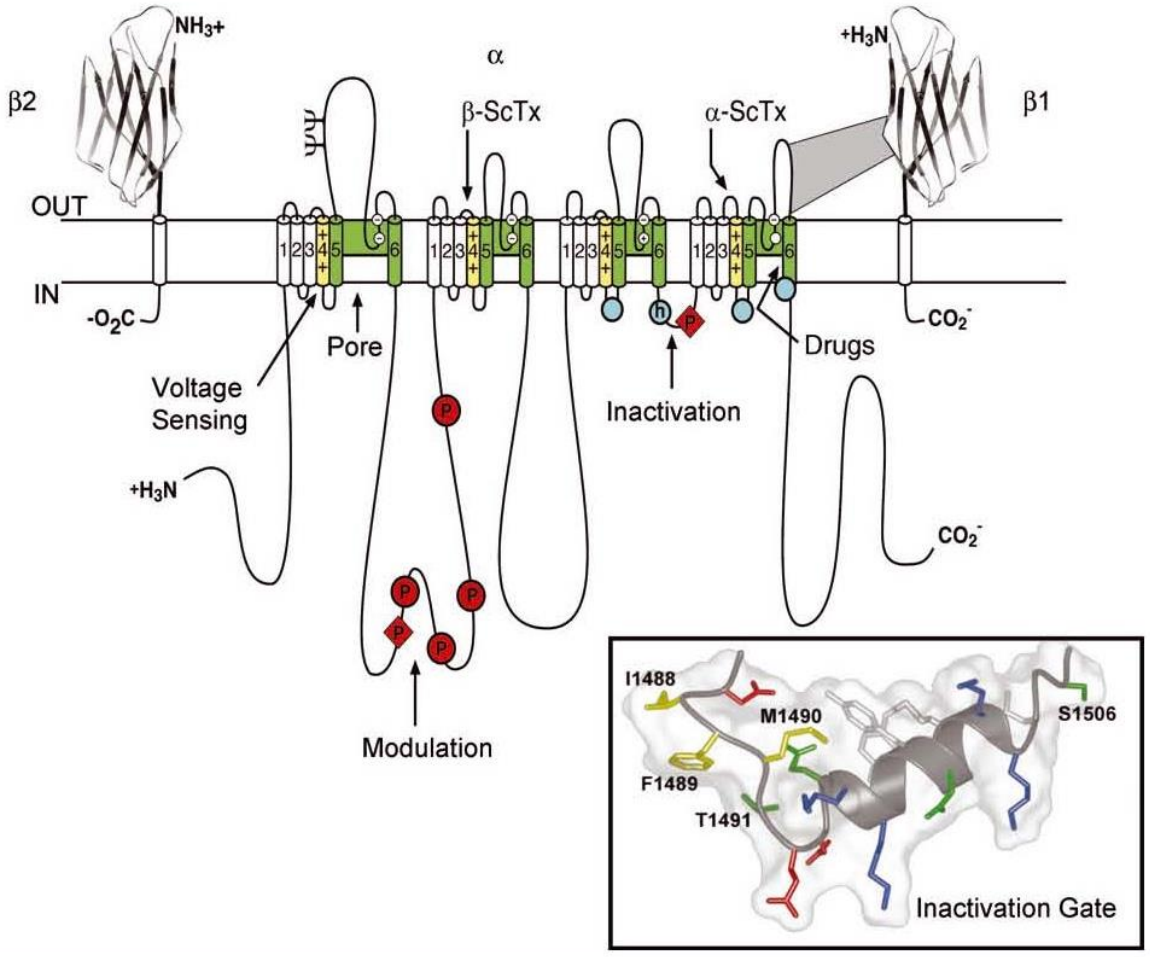
Inactivation of Voltage-gated channels

From the molecular point of view, inactivation is due to intracellular component of the inactivating channels.
Both K+v Shaker and Na+v present the inactivating domain at the N term of the domain = BALL AND CHAIN mechanism



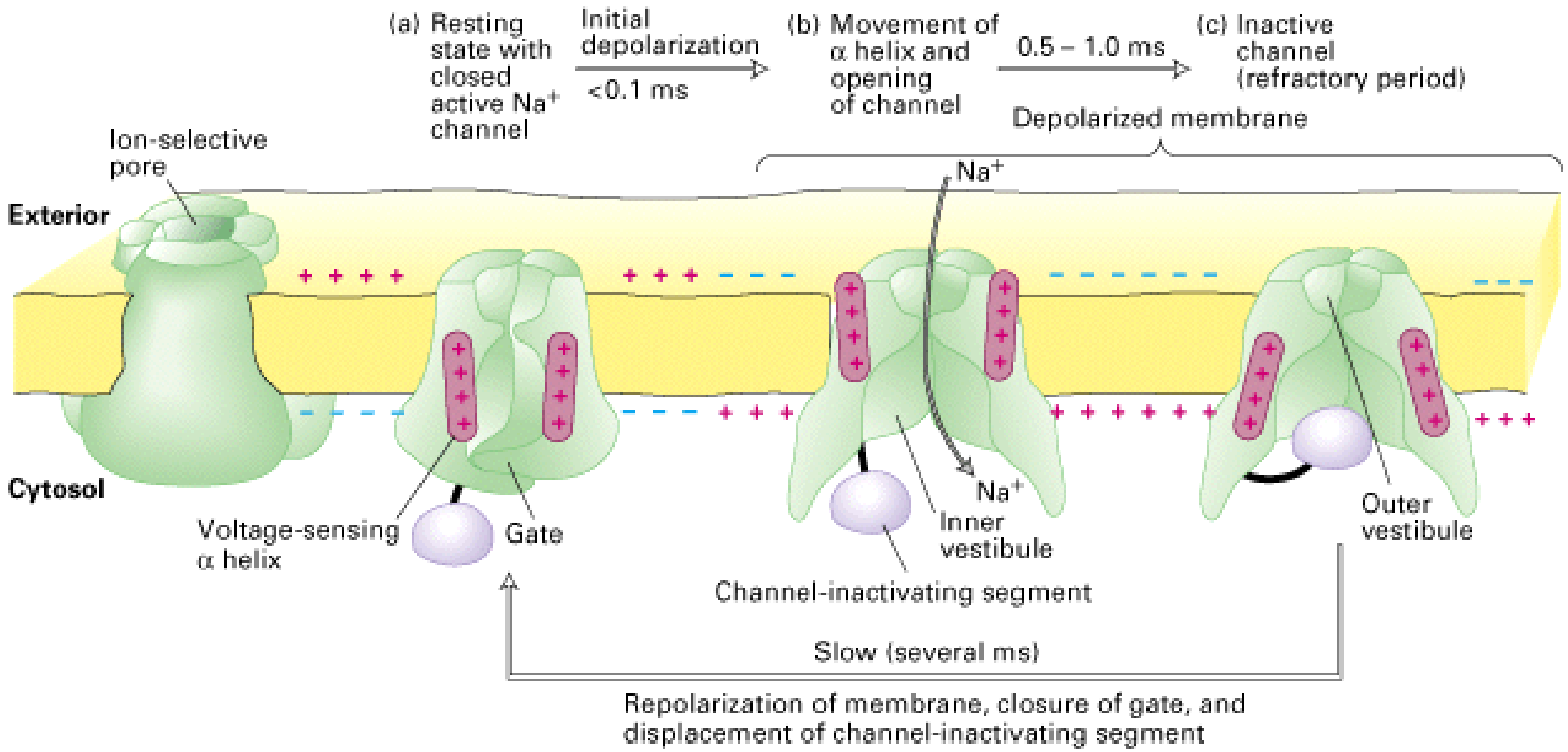
Inactivation of Voltage-gated channels

As regarding Na+v the inactivation domain is located between the third and fourth domain



Catterall WA, J Physiol 2012

Structure and function of Na+ Voltage-gated channel



Gating mechanisms : Ligand-dependent gating

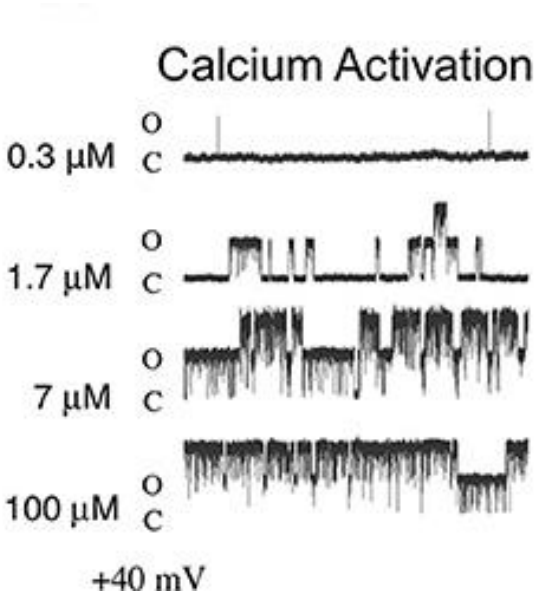
- For ligand-gated Ion channels, the conformational transitions and p_o are controlled by agonists.
- The ligand can be extracellular as in the case of channels activated by neurotransmitters
- Or intracellular as in the case of K+ channels activated by Ca2+ (K_{Ca}).

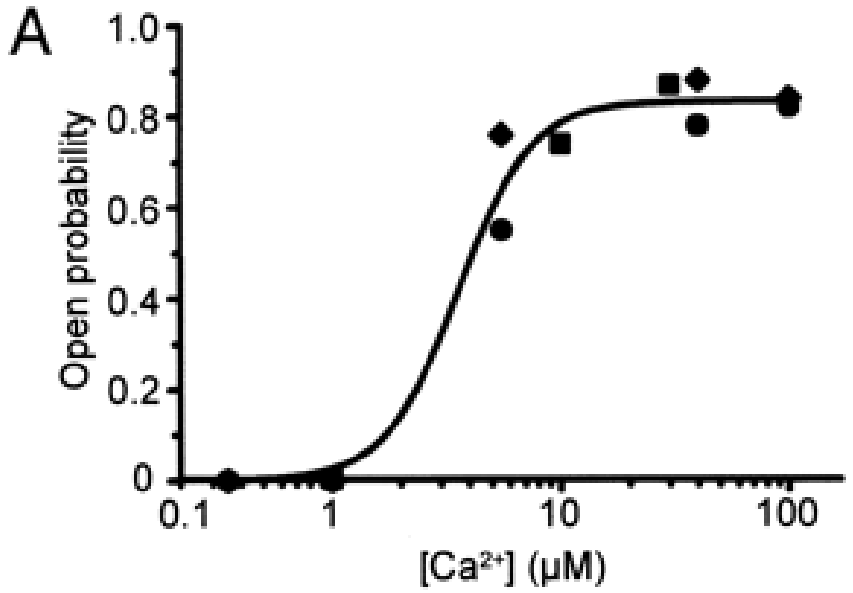
Gating mechanisms : Ligand-dependent gating

- Example:

BK = $K_{Ca\text{-}BK}$

Vm is constant and [Ca2+]i chages. Increasing [Ca2+]i po icreases





Gating mechanisms : Ligand-dependent gating

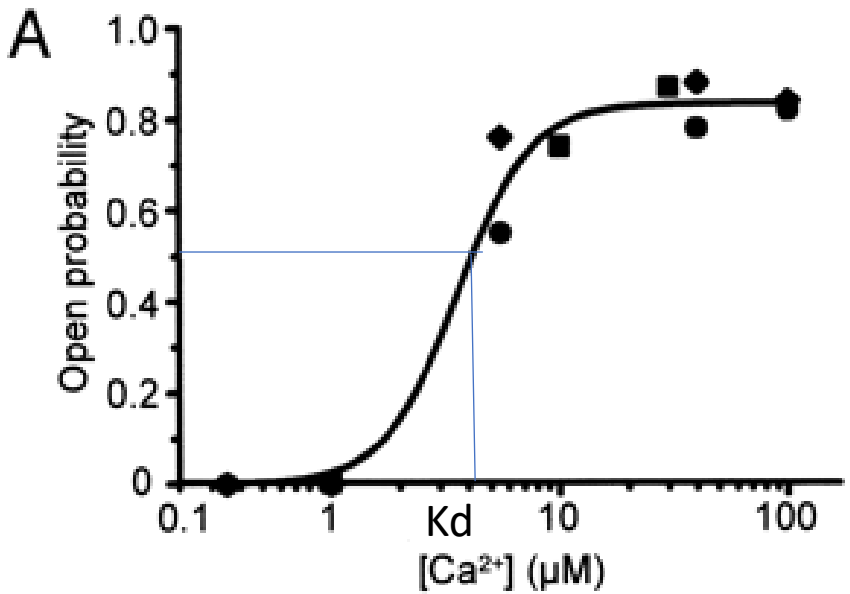
- *Po*- [Ca^{2+}] relation is well described by *Hill equation*

$$Po = \frac{1}{1 + \left(\frac{Kd}{c_A}\right)^n}$$

C_A = Agonist Concentration

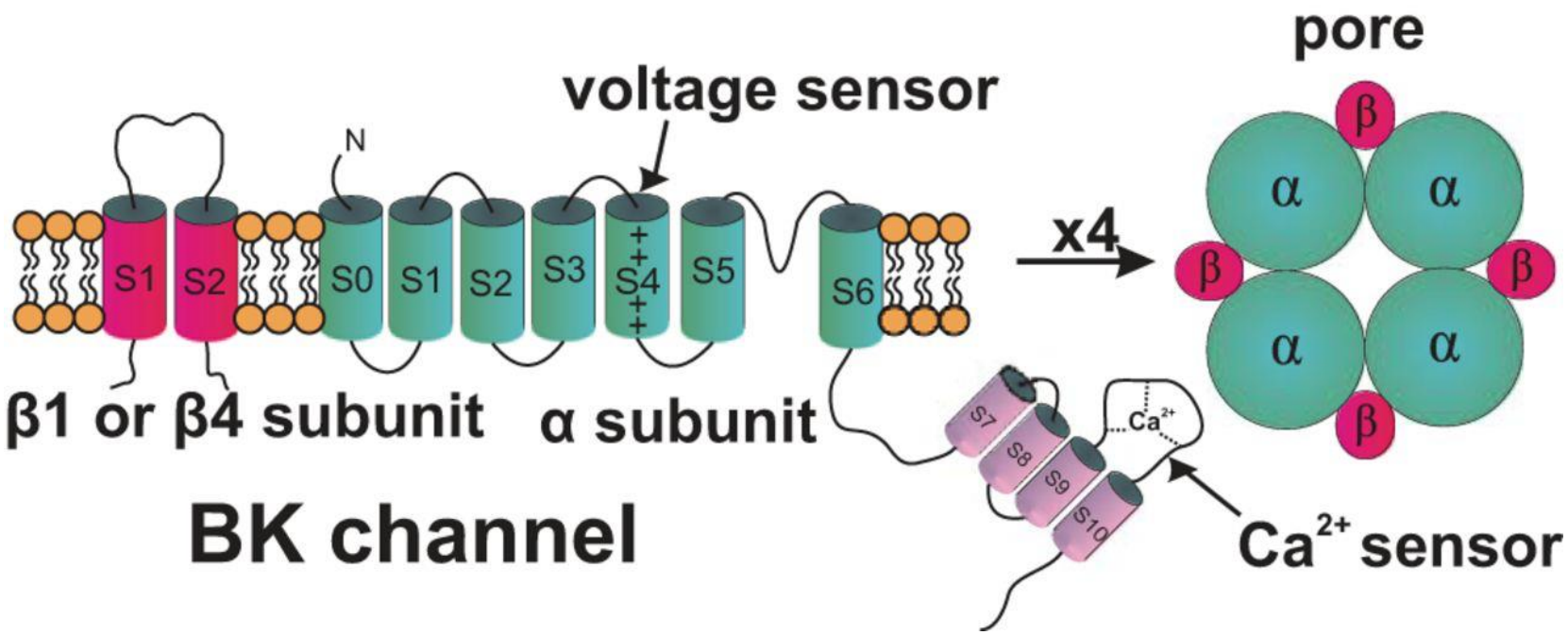
Kd = Dissociation constant

n = constant indicating the speed of the relation. The bigger is n the shorter is the concentration interval required to take Po to 1



Gating mechanisms : Ligand-dependent gating

- Example: **BK = $K_{Ca\text{-}BK}$**



Molecular characteristics

- 7 TM domains. S0 at the N-term in addition to S1-S6 also present in V-gated channels
- Intracellular C-term with 4 α-helics (S7-S10) =responsible of Ca2+ sensitivity of the channel. Present high negative charges.

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Thank you