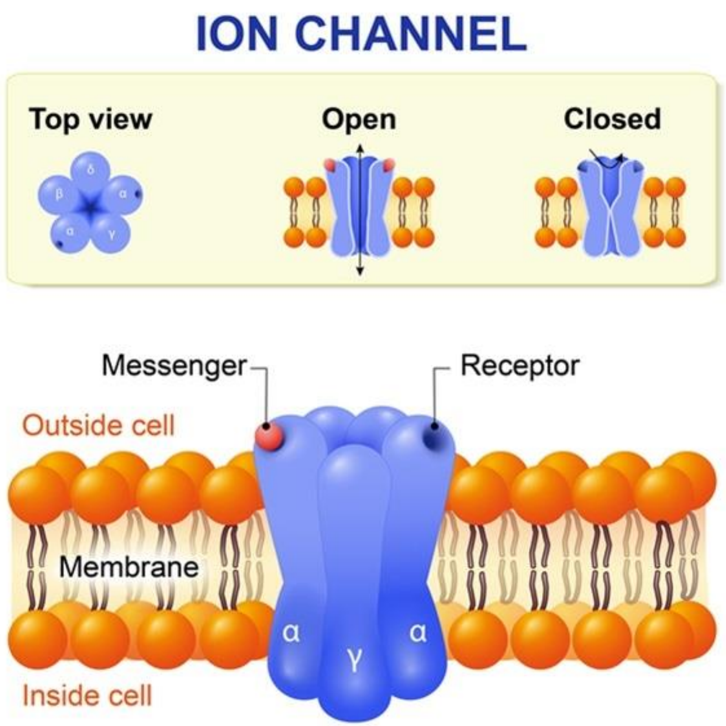
Ion Channels

1- Voltage Gated Ion Channels

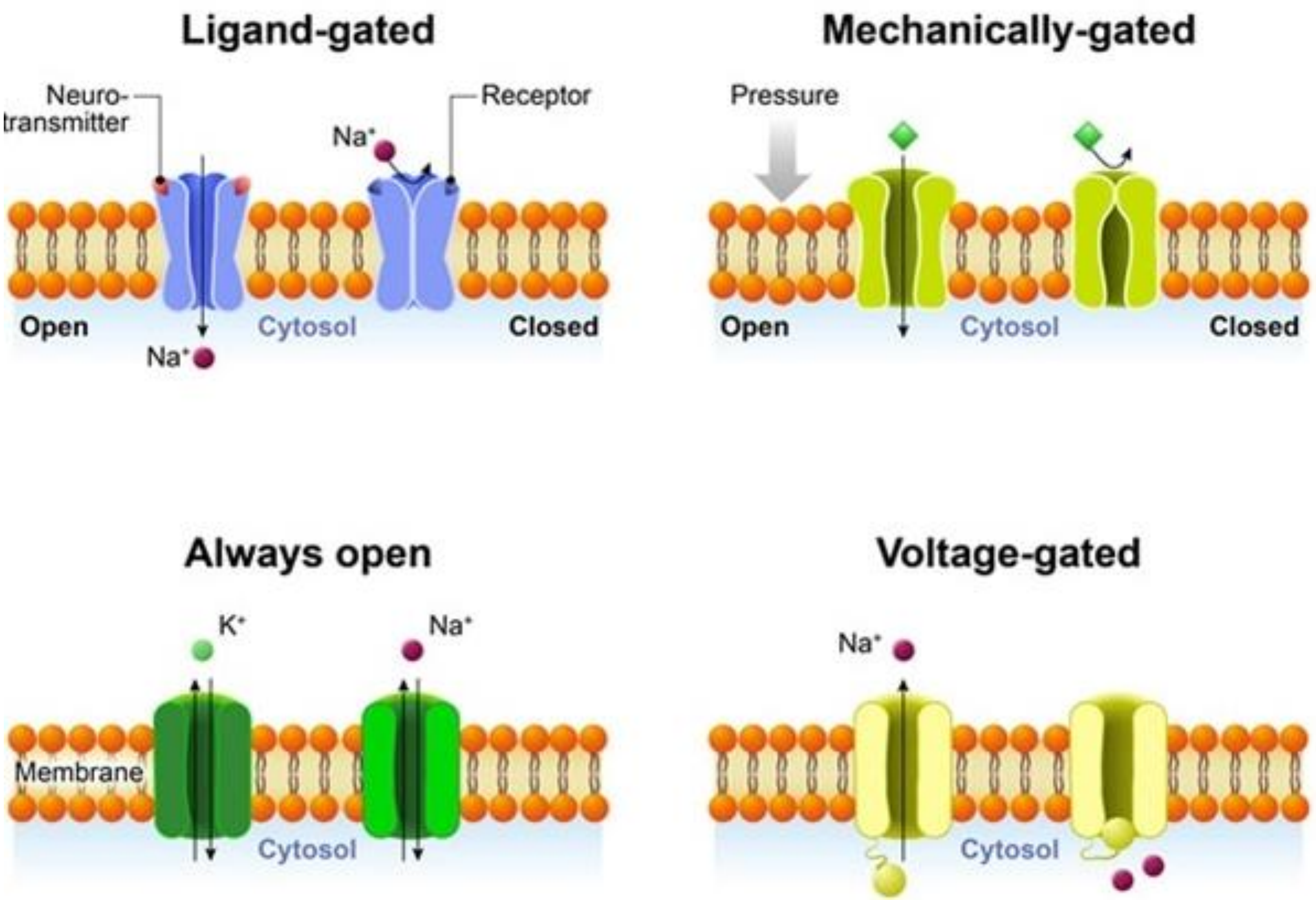
Ion Channels

Ion channels are pore-forming membrane proteins whose function is
- establishing a resting membrane potential,
- shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane,
- controlling the flow of ions across membranes,
- regulating cell volume.

Their activation translates into a rapid physiological effect

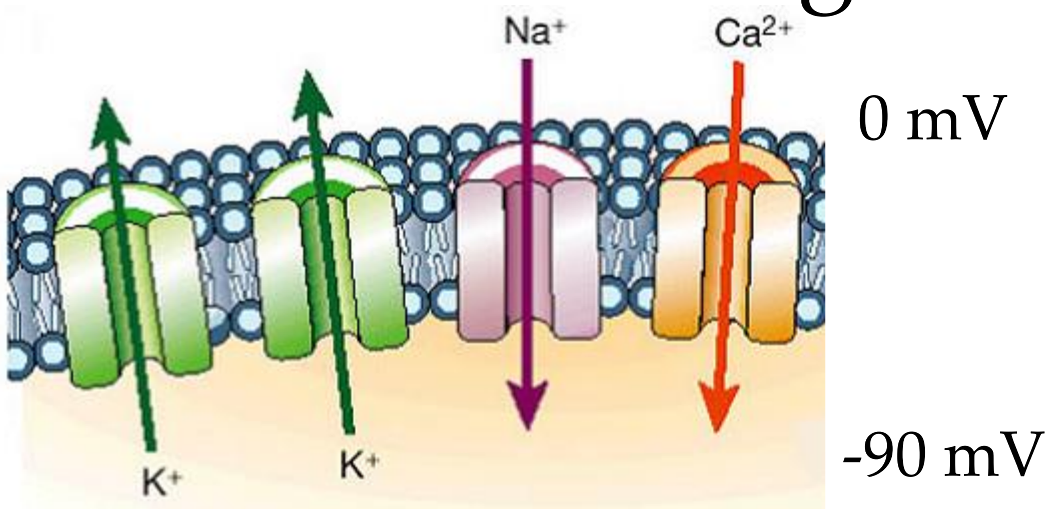


Different classes of ion channels



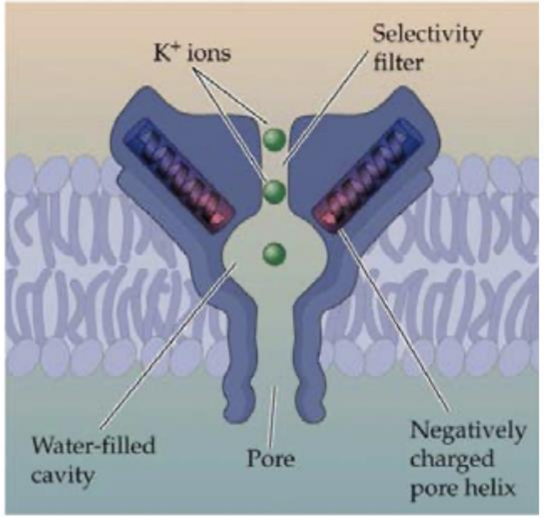
Which force moves the ions?

The electrochemical gradient



Ion	Intracellular concentration (mM)	Extracellular concentration (mM)
K+	140	4
Na+	15	145
Cl-	4	110
Ca2+	0.0001	5

What are Ion Channels ?



- **Ion channels - structure**

– are proteins that span (or traverse) the membrane
– have water-filled 'channel' that runs through the protein
– ions move through channel, and so through membrane

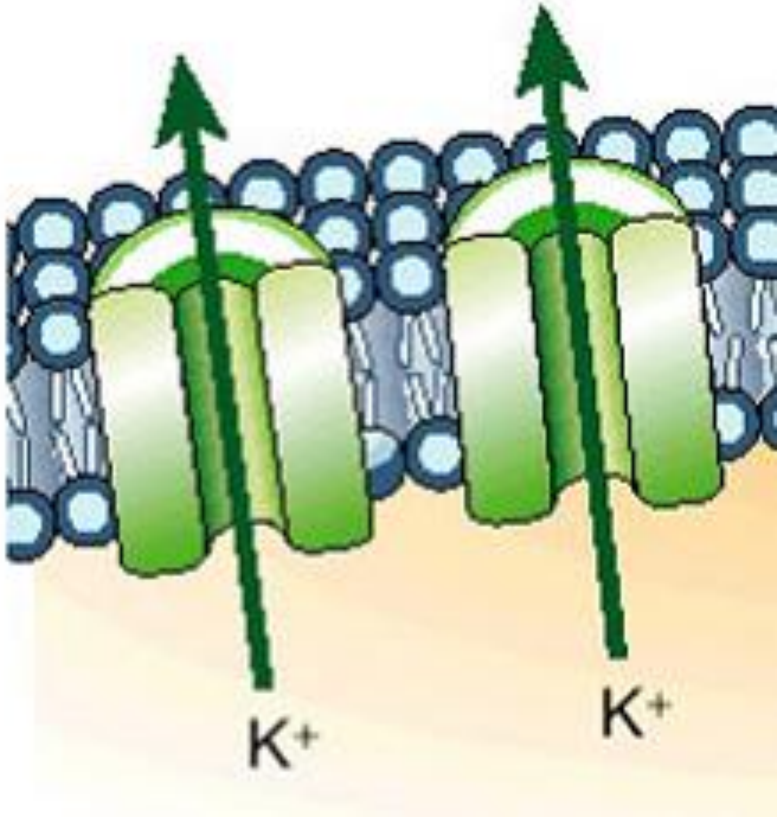
- **Ion channel - properties**

– Selectivity: Each specific ion crosses through specific channels
– Gating: transition between states (closed ↔ open ↔Inactivated)
Voltage-gated ; Ligand-gated
– Channels mediate ion movement down electrochemical gradients.
– Activation of channel permeable to ion X shifts membrane potential towards to its Equilibrium Potential, E_X

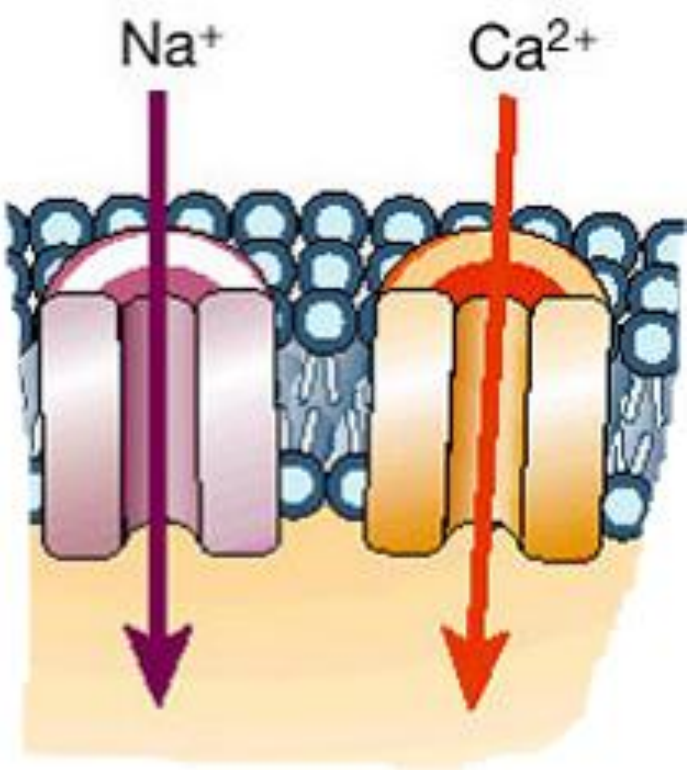
Physiological Function of Ion Channels

- **Maintain cell resting membrane potential:** inward rectifier K and Cl channels.
- **Action potential and Conduction of electrical signal:** Na, K, and Ca channels of nerve axons and muscles
- **Excitation-contraction (E-C) coupling:** Ca channels of skeletal and heart muscles
- **Synaptic transmission at nerve terminals:** glutamate, Ach receptor channels
- **Intracellular transfer of ion, metabolite,** propagation: gap junctions
- **Cell volume regulation:** Cl channel, aquaporins
- **Sensory perception:** cyclic necleotide gated channels of rods, cones
- **Oscillators:** pacemaker channels of the heart and central neurons
- **Stimulation-secretion coupling:** release of insulin formpancreas (ATP sensitive K channel)

What happens when ions move?



Vm -> -90 mV



Vm -> +50 mV

Equilibrium Potential or Nernst Potential

The voltage at which there is zero net flux of a given ion
(Electrical gradient = a chemical concentration gradient)

For K^+ : ~ -90 mV

$$E_K = \frac{RT}{ZF} \ln \frac{[K^+]_o}{[K^+]_i}$$

Hermann (Walther) Nernst
1864-1941
1920 Nobel Prize for chemistry

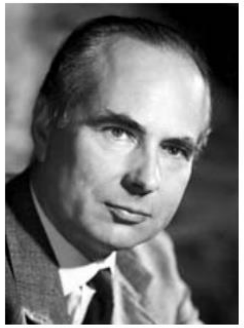
- R = gas constant
- F = Faraday constant
- T = temperature (K)
- Z = valence (charge) of ion ~

K current (I_{K1}) is the major contributor for RMP

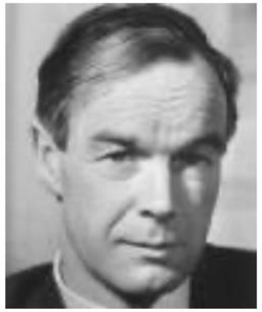
Four Milestones in Ion Channel Research

1. Ionic conductance

Noble 1963 (Physiol/Medicine)



Alan L. Hodgkin



Andrew F. Huxley

2. Patch clamp methodology

Noble 1991 (Physiol/Medicine)

Erwin Neher

Bert Sakmann

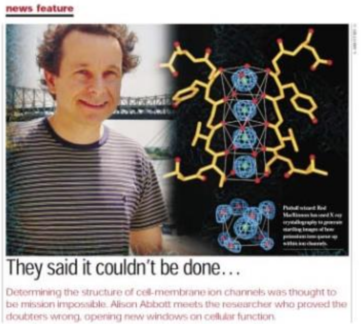
3. Channel cloning sequencing (Ach receptor, Na, Ca channels)

Japan Academy Prize 1985

Shosaku Numa (沼 正作)

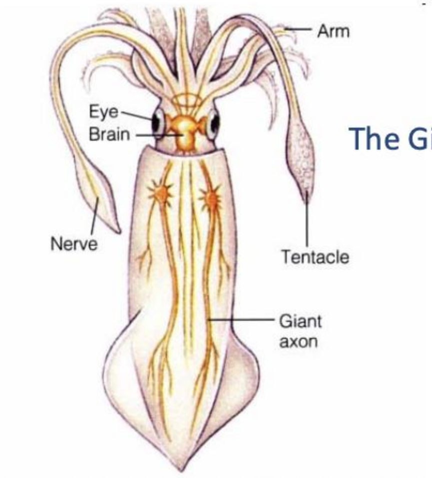
4. K channel structure

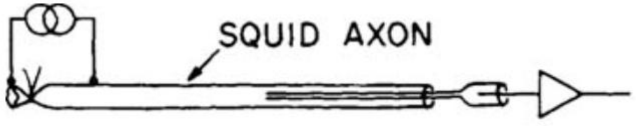
Noble 2003 (Chemistry)



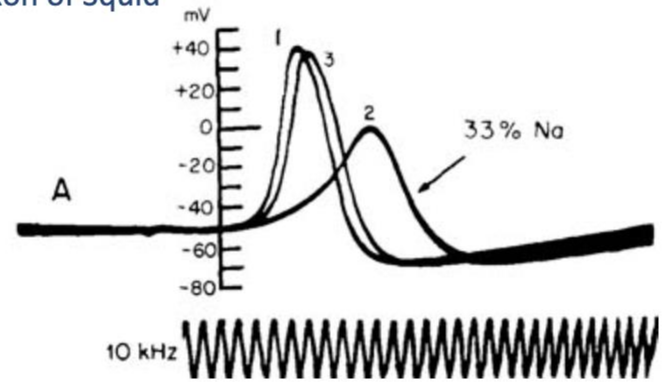
Rod MacKinnon

Hodgkin-Huxley Model Predicted the Existence of Ion Channels





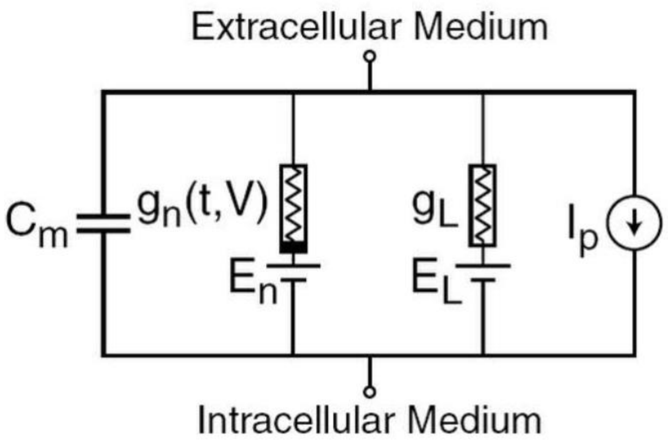
The Giant Axon of Squid

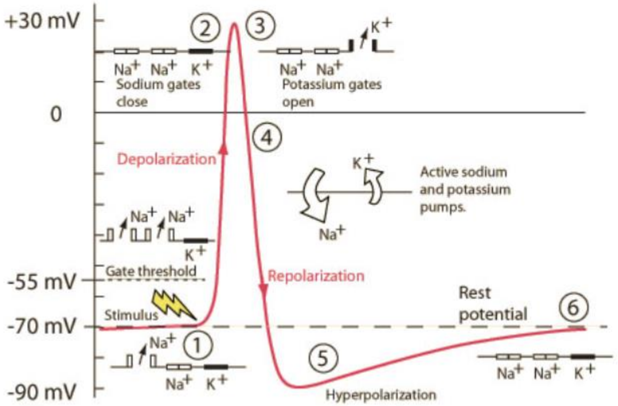


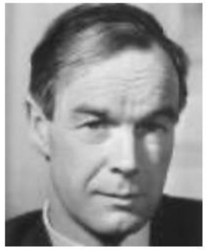
$$C\frac{dV}{dt} = \bar{g}_{Na} m^3 h (V_{Na} - V) + \bar{g}_K n^4 (V_K - V) + g_L (V_L - V) + I_{ext}$$

Na channel gating

K channel gating



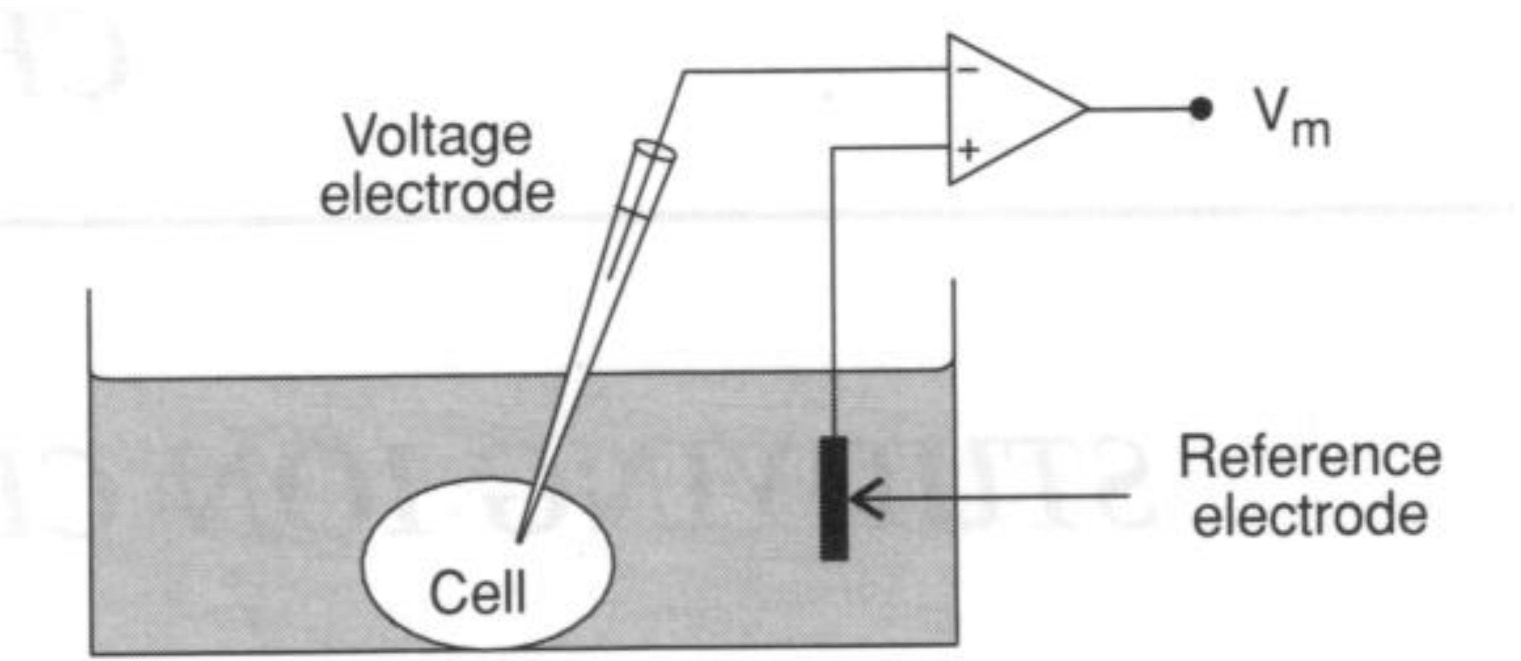




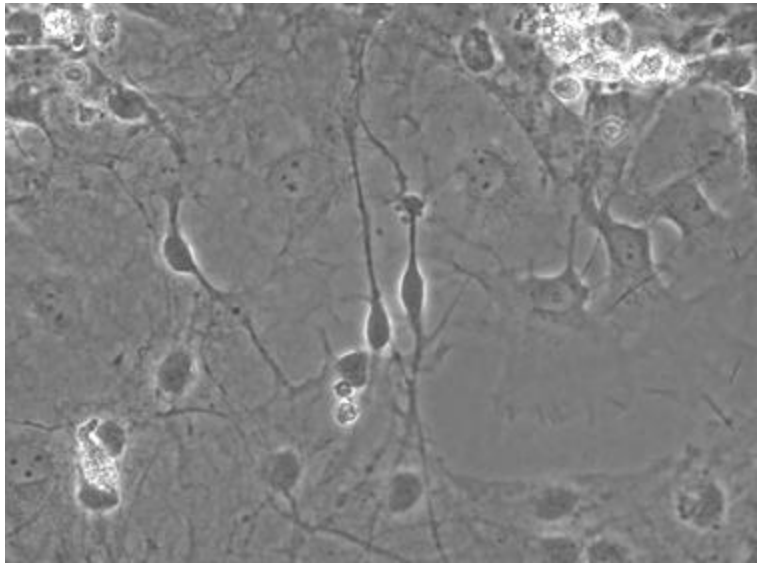
1963 noble Prize

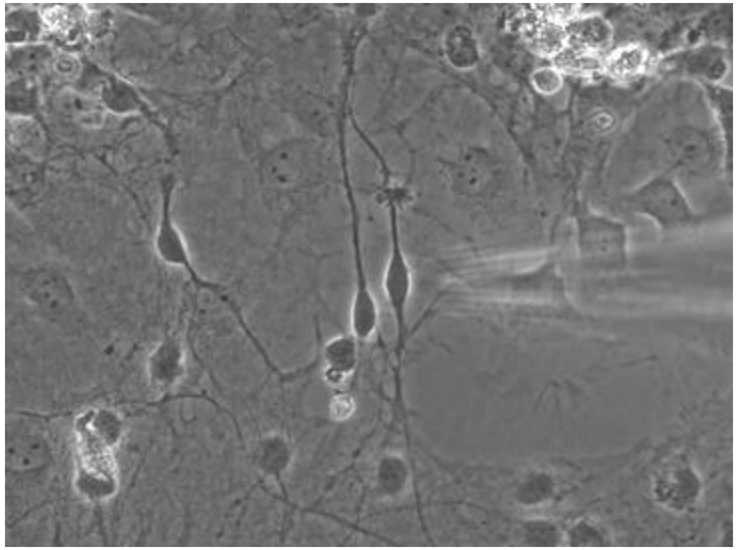
How to study ion channels?

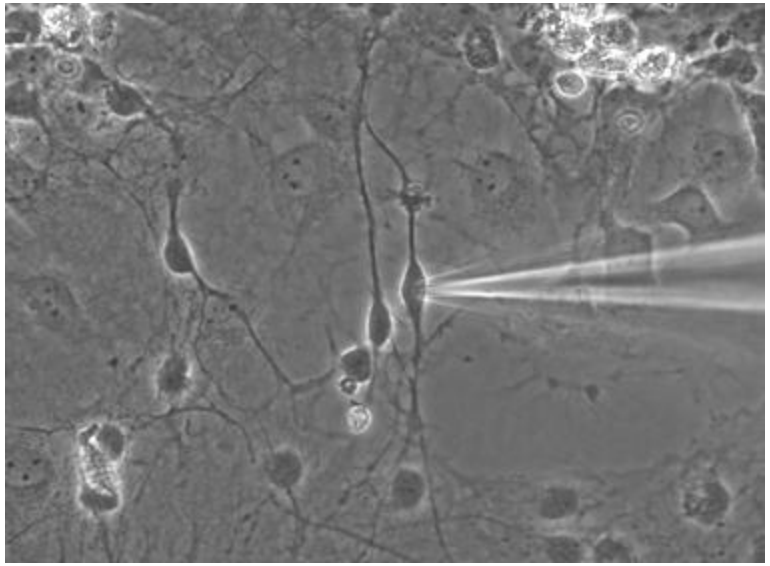
How to study ion channel function? Electrophysiological approaches

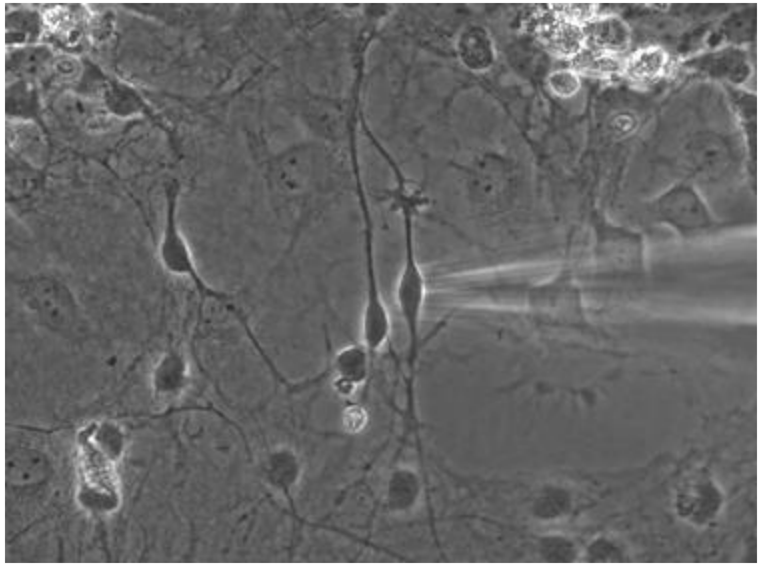


Electrophysiological recordings

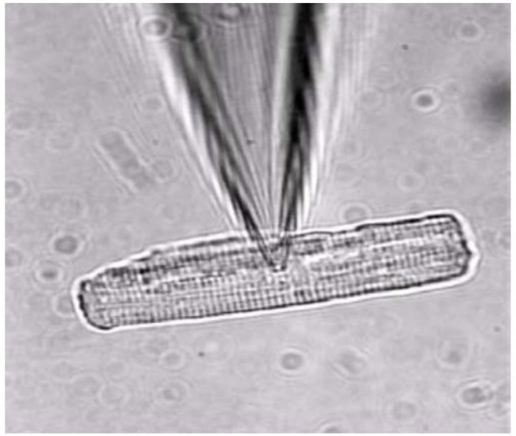


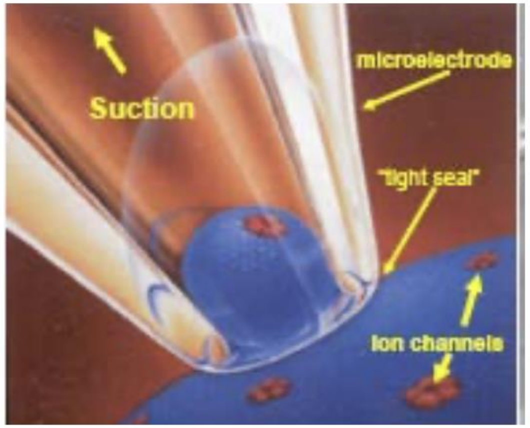


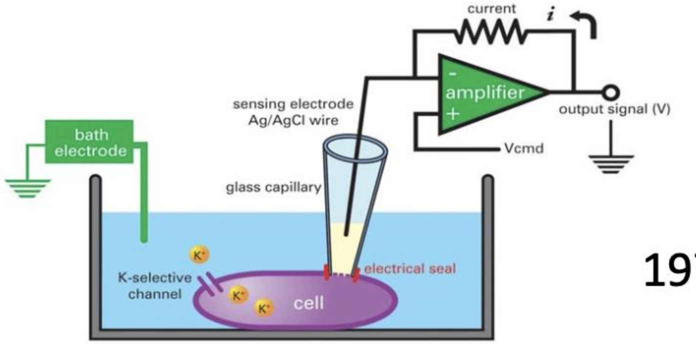




Patch-Clamp Technique

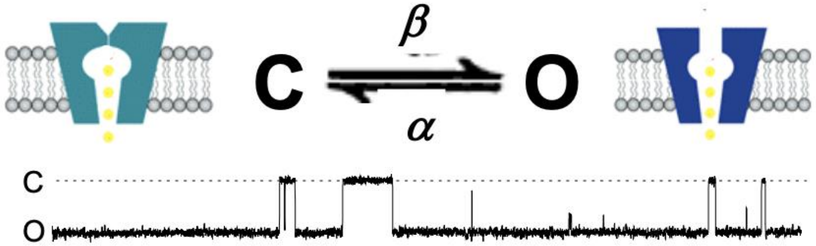






1976

Erwin Neher & Bert Sakmann
Nobel prize for medicine in 1991
for the development of the patch-clamp technique making possible the characterization of single ion channels



1991 Nobel Prize

Patch-Clamp Technique

The Patch Clamp Method

How to study ion channel function?
Molecular approaches

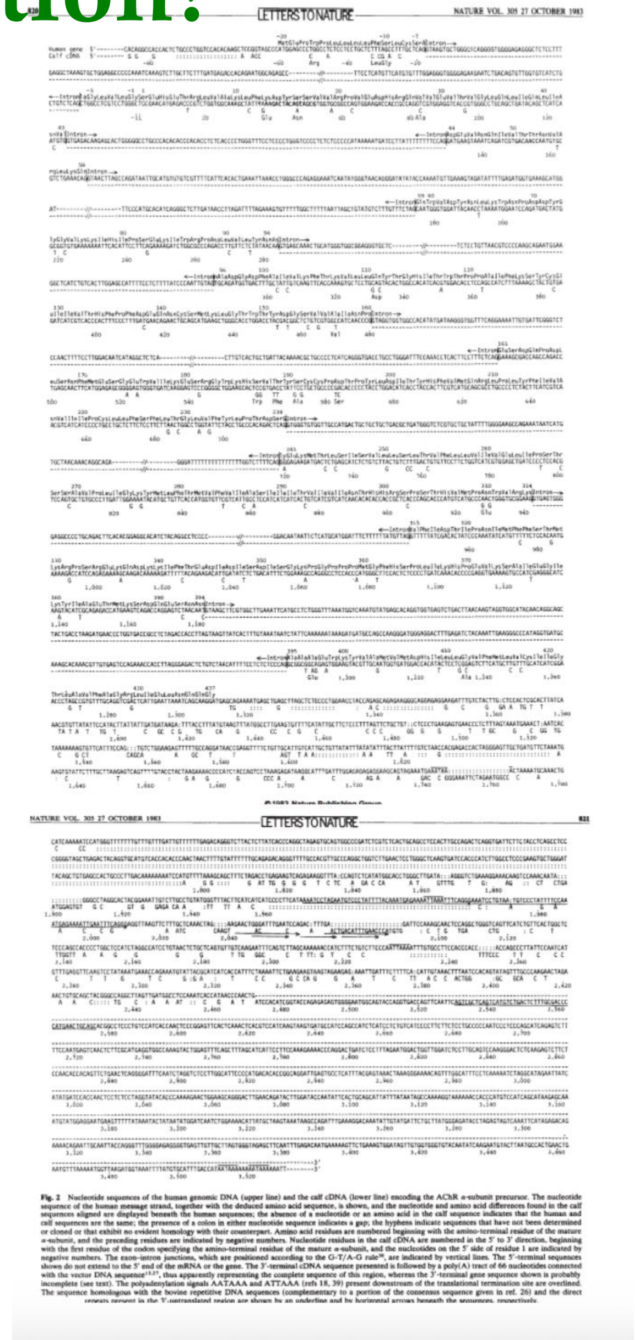
NATURE VOL. 305 27 OCTOBER 1983

Cloning and sequence analysis of calf cDNA and human genomic DNA encoding α-subunit precursor of muscle acetylcholine receptor

Masaharu Noda, Yasuji Furutani, Hideo Takahashi, Mitsuyoshi Toyosato, Tsutomu Tanabe, Shin Shimizu, Sho Kikyotani, Toshiaki Kayano, Tadaaki Hirose*, Seiichi Inayama* & Shosaku Numa

Department of Medical Chemistry, Kyoto University Faculty of Medicine, Kyoto 606, Japan
* Pharmaceutical Institute, Keio University School of Medicine, Tokyo 160, Japan

Sakmann

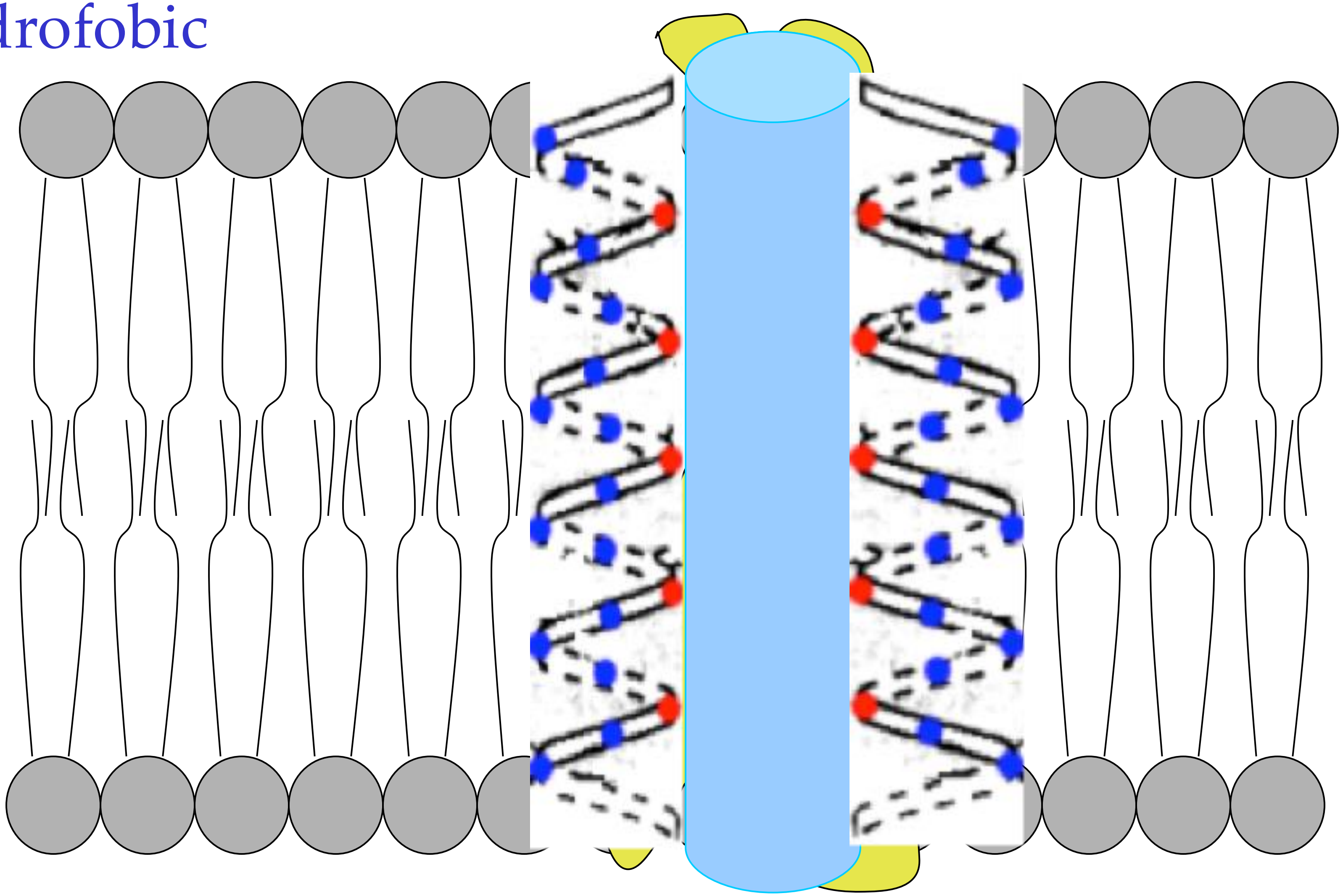


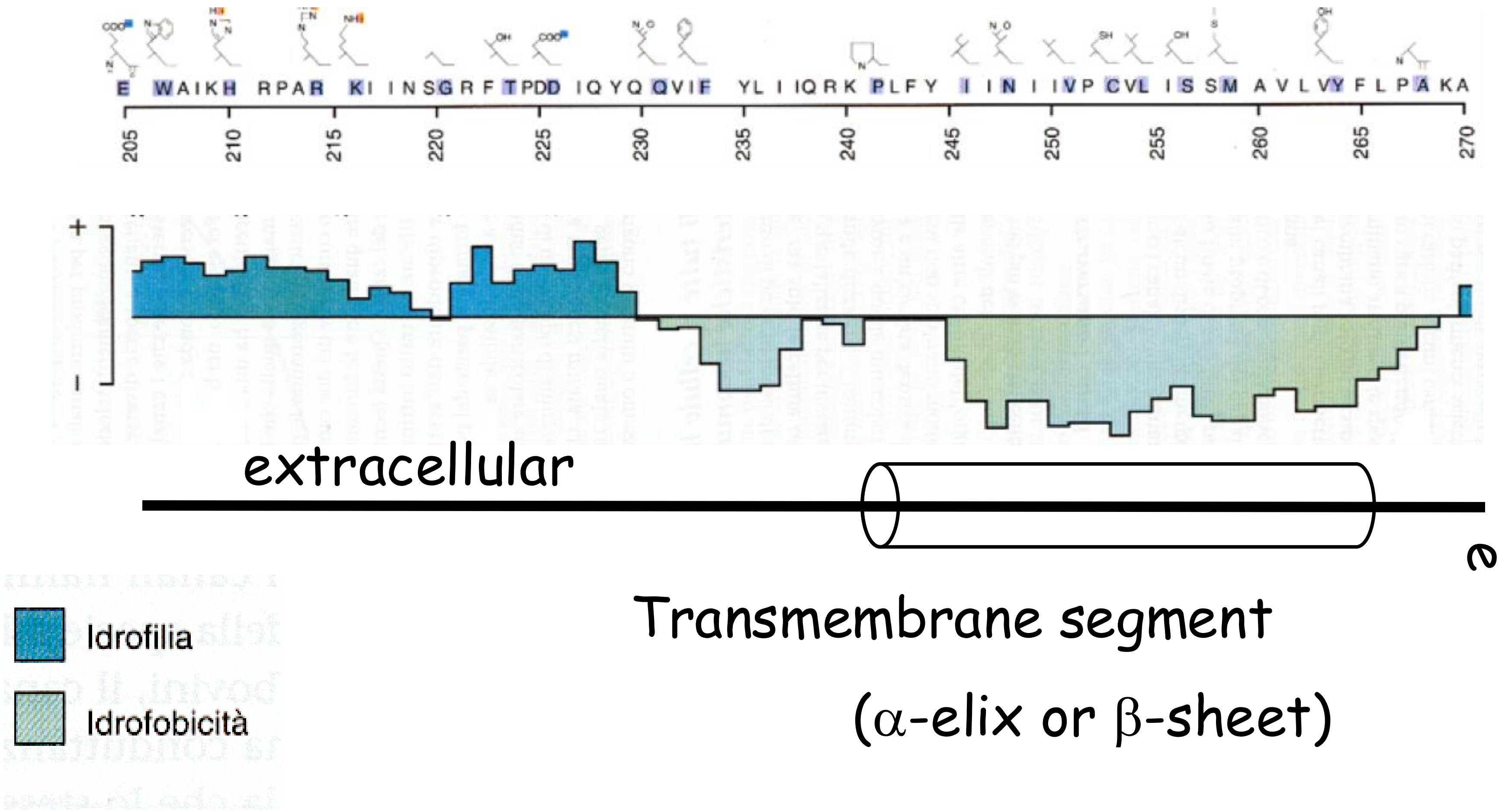
How to study ion channel function?
Structural approaches

Hydrophilic
Hydrofobic

Predictions…

Hydrophilic
Hydrofobic





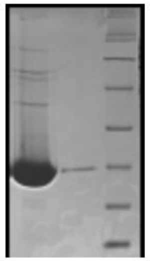
E WAIKH RPAR KI INSGRFTPDD IQYQ QVIF YL I IQRK PLFY I IN I IVP CVL IS SM AVLVY FLPA KA
205 210 215 220 225 230 235 240 245 250 255 260 265 270
+
-
extracellular
Transmembrane segment
(α-elix or β-sheet)
Idrofilia
Idrofobicità

Structure

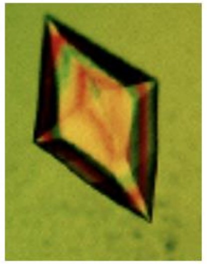
Crystal structure of ion channels

Protein x-ray crystallography

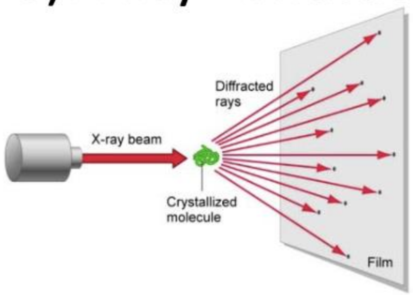
1) Purification

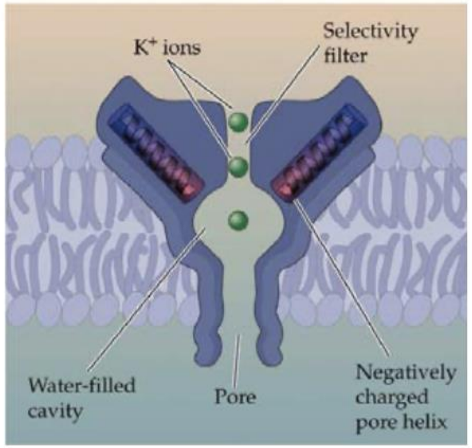


2) Crystallization



3) X-Ray Diffraction





The Nobel Prize in Chemistry 2003
Peter Agre, Roderick MacKinnon

Classification of Ion Channels

1) Based on ion selectivity:

K^+, Na^+, Ca^{2+}, Cl^- channels

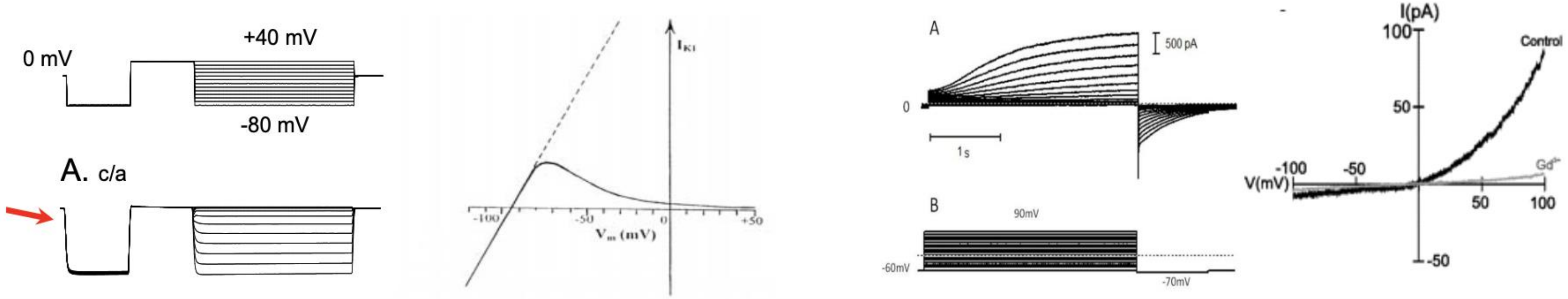
2) Based on gating:

Voltage-gated : ions

Ligand-gated: Glutamate, GABA, ACh, ATP, cAMP

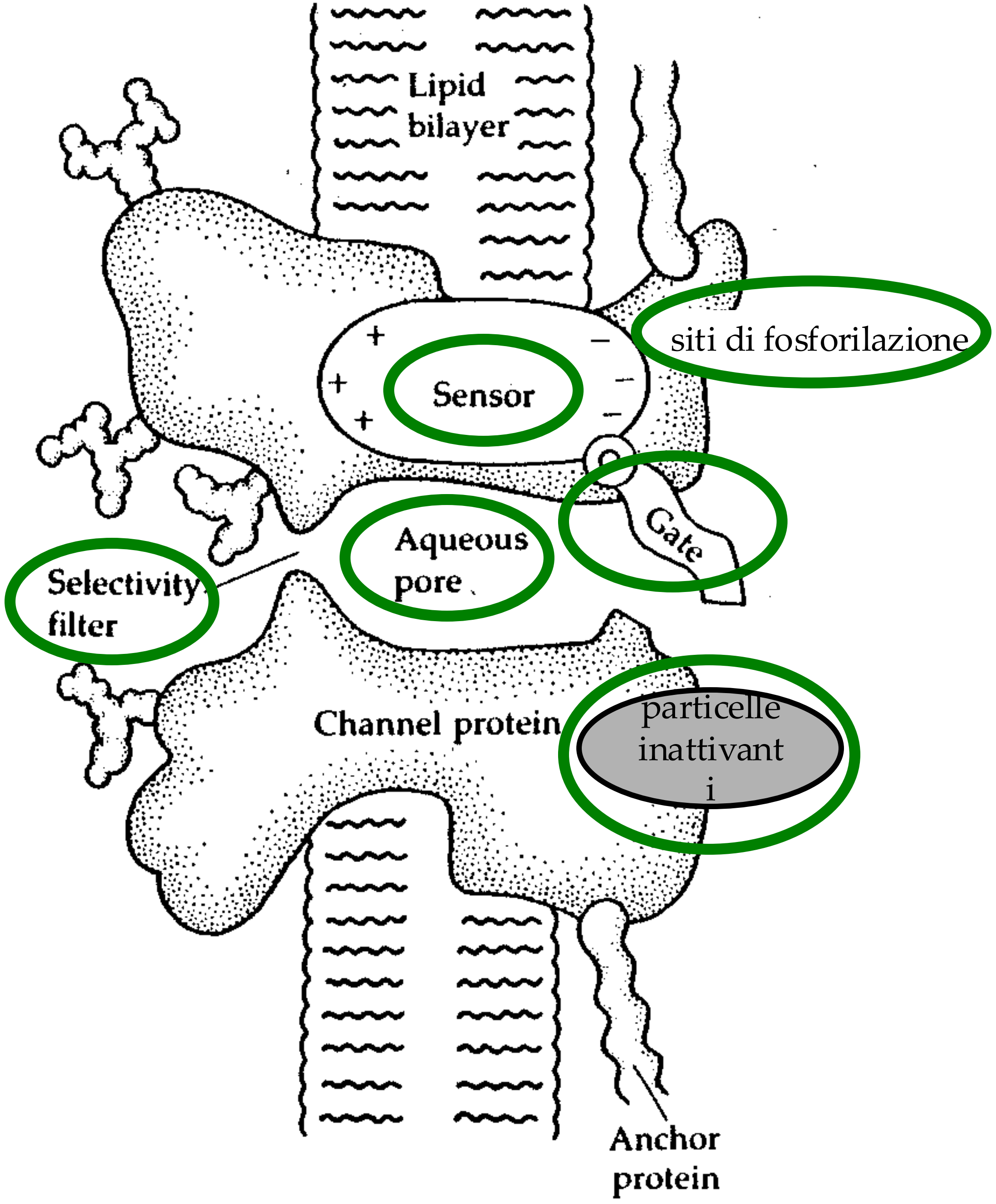
3) Based on rectification:

Inwardly or outwardly rectifying



Structural motifs

ie: what's in all the channels?



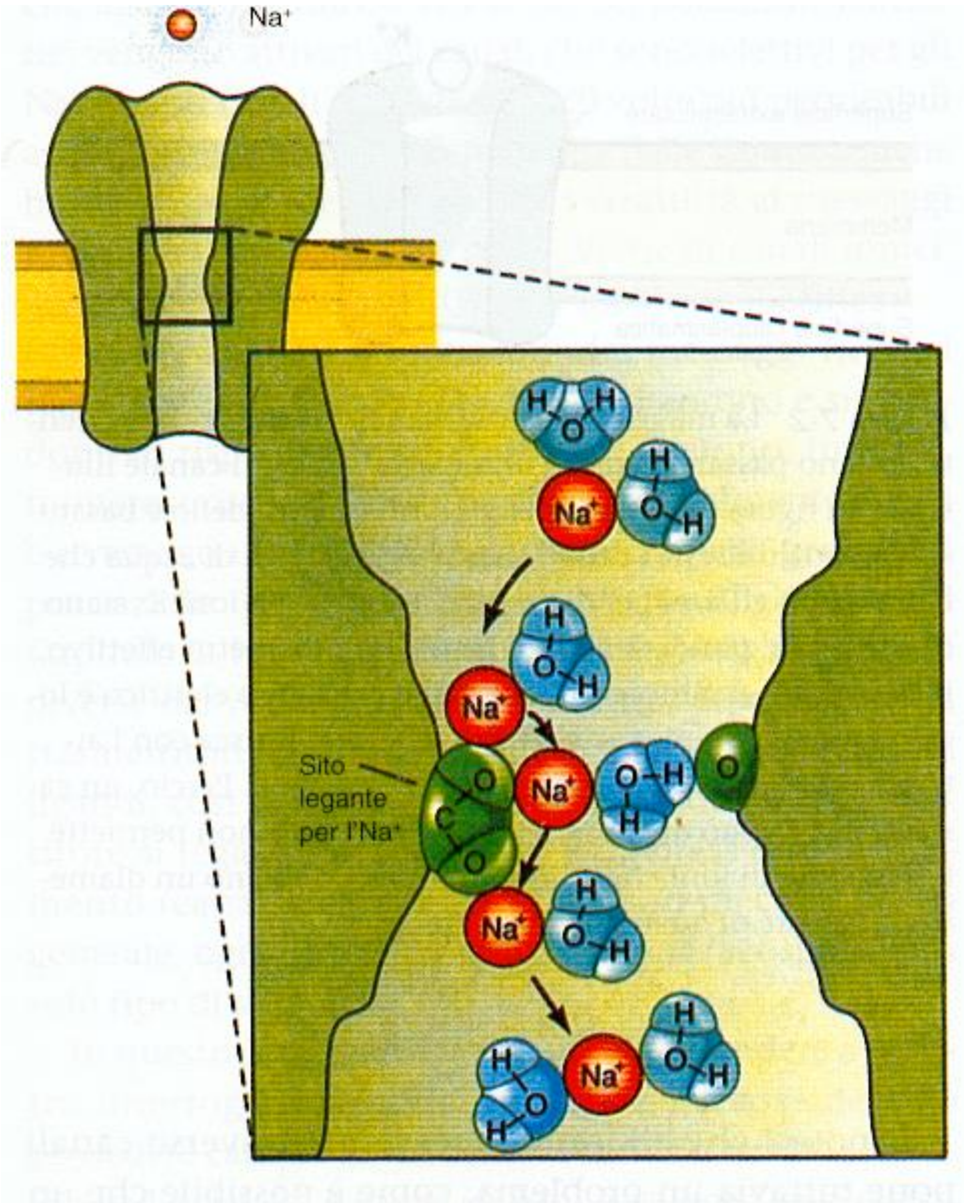
Selectivity filter (3/4 aa)

Ions interact with charged aa

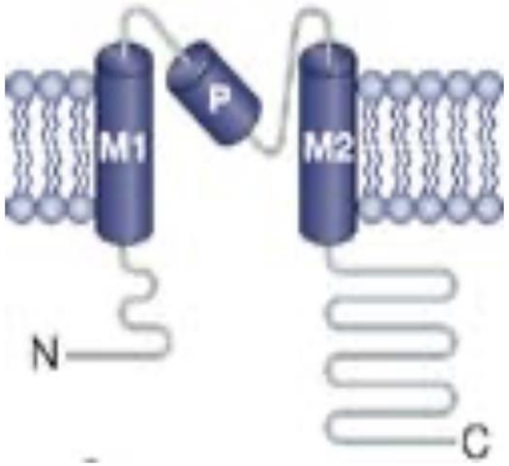
1) Each channel excludes ions of dimensions> characteristic value. Es. K 3A°, Ca 5A°…

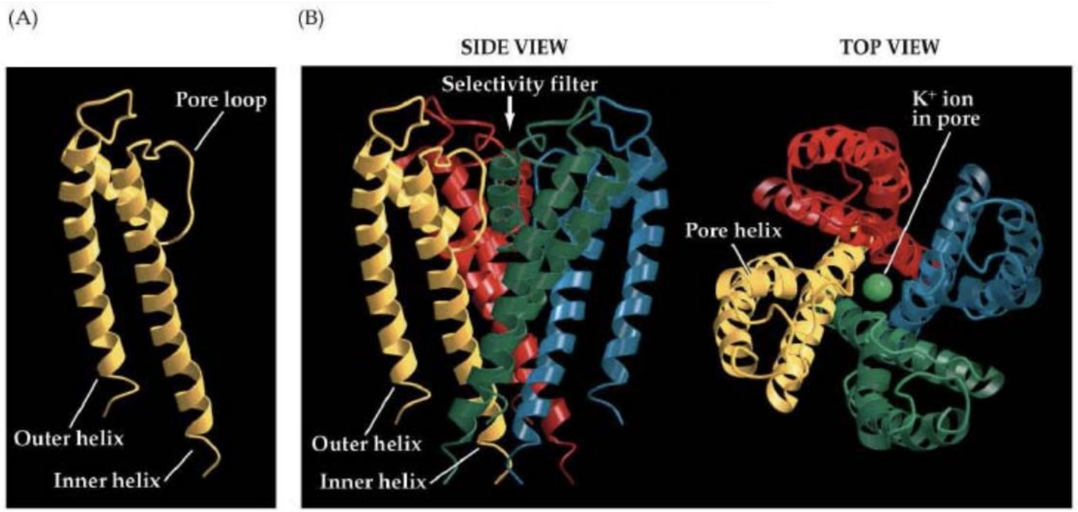
2) Important charges aa are cations binding sites - and exclude ion water hydration molecules

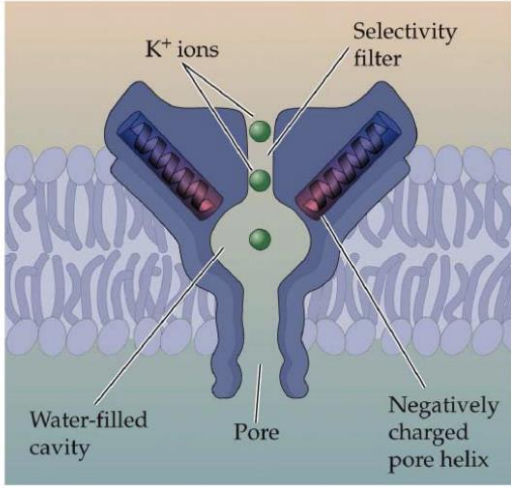
3) Due to the reduced size of the filter important steric effects occur, more ions occupy -greater repulsion (2 Ca or 4 Na neutralize 4 + charges)



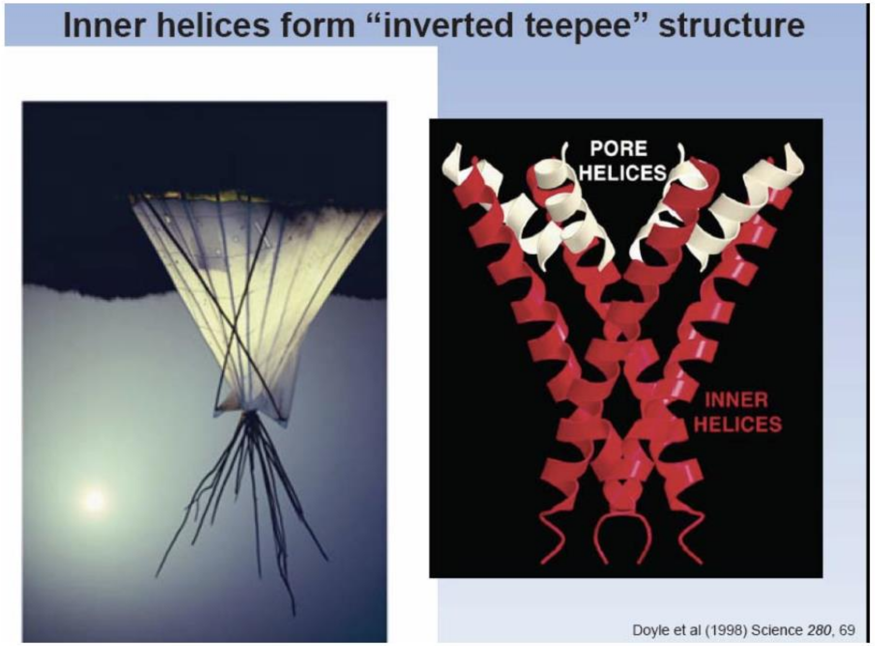
Structure of KCSA Channels: Selectivity Filter and Gating





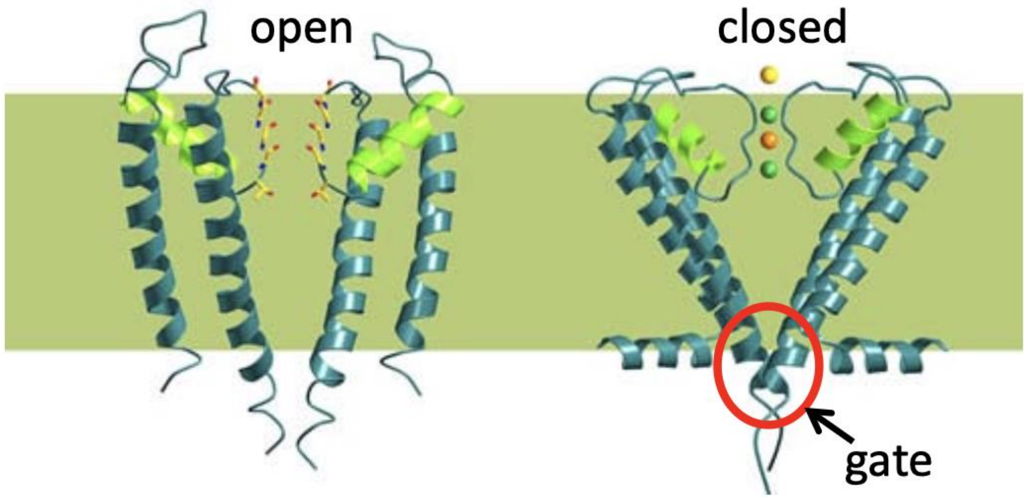


profile



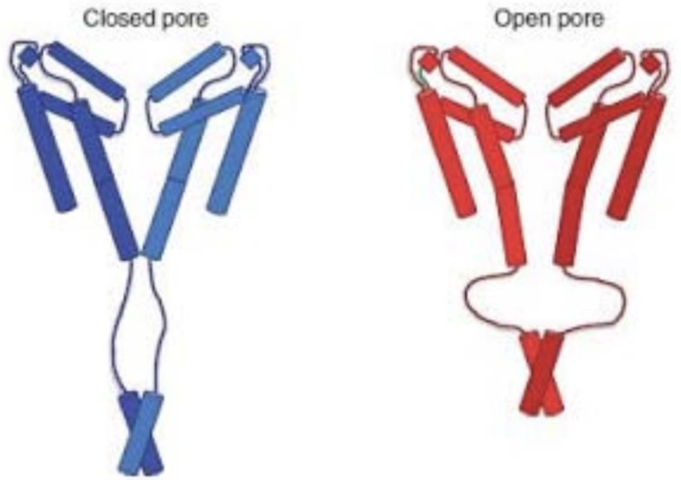
Doyle *et al*. Science 1998;

Open-Close Gating



Doyle *et al*. Science 1998;

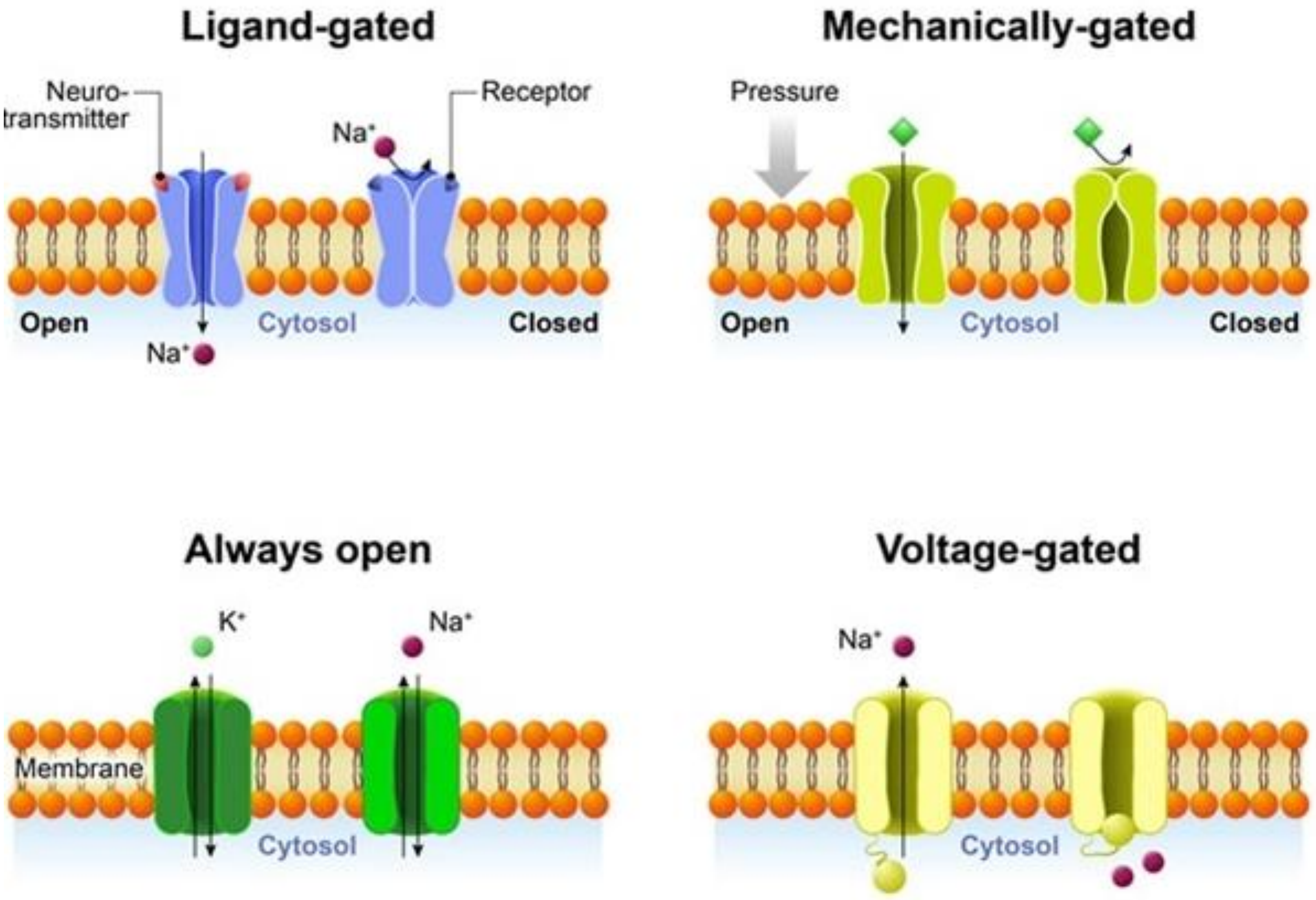
Bacterial K channel
selective filter: P-loop; **Gating: intracellular side of the pore bundle crossing**



Bacterial Na channel pore in the closed and "open" conformation

How may we Open/Close a channel?

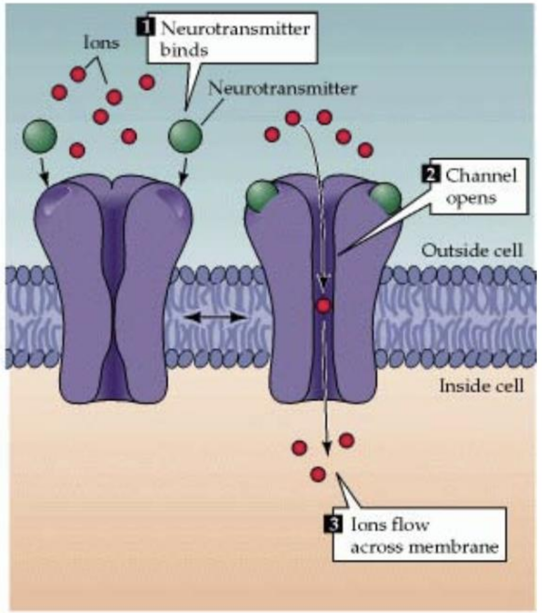
Different classes of ion channels



Ligand-Gated Ion Channels

ACh receptor channel

ACh



ATP-sensitive K channel

$$C \underset{+\,ATP}{\overset{-ATP}{\rightleftharpoons}} O$$

Also a weak inward rectifier

- Open when a signal molecule (ligand) binds to an extracellular receptor region of the channel protein.

- This binding changes the structural arrangements of the channel protein, which then causes the channels to open or close in response to the binding of a ligand such as a neurotransmitter.

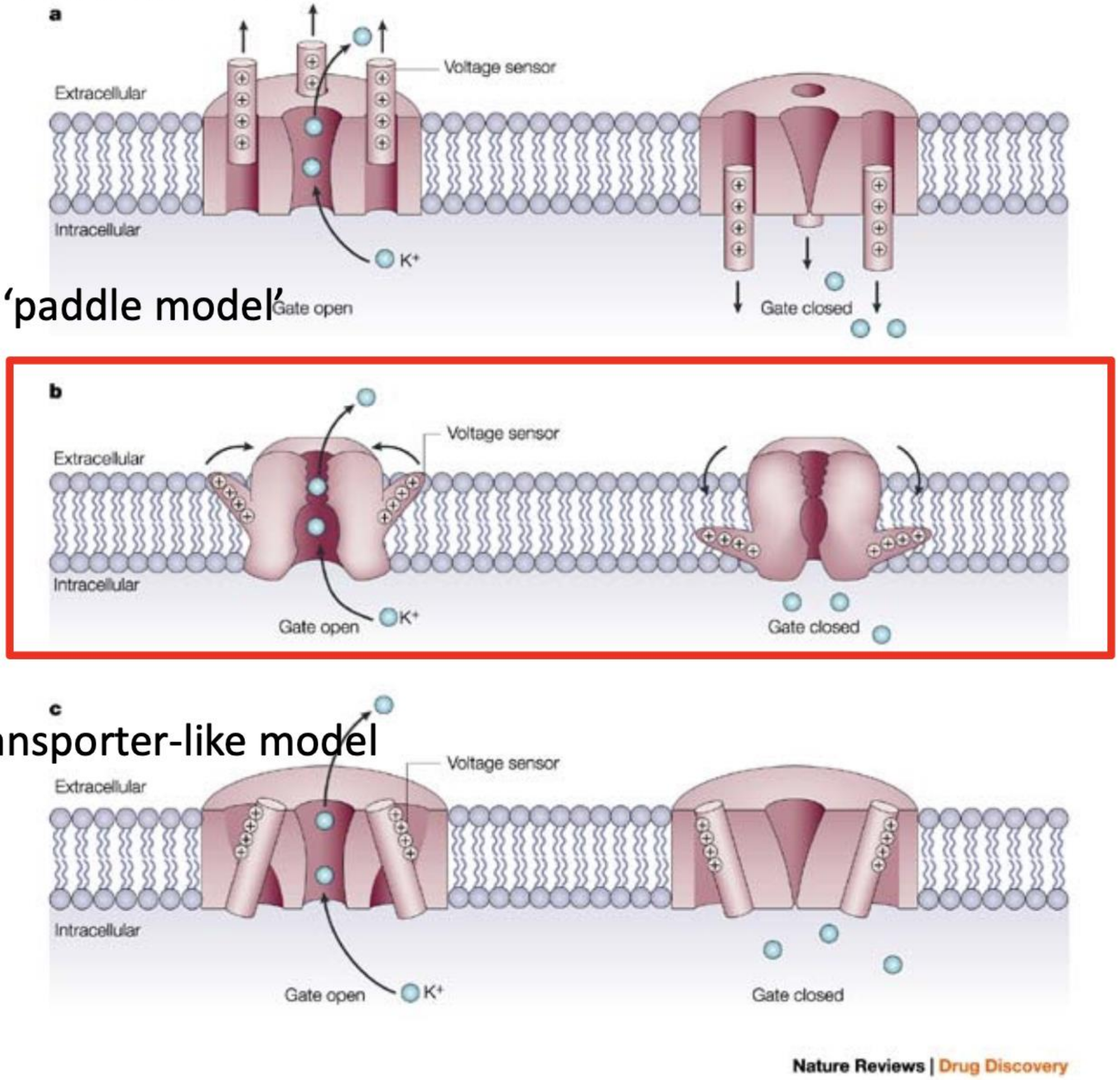
- This ligand-gated ion channel, allows specific ions (Na+, K+, Ca2+, or Cl-) to flow in and out of the membrane.

Models for Voltage Gating

The conventional model

A new ‘paddle model’

The transporter-like model



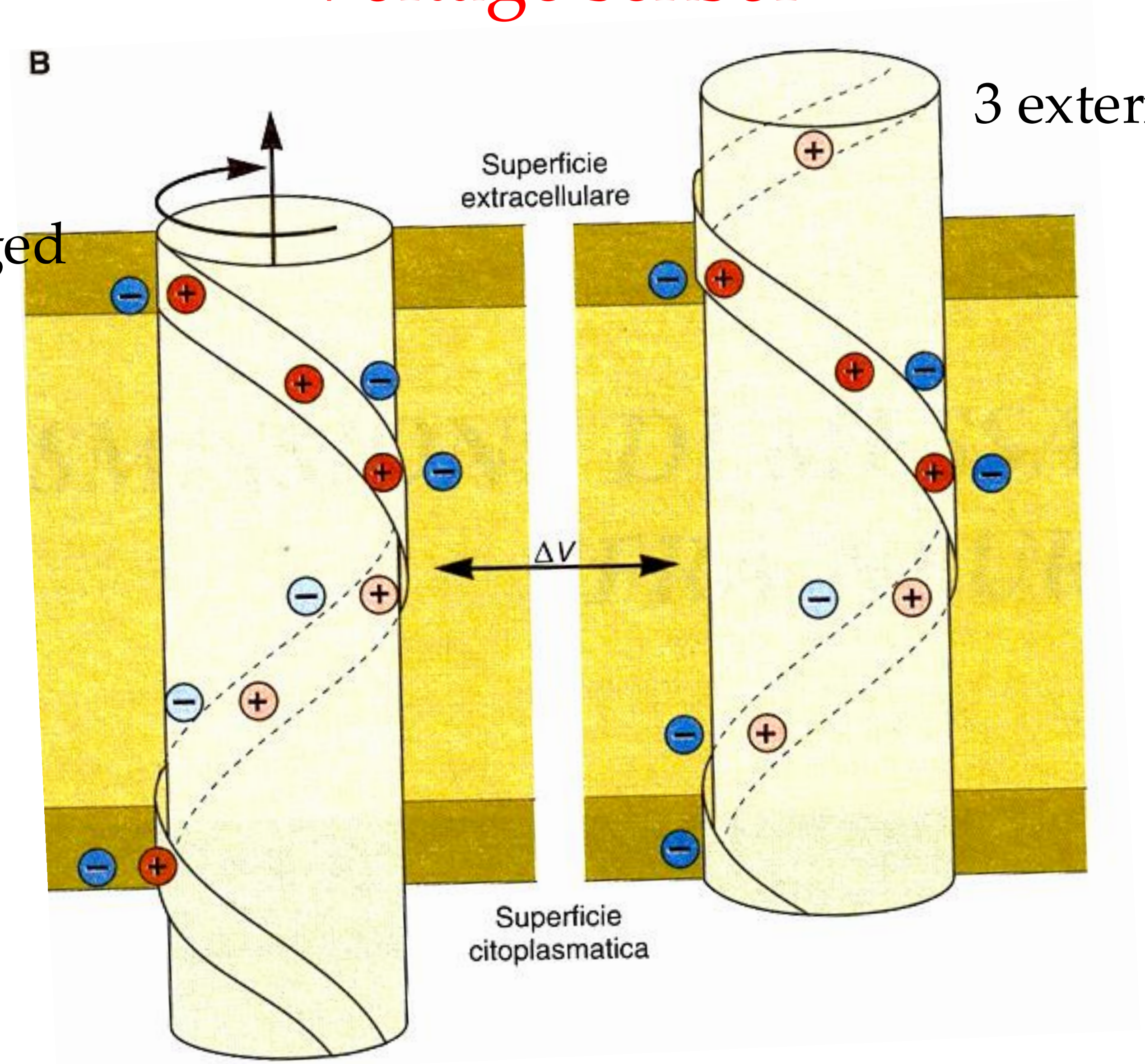
the S4 segment is responsible for detecting voltage changes.
The movement of positively-charged S4 segments within the membrane electric field

Voltage sensor

S4

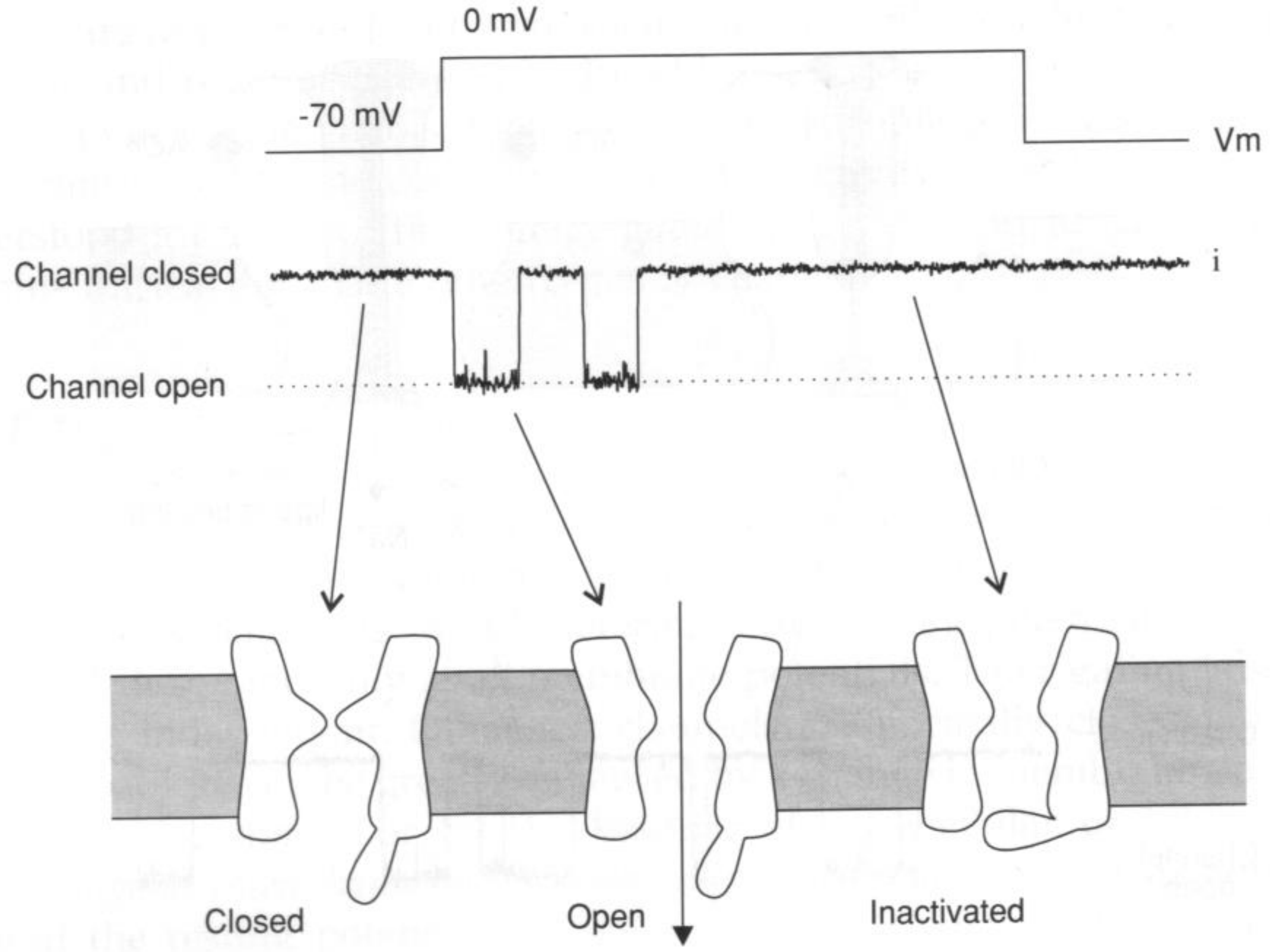
4-8 aa charged

3 external charges

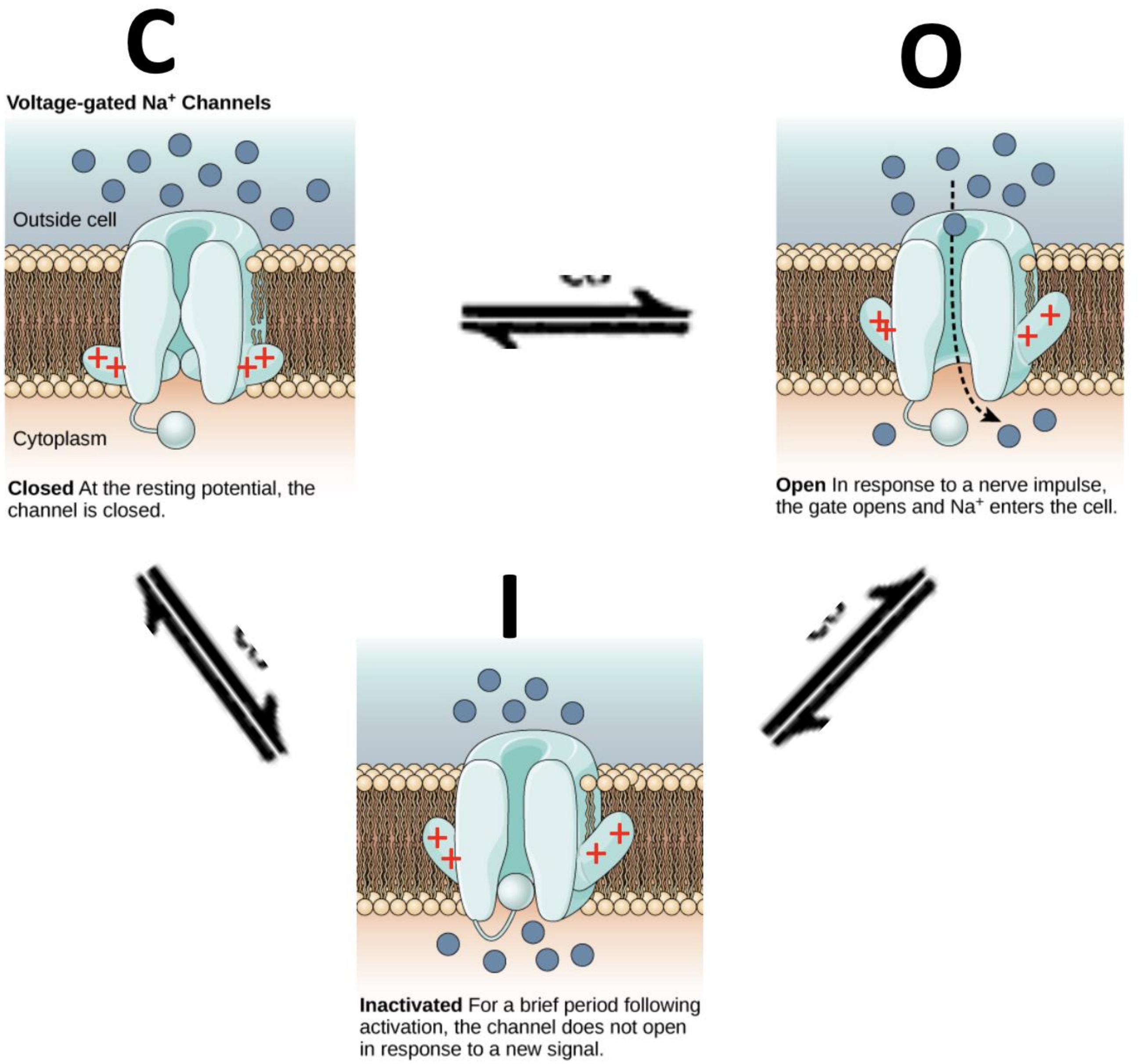


charged groups move in the direction of the electric field
(shown with transient currents)

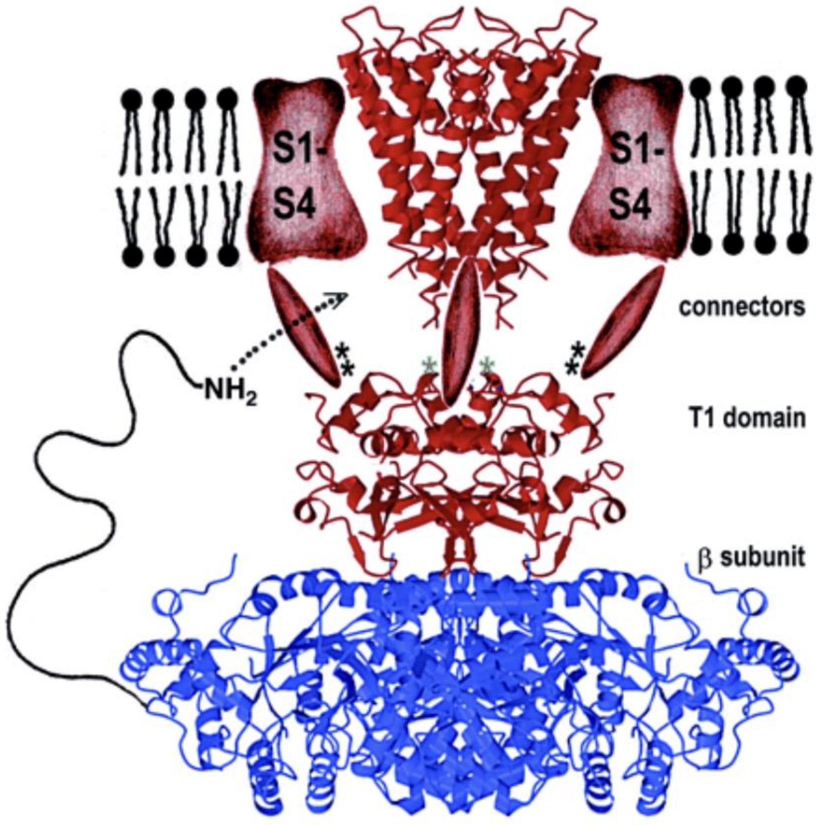
Transition between Closed and Open States… and more..



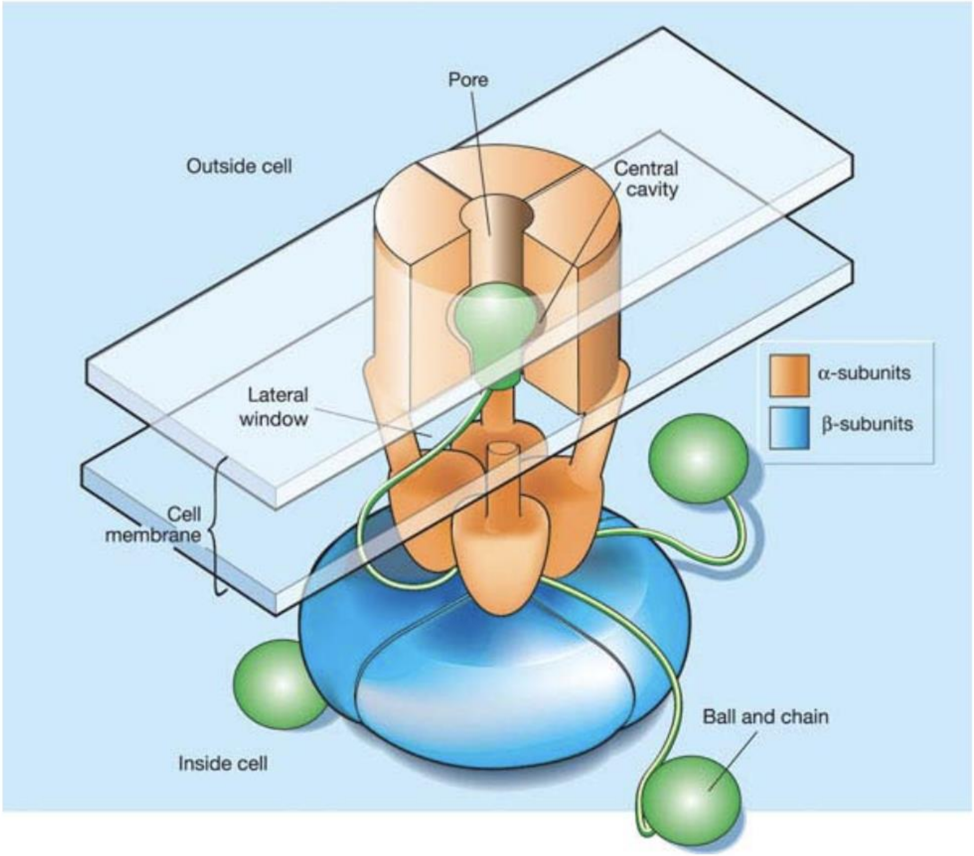
Transition between Closed, Open and Inactivated States



Inactivation Gating of Voltage-Gated Channels -Ball and Chain



(Gulbis et al, Science 2000)



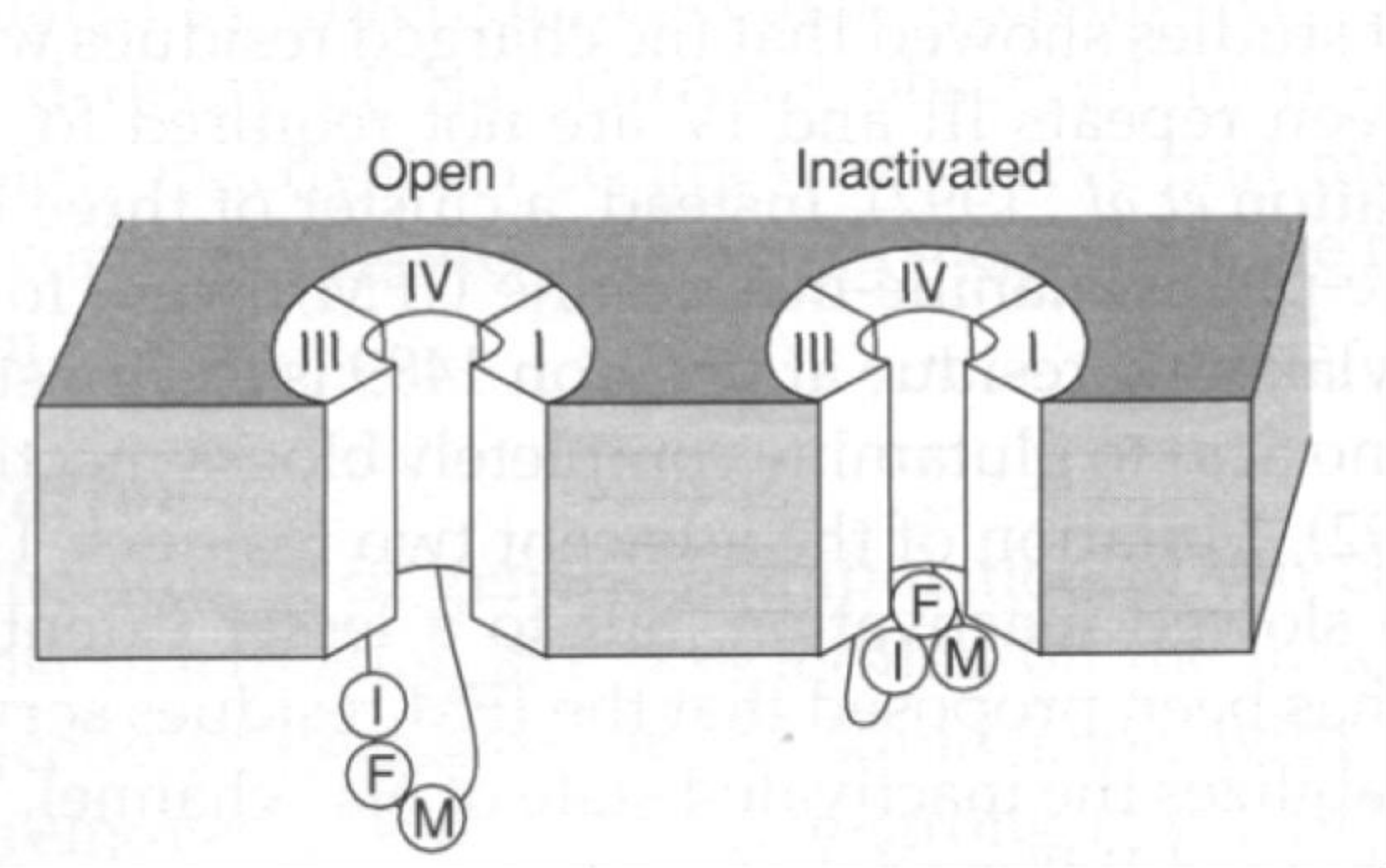
N-terminal inactivation gate

A positively charged inactivation particle (ball) has to pass through one of the lateral windows and bind in the hydrophobic binding pocket of the pore's central cavity.

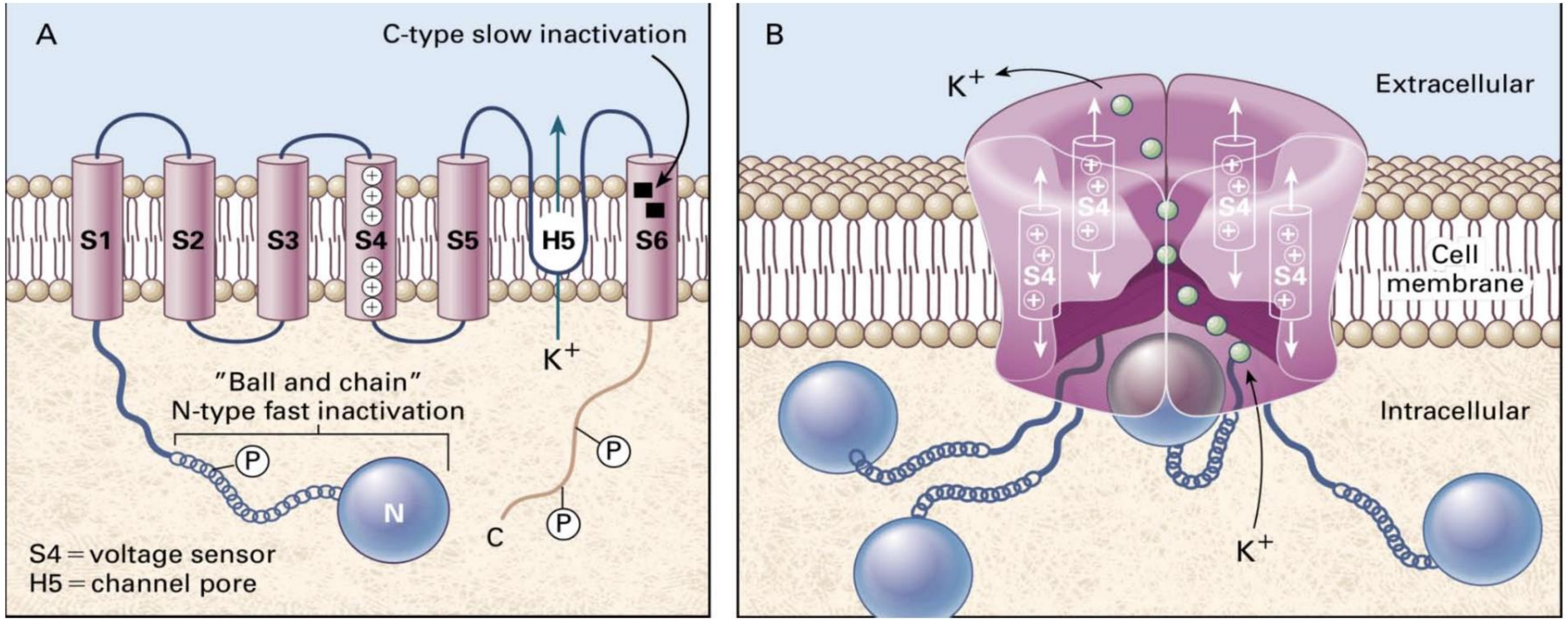
This blocks the flow of ions through the pore.

There are four balls and chains to each channel, but only one is needed for inactivation.

Inactivating particles



Structural Basis of Gating in a Voltage-gated Channel



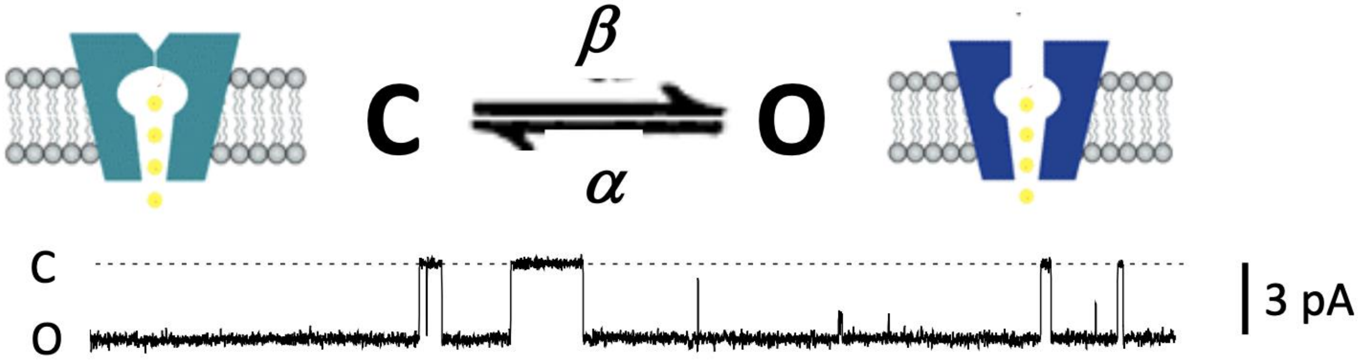
A: a subunit containing six transmembrane-spanning motifs. S5 and S6 and the pore loop are responsible for ion conduction (channel pore).
S4 is the the *voltage sensor, which* bears positively charged amino acids (Arg) that relocate upon changes in the membrane electric field.
N-terminal ball-and-chain is
responsible for inactivation

B: four such subunits assembled to form a potassium channel.

Channel Function:
Single Channel and Whole-cell Current

Ion channels are not open continuously but open and close in a stochastic or random fashion.

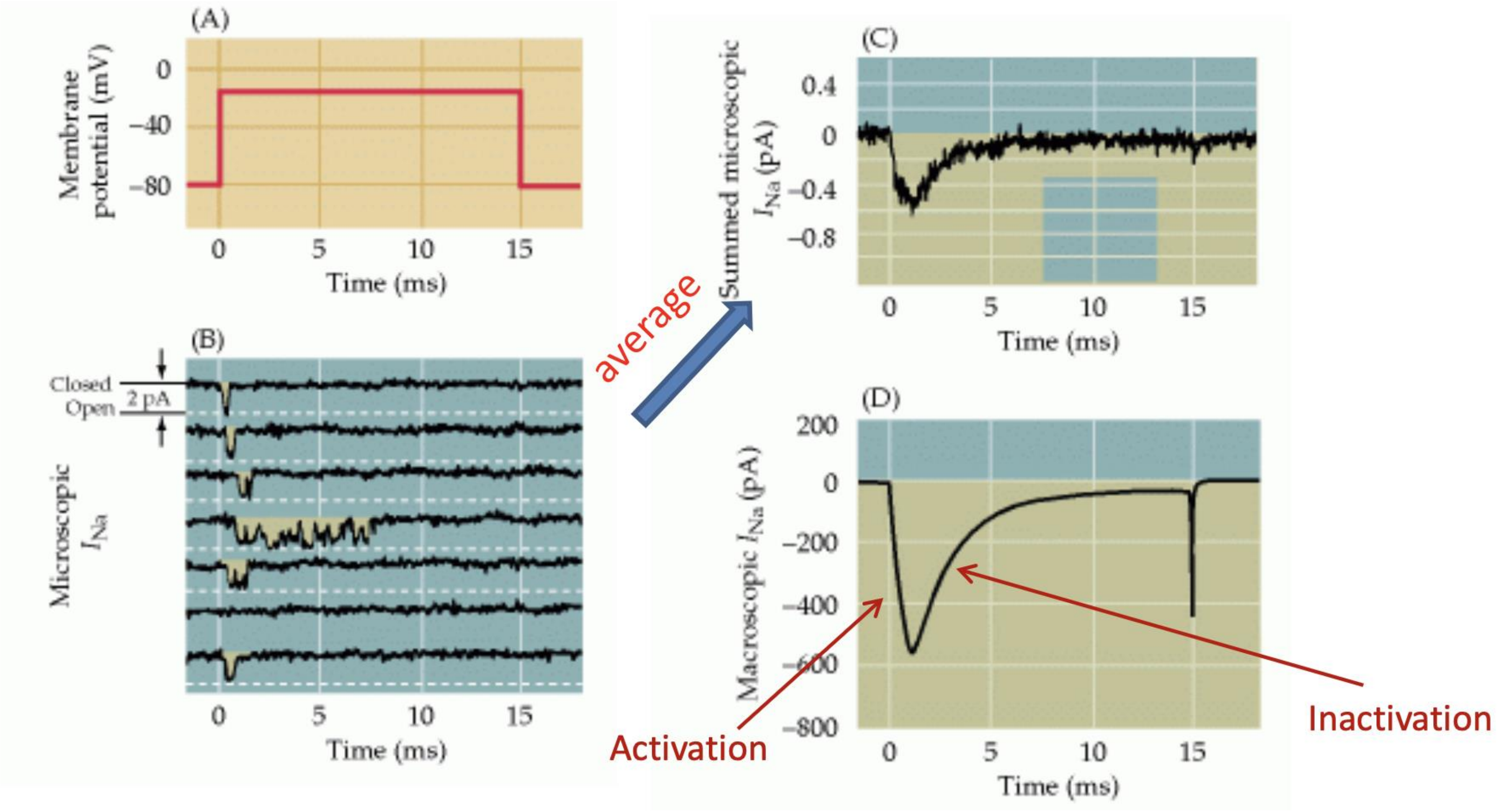
- Ion channel function may be decreased by
  - – decreasing the open time (O),
  - – increasing the closed time (C),
  - – decreasing the single channel current amplitude (i)
  - – or decreasing the number of channels (n)



$$I = n * P_o * i$$

$$P_O = \frac{\tau_o}{\tau_o + \tau_c}$$

Channel Function: Single Channel and Whole-cell Current



Depolarizing voltage pulses result in brief openings in the seven successive recordings of membrane current

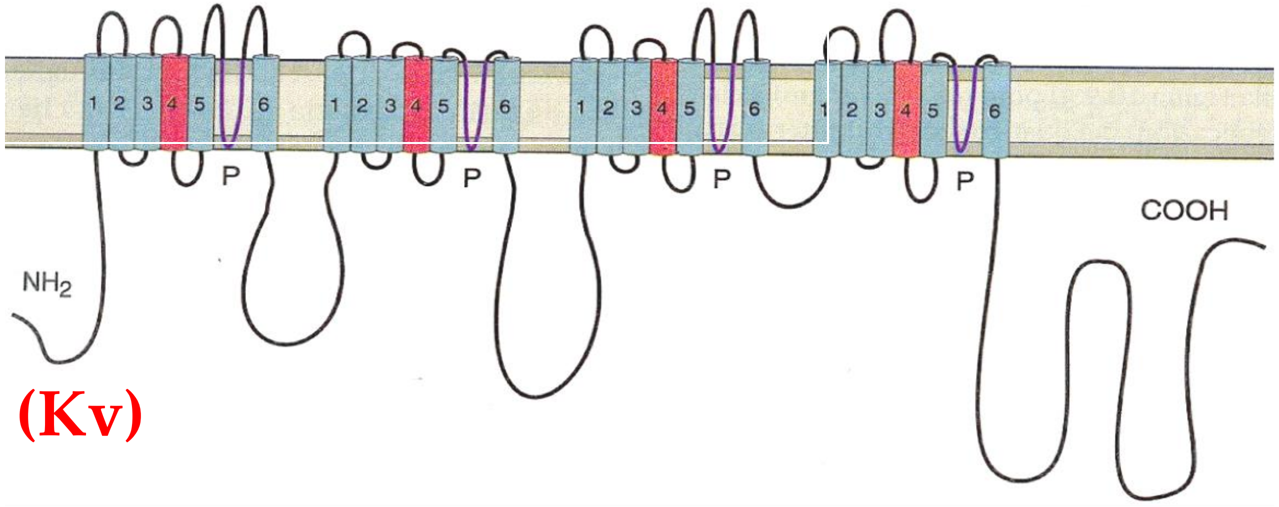
Close correlation between the time courses of microscopic and macroscopic Na+ currents

Voltage-gated Ion Channels

- sodium: I, II, III, μ1, H1, PN3
- potassium: K_A, K_v (1-5), K_v(r), K_v(s),K_{SR}, BK_{Ca}, IK_{Ca}, SK_{Ca}, K_M, K_{ACh}
- calcium: L, N, P, Q, T
- chloride: ClC-0 - ClC-8

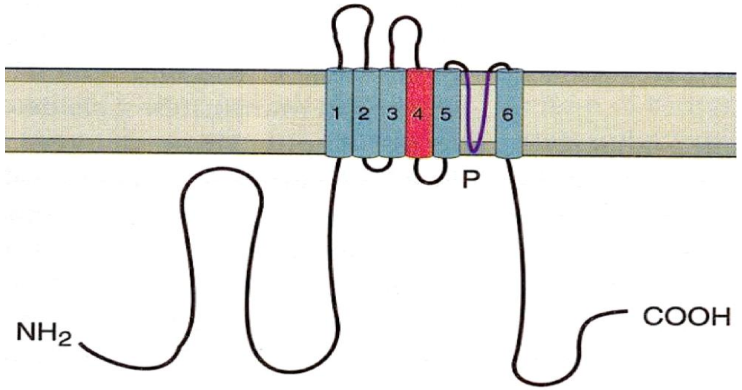
Sodium and calcium channel structure

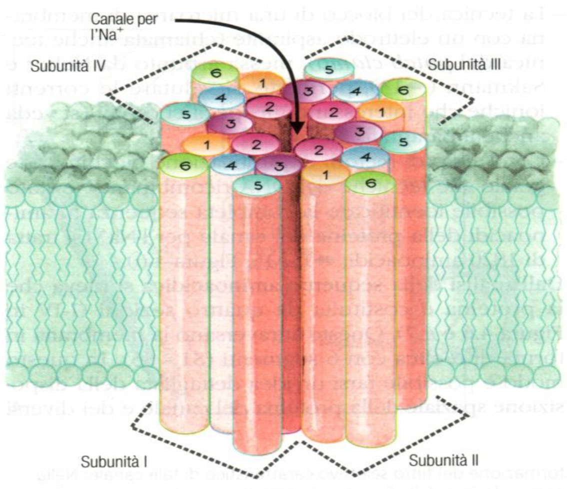
Large protein, α polypetide chain with 4 homologous domains (I-IV), each with 6 hydrophobic segments (S1-S6), P-handle between S5-S6.S4 aa basic loads = voltage sensor 6TM



Potassium Channel Structure (Kv)

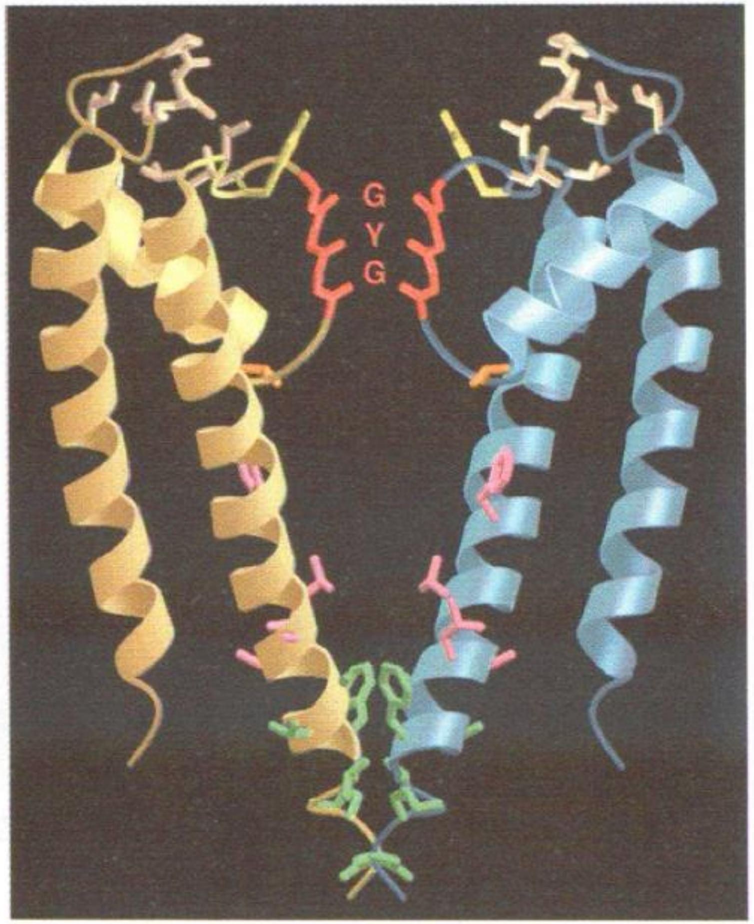
4 polypetide chains aggregated in tetrameric structure. Each with a similar domain to sodium and potassium 6TM (S1-S6)



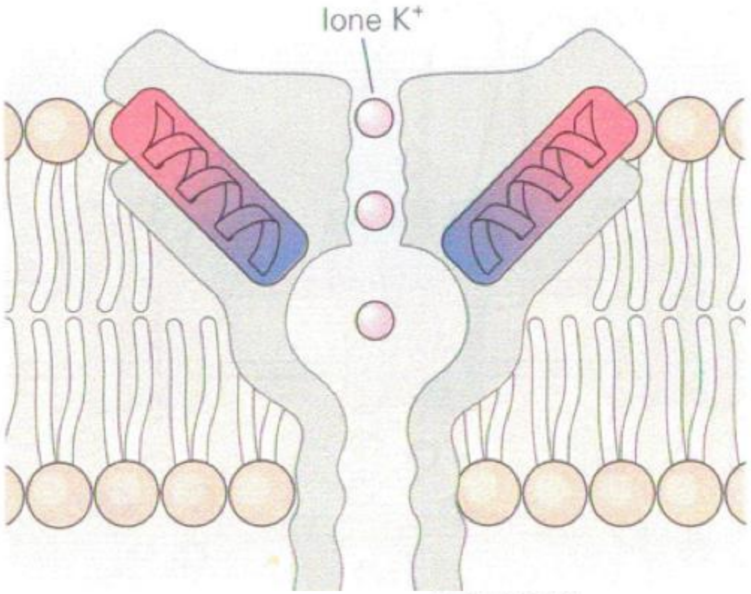


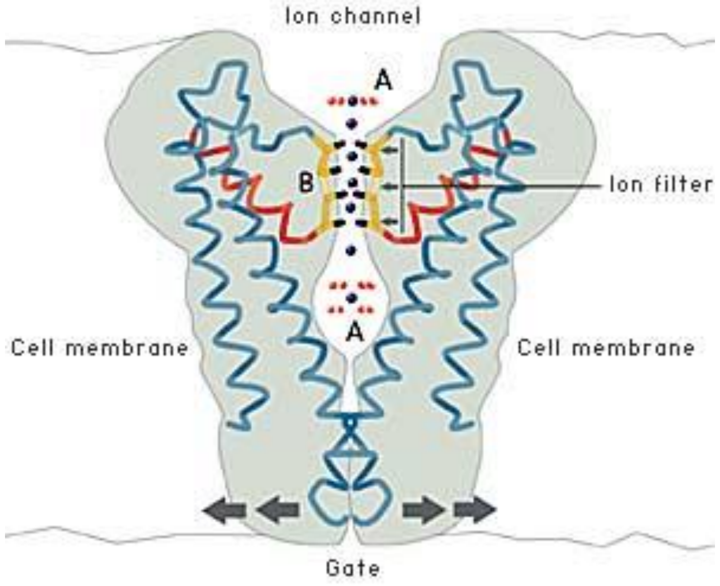
Bacterial K+ channel KcsA

MacKinnon, 1998



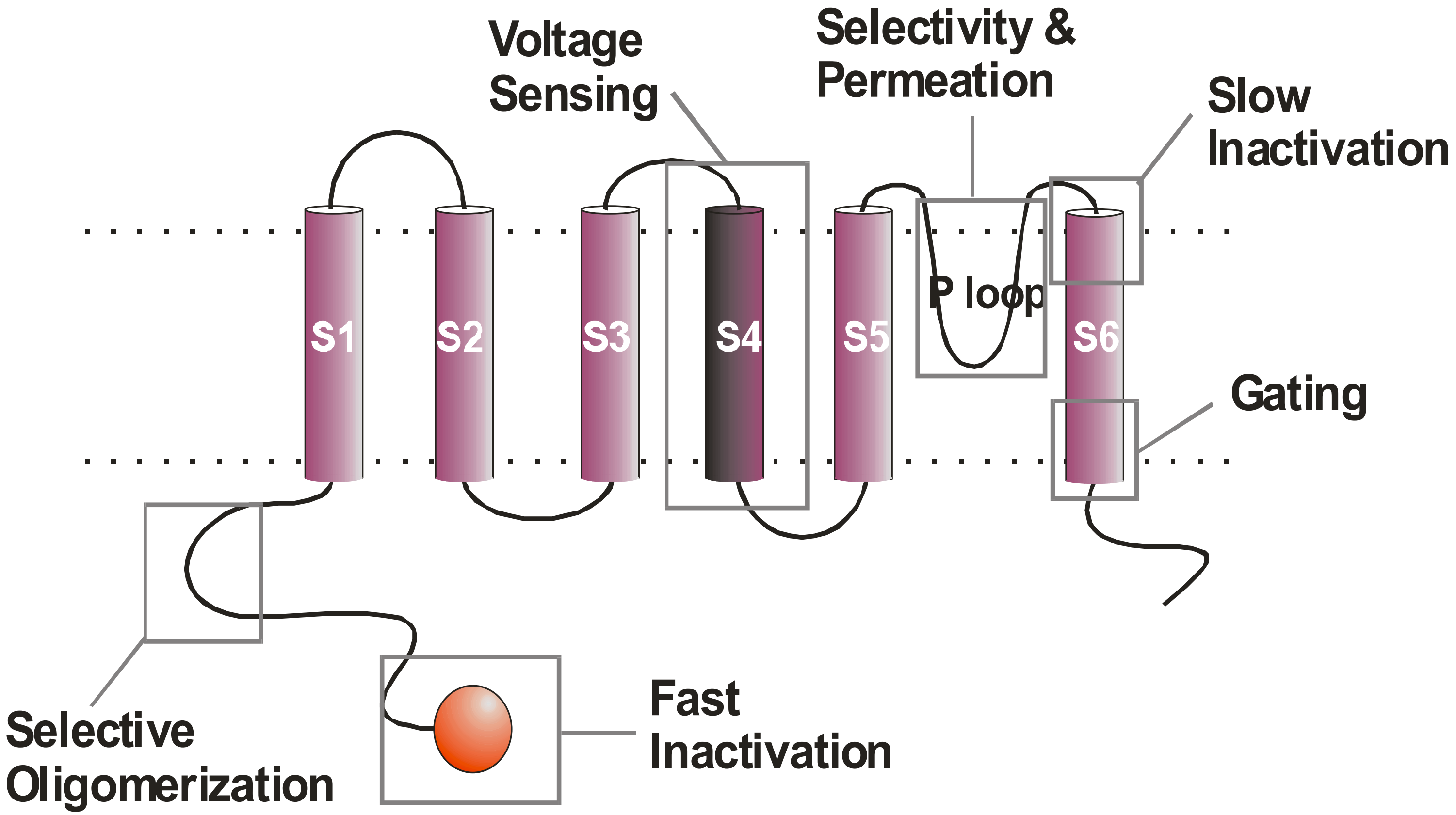
D





(4 subunits with two transmembrane segments connected by loop P, pore channel)

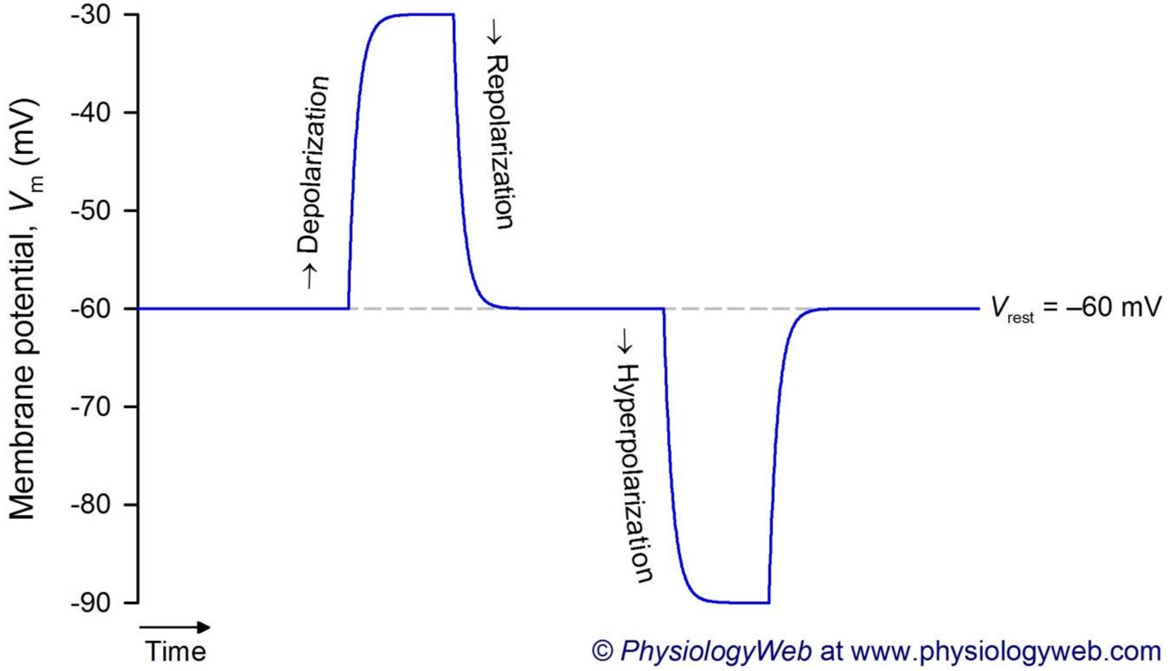
Voltage-gated Ion Channels
General structure



Two classes of Voltage-dependent Ion Channels

channels open by depolarization

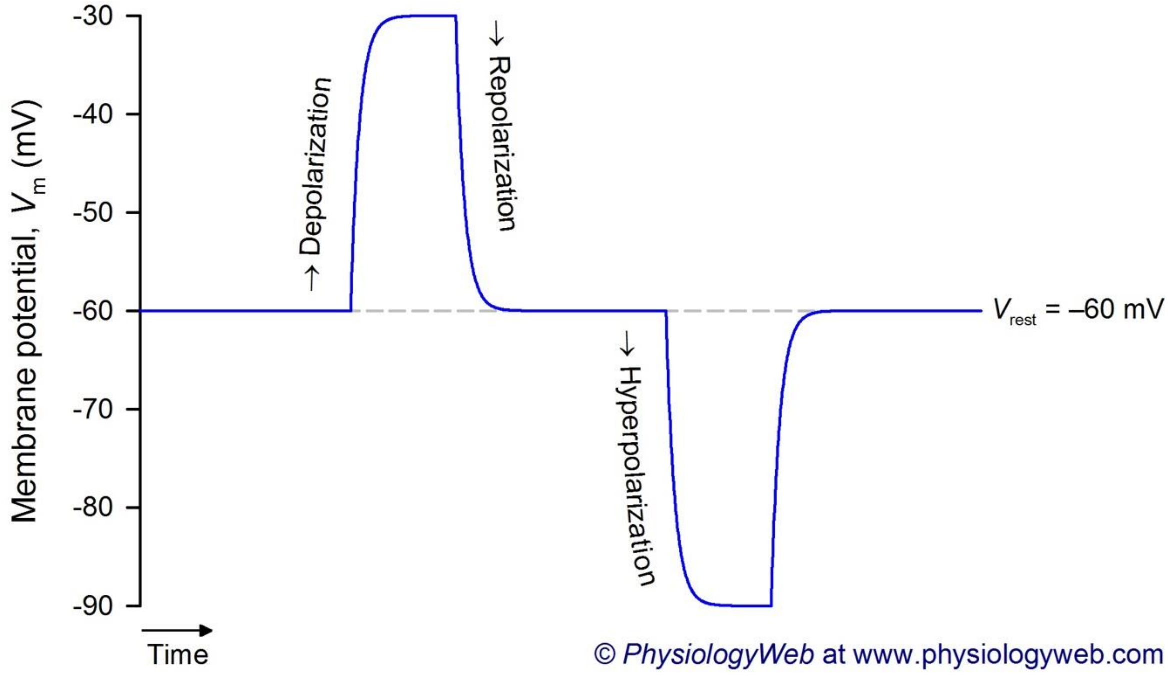
channels open by hyperpolarization



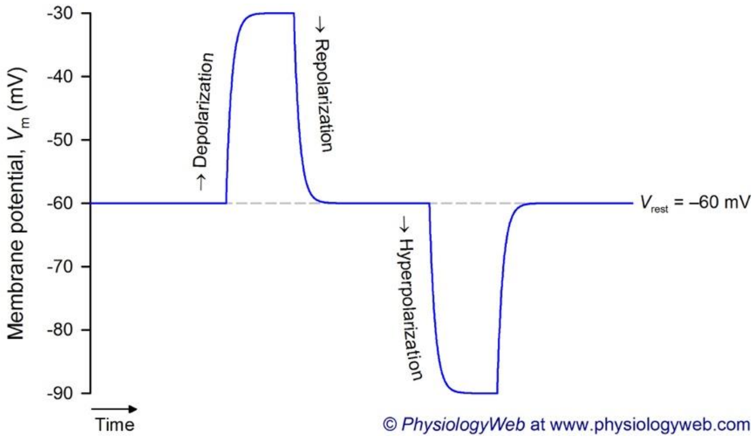
- **Polarization**: a state in which membrane is polarized at rest, negative inside and positive outside.
- **Depolarization**: the membrane potential becomes less negative than the resting potential (close to zero).
- **Hyperpolarization**: the membrane potential is more negative than the resting level.

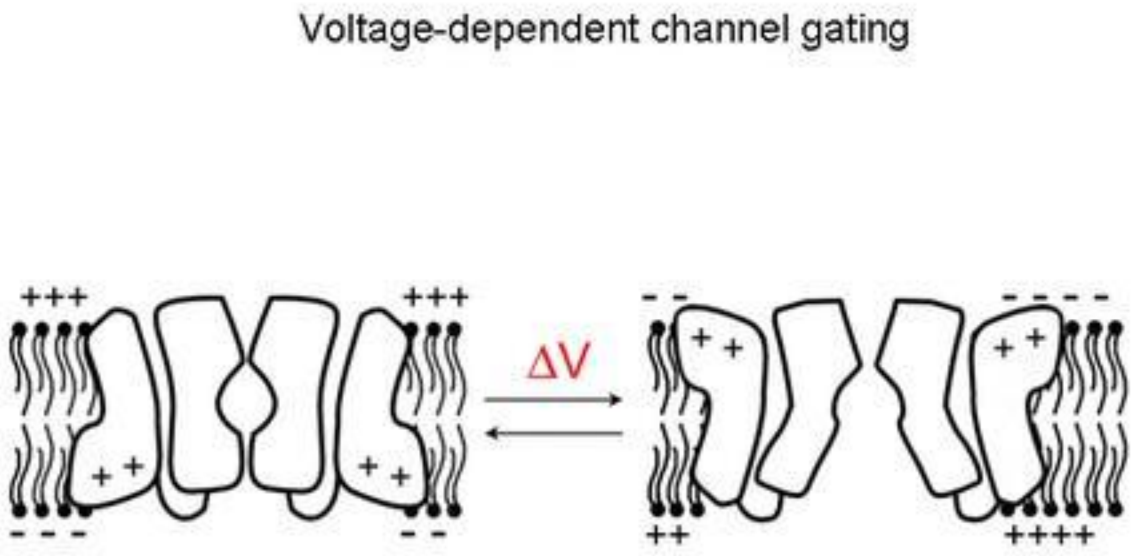
Voltage-dependent Ion Channels

channels open by depolarization



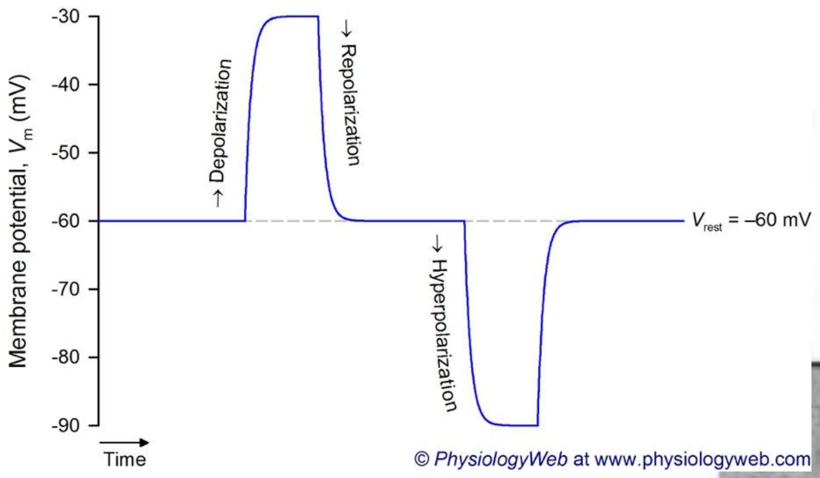
Channels Open by Depolarization

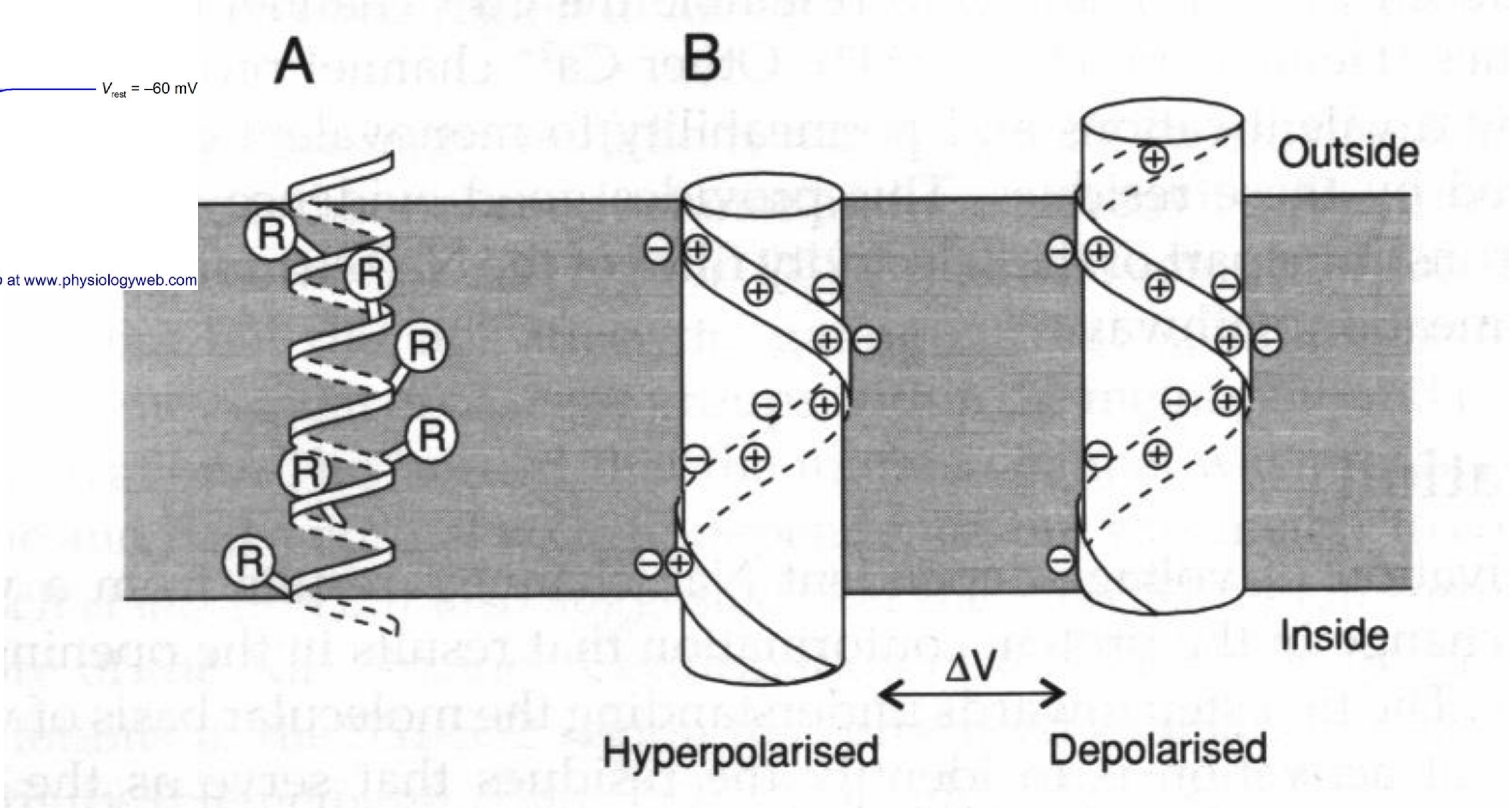




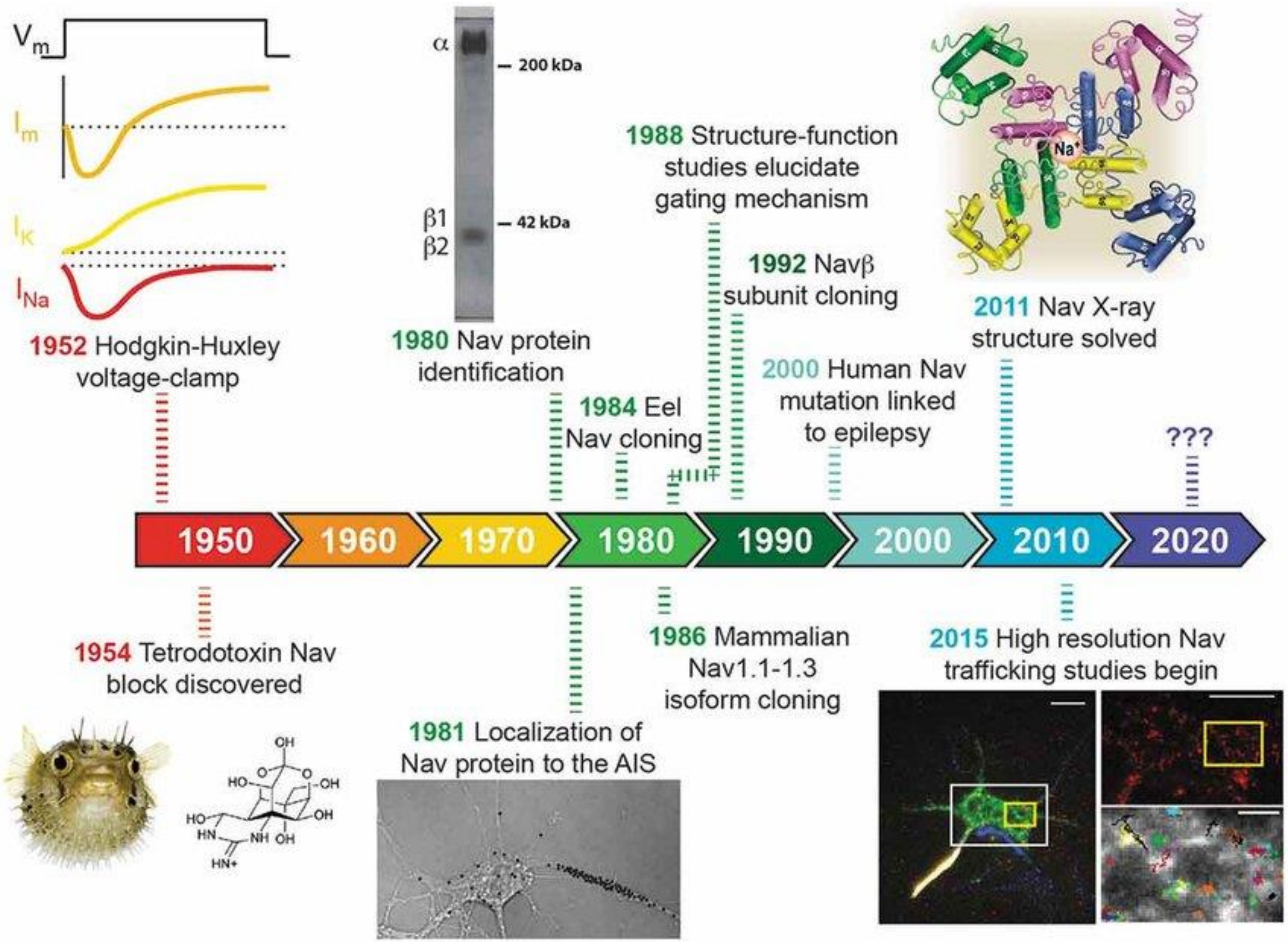
They are a family of selectively permeable channels for Na^+, K^+, Ca^{2+}

Channels Open by Depolarization - Gating



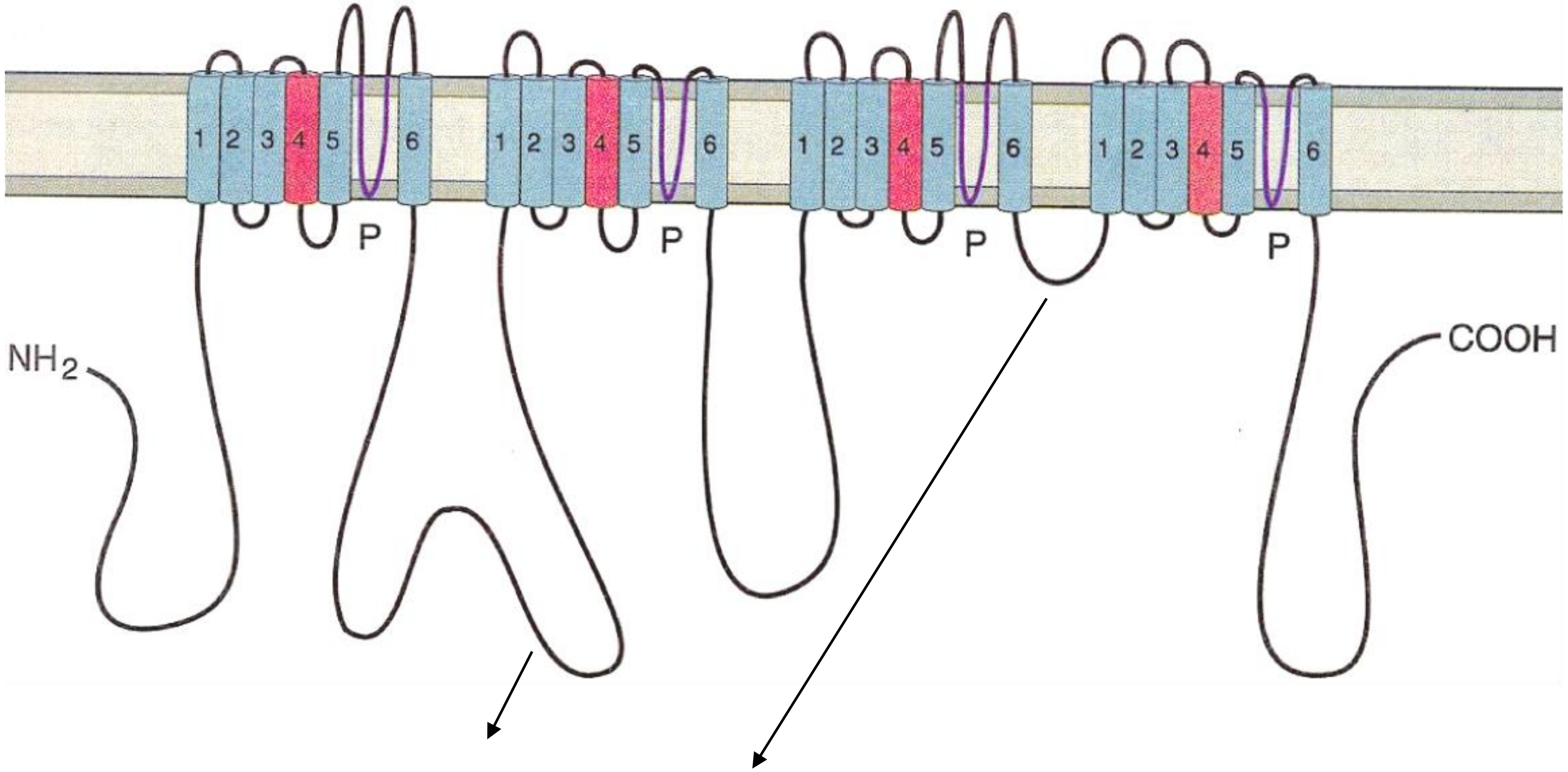


Voltage gated Na^+ channels

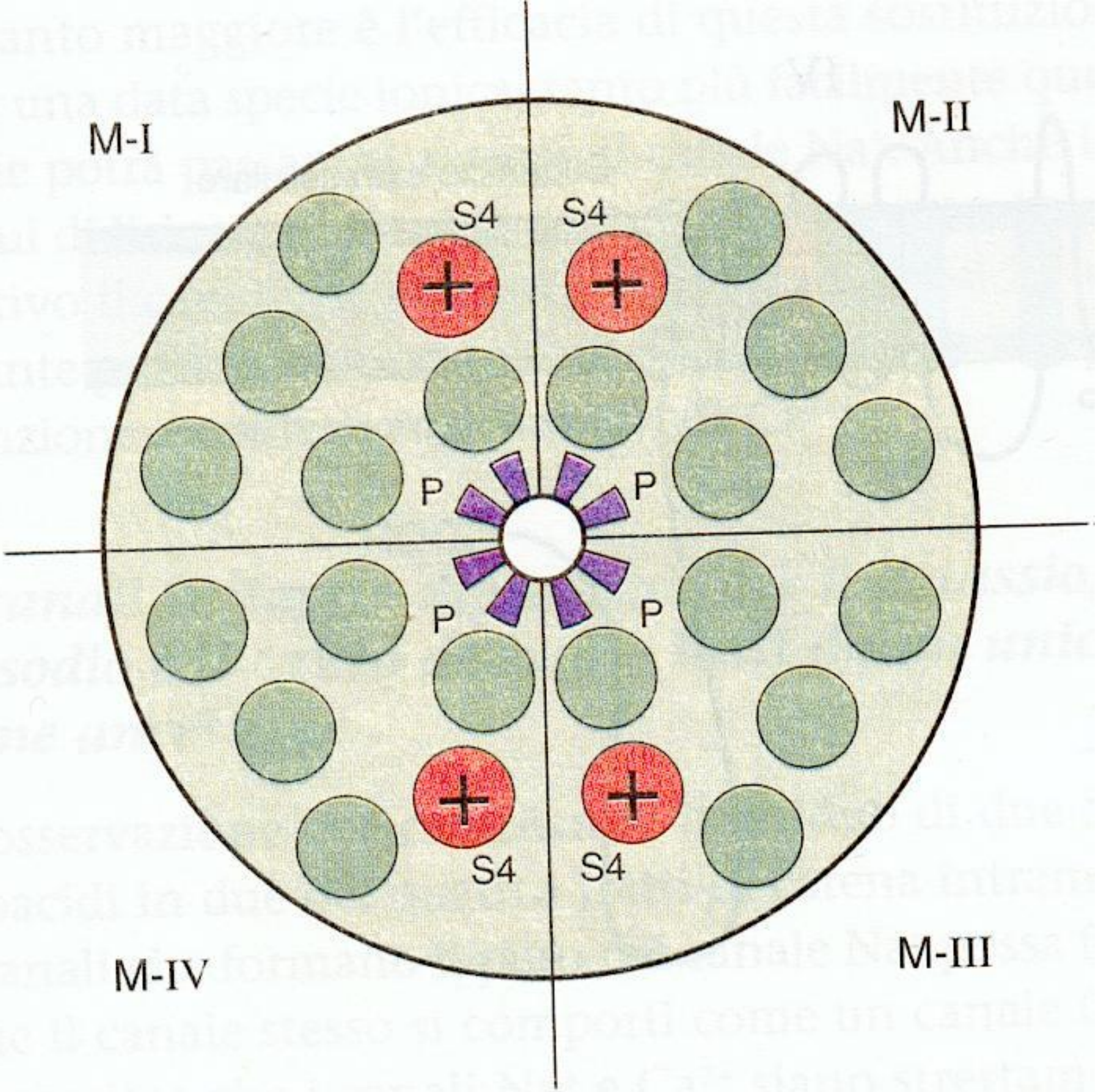


Voltage gated Na^+ channels

alpha subunit
(+ 1 o 2 beta accessorial subunits)

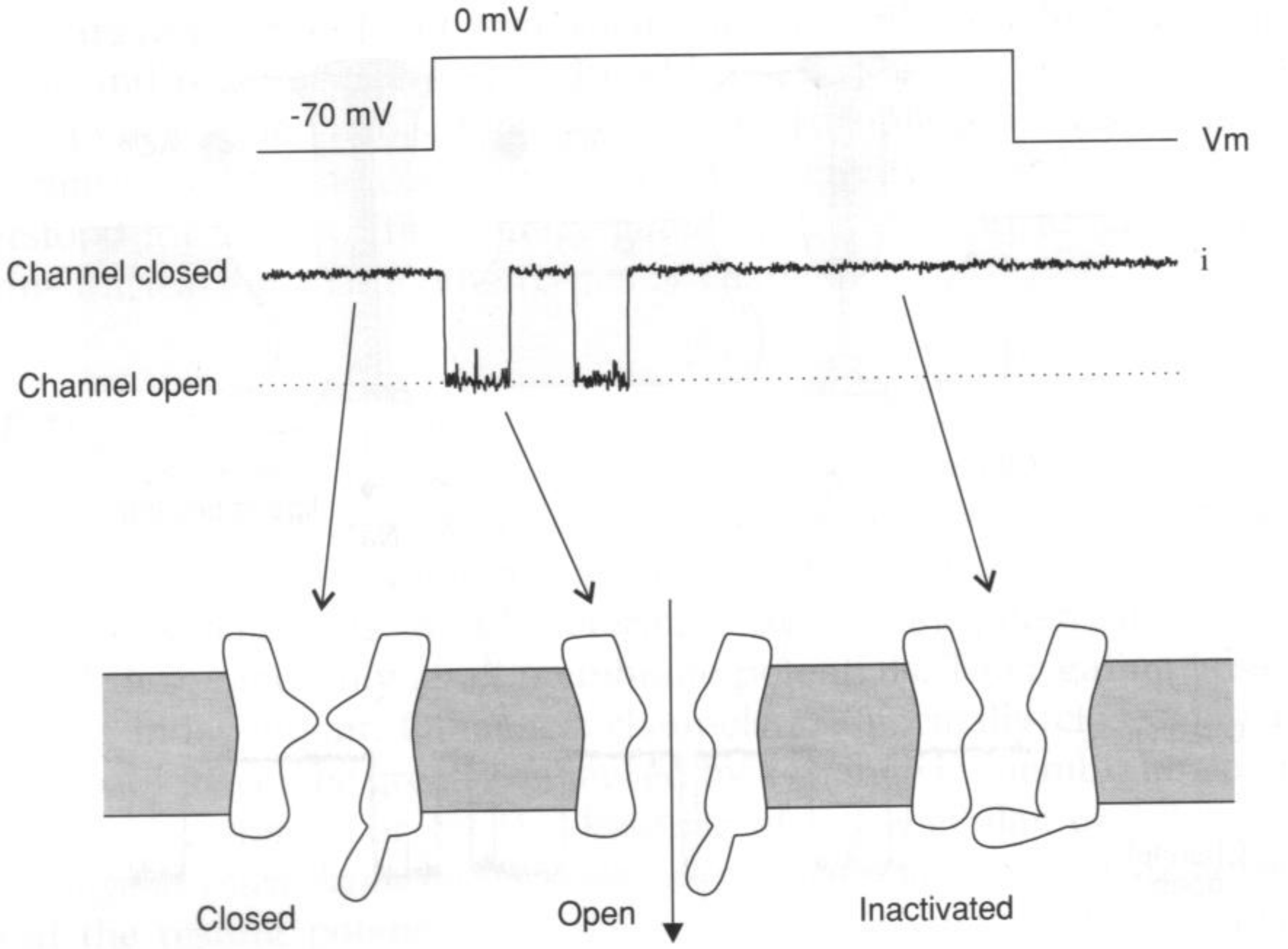


PKA and PKC phosphorylation sites



M-I
M-II
S4
S4
+
+
P
P
P
P
+
+
S4
S4
M-IV
M-III

Channel open = current flow

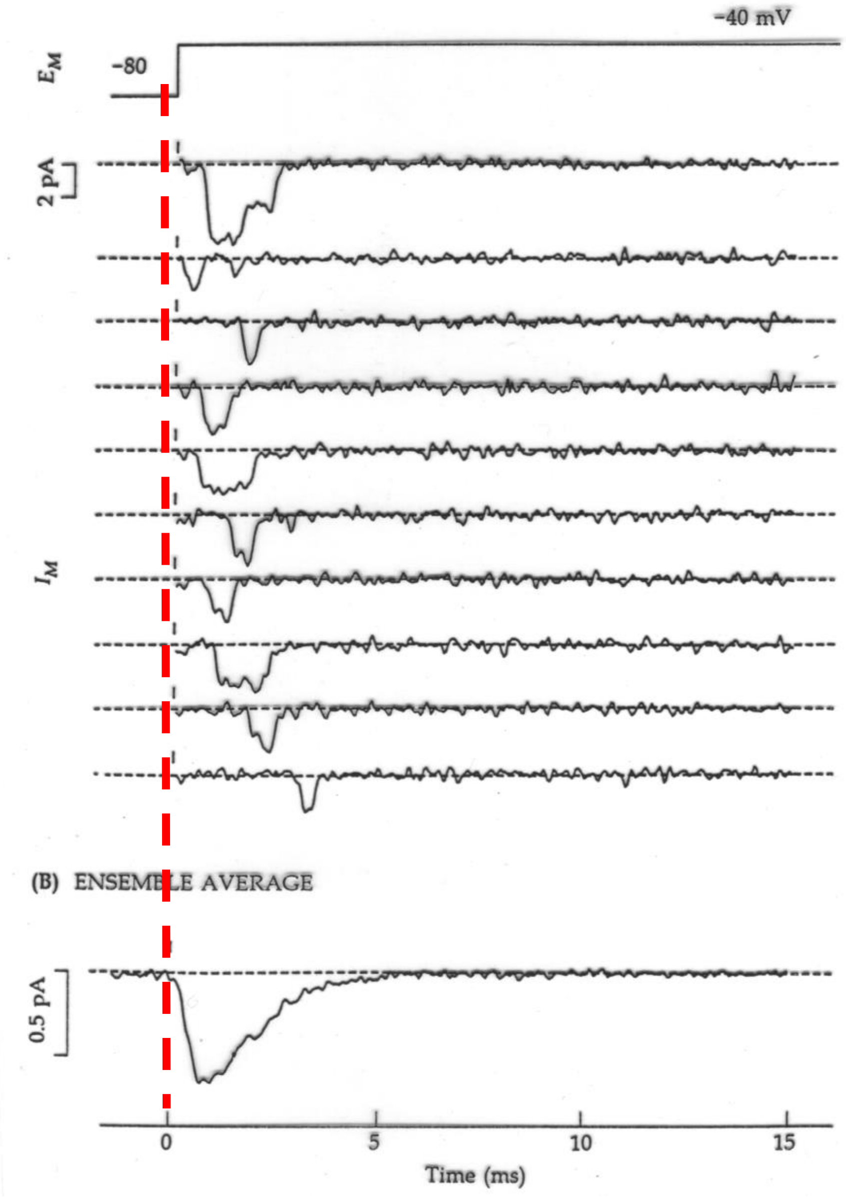


Voltage gated Na^+ channels

Channels selectively permeable to

Na^+

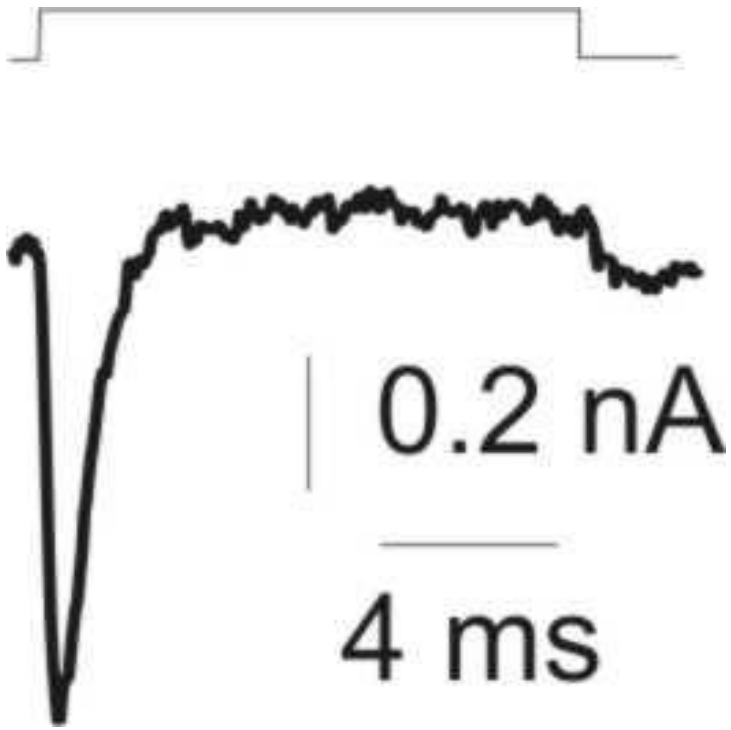
- Rapid activation
- Rapid inactivation



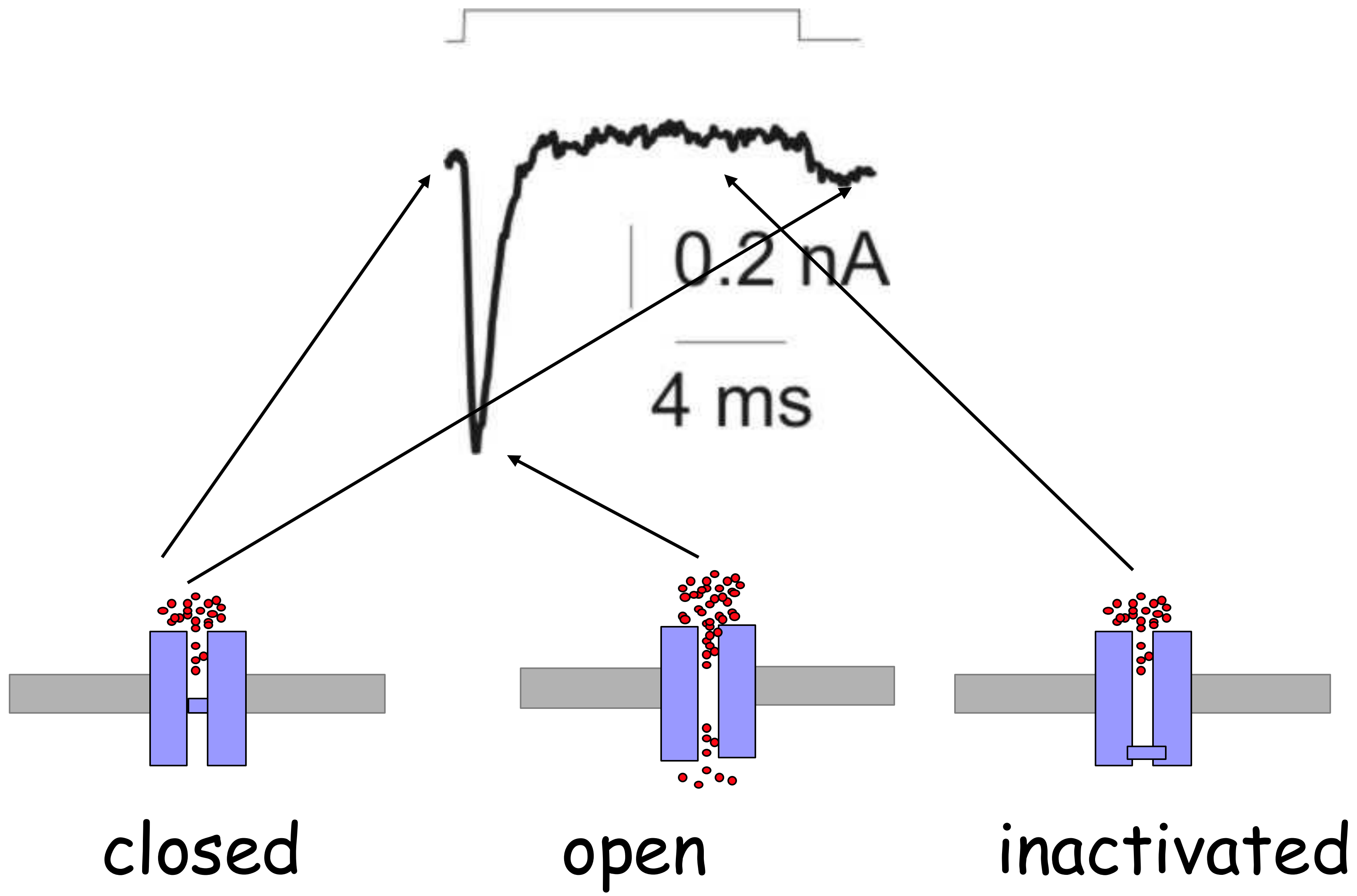
Voltage gated Na^+ channels

Channels selectively permeable to Na^+

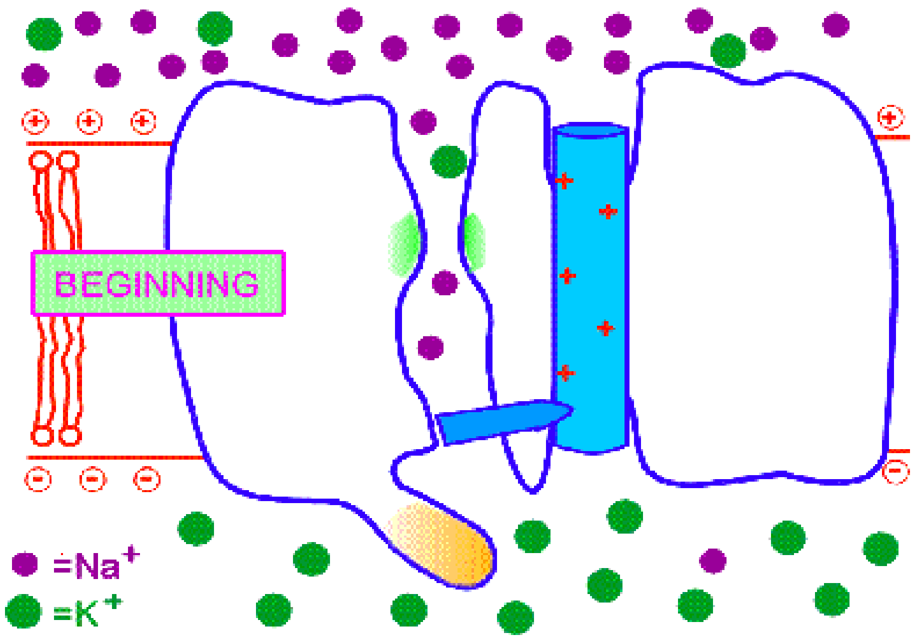
- Rapid activation
- Rapid inactivation

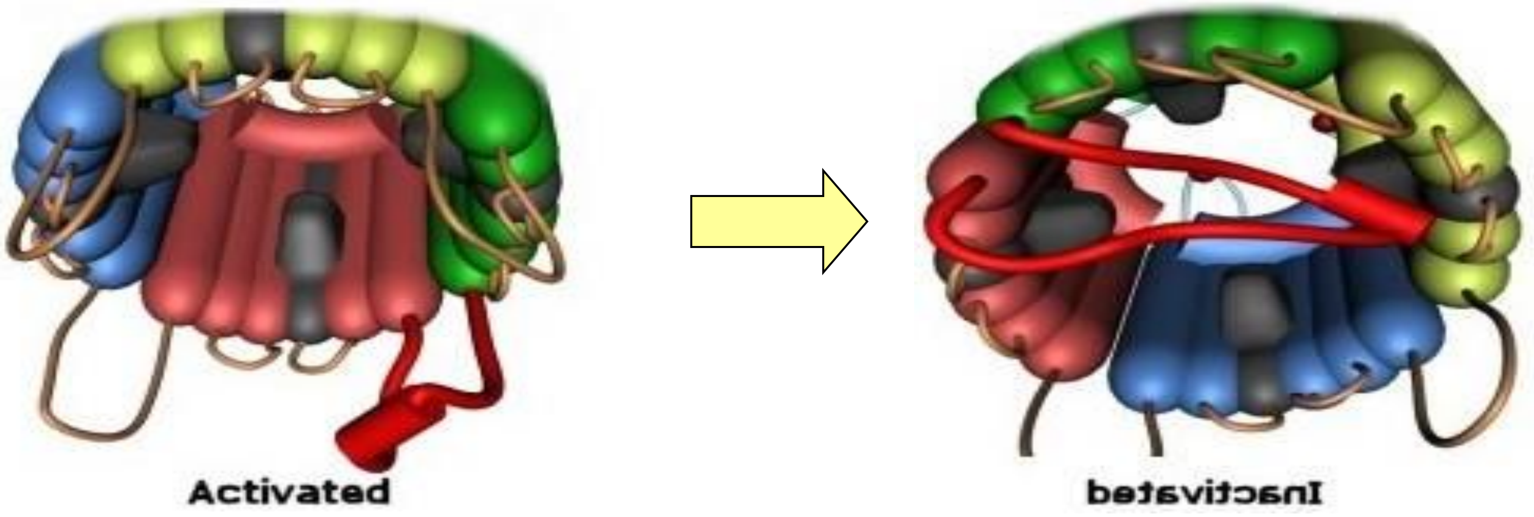


Voltage gated Na$^+$ channels - Inactivation

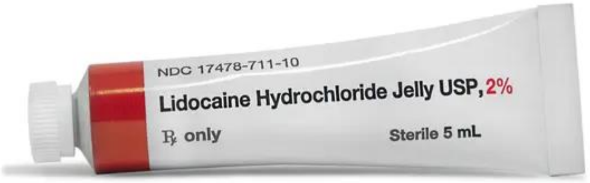


Voltage gated Na^+ channels - Inactivation

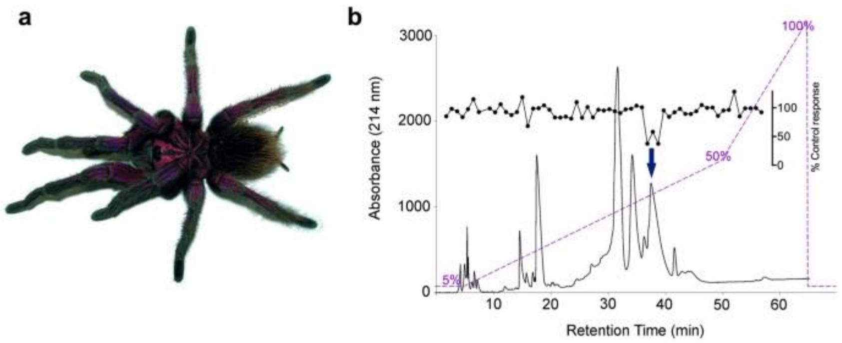




Voltage gated Na^+ channels - Blockers



- Tetrodotoxin (TTX)
- Amioderone
- Lidocaine
- Procainamide
- Mexilitine
- Ketamine
- Many, many others

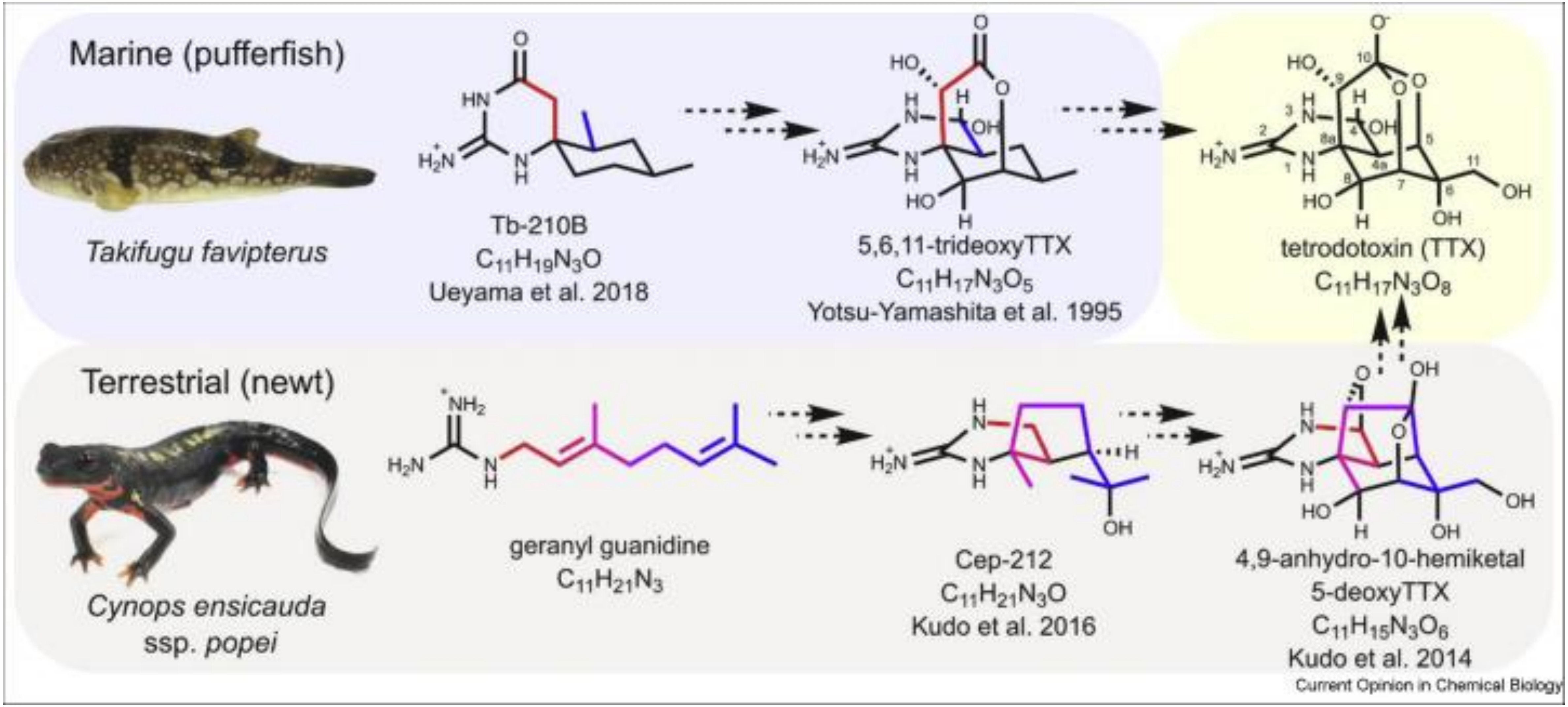


c

Toxin name	Sequence	Observed Mass (Da)	Calculated Mass (Da)
μ-TRTX-Pn3a	DCRYMFGDCEKDEDCCKHLGCKRKMKYCAWDFTFT	4268.5	4268.8
μ-TRTX-Pn3b	GCRYMFGDCEKDEDCCKHLGCKRKMKYCAWDFTFT	4210.5	4210.8

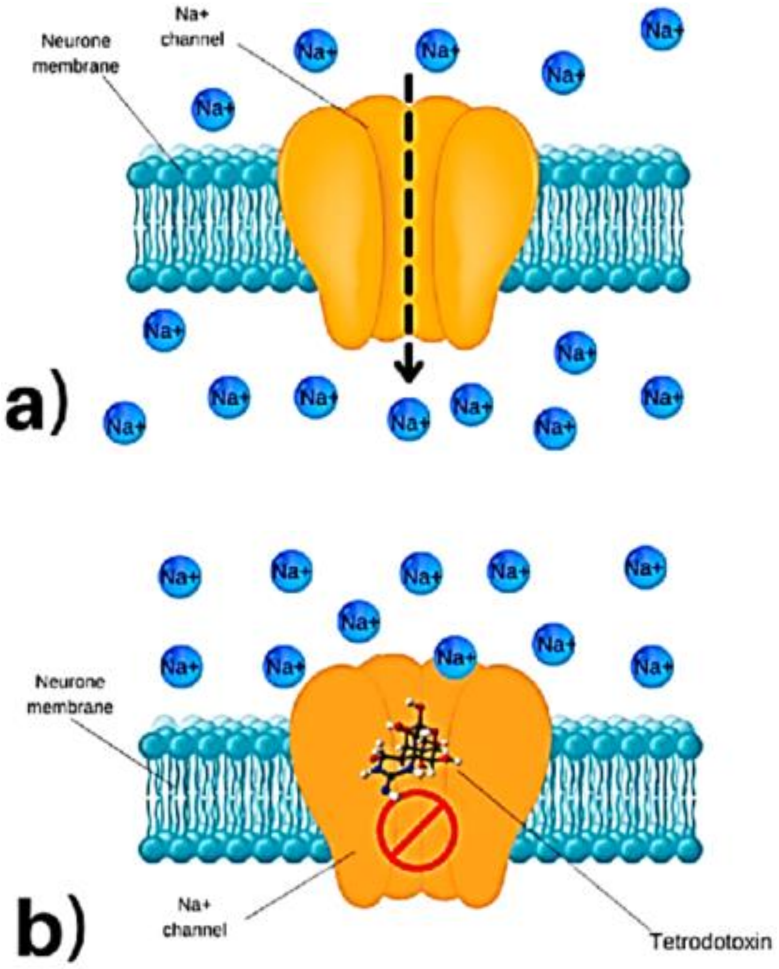
Voltage gated Na⁺ channels - Blockers

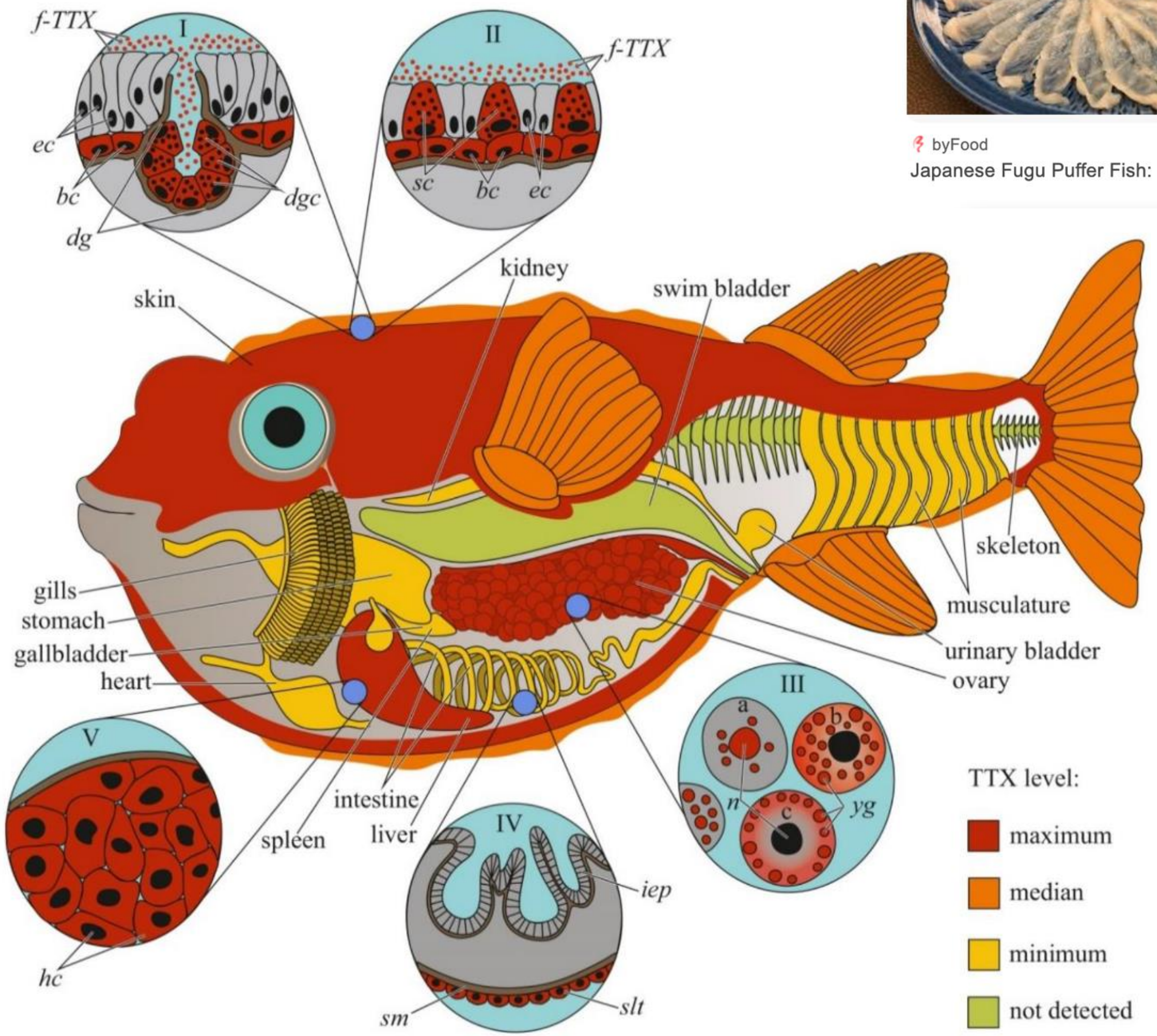
- **Tetrodotoxins (TTX)**



Voltage gated Na$^+$ channels - Blockers

- **Tetrodotoxin (TTX)**





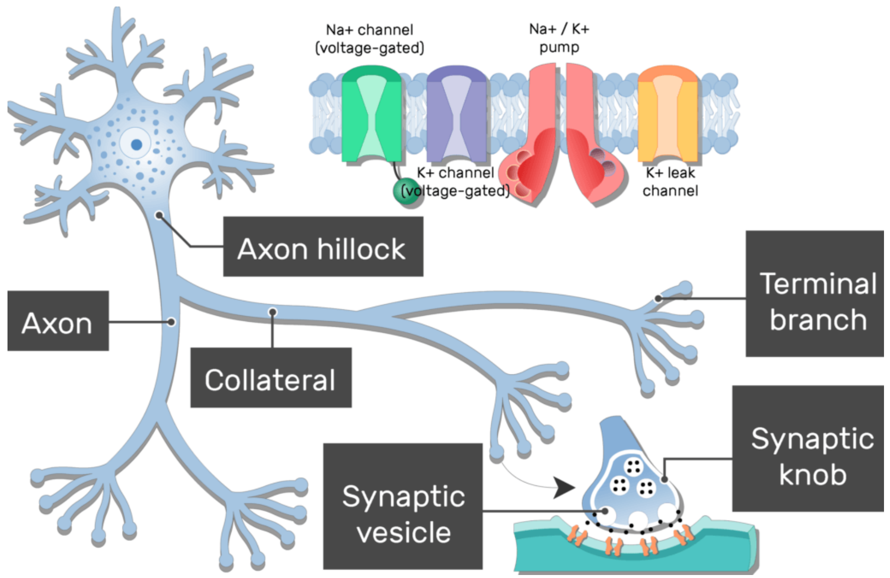
byFood
Japanese Fugu Puffer Fish: Dangers, Prep…

Voltage gated Na^+ channels - Functions

Opening of voltage gated Na^+ channels ◎ depolarization (E_{Na}=+55 mV)

Na$^+$ channels in:

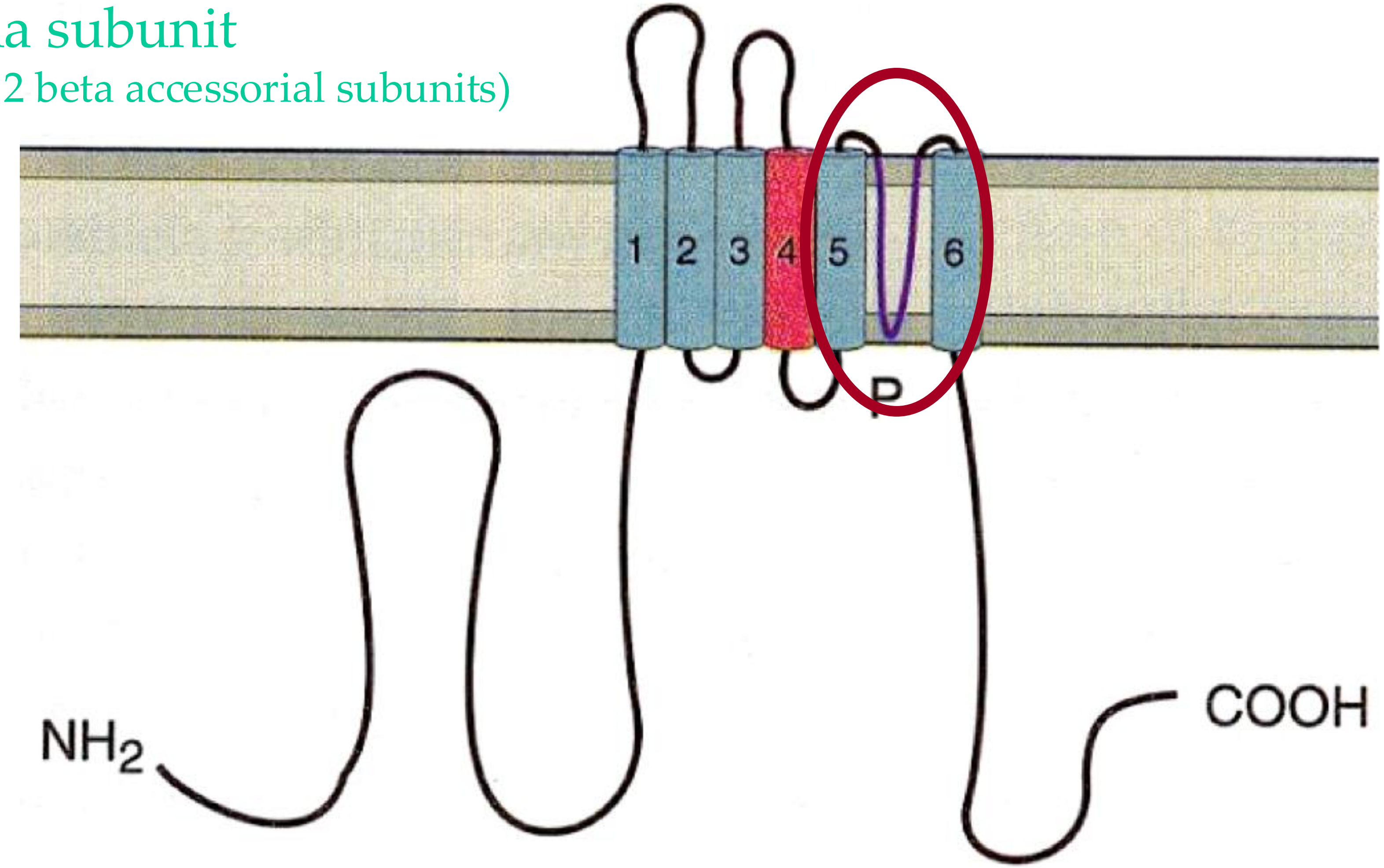
- skeletal muscle
- working myocardium
- Axons



Important in
1)Generation and propagation of acton potential
2)Depolarization post action potential
3)Discharge frequency regulation

Voltage gated K+ channels

alpha subunit
(+ 1 o 2 beta accessorial subunits)



Voltage gated K+ channels

Selectively permeable to K^+

There are various channel families for K +

they are found in all cell types

Channel opening K^+

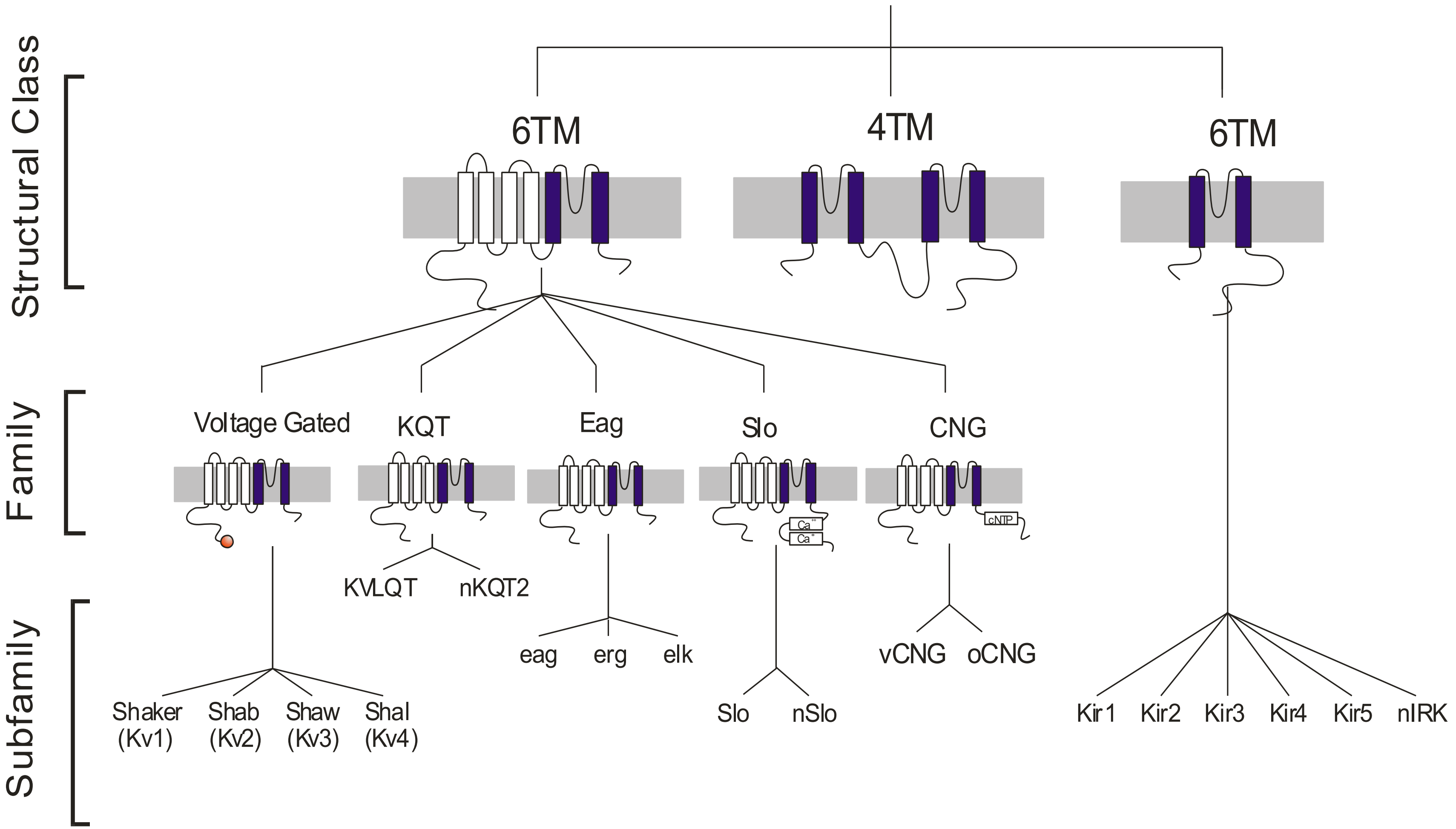
repolarization (E_K=-90 mV)

Important in excitable cells for:

1) They contribute to the resting potential
2) They support the repolarizing phase of the action potential
3) Inhibitively contribute to synaptic integration

Voltage gated K+ channels

more than 100 genes!!!

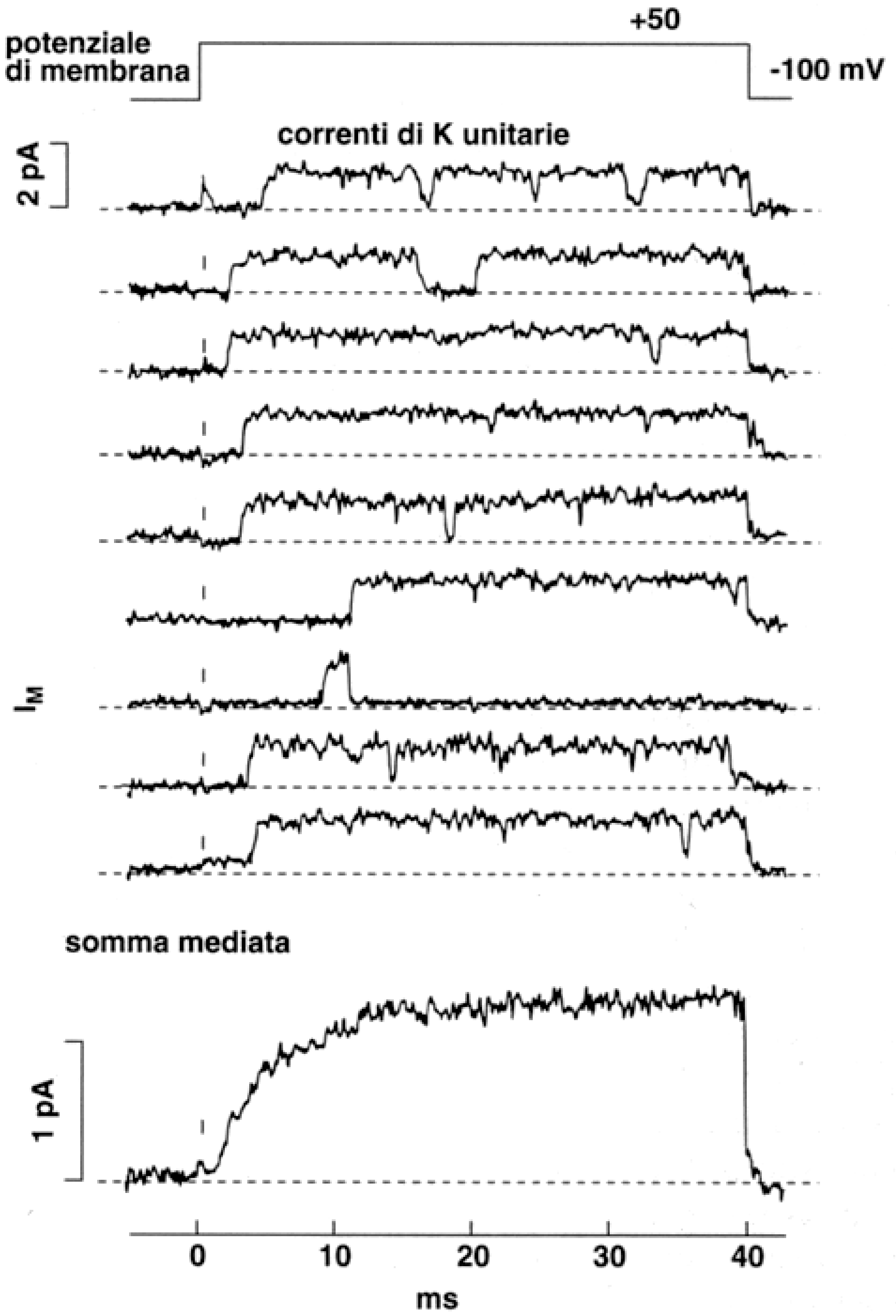


Voltage gated K+ channels

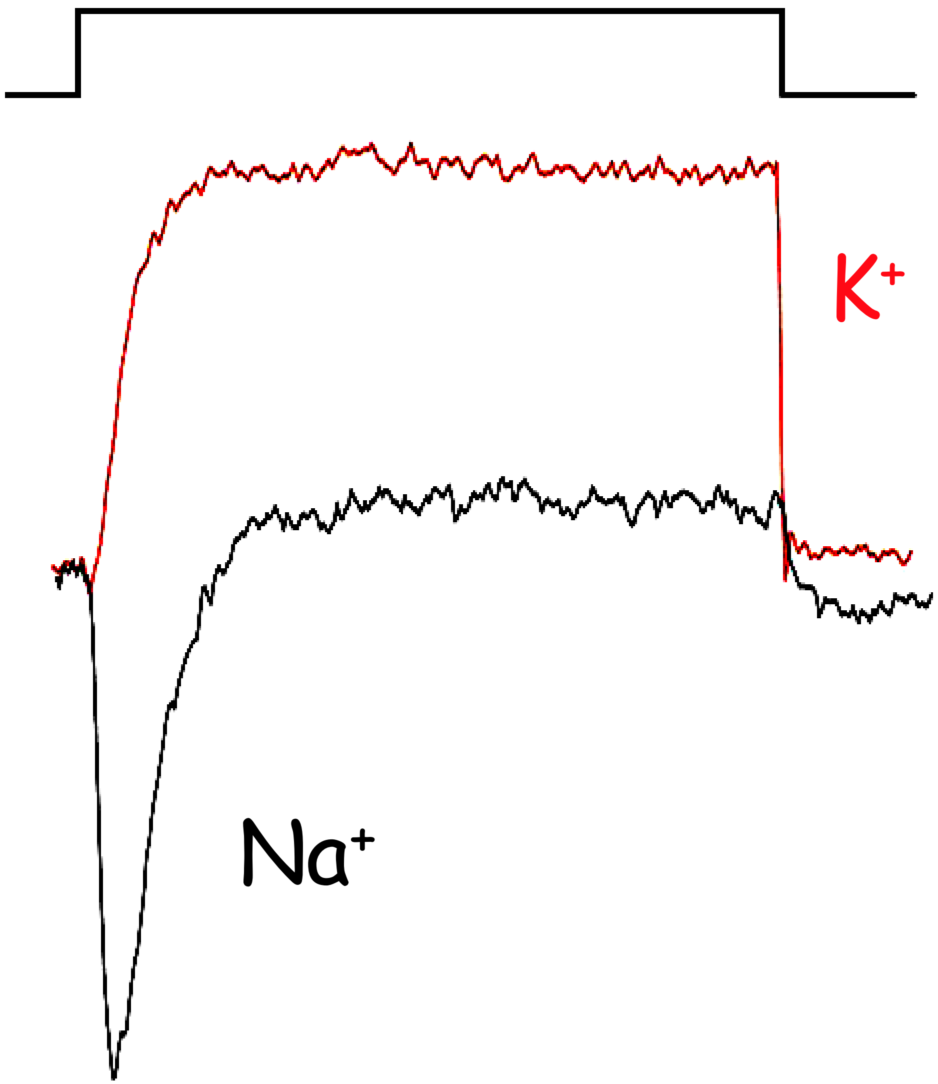
K^{+} Channels in the axons

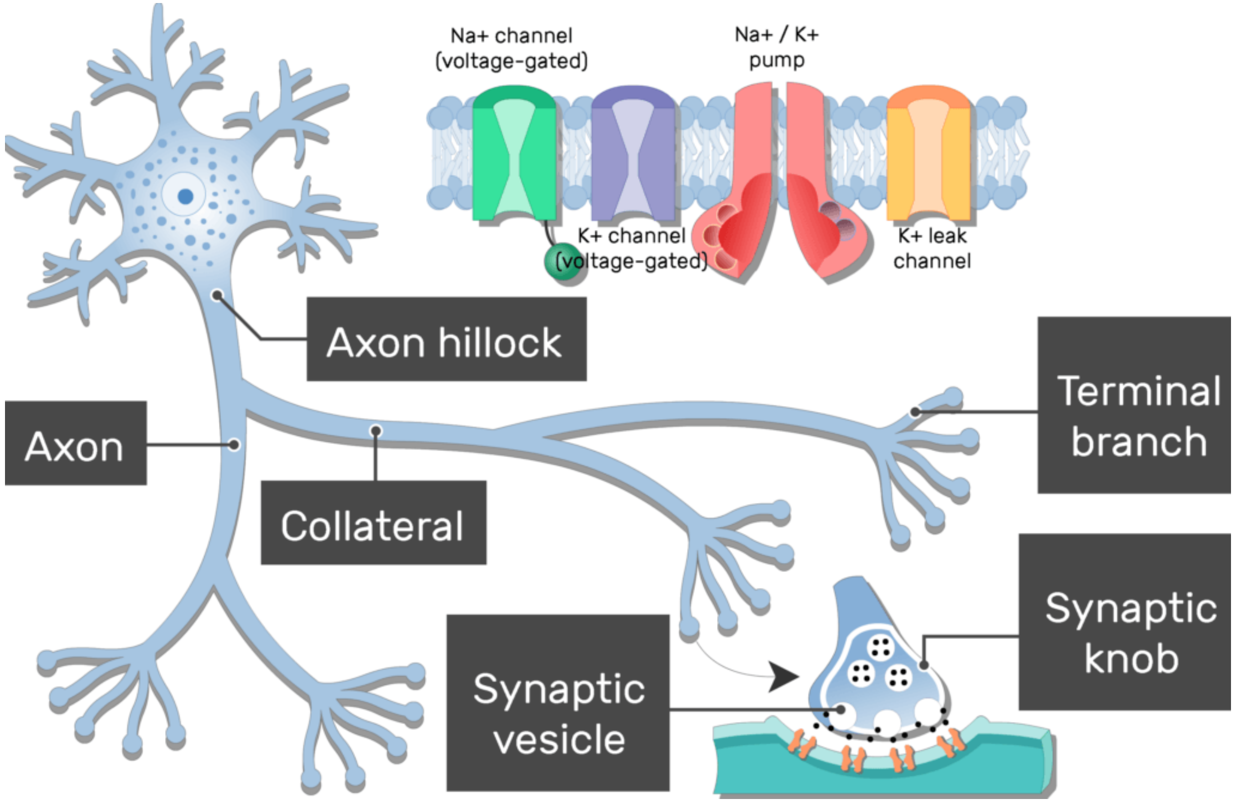
Channels selectively permeable to K^{+}

•Slow activation
•No inactivation

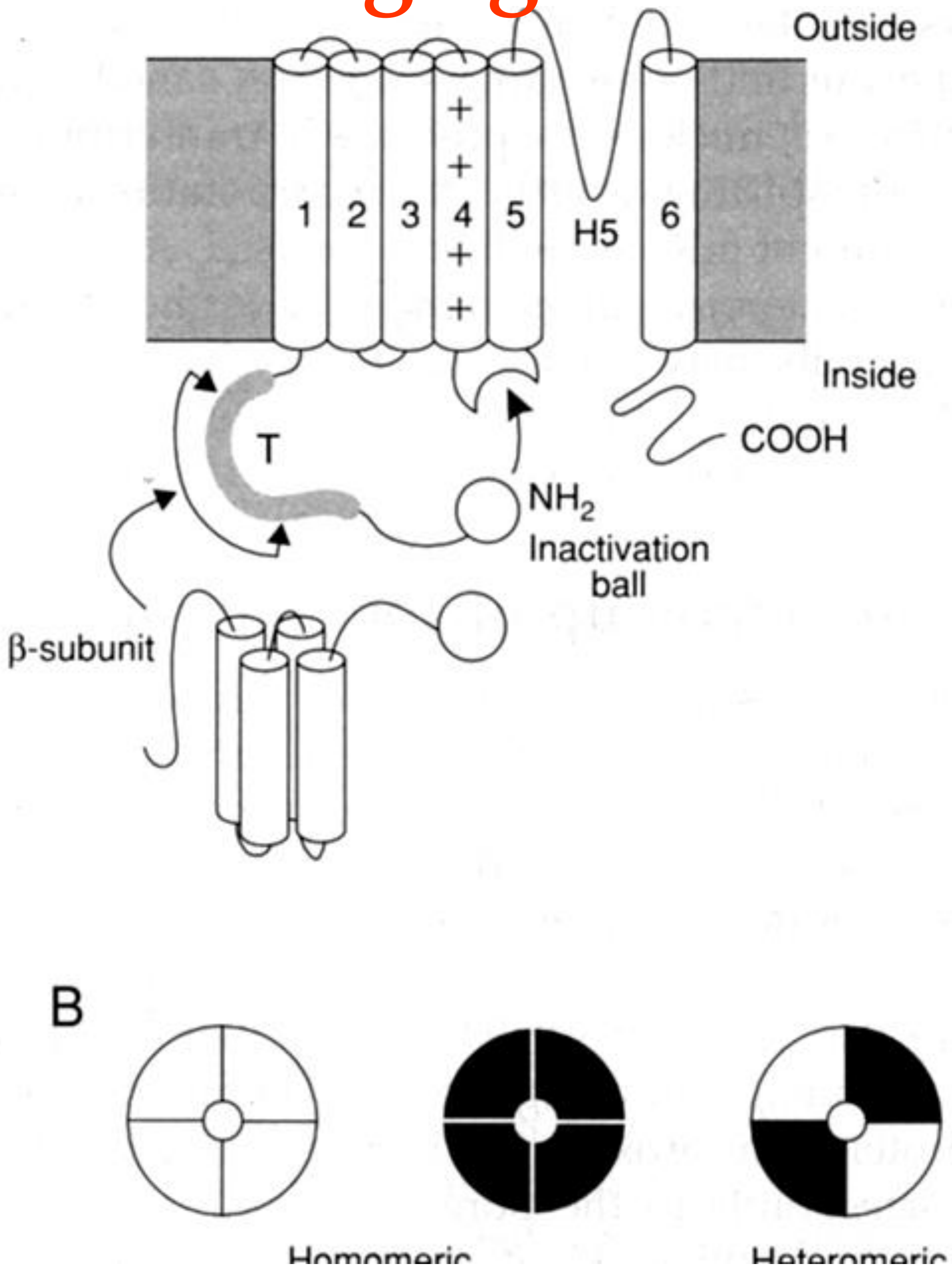


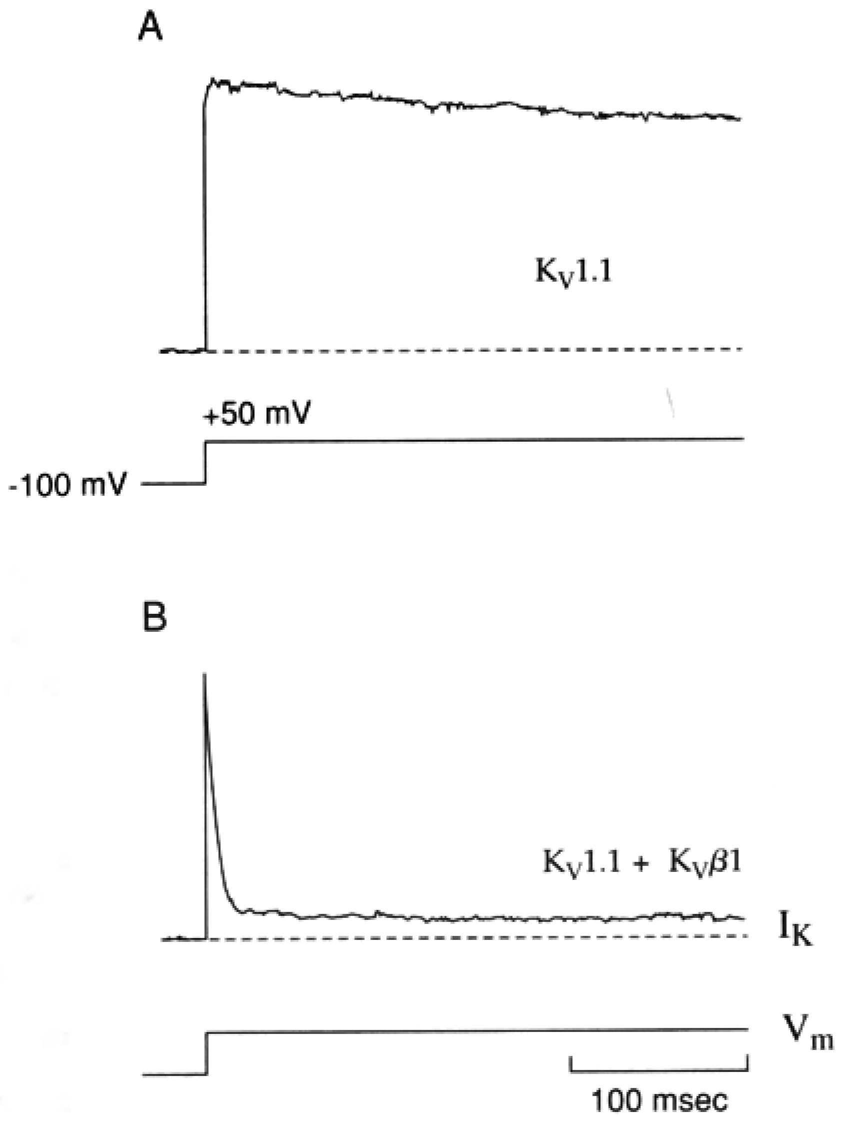
Voltage gated K+ channels vs Voltage gated Na+ channels





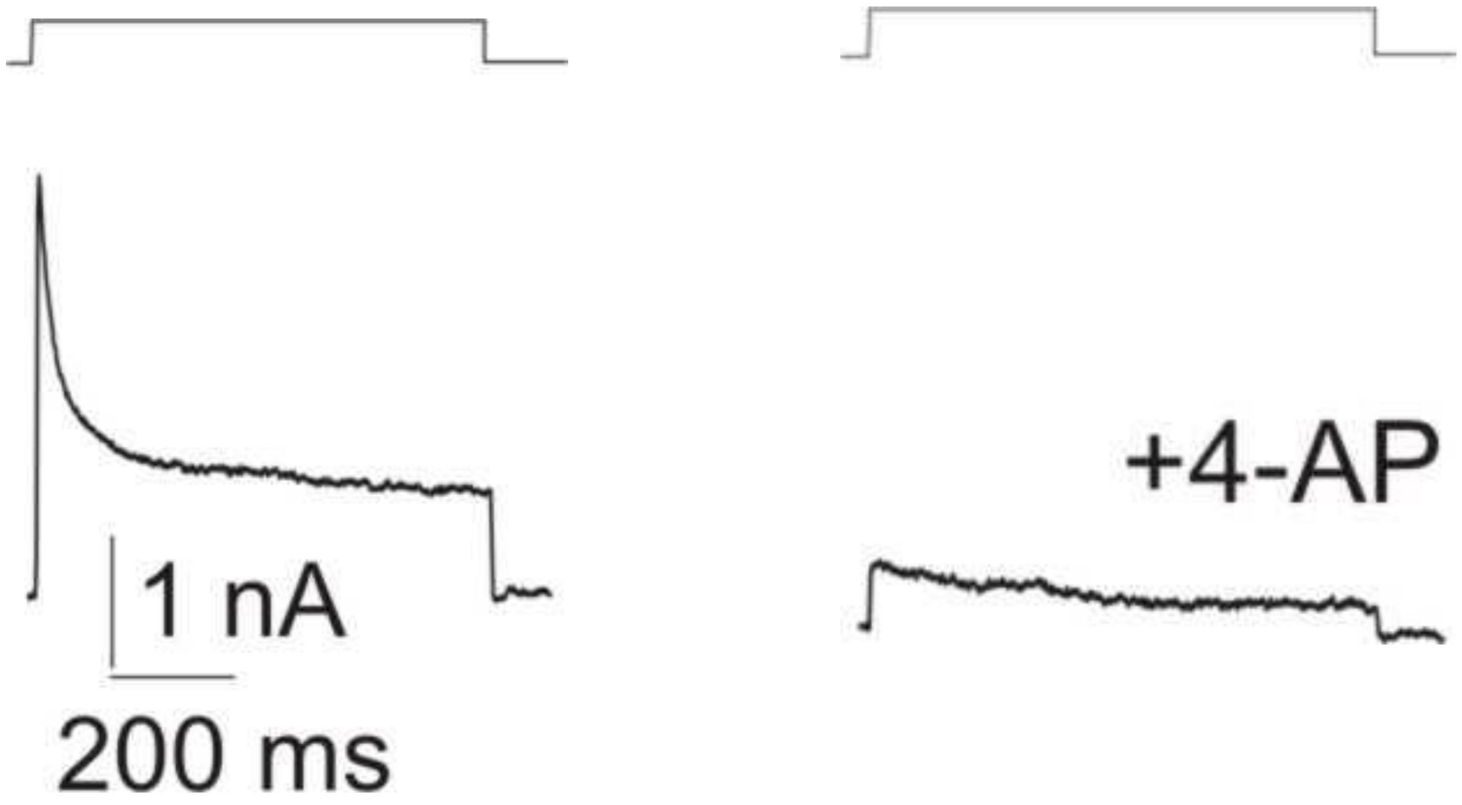
Different Voltage gated K+ channels



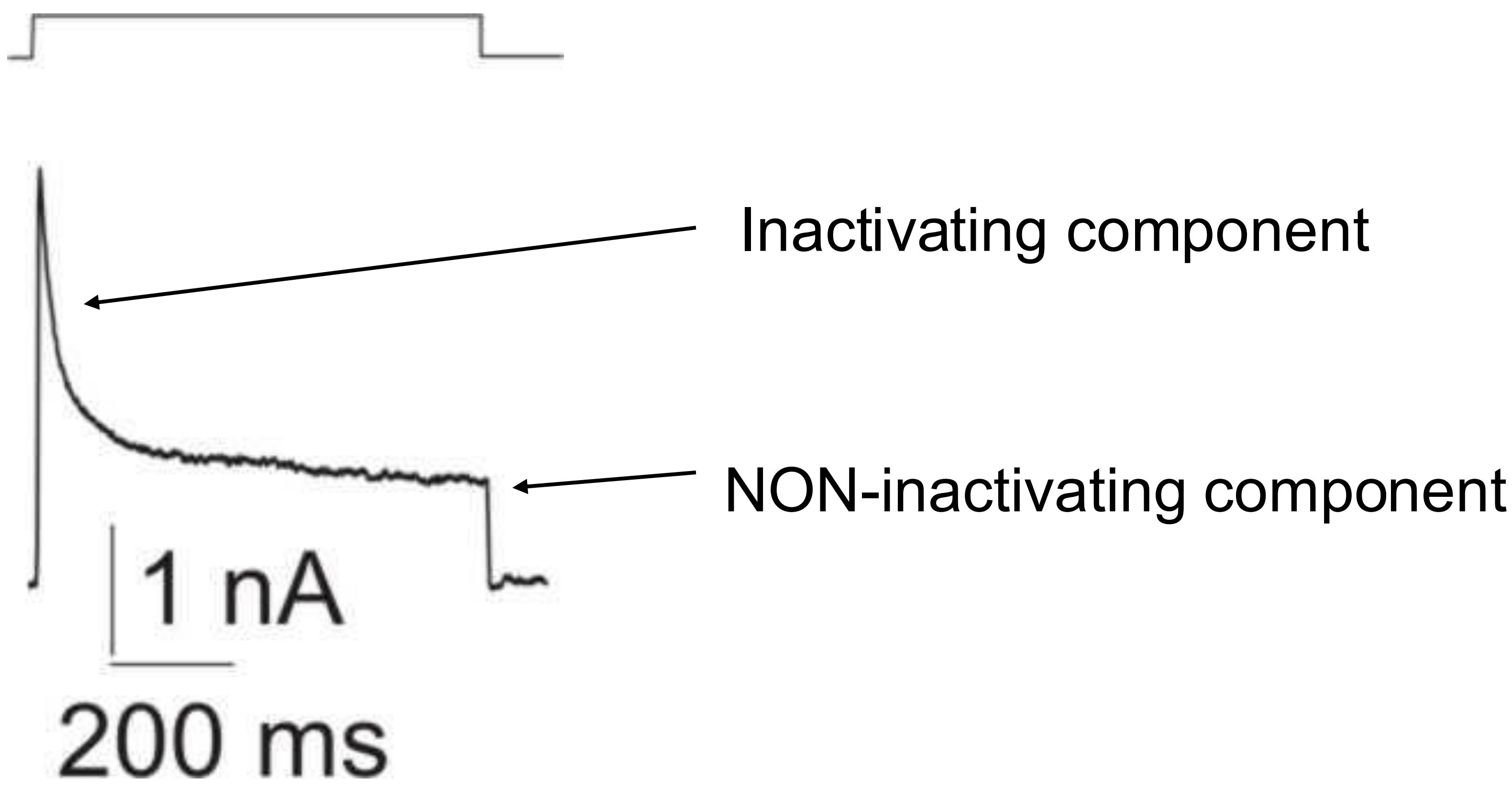


Different Pharmacology of Voltage gated K+ channels

Many types of K + channels are blocked by 4 aminopyridine

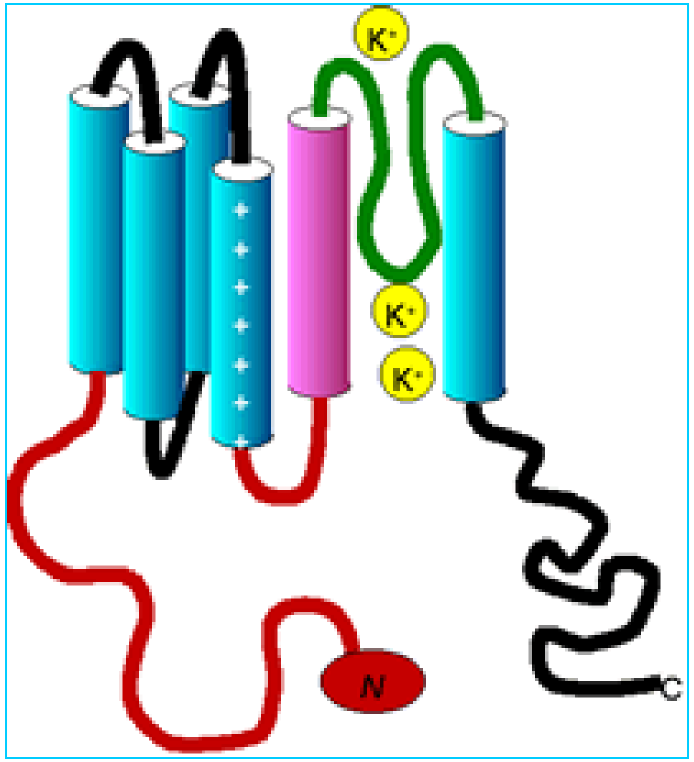


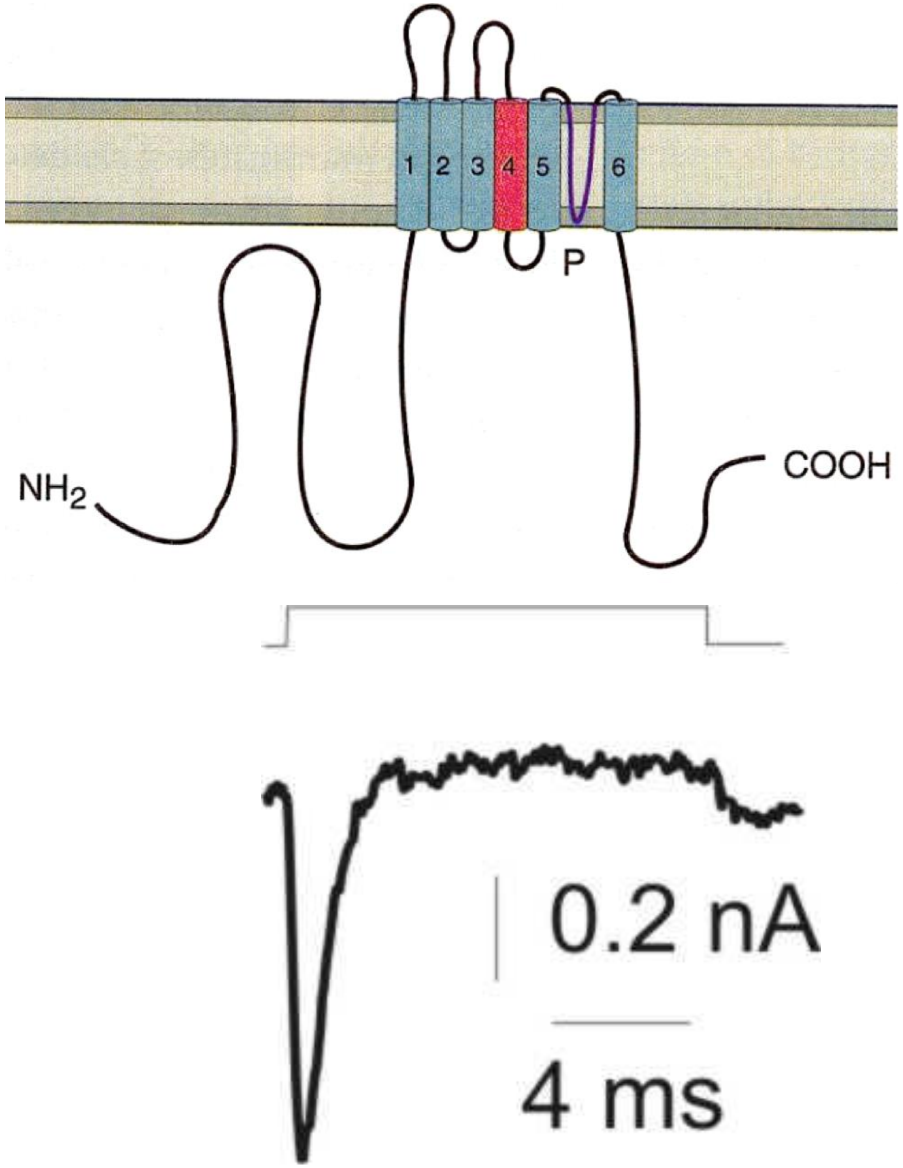
Different Inactivation of Voltage gated K+ channels



Depends on the different combinations of subunits

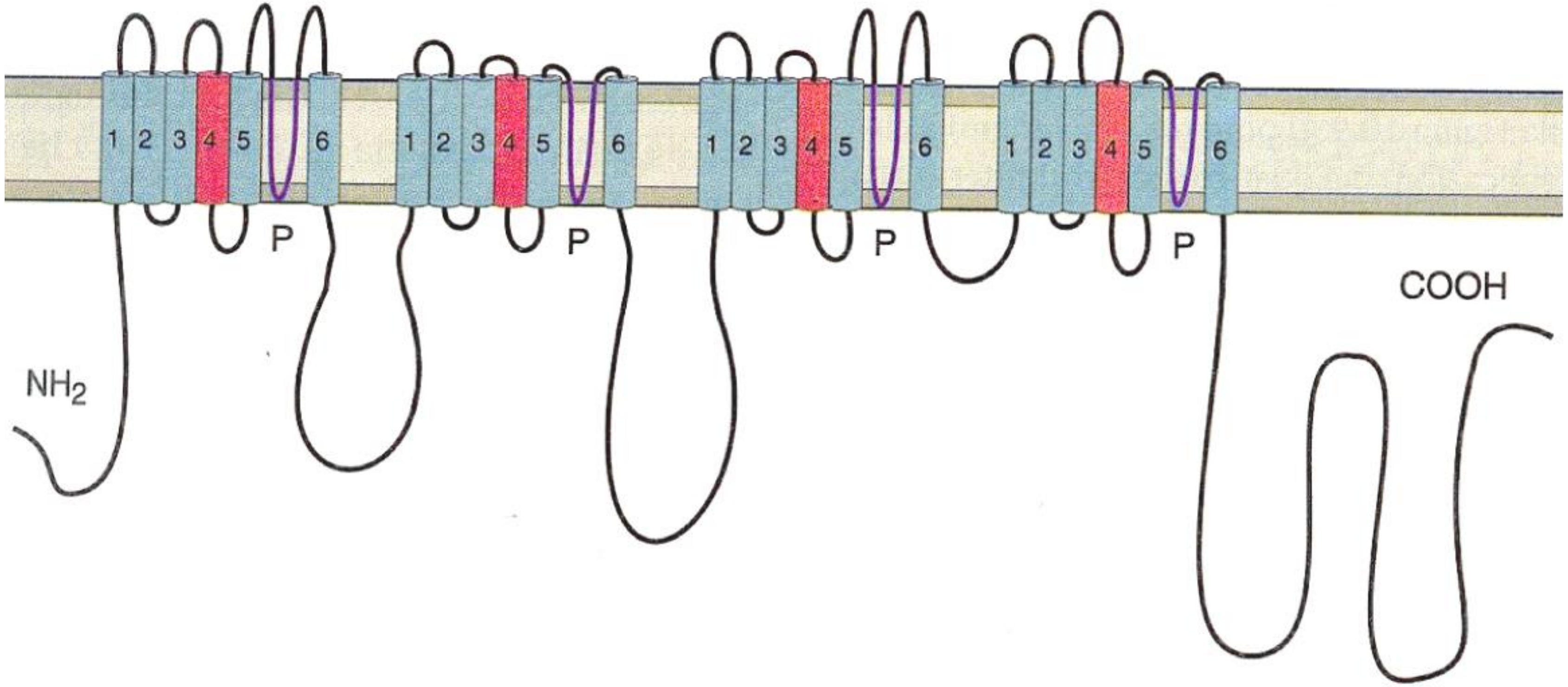
Inactivating Voltage gated K+ channels





Voltage gated Ca2+ channels

Alpha Subunit
(+ 1 beta, alpha2, delta and gamma)



They transduce electrical signals into metabolic signals

Intracellular and extracellular Ca2+ concentrations

Intracellular

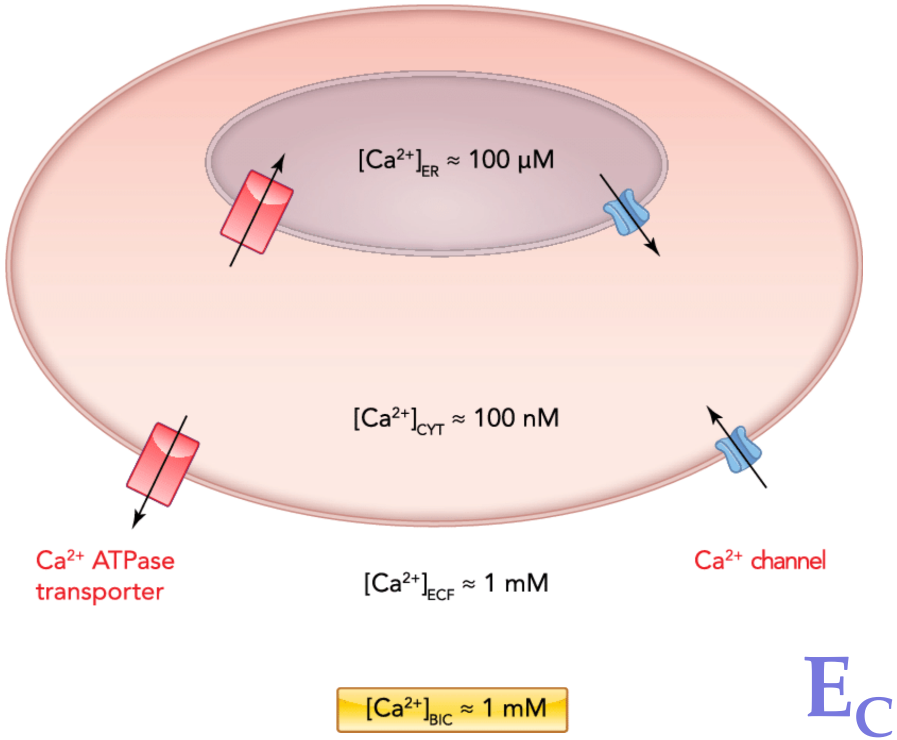
$[Ca^{2+}]_{rest} = 100$ nM

$[Ca^{2+}]_{stim} = 1000$ nM

(1 μM)

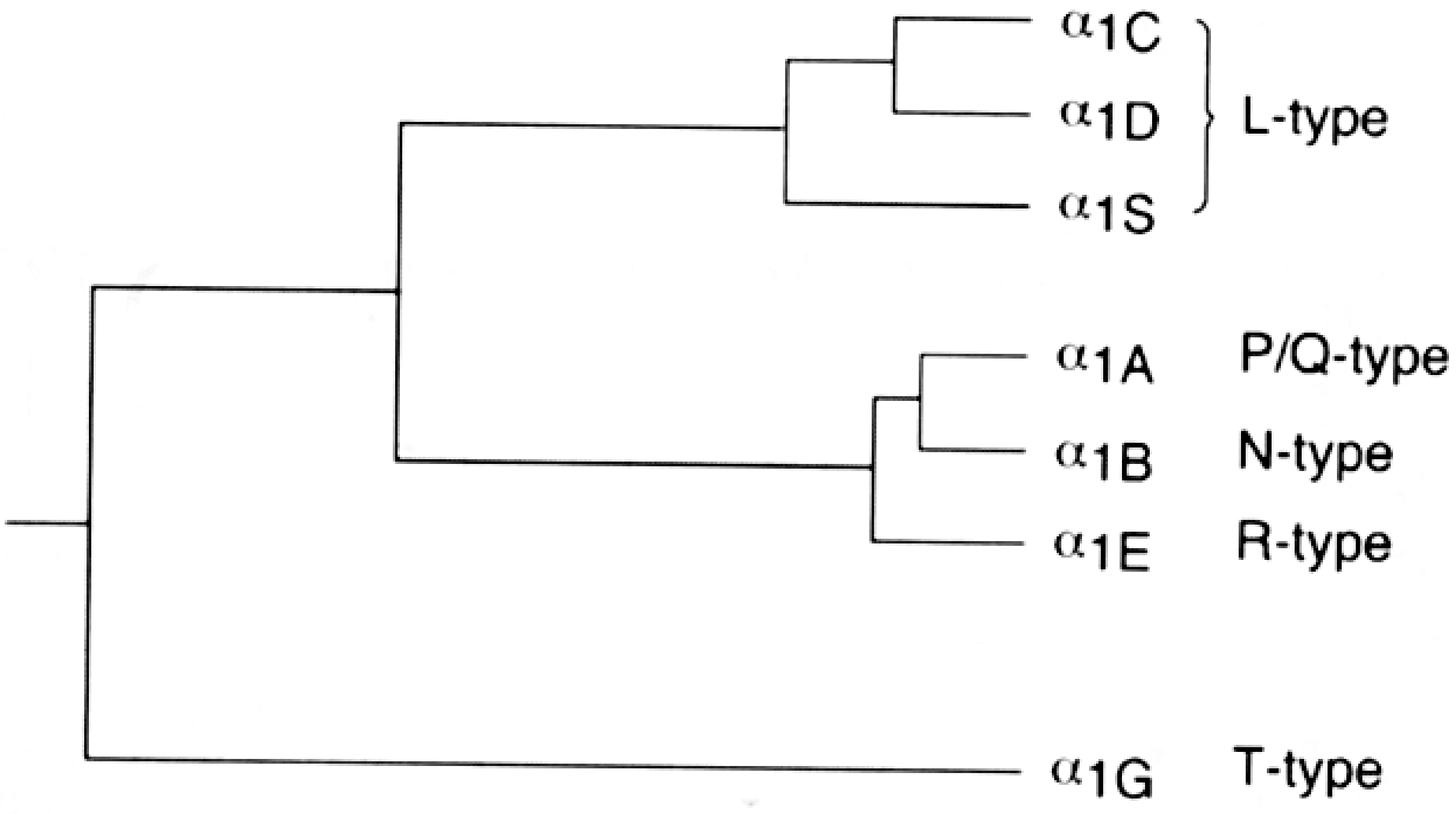
Extracellular

$[Ca^{2+}] = 1.2$ mM



$E_{Ca} = +110$ mV

Voltage gated Ca2+ channels



Voltage gated Ca2+ channels

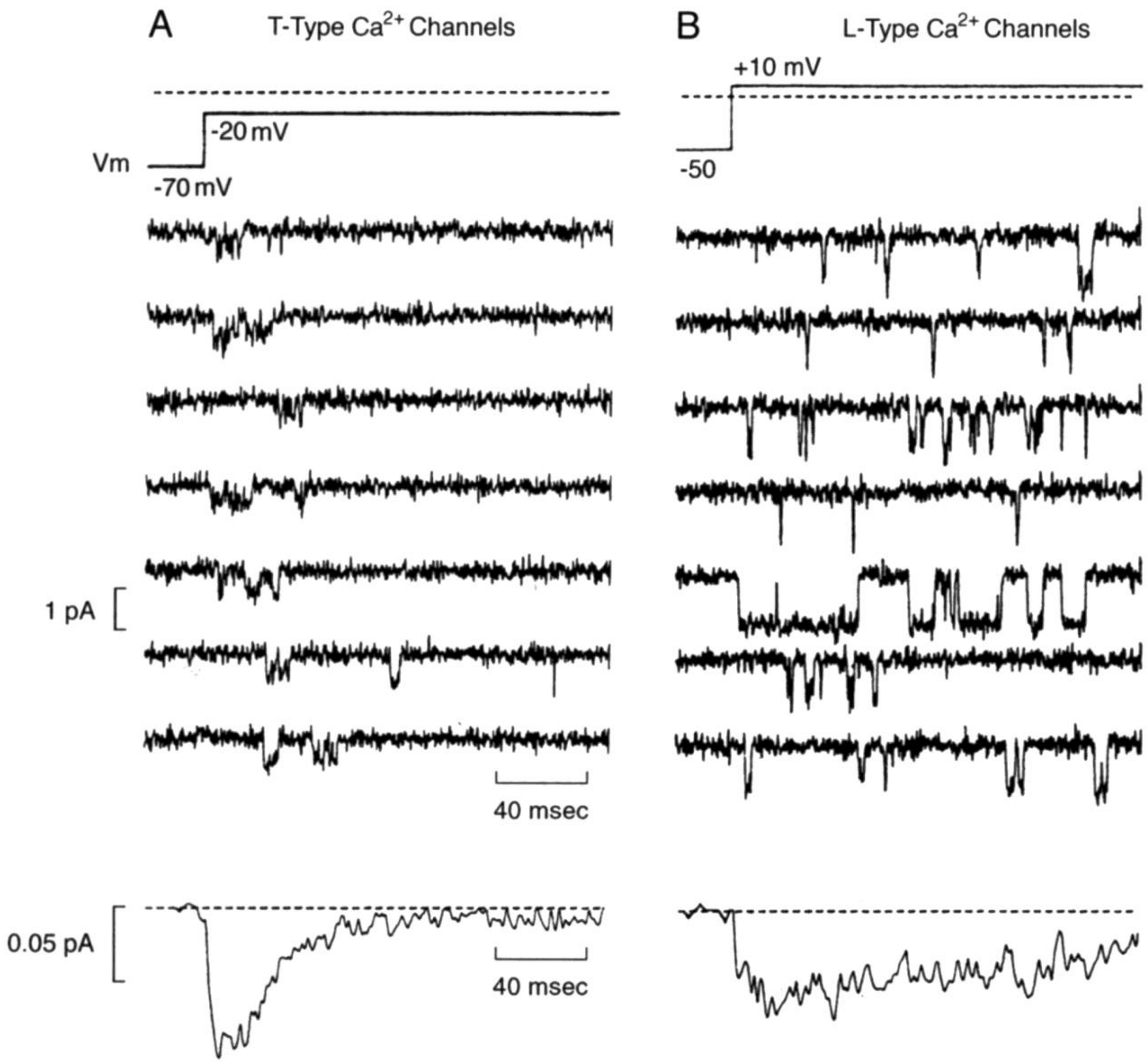
There are 2 types of voltage-dependent channels for Ca2+:

HIGH threshold
(open for large depolarizations)

LOW threshold
(open with slight depolarizations)

Low threshold and High threshold Voltage gated Ca2+ channels

$E_{Ca} = +110\ mV$



Low threshold and High threshold Voltage gated Ca2+ channels

Low threshold

In posthumous hyperpolarization
Repeated discharge control
Generation of action potentials independent of Na

Inhibite: amiloride, low nickel conc

High threshold

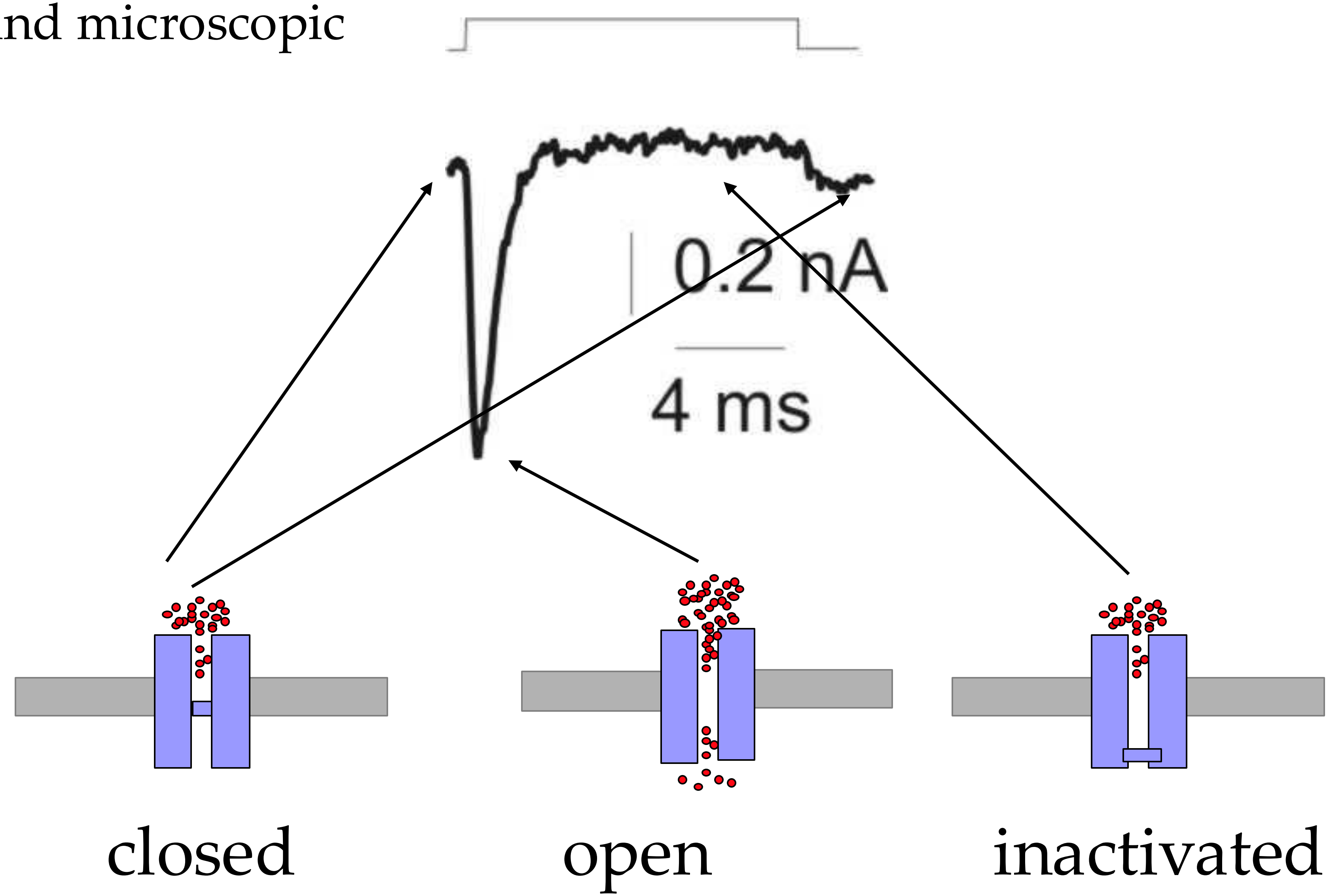
Activate Ca-dip potassium channels
Vesicular exocytosis

Dihydropyridine inhibitors: L channels
Not inhibited by dihydropyridines: non-L channels (N, R, P/Q)

Parameters that characterize Ion channels

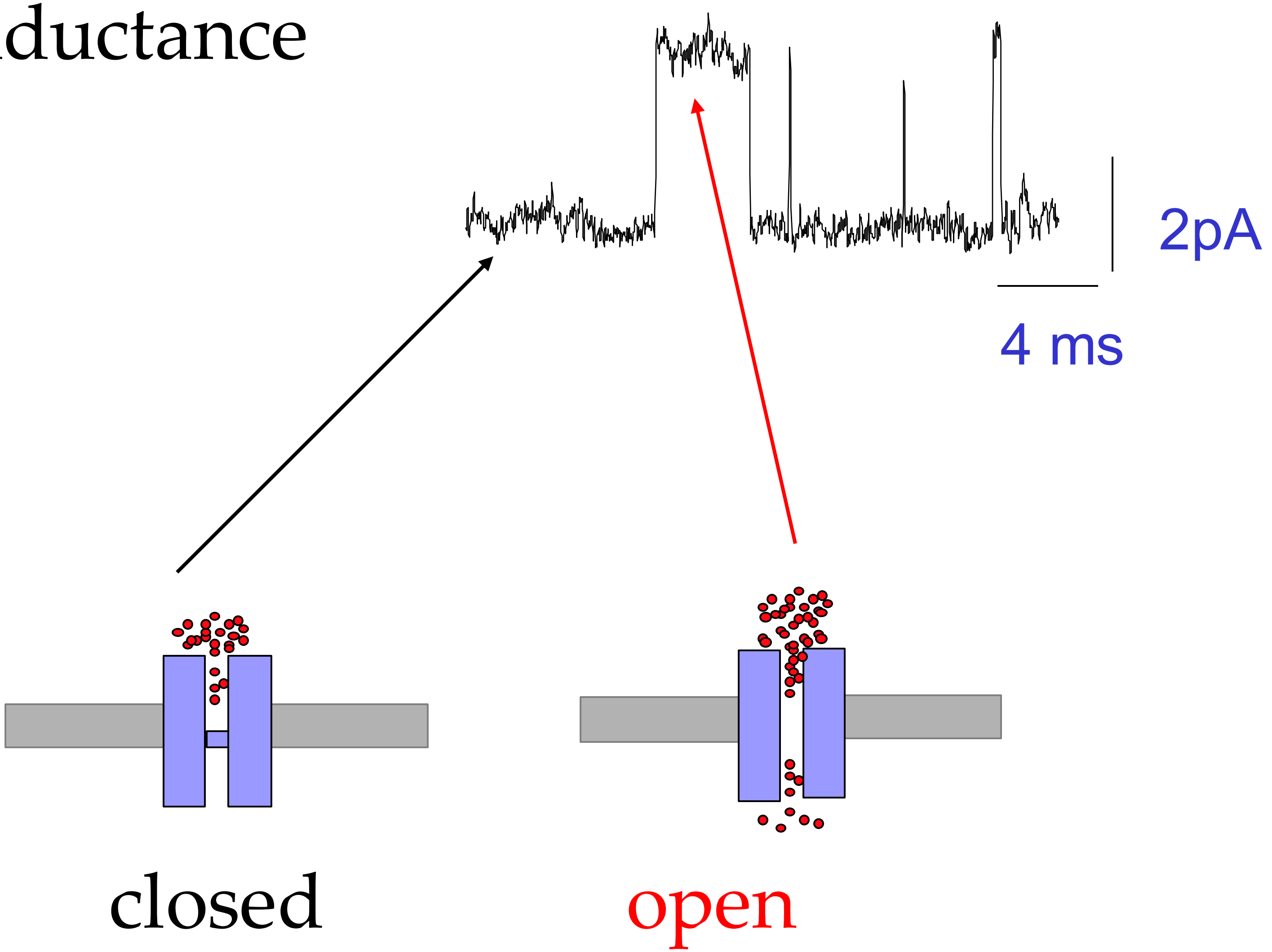
Single Channel and Whole-cell Current

Macroscopic and microscopic



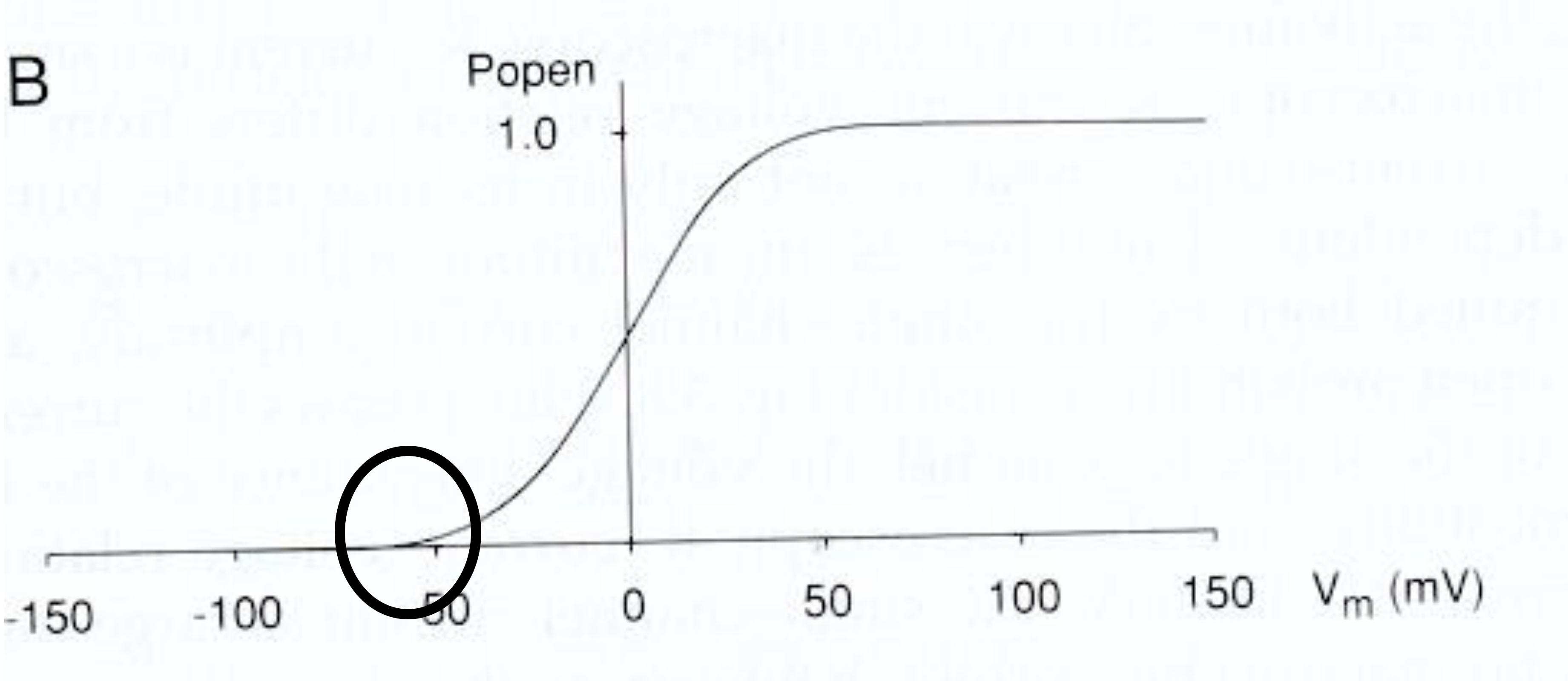
Single Channel Current

Conductance



Single Channel and Whole-cell Current

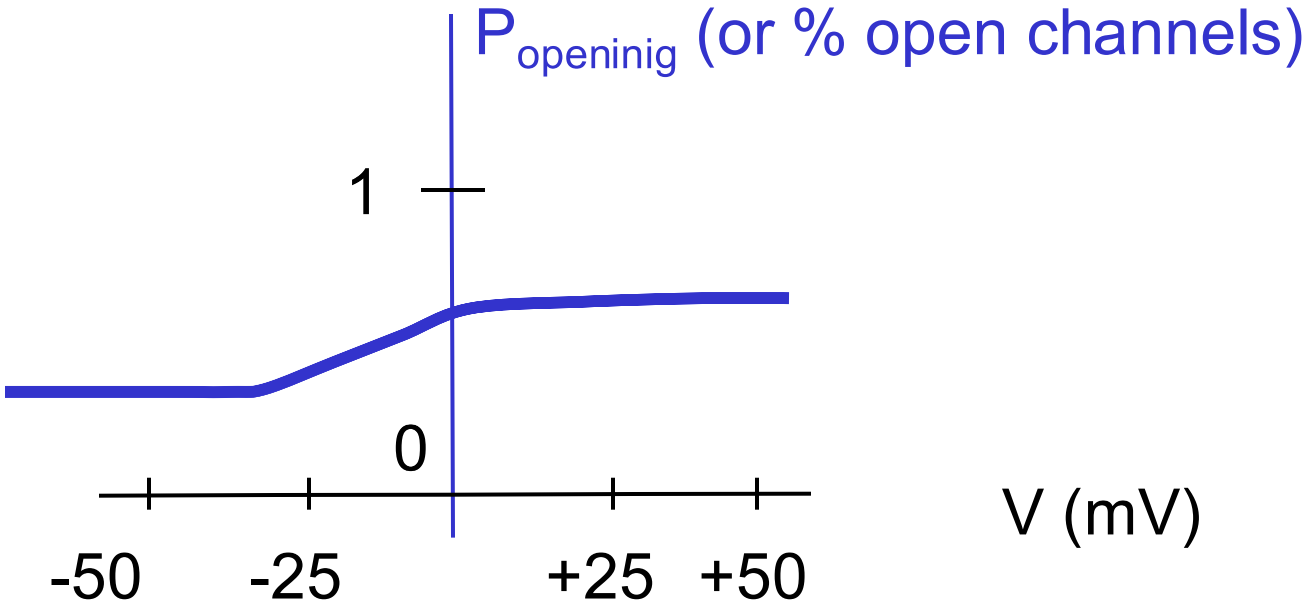
"threshold"
potential



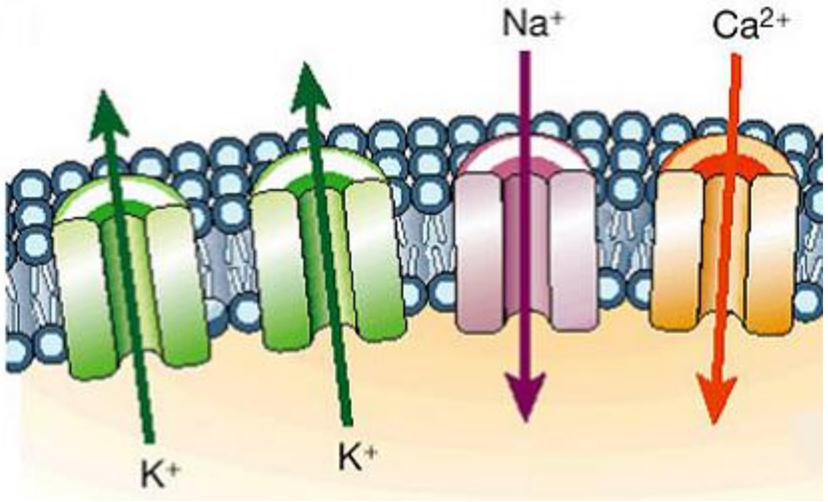
Current-voltage relashionship

Single Channel and Whole-cell Current

Opening probability



Single Channel and Whole-cell Current



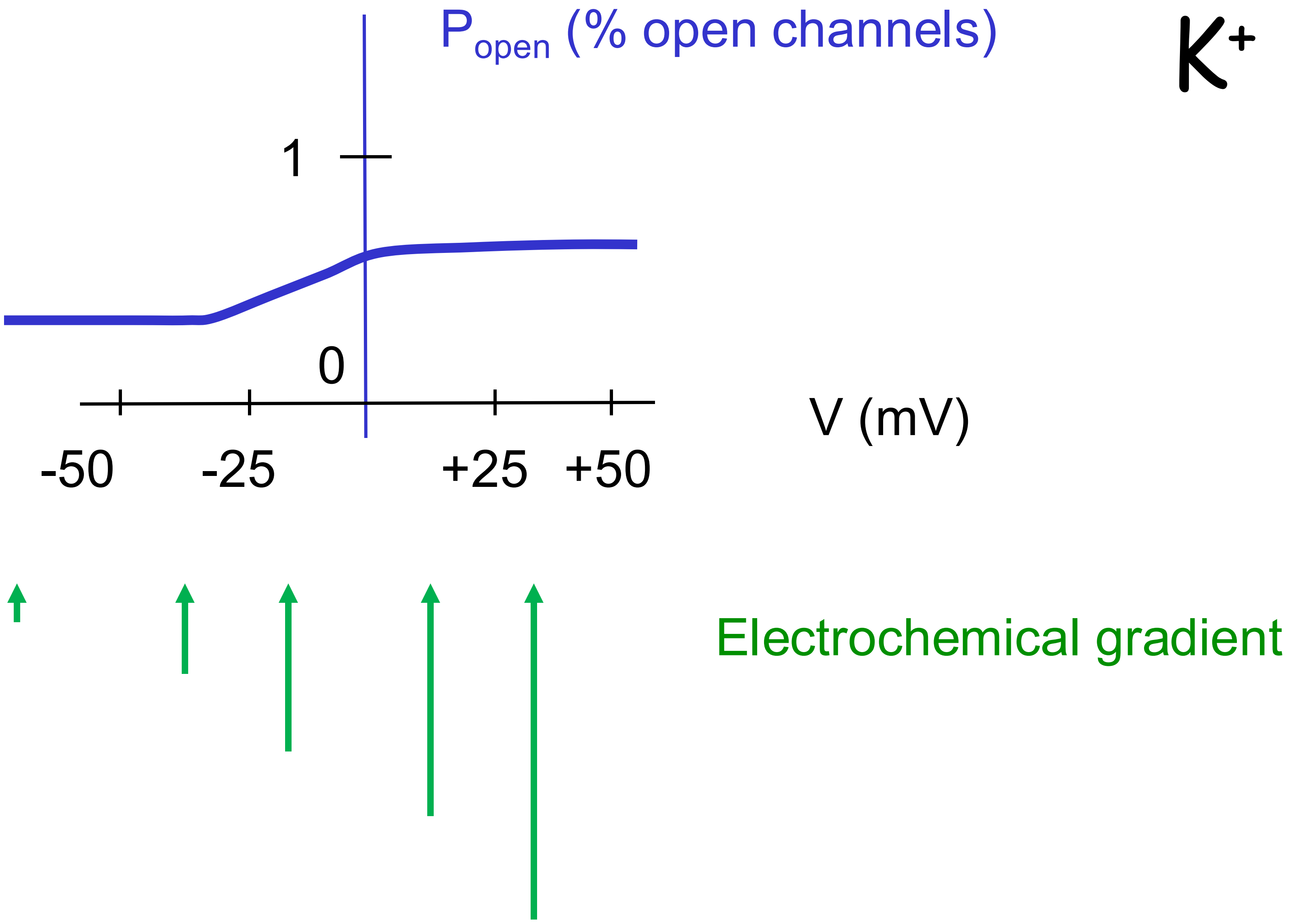
Electrochemical gradient

$$I = 1/R \times V$$

$$I = \text{conductance} \times df$$

current = No. of open channels x (channel conductance) x df

current = No. of open channels x (channel conductance)
x (Vm -ENa)

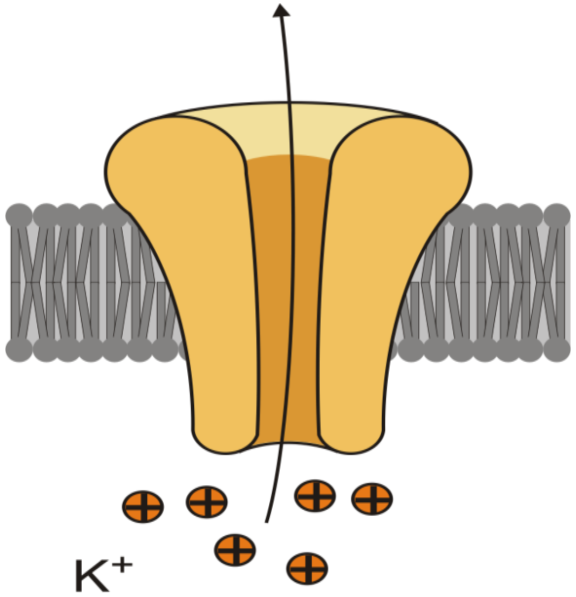


Current = N. open channels x (V_m -E_K) x (conduttanza canale)

The outward flux of K +

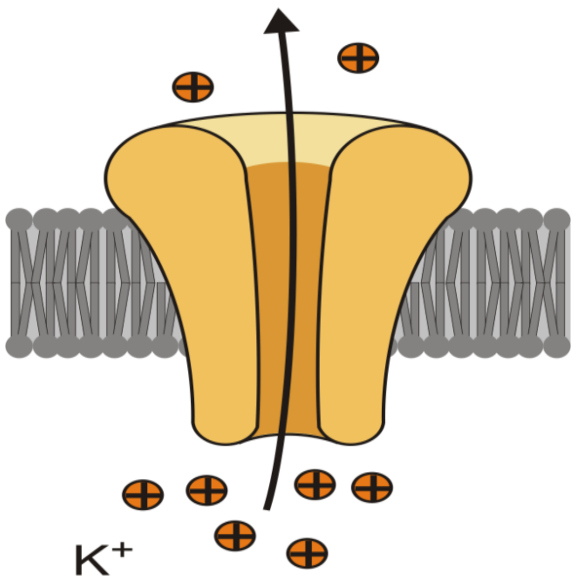
$DF = V_m - E_K$ $E_K = -80mV$

The total current of K + which leaves the cell depends not only on f.e.m. also from the number of channels open to a certain potential, or from the conductance (gK):



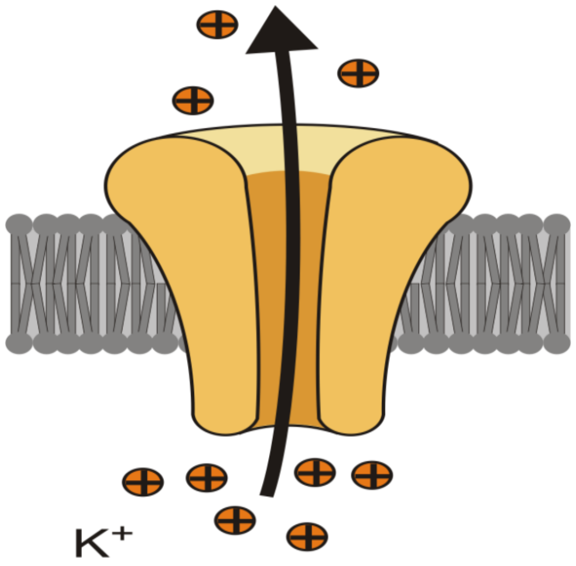
--35 mV

4% open channels
Small Driving Force
Small K^+ **flux**
Total I_K low



0 mV

70% open channels
Average Driving Force
Average K^+ **flux**
Total I_K totale **average**



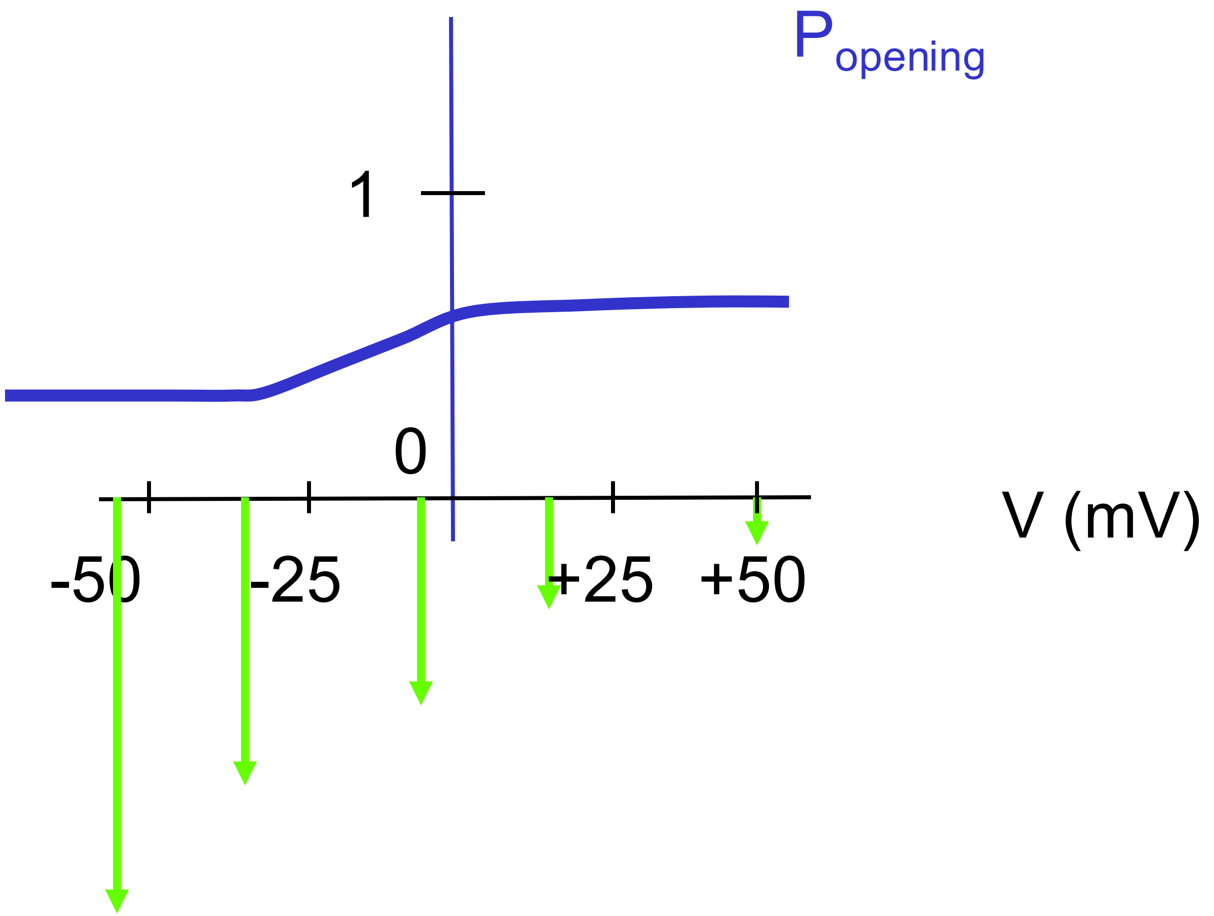
+63 mV

100% open channels
Huge Driving Force
Big K^+ **flux**
Total I_K totale **Big**

Single Channel and Whole-cell Current

Electrochemical gradient

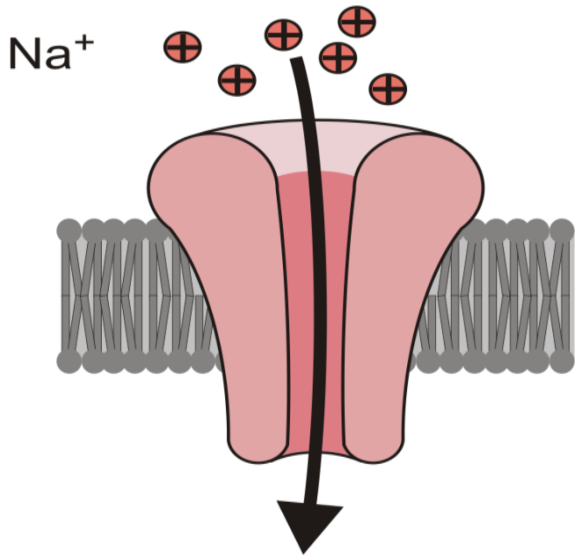
Na^+



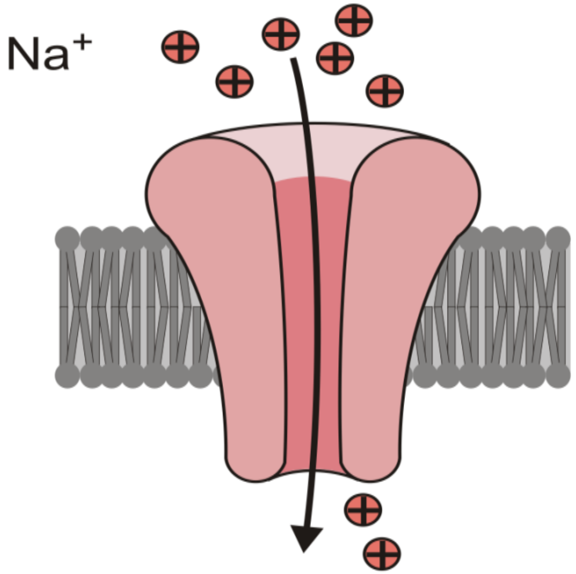
The inward flux of Na +

$DF = V_m - E_{Na}$ $\qquad$ $E_{Na} = +63 mV$

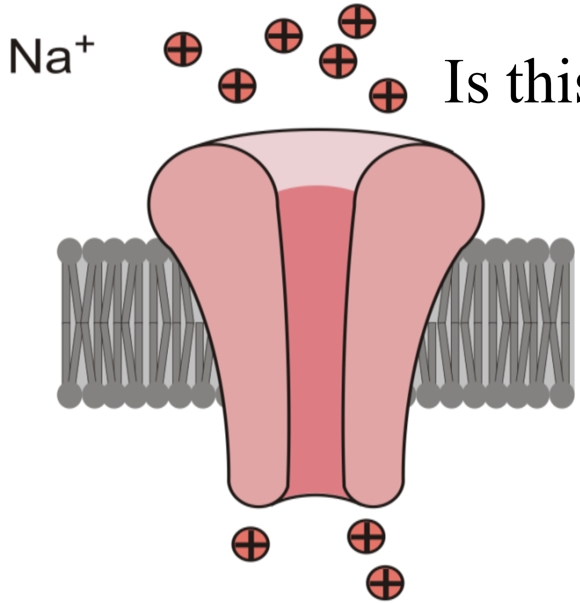
The total current of Na + which enters the cell depends not only on f.e.m. also from the number of channels open to a certain potential, or from the conductance (gNa):



-35 mV



0 mV



Is this correct?

+63 mV

10% open channels
Huge driving force
Big Na+ **flux**
Total I_{Na} **small**

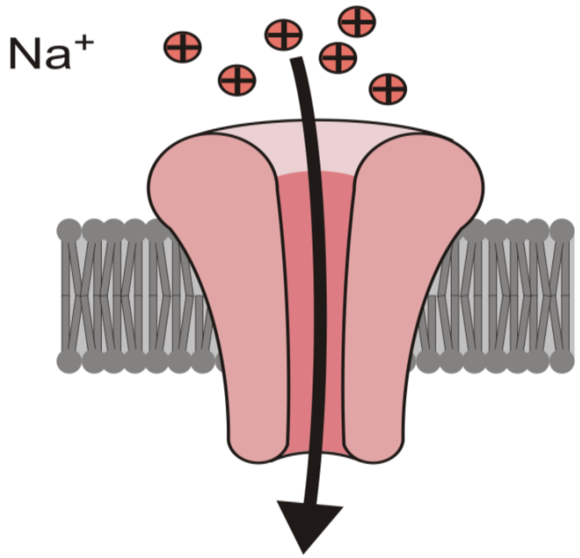
80% open channels
Average Driving force
Average Na+ **flux**
Total I_{Na} **Higest**

100% open channels?
Zero Driving force
No Na+ **Flux**
Total I_{Na} **zero**

The inward flux of Na +

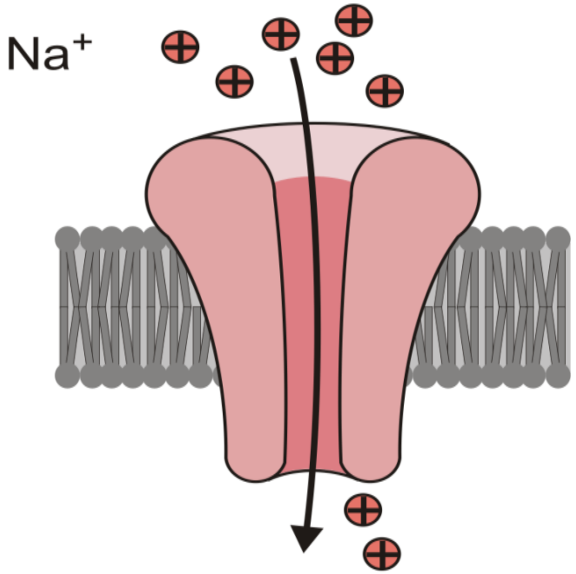
$$DF = V_m - E_{Na} \qquad E_{Na} = +63mV$$

The total current of Na + which enters the cell depends not only on f.e.m. also from the number of channels open to a certain potential, or from the conductance (gNa):



-35 mV

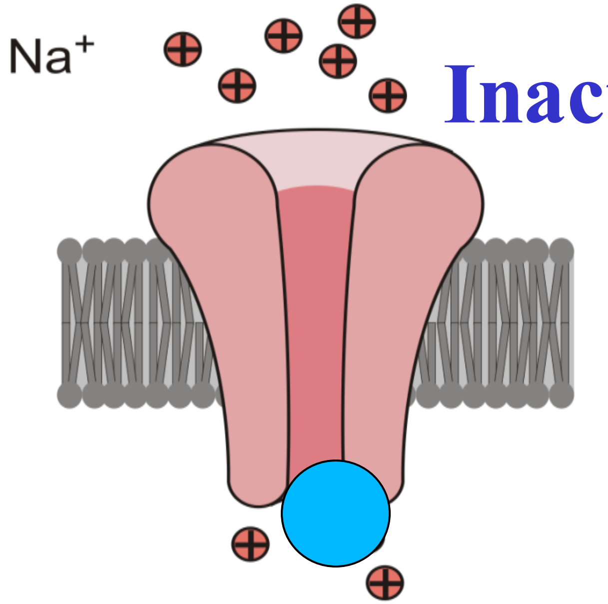
10% open channels
Huge driving force
Big Na+ **flux**
Total I_{Na} **small**



0 mV

80% open channels
Average Driving force
Average Na+ **flux**
Total I_{Na} **Higest**

Inactivated



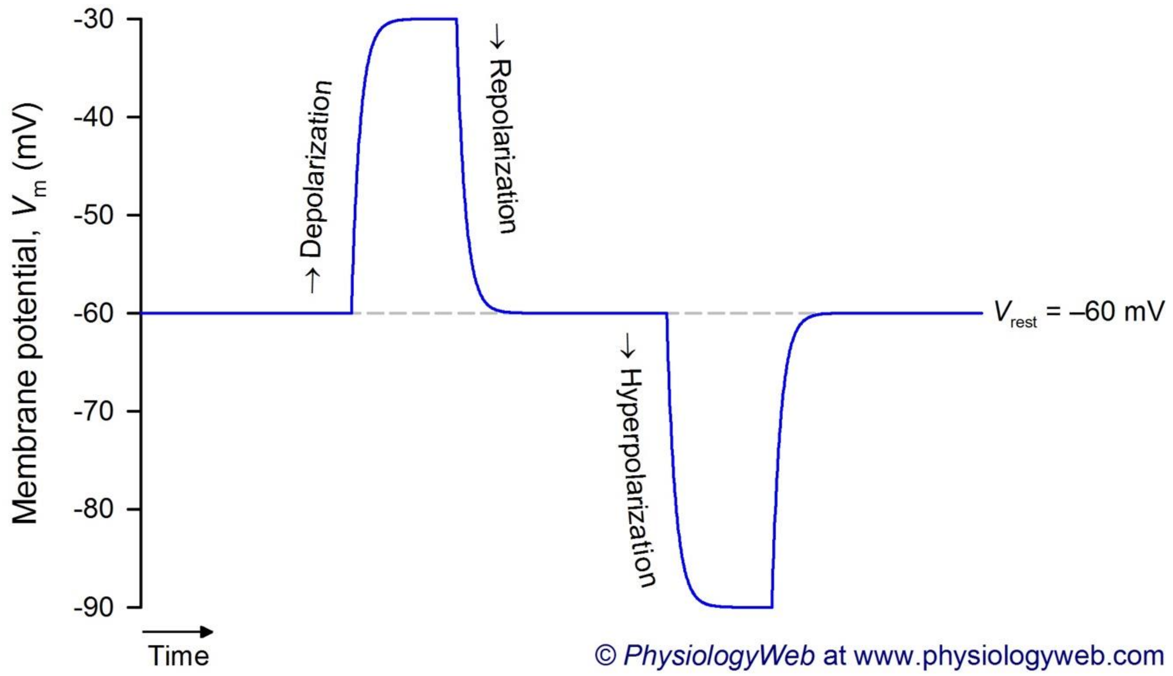
+63 mV

100% Inactivated channels
No Na+ **Flux**
Total I_{Na} **zero**

channels open by hyperpolarization

Voltage-dependent Ion Channels

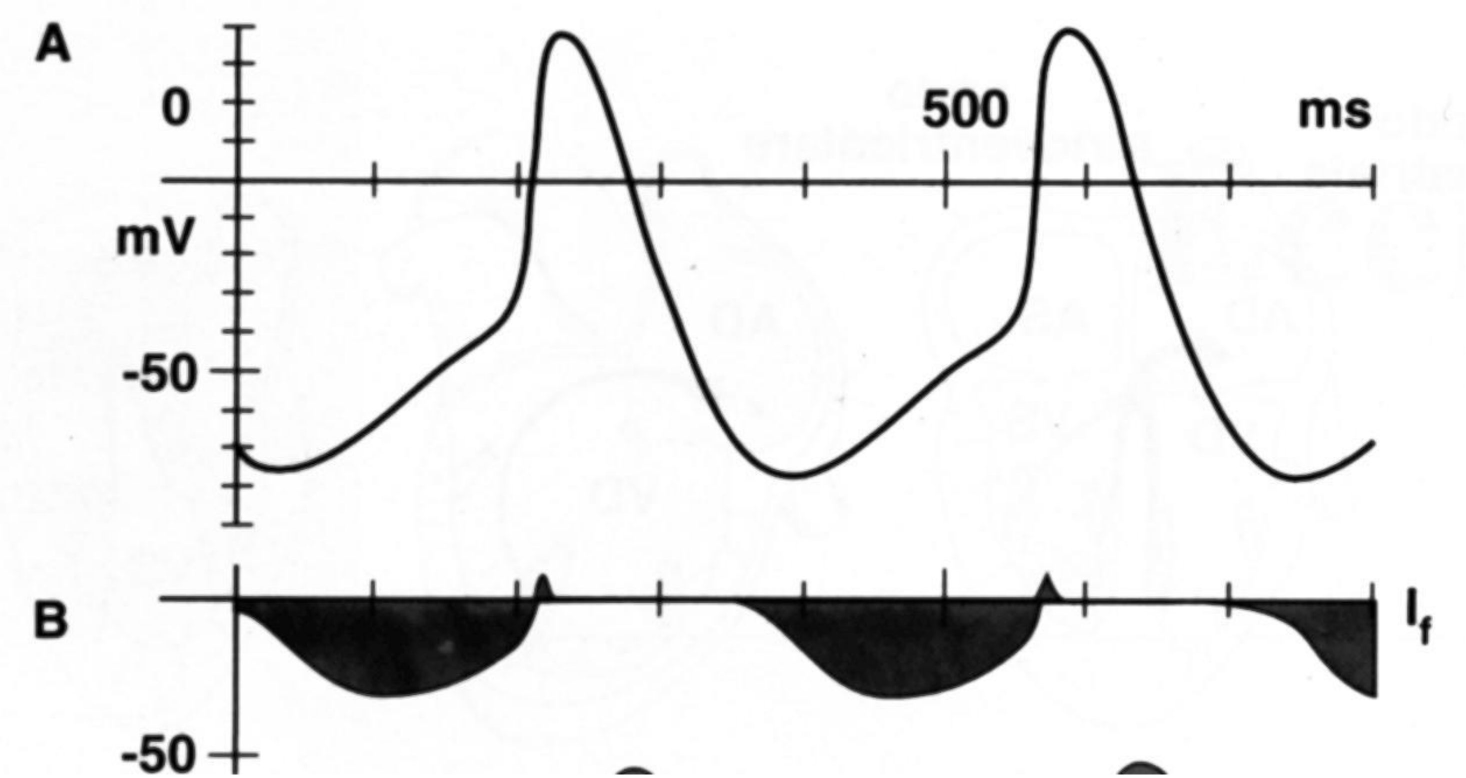
channels open by hyperpolarization



I_{funny}

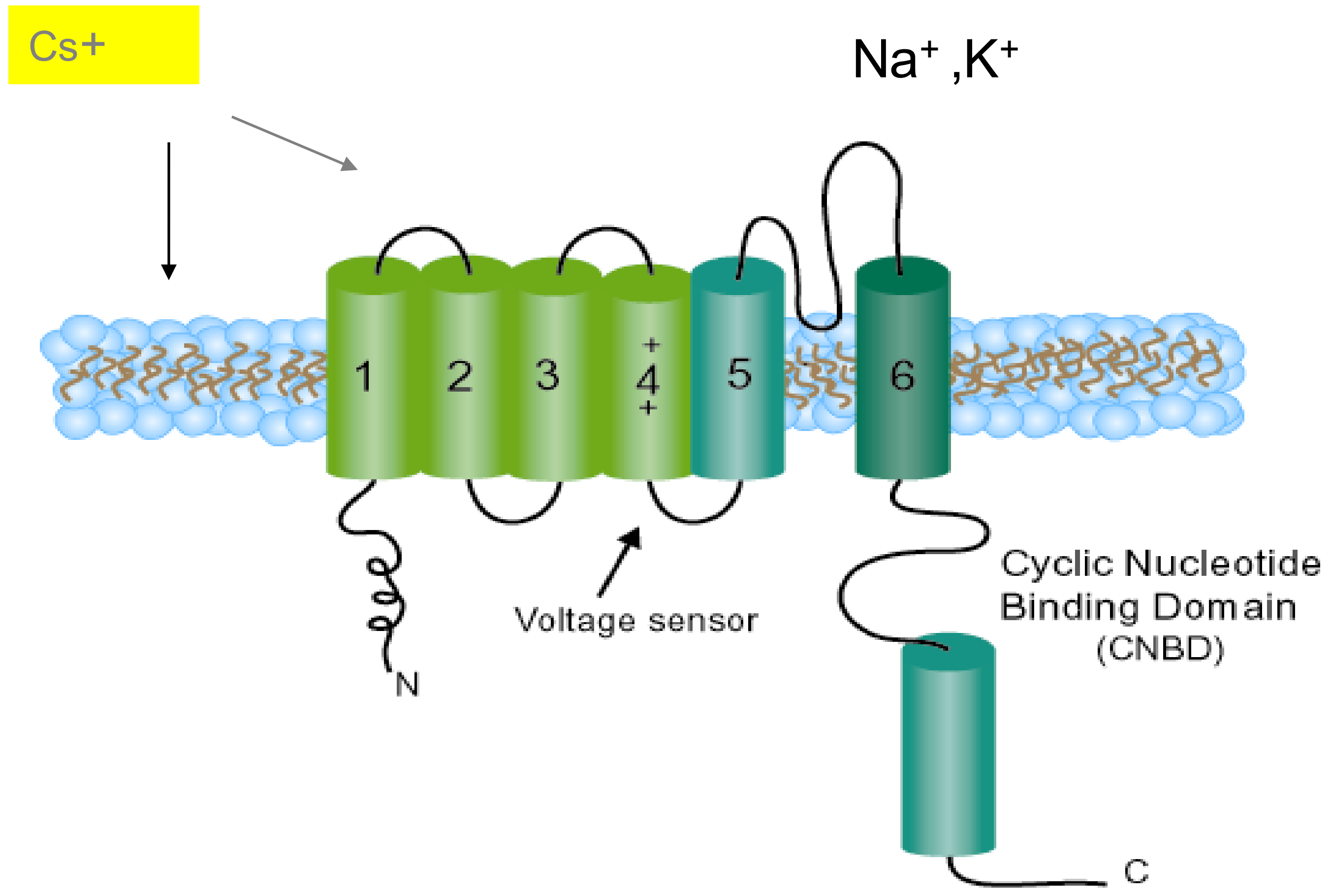
They are cationic channels permeable to Na^+ and K^+

They determine rhythmic variations of Vm



Structure of HCN channels

similar to V gated K channels



The current If has singular characteristics

1) It is a voltage dependent current that is activated in hyperpolarization: the channel opens when the others close

2) The opening of the channels induce a very slow current, entering "inward" that begins at the end of the action potential after the cell has reached its negative potential

3) Both sodium and potassium flow in the channel, it has a mixed conductance

4) The channel is modulated directly by the cyclic nucleotides and in particular by cAMP and blocked by Cesium

If has helped to found a new family of channels whose exact definition is "activated by hyperpolarization and modulated by nucleotides (HCN)" whose recognition occurred in 1998 with the identification of the genes coding for these proteins.

To this family belong similar currents to If discovered in neurons and photoreceptors.

I_{funny}

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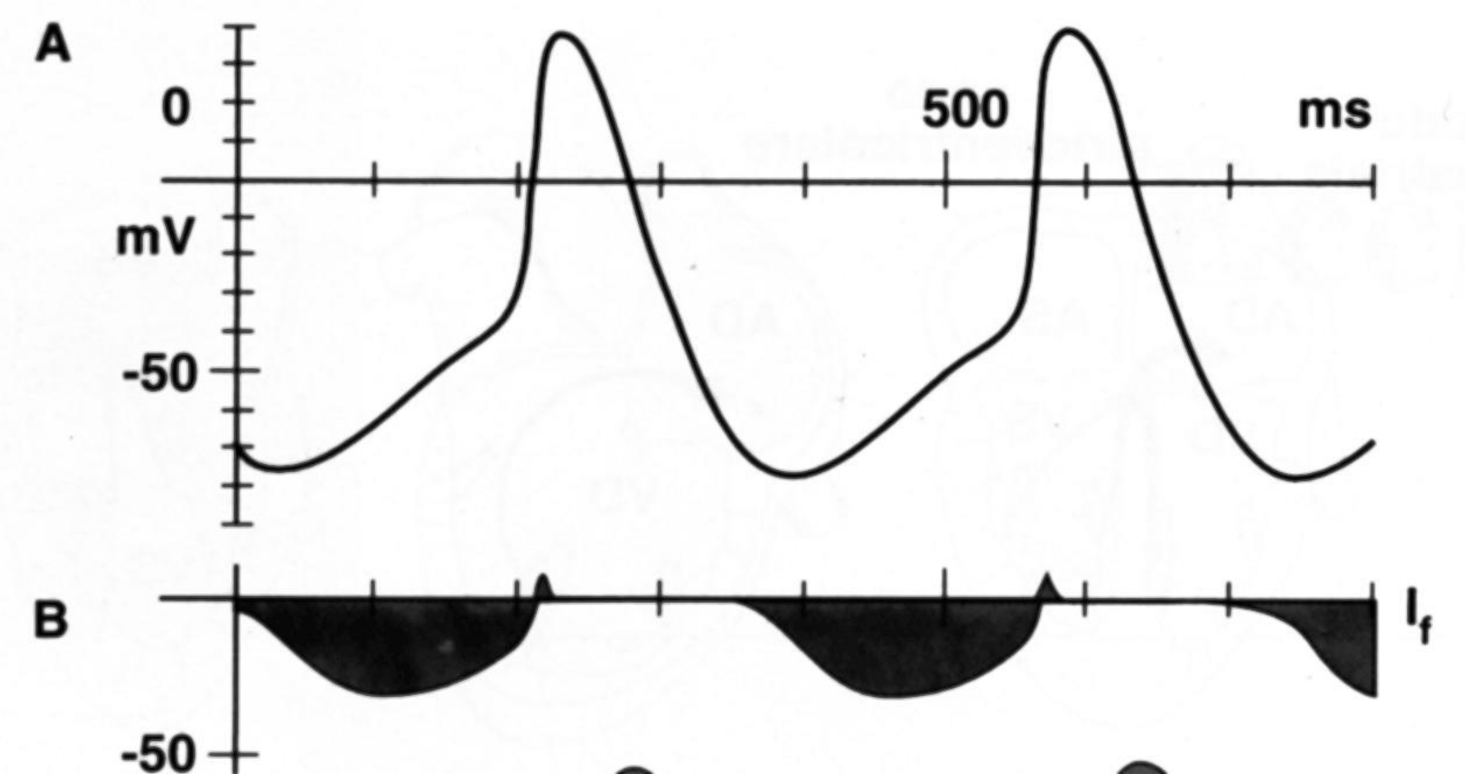

Action potential in the Seno-atrial node

1) Starter tissue cells (pacemakers) have a low membrane potential of -60 mV
2) To this Vm is active the cationic current depolarizing If permeable to Na + and to K +.
3) After a first depolarization due to If follows a current of voltage-dependent Ca2 + which at a certain point becomes strongly regenerative. Depolarization inhibits If.
4) Depolarization activates a current to K + that repolarizes the cell.
5) Repolarization closes the channels to the Ca2 + and activates the If

Action potential in the Seno-atrial node

- I_f : activated by repolarization
- $\mathbf{I}_{Ca2+}$: activated by depolarization, transient current
- I_{k+} : activated by depolarization