Ion Channels

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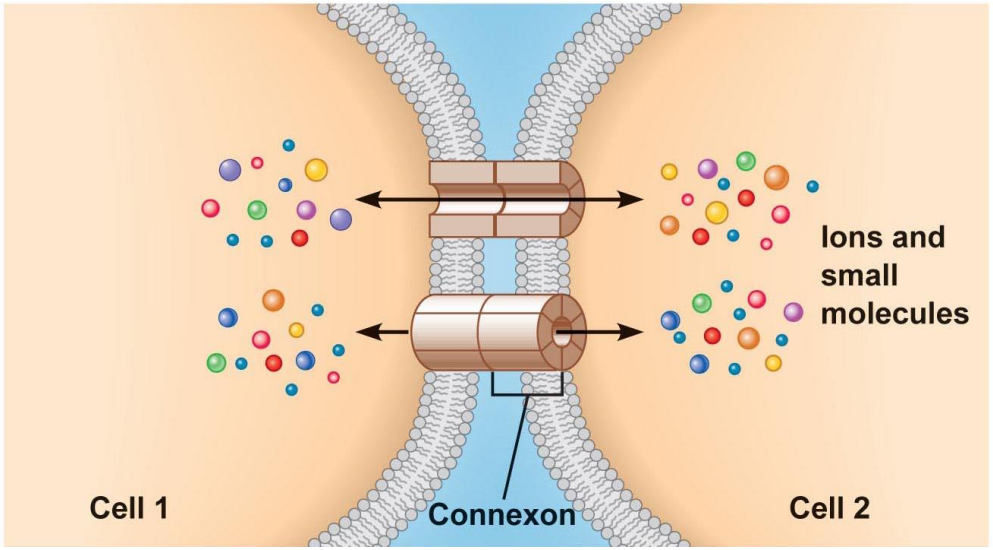
- Channel proteins differ from transporter proteins in that :
- they form a hydrophilic pore through the membrane and allow passage of ions through diffusion.

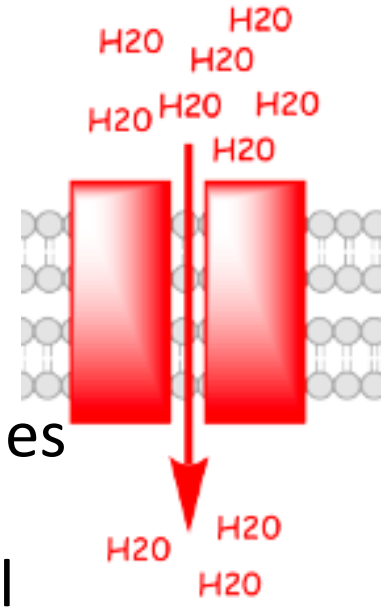
- Gap junctions
- Porines
- Ion channels

Gap Junctions



- Hydrophilic channels
- Combine two adjacent cells
- Each adjacent cell contributes to the channel formation equally
- They unify two cytoplasm

Porins



- Hydrophilic channels
- Large diameter and high permeability
- Transport medium-sized or charged molecules across, a water-filled channel or pore
- Porins typically control the diffusion of small metabolites like sugars, ions, and amino acids.
- In gram-negative bacteria outer membrane contains porins, which render it largely permeable to molecules less than about 1500 daltons. Many bacterial toxin acts through porins.

- Ion channels

- Two important properties distinguish ion channels from simple aqueous pores.
- *ion selectivity*
- *they are gated*

Ion channels regulate information traffic

- Fast information transport
- Transport effficacy is 10^5 x higher than transporter proteins
- Approx. 10^8 ions/s can be transmitted at each opening
- Especially nerve cells can display high response to the small stimuli

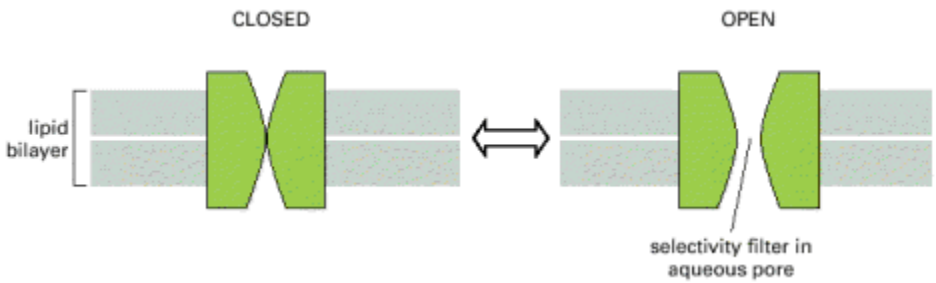
- Excitable cells
- Contraction
- Secretion
- Sensation
- Brain processing
- Transmission of brain output to the periphery

- Non excitable cells
  - Secretion
  - Gen expression
  - Cell division
  - Osmotic regulation

Voltage gated channels takes part in all these processes

- Transport is due to the passive electrochemical gradients
- They provide fast transport of inorganic ions like Na, K, Ca, Cl
- Despite its high rate, transport is highly specific

- (there are channels permit passage of the several types of ions, but most of them are specific to the one type)



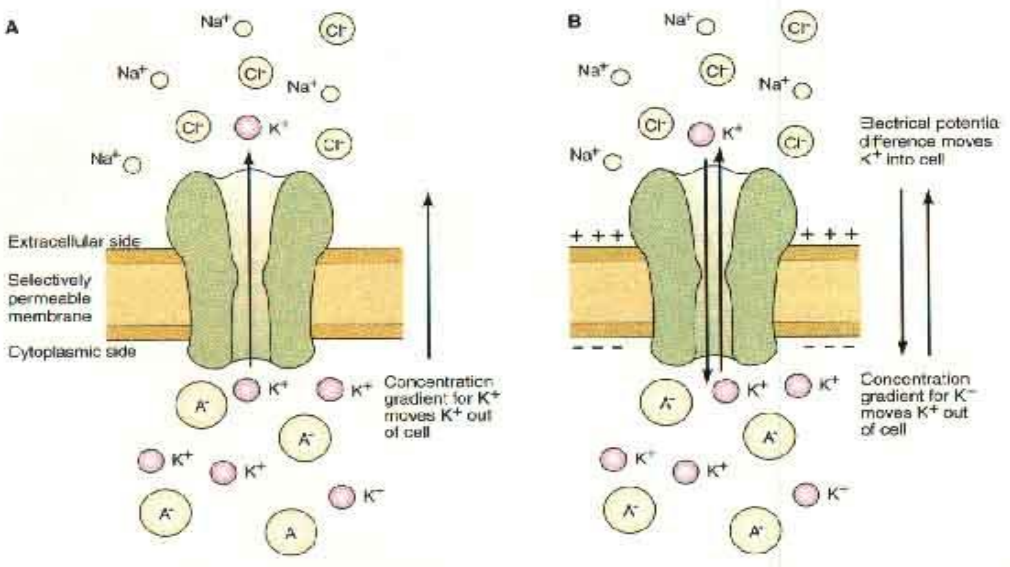
A typical ion channel fluctuates between closed and open conformations. This is called «gating»

Polar groups are thought to line the wall of the pore, while hydrophobic amino acid side chains interact with the lipid bilayer The pore narrows to atomic dimensions in one region (the selectivity filter), where the ion selectivity of the channel is largely determined.

- The main types of stimuli are:
- a change in the voltage across the membrane *(voltage-gated channels)* e.g. Na_v , K_v
- a mechanical stress *(mechanically gated channels),*
- the binding of a ligand *(ligand-gated channels).*
- The ligand can be either an extracellular mediator—specifically, a neurotransmitter *(transmitter-gated channels)*—e.g. GABA or glycine
- an intracellular mediator, such as an ion *(ion-gated channels)* or a nucleotide *(nucleotide-gated channels)*. e.g. Ca^{2+}, cAMP, cGMP or PI
- The activity of many ion channels is regulated, in addition, by protein phosphorylation and dephosphorylation
- With prolonged (chemical or electrical) stimulation, most channels go into a closed “desensitized” or “inactivated” state, in which they are refractory to further opening until the stimulus has been removed, as we discuss later.

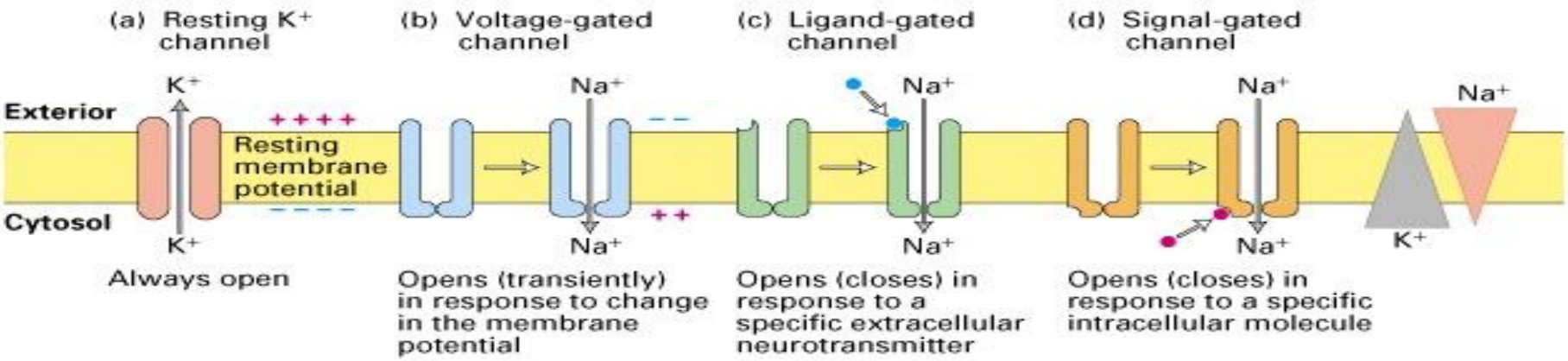
Leak Channels

- There are also «ungated» ion channels called leak channels
- They are always open
- Since there are many leak channels of K^+
- in the membrane.
- membrane is
- highly
- permeable to
- potassium



Ion Channels

- ion channels in the PM of neurons and muscles contributes to their excitability
- when open - ions move down their concentration gradients
- channels possess gates to open and close them
- two types: **gated and non-gated**



1. Leakage (non-gated) or Resting channels: are always open, contribute to the resting potential

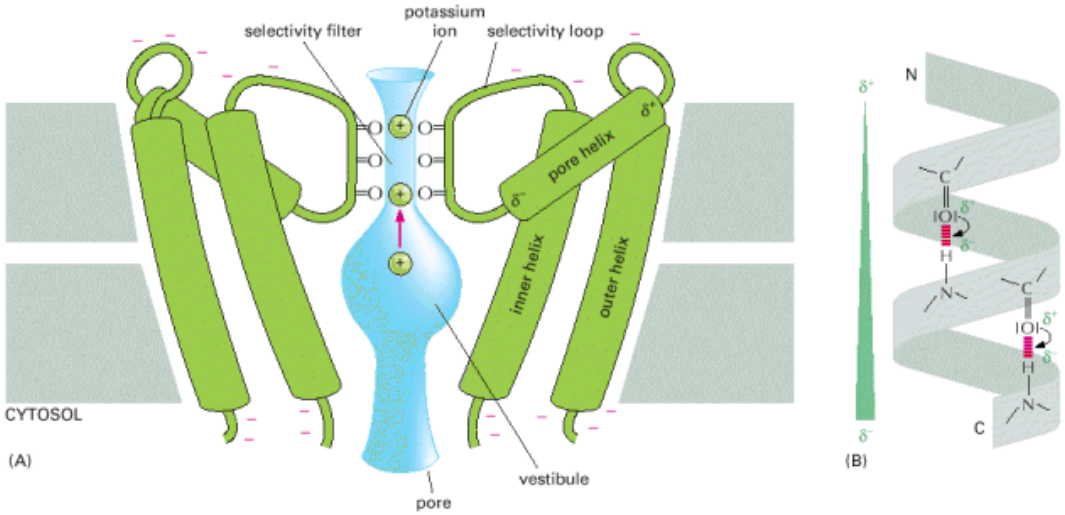
-nerve cells have more K+ than Na+ leakage channels
-as a result, membrane permeability to K+ is higher
-K+ leaks out of cell - inside becomes more negative
-K+ is then pumped back in

2. Gated channels: open and close in response to a stimulus

A. voltage-gated: open in response to change in voltage - participate in the AP
B. ligand-gated: open & close in response to particular chemical stimuli (hormone, neurotransmitter, ion)
C. mechanically-gated: open with mechanical stimulation

Selectivity of channel

- Na^+ (180 pm)v and K^+ (220 pm) are very close in size, but still they have high selectivity and conductance
- K leak channels permeates K^+ 10000 more than Na^+
  - Difference originates from the hydration layer of molecules. Since it is smaller Na has a higher electrical density and stronger interaction with water molecules

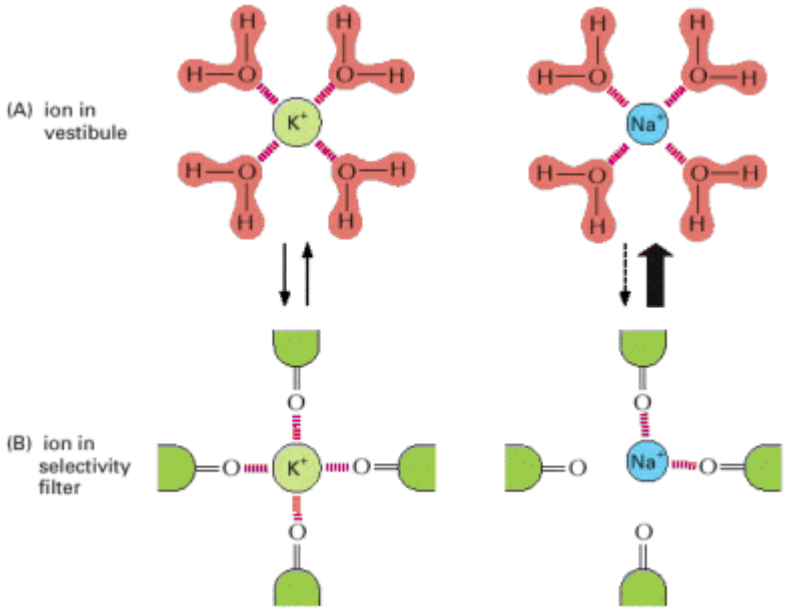


The structure of a bacterial K+ channel.

Only two of the four identical subunits are shown. From the cytosolic side, the pore opens up into a vestibule in the middle of the membrane. The vestibule facilitates transport by allowing the K+ ions to remain hydrated even though they are halfway across the membrane.

The narrow selectivity filter links the vestibule to the outside of the cell. Carbonyl oxygens line the walls of the selectivity filter and form transient binding sites for dehydrated K+ ions.

Two K+ ions occupy sites in the selectivity filter, while a third K+ ion is located in the center of the vestibule, where it is stabilized by electrical interactions with the more negatively charged ends of the pore helices. The ends of the four pore helices point precisely toward the center of the vestibule, thereby guiding K+ ions into the selectivity filter.



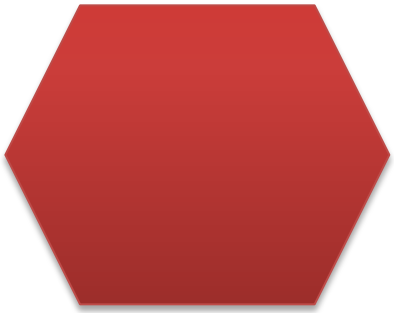
K+ specificity of the selectivity filter in a K+ channel.

In the vestibule, the ions are hydrated. In the selectivity filter, the carbonyl oxygens are placed precisely to accommodate a dehydrated K+ ion. The dehydration of the K+ ion requires energy, which is precisely balanced by the energy regained by the interaction of the ion with the carbonyl oxygens that serve as surrogate water molecules.

Because the Na+ ion is too small to interact with the oxygens, it could enter the selectivity filter only at a great energetic expense. The filter therefore selects K+ ions with high specificity.

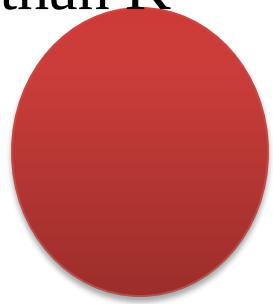
Proposed Mechanisms for Channel Ion Selectivity

Non-specific cation channel, i.e. little selectivity other than for cations



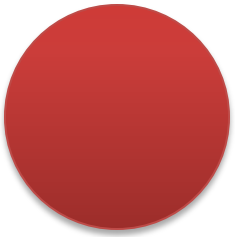
Ach receptor channel - 6.5 A in diameter

10-20 X more Na^+ than K^+



Voltage-gated Na^+ channel - 4 A in diameter

100 X more K^+ than Na^+



Voltage-gated K^+ channel – 3.3 A in diameter

Proposed Mechanisms for Channel Ion Selectivity by Channels: Ionic size

Non-specific cation channel, i.e. little selectivity other than for cations

Ach receptor channel - 6.5 A in diameter

10-20 X more Na^+ than K^+

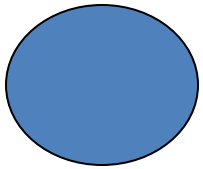
Voltage-gated Na^+ channel - 4 A in diameter

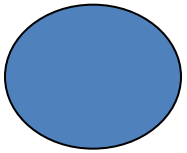
100 X more K^+ than Na^+

Voltage-gated K^+ channel – 3.3 A in diameter

Non-hydrated Na^+ ion = 1.9 A in diameter

Non-hydrated K^+ ion = 2.7 A in diameter





If ionic size explains channel selectivity, why is the K^+ channel so selective for K^+ since Na^+ is smaller?

Proposed Mechanisms for Ion Selectivity by Channels: Ionic size

Non-specific cation channel, i.e. little selectivity other than for cations

Ach receptor channel - 6.5 A in diameter

10-20 X more Na^+ than K^+

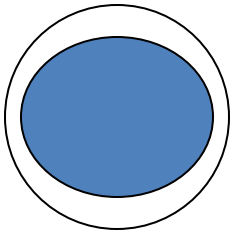
Voltage-gated Na^+ channel - 4 A in diameter

100 X more K^+ than Na^+

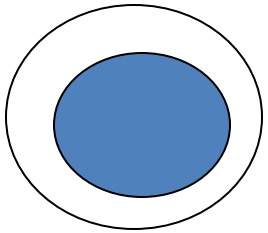
Voltage-gated K^+ channel – 3.3 A in diameter

Modified Model = perhaps channels select based on hydrated ionic radius?

Hydrated K^+ ion = 3.3 A in diameter



Hydrated Na^+ ion = 3.3-4 A in diameter



(K^+ is larger, has a lower charge density and so attracts fewer waters of hydration.)

- **Voltage-gated Cation Channels Generate Action Potentials in Electrically Excitable Cells**

- In nerve and skeletal muscle cells, a stimulus that causes sufficient depolarization promptly causes **voltage-gated Na^+ channels** to open, allowing a small amount of Na^+ to enter the cell down its electrochemical gradient.

- The influx of positive charge depolarizes the membrane further, thereby opening more Na^+ channels, which admit more Na+ ions, causing still further depolarization.

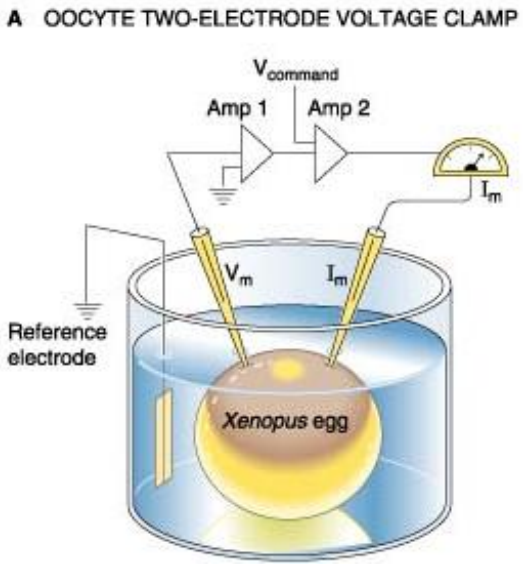
- This process continues in a self-amplifying fashion until, within a fraction of a millisecond, the electrical potential in the local region of membrane has shifted from its resting value of about -70 mV to almost as far as the Na+ equilibrium potential of about +50 mV

- At this point, when the net electrochemical driving force for the flow of Na^+ is almost zero, the cell would come to a new resting state, with all of its Na^+ channels permanently open, if the open conformation of the channel were stable. The cell is saved from such a permanent electrical spasm by two mechanisms that act in concert: inactivation of the Na+ channels, and opening of voltage-gated K^+ channels.

A brief history of voltage gated ion channels

At 1950s Hodgkin-Huxley published a study that provided us to understand electrical stimulation and transmission in the nerve cells through voltage clamp technique.

They were able to observe direct ionic currents by stimulating axons to a certain potential.

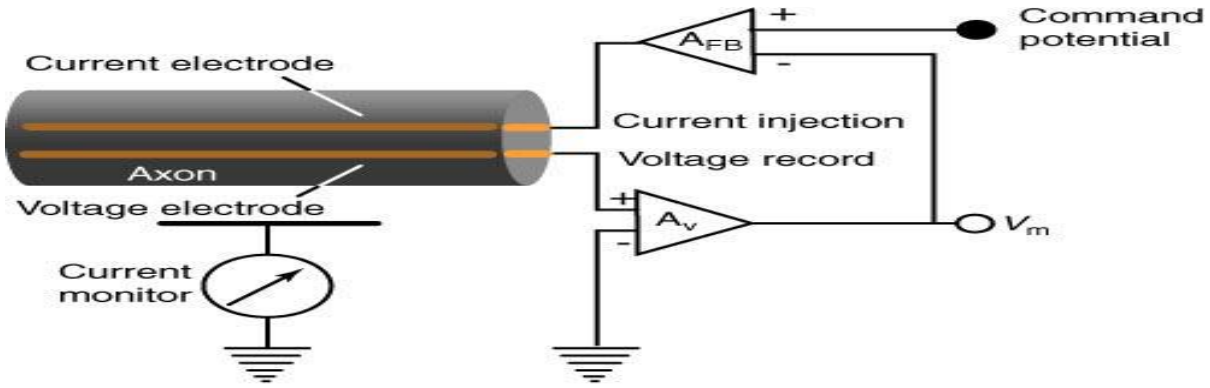


- To define the types of currents, they used various toxins known to specificaly block certain channels.
- TTX-tetrodoxin ve TEA-tetraethylammonium blocks Na+ ve K+currents.

- Different kinetics of Na+ ve K+ currents showed that they are going through the different types of proteins.

- These voltage regulated proteins later called as «voltage gated ion channels»

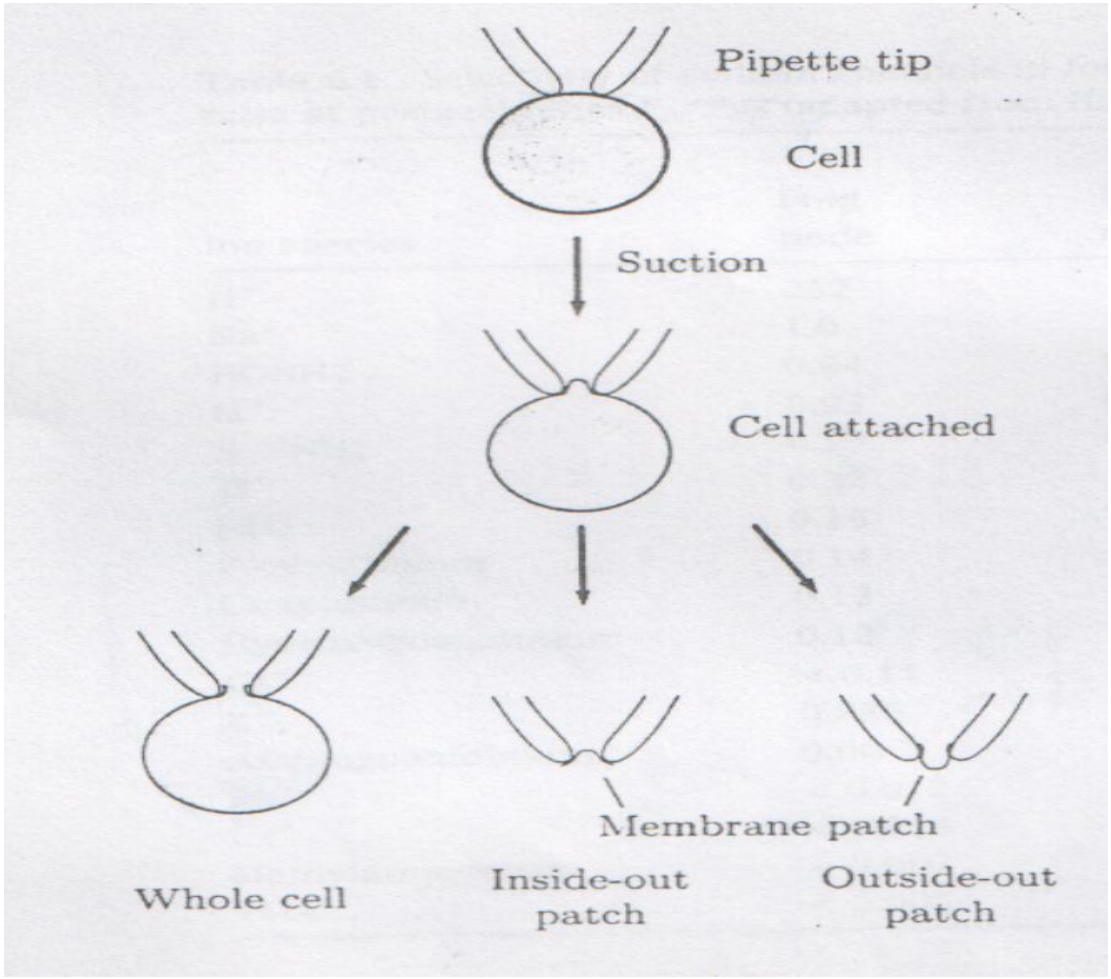
Voltage Clamp Technique



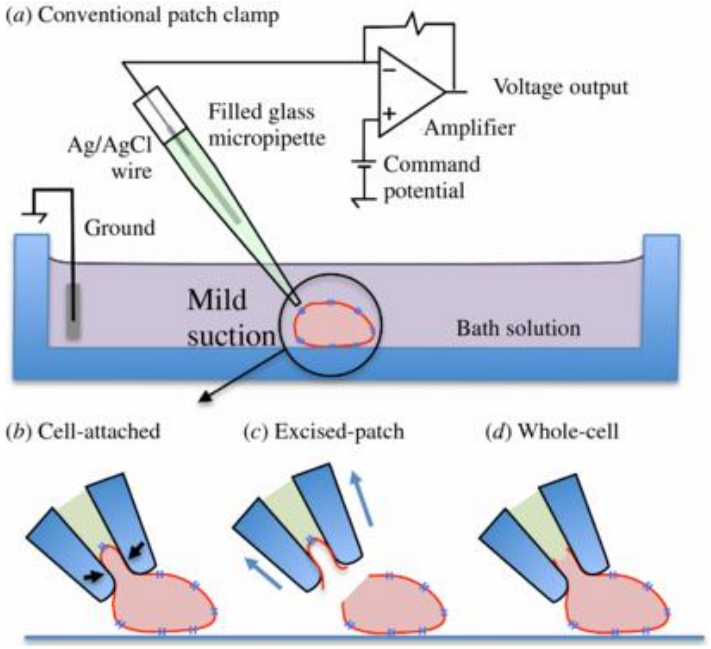
- Squid giant axon was used to measure ion currents
- Membrane potential kept constant by a feedback mechanism
- As the voltage up- or down- regulated, ionic currents through the membrane was observed

- Patch Clamp Technique
  - A similar technique deveoped later on was the Patch-Clamp, inventors of the technique, Neher and Sakman were awarded with Nobel prize.
  - This technique provided to measure current through the one channels.
  - Molecular cloning studies supported these studies and helped us to learn 3-D structures of ion channels and their localizations in the membrane.
  -

Patch-Clamp Tekniği : A patch micropipette (ϕ 1 μm) is attached to the membrane by suction, where a high resistance develop (10-100 GΩ).



- Whole cell
  - membrane is disrupted by suction
  - current changes in whole cell is measured



- Cell attached
  - electrode is attached to the cell but the membrane is not broken, records the summed current of many single channels in a patch of membrane, and spontaneous cell firing activity

- If membrane is torn out in whole-cell or cell-attached positions, we can do inside-out and outside-out measurements.
- Inside-out ve outside-out
- a small patch of membrane is torn out and placed into the solution containing the materials of interest.
- Then currents through the channels are measured

- Remember! Ion channels do not just permit to one type of ion, but their permeability for one type is much higher than the others.

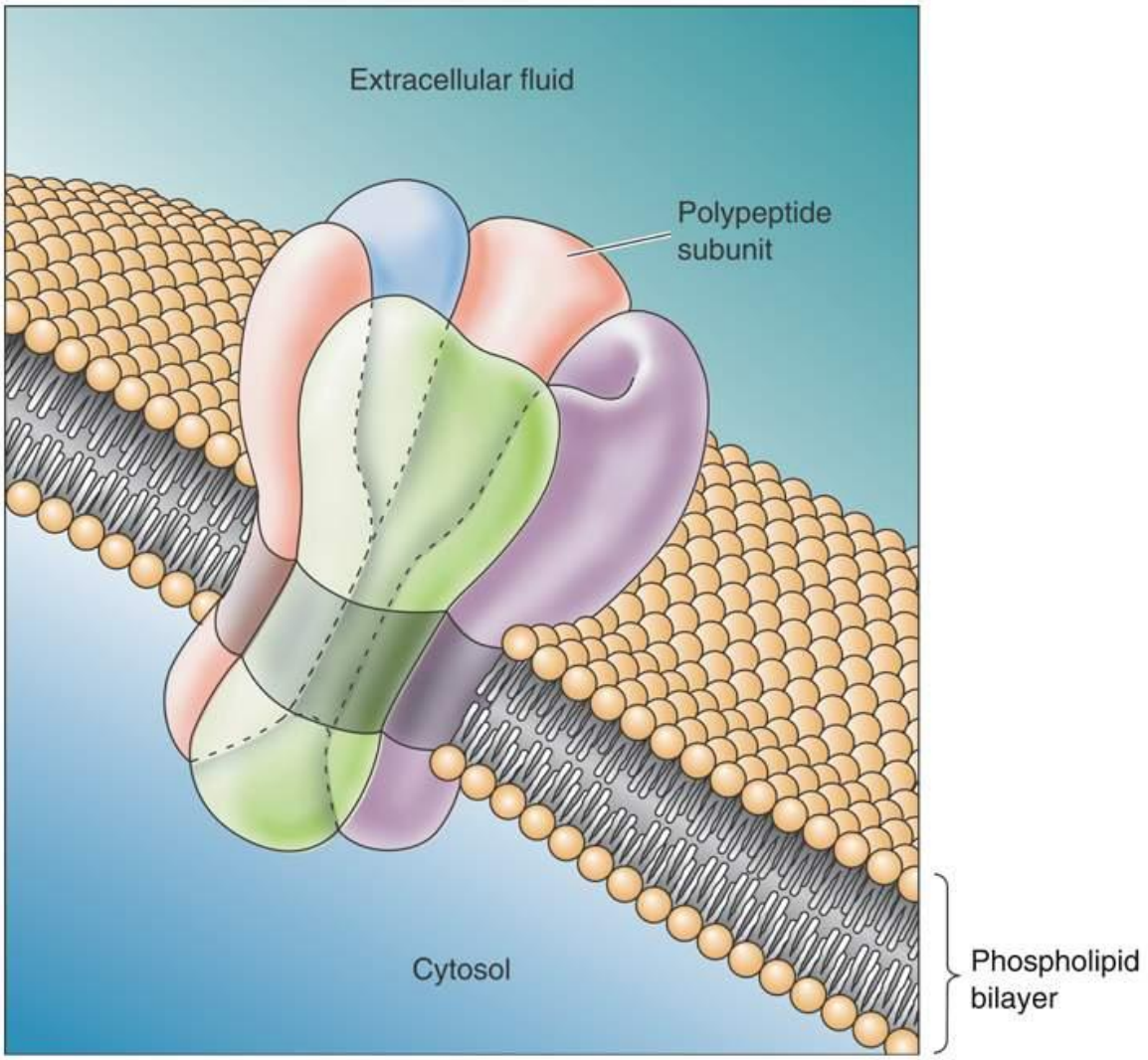
Table 6.1 Selectivity of sodium channels in four different cell types expressed as the ratio of permeabilities P_n/P_{Na} (adapted from Hille, 1992, Table 13-2).

	Permeability ratio			
Ion species	Frog node	Frog muscle	Squid axon	*Myxicola* axon
H^+	252	—	> 2	—
Na^+	1.0	1.0	1.0	1.0
$HONH_3^+$	0.94	0.94	—	—
Li^+	0.93	0.96	1.1	0.94
$H_2NNH_3^+$	0.59	0.31	—	0.85
Tl^+	0.33	—	—	—
NH_4^+	0.16	0.11	0.27	0.20
Foramidinium	0.14	—	—	0.13
Guanidinium	0.13	0.093	—	0.17
Hydroxyguanidinium	0.12	—	—	—
Ca^{++}	< 0.11	< 0.093	0.1	0.1
K^+	0.086	0.048	0.083	0.076
Aminoguanidinium	0.06	0.031	—	0.13
Rb^+	< 0.012	—	0.025	—
Cs^+	< 0.013	—	0.016	—
Methylammonium	< 0.007	< 0.009	—	—
TMA	< 0.005	< 0.008	—	—

What do we know about the structure of gated ion channels?

A. Biochemical Information –
   1. MWs range from 25-250 kDal.
   2. They are integral membrane glycoproteins.
   3. They usually consist of 2 or more subunits.
   4. The genes that code for the proteins have been isolated, cloned and sequenced. These sequences have been grouped into 6-7 protein families.
   5. The primary (amino acid) sequences of these channels is known.

- Most channels have this basic structure: multimeric (quaternary structure: homo- or hetero-), membrane-spanning, and, by definition, have a pore running longitudinally through the structure.
- Vary in the number of subunits and complexity.

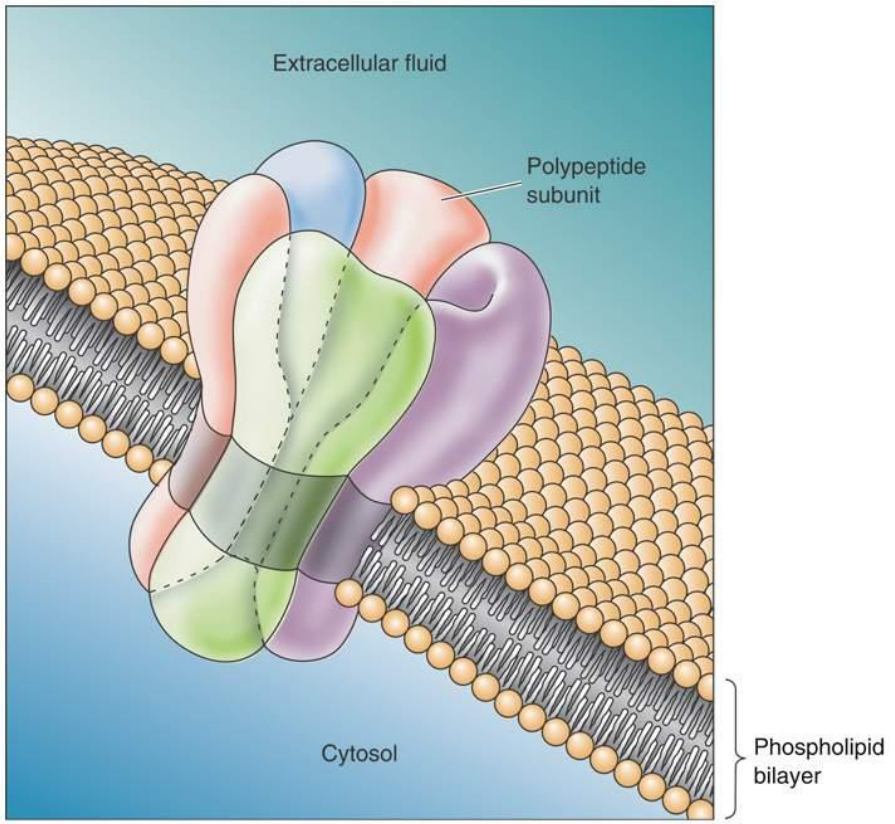


Remember your amino acids?

- Primary, secondary, and tertiary structures of proteins.
- In addition, recall that multimeric proteins are formed from the attraction of individual subunits, forming the *quarternary* structure.
- Recall the structure and ionization of the each of the amino acid side-chains (R).

  -It wouldn't hurt if you reviewed what a pI is.

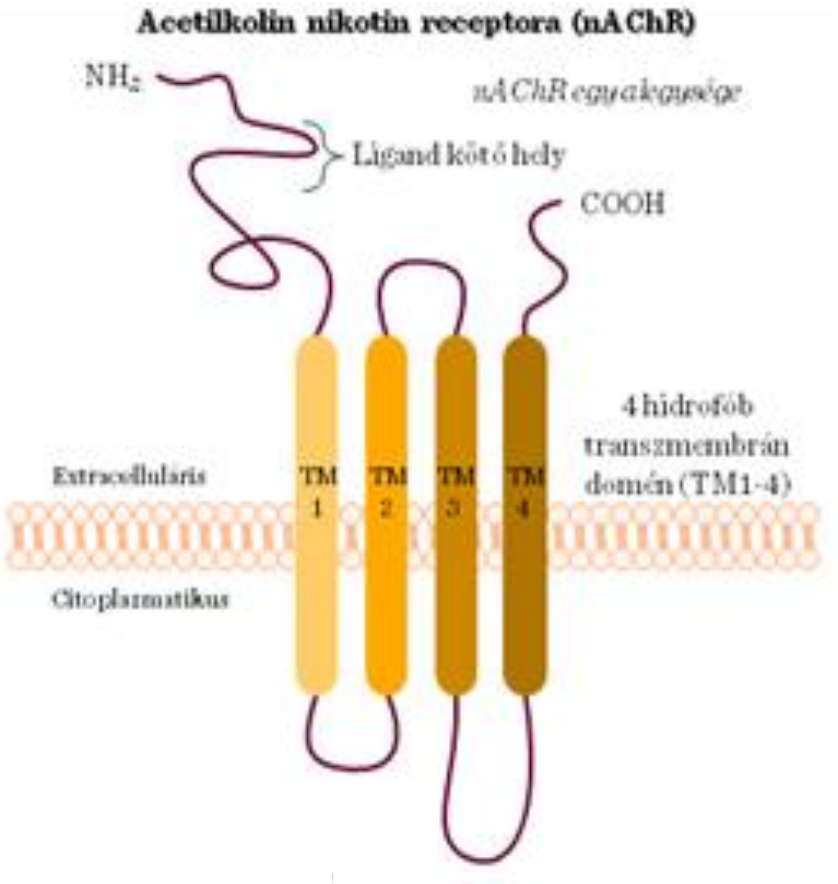
- The primary amino acid sequence and higher –order structures determine the channel topology.
- Interior of the channel will be lined with hydrophilic amino acids.
- Exterior of the channel will be lined with hydrophobic amino acids.

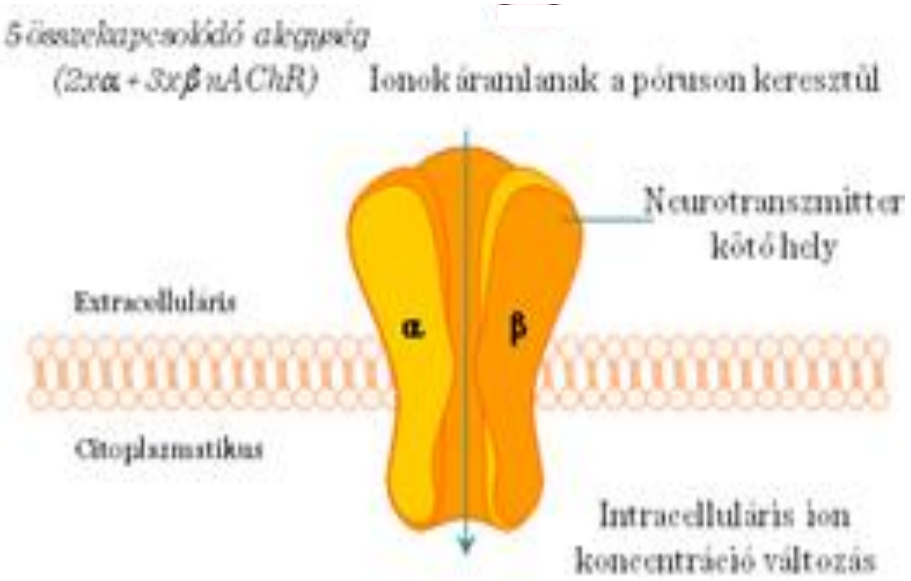


Transmembrane regions mostly contains hydrophobic amino acids

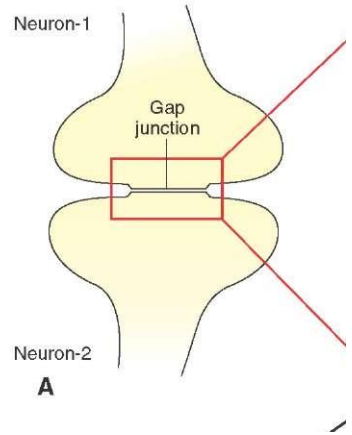
Examples

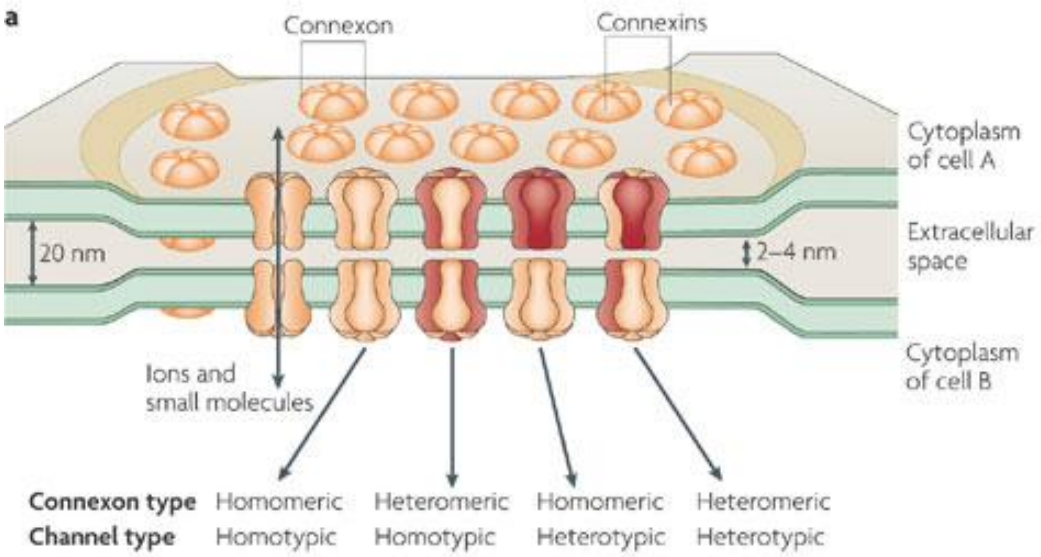
- 1.Heteromultimetric- nicotinic receptors in nerve-muscle junction

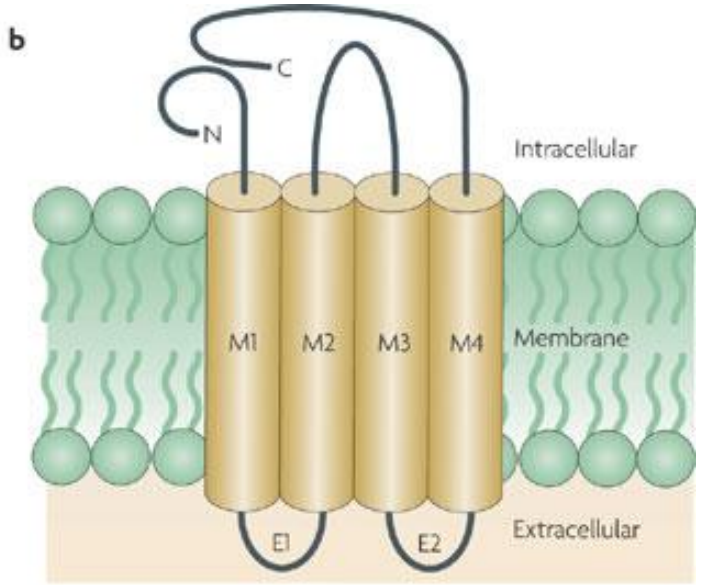




- 2.Homomultimerik- Gap junctions







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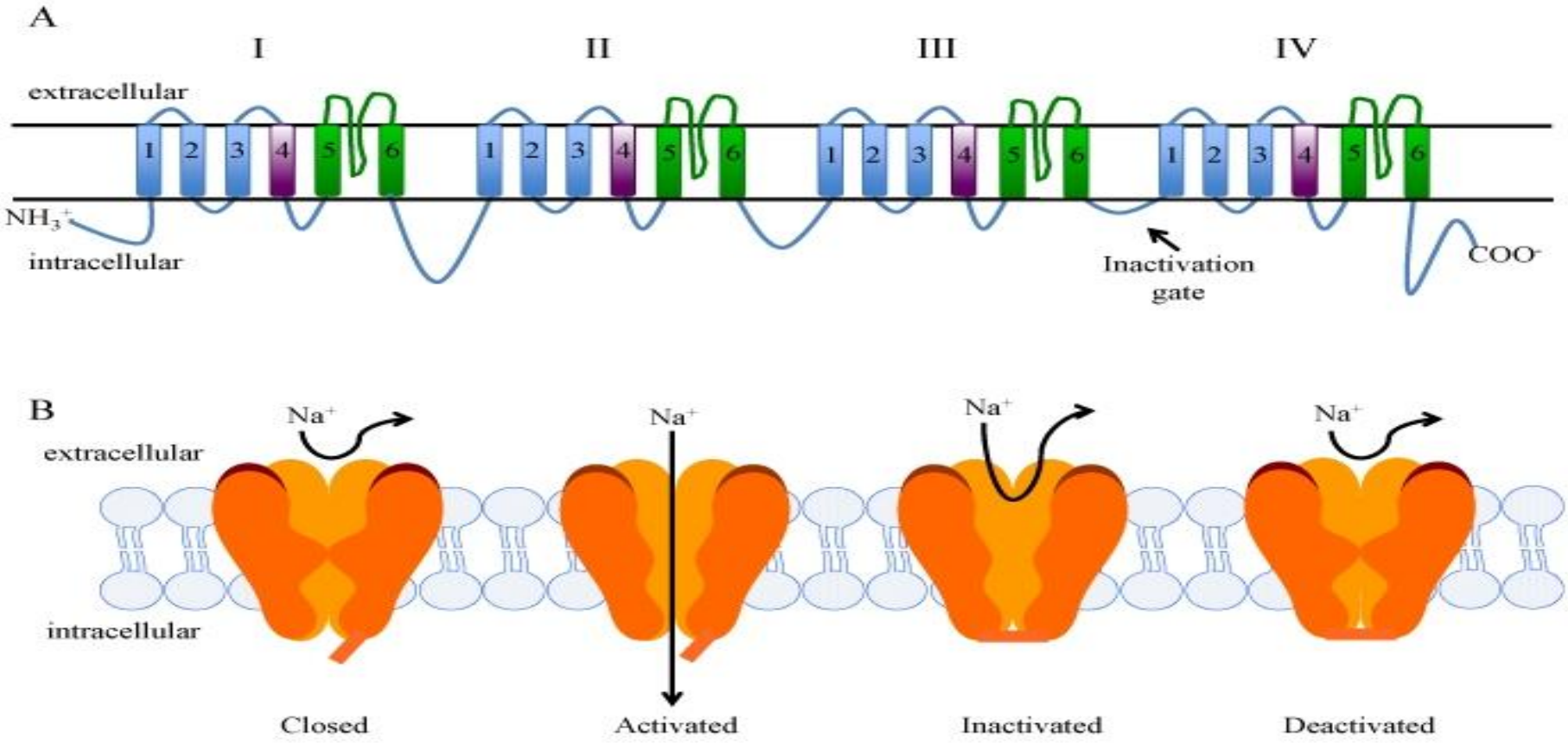
- 3. Na-channel consisted of one α peptide chain



- Structure of voltage-gated channels
- Voltage gated ion channels are coded by 143 genes in human genome.
- They are the main target for many pharmacologic agents.

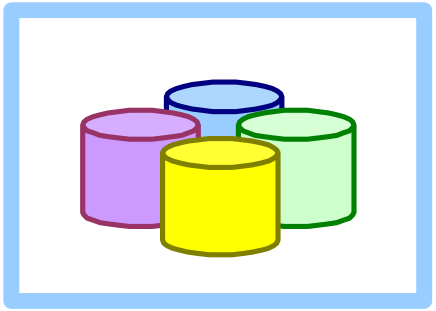
- Voltage gated K channels returns the nerve cell to its resting situation
- Since their response is late, they are called «delayed K channels»

Voltage-gated K+ channels mediate outward K+ currents during nerve action potentials.

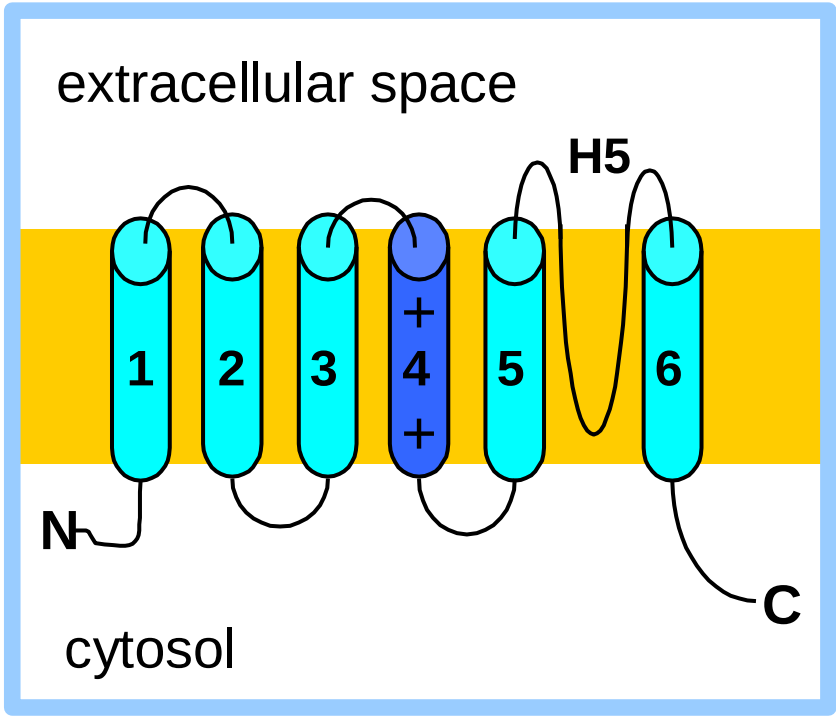
Important advances in understanding have come from:

- physiological studies, including the use of patch clamping
- mutational studies of the Drosophila voltage-gated K+ channel protein, product of the Shaker gene
- crystallographic analysis of the structure of bacterial K+ channels.
- molecular dynamics modeling of permeation dynamics.

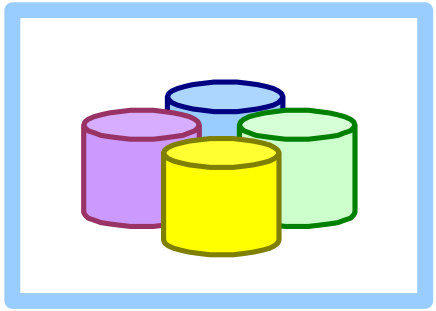
4 identical copies of the K+ channel protein, arranged as a ring, form the channel walls.

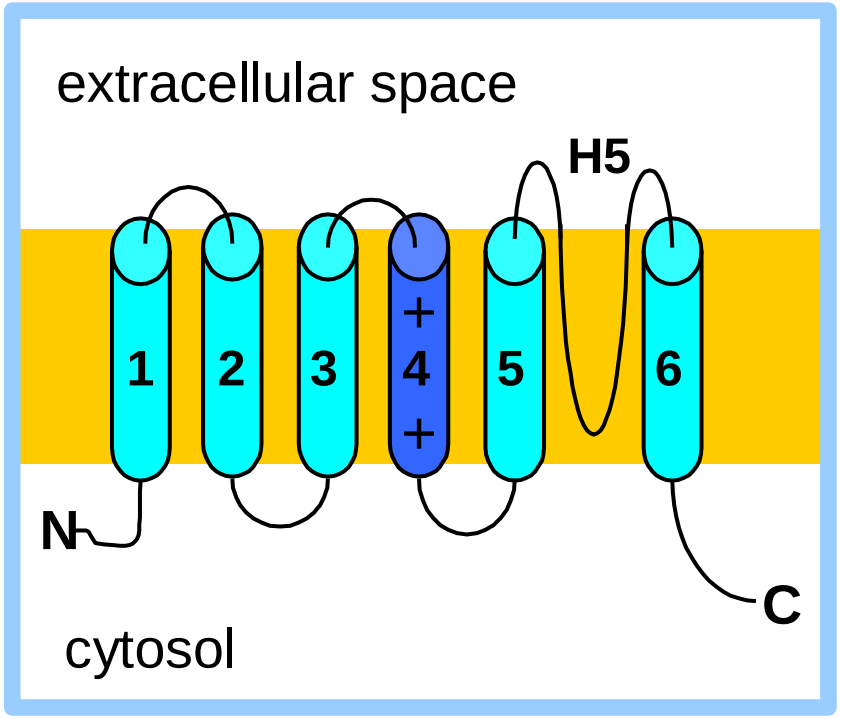


Hydropathy analysis & topology studies predicted the presence of 6 transmembrane a-helices in the voltage-gated K+ channel protein.



The core of the channel consists of helices 5 & 6 & the intervening H5 segment of each of the 4 copies of the protein.





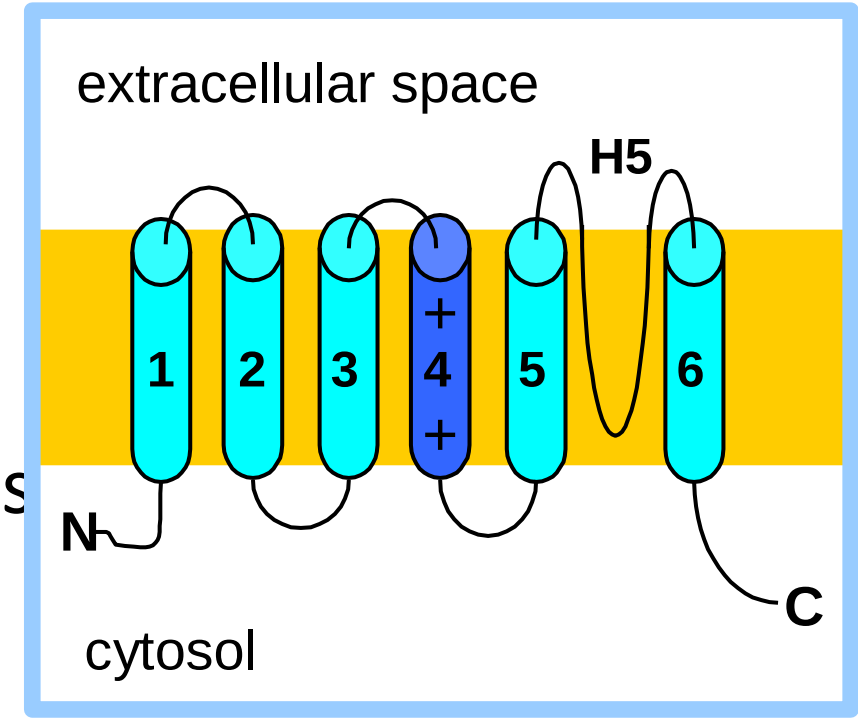
Helices 1-4 function as a **voltage-sensing domain**, with **helix #4** having a special role in voltage sensing.

This domain is absent in K^+ channels that are not voltage-sensitive.

Voltage sensing:

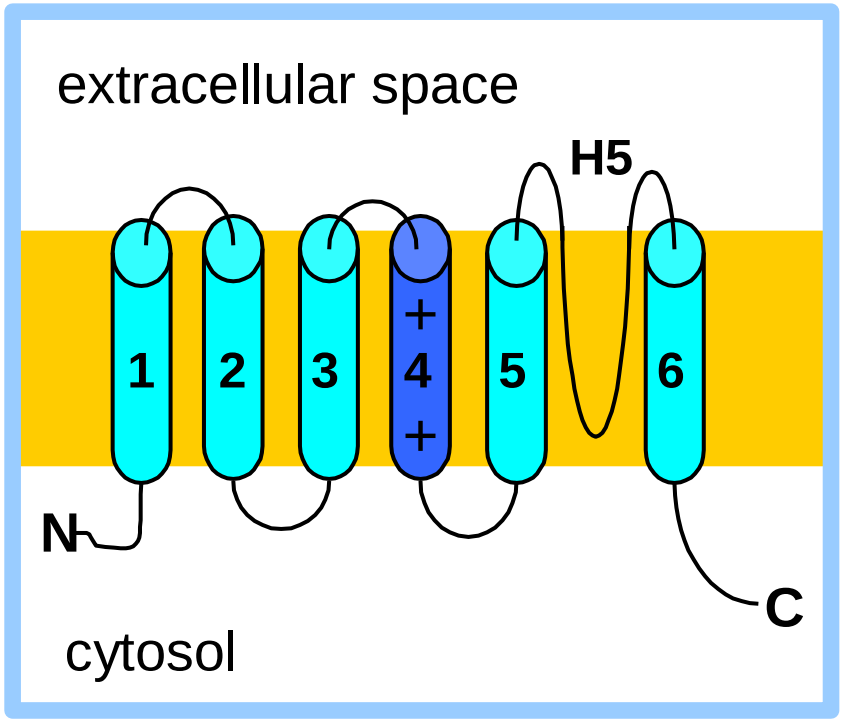
Mutational analysis showed (+) residues in helix #4 to be essential for voltage gating.

In helix #4 every 3rd residue is Arg or Lys, & intervening residues are hydrophobic.



Decreased transmembrane potential causes **helix #4** to change position, resulting in more of its (+) charges being accessible to the aqueous phase outside the cell.

A small "**gating current**" is measurable, as **(+) charges** effectively move **outward**.



The ***N*-terminus** of the Shaker channel (or part of a separate subunit in some voltage-activated channels) is **essential** for **inactivation**.

Mutants that lack this domain do not inactivate.

Adding back a peptide equivalent to this domain restores the ability to inactivate.

Selectivity:

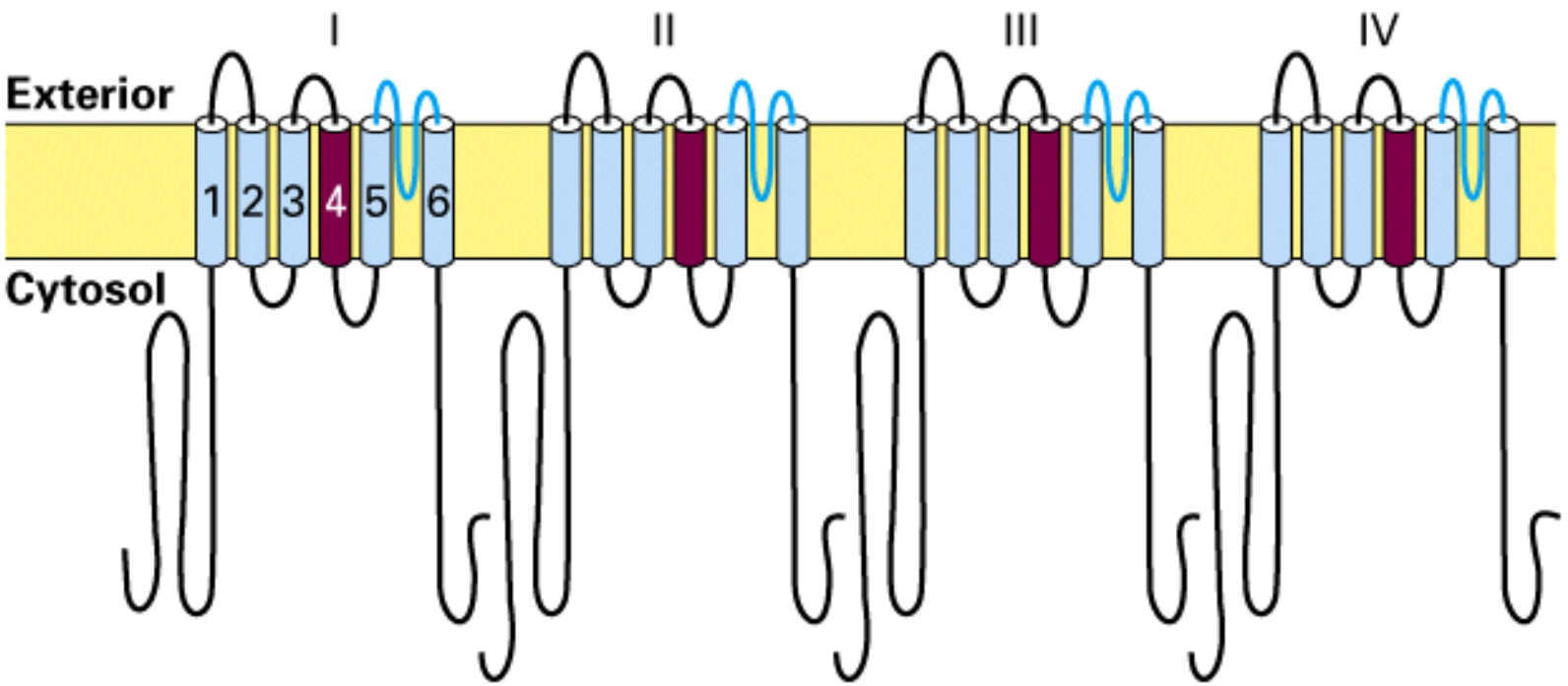
K+ channels are highly selective for K+, e.g., relative to Na+.

The selectivity filter that determines which cation can pass through a channel is located at the narrowest part.

Mutation studies showed that the H5 segment is essential for K+ selectivity.

H5 includes a consensus sequence (Thr-Val-Gly-Tyr-Gly) found in all K+ channels, with only minor changes through evolution.

(a) Voltage-gated K^+ channel protein (tetramer)

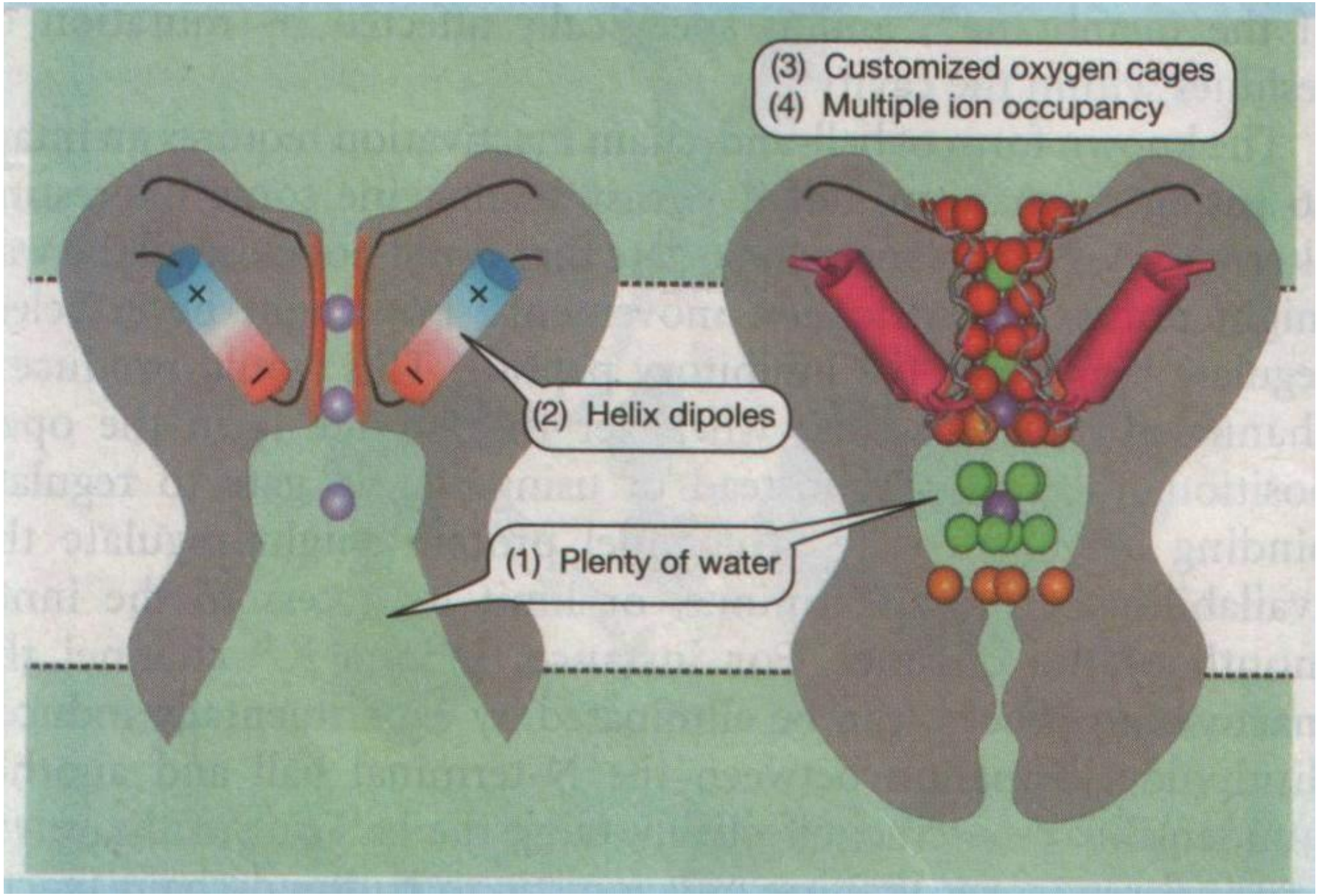


Important elements in the structure:

-Channel has sufficiently large to accomodate water molecules together with K+, to maintain hydration for stability.

-Negative charges line inside wall of the channel to provide electrostatic stability.

- In selectivity filter, oxygen atoms lined in a way to mimic hydrated form of K+ ion.


(3) Customized oxygen cages
(4) Multiple ion occupancy
(2) Helix dipoles
(1) Plenty of water

Voltage sensitivity and inactivation of K+ channels

- There are 3 ways to regulate currents throught the channels:
- 1-Transcriptional regulation of number of channels –which requires long time.
- 2-traficking to the membrane - occurs in shorter time
- 3-Regulation of current by regulation of opening time- which necessary in excitable cell, at the time of action potential, for a very quick response.

- In excitable cells (muscle and nerves), there are three conformation of voltage gated channels:

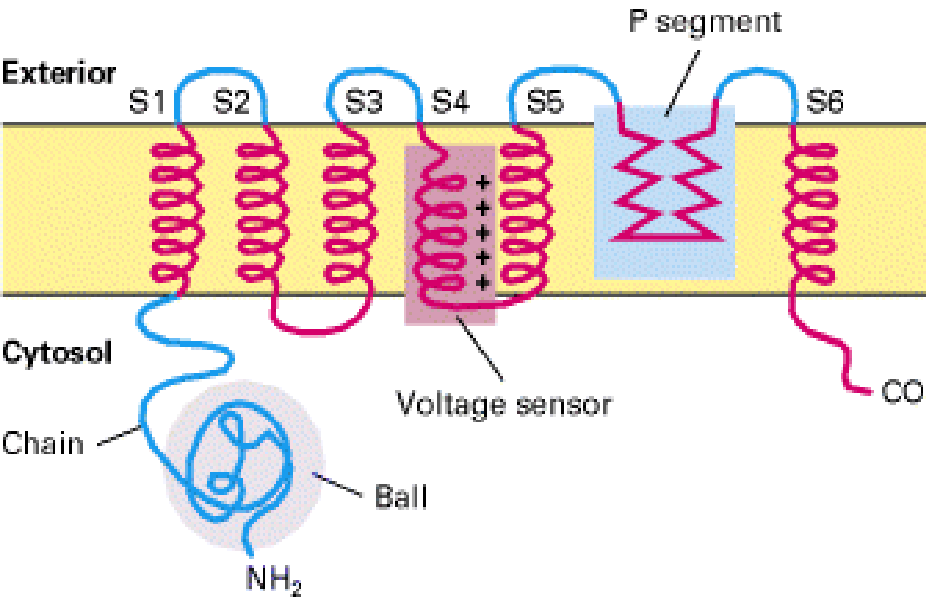
- Closed- Open- Inactive

- For re-opening of the channel, channel should first return to closed form from inactive form.

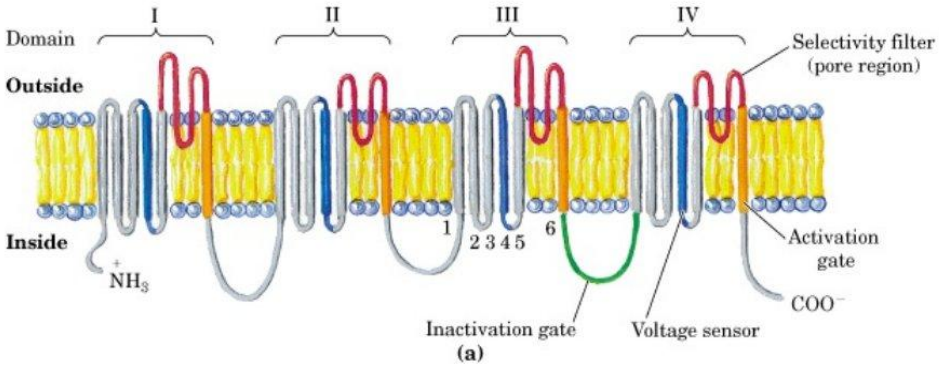
- There is no direct transition from inactive to open form.

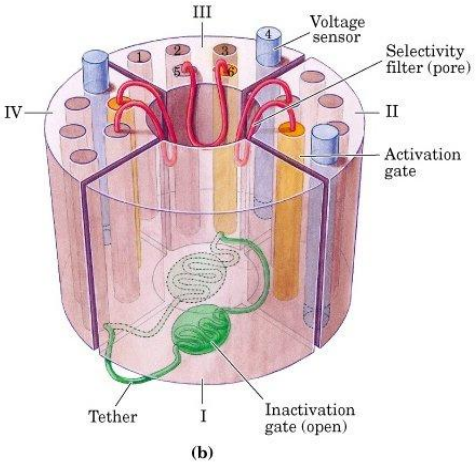
- Transition from closed to open form requires conformational changes in voltage sensitive domain of the channel. This transition occurs in milliseconds.

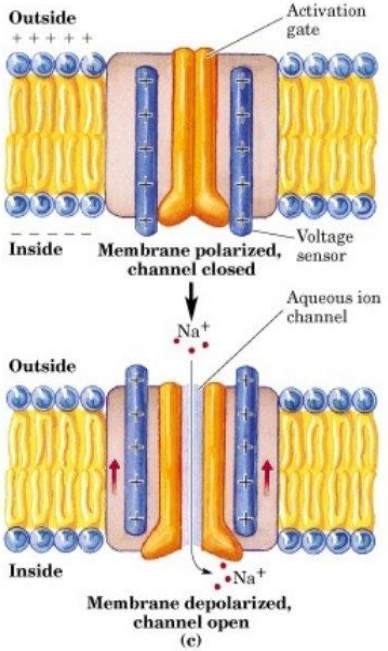
- Voltage sensitivities and time intervals for transition from open to inactive state changes with the channel type and time can be in the level of seconds.



A Voltage-gated Channel

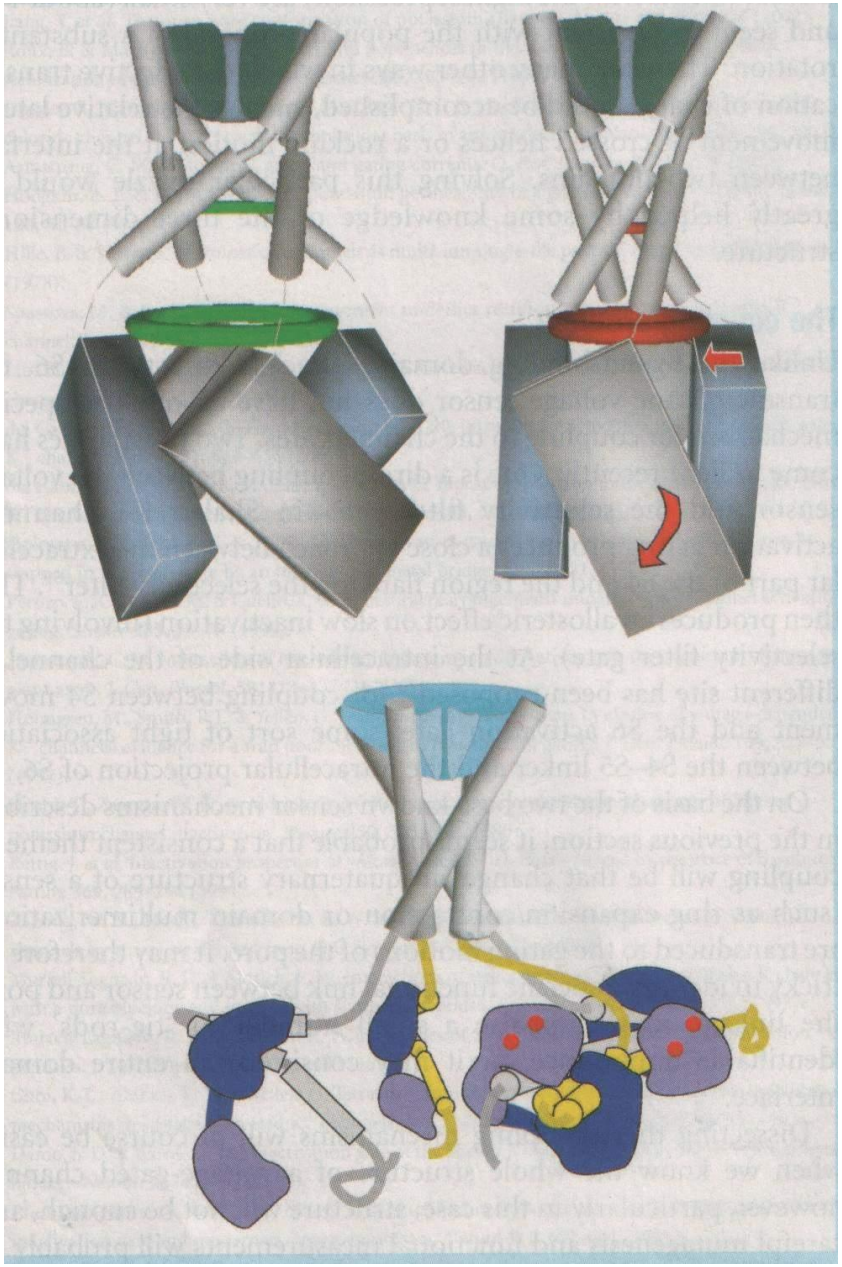






K+ channels has 3 mechanisms for «gating»

- **1- Channel closes with conformational changes in the cytoplasmic side of** S6 TM region.
- **2-** (Ball and Chain gating) Inactivation provided by the blockage of the channel by a polypeptide in the N-terminıus of S6 and becomes transiently inactivated.
- **3-Selectivity filter is regulated according to the voltage sensitive changes in S6 domain.**".



In voltage sensitive channels 4 charges in S4 domain will be shifted to open the channel

Rectifying channels

- Most of the voltage sensitive channels have recitfying property.

- This means that they show high permeability in one direction, but very high resistance in reverse direction.

Kinetik

- Voltaj kapılı kanalların açılma, inaktif duruma geçme kinetiği büyük değişkenlikler gösterirler.

- Bu zaman dilimleri bazı kanallar için mikrosaniye mertebesinde iken diğer bazı kanallar için birkaç saniye olabilmektedir.

- Unlike to the Na^+ channels, K^+ ion channel family is a large one showing high variabilities.
- Besides ligand gated channels (such as Ca^{++} , ATP , serotonin, acethylcholine, NMDA dependent) , there are three subfamilies of voltage-dependent K^+ channels. Still there are some others out of this classification.

- Delayed Rectifier K+ Channels
- Fast-response K+ Channels
- Inwardly rectifying K+ Channels
- Ca++ activated K+ Channels

Crystal structures have been determined for:

- a bacterial voltage-gated K+ channel KvAP
- a mammalian equivalent of the Shaker channel designated Kv1.2.

The core of both voltage-gated channels (selectivity filter & two transmembrane α-helices of each of four copies of the protein) is **similar** to that of other K^+ channels.

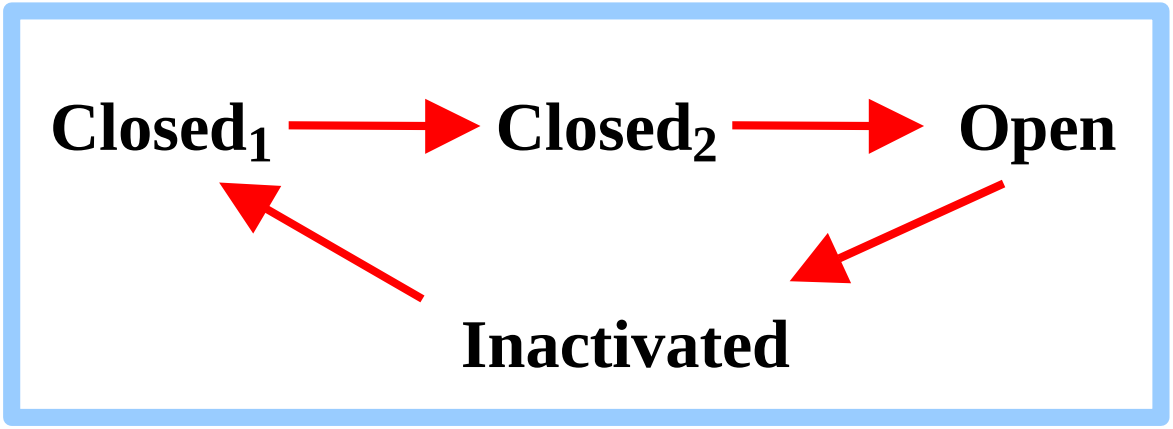
.

According to current models, a voltage change drives movement of each positively charged voltage sensor paddle complex across the membrane.

This exerts tension, via a linker segment, on the end of each inner helix of the channel core to promote **bending**, and thus channel opening.

Recent high-resolution structural studies permit predictions of how **acidic residues** may **stabilize positive charges** on the paddle as it moves within the membrane.

Inactivation:



- Many channels have **multiple open &/or closed states**.

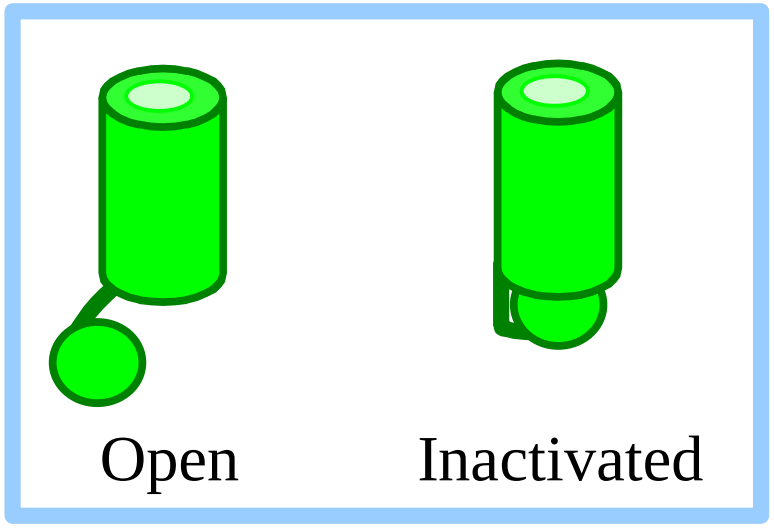
  There may be an **inactivated state**, as in the hypothetical example above.

- Voltage-gated K^+ channels undergo transient **inactivation** after opening.

  In the inactivated state, the channel cannot open even if the voltage is favorable.

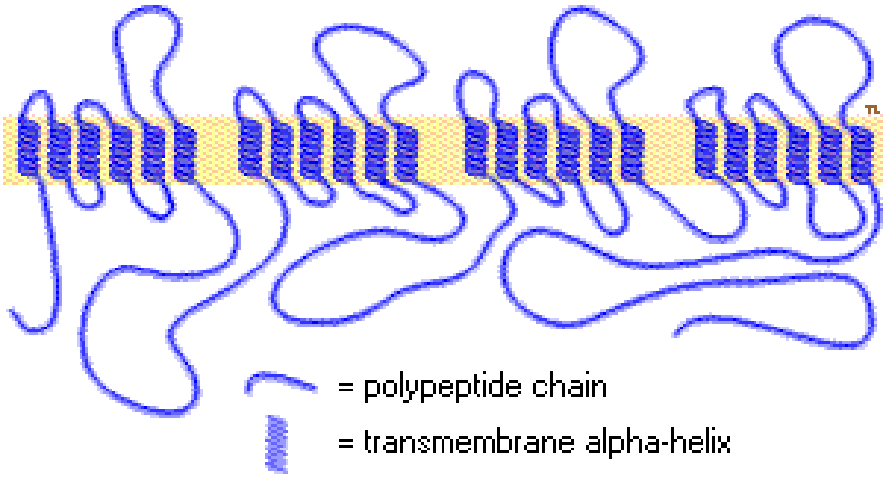
  This results in a **time delay** before the channel can reopen.

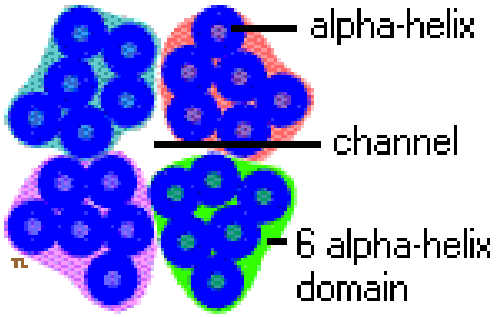
A "ball & chain" mechanism of inactivation has been postulated, in which the ***N*-terminus** of one of the 4 copies of the channel protein enters the channel from the cytosolic side of the membrane to inhibit ion flow.



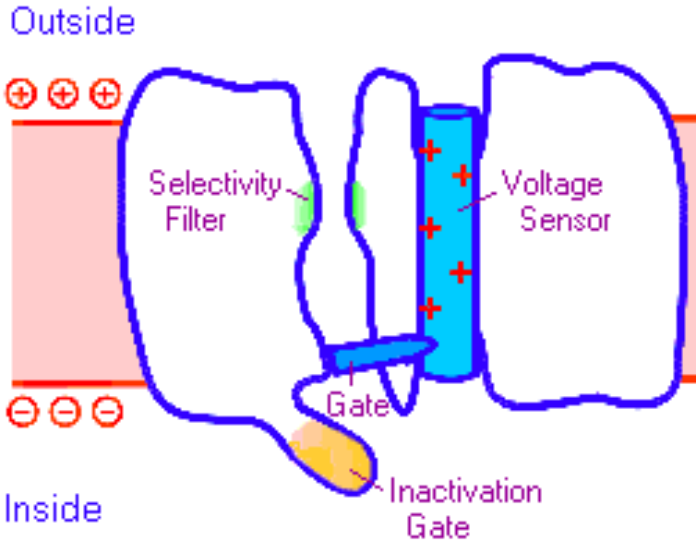
In some voltage-gated K^+ channels, entrance of the *N*-terminus into the channel is followed by a **conformational change** in the **selectivity filter** that contributes to the process of inactivation.

- **Sodium Voltage-Gated Ion Channels**:
- In 1978, purified from electric eel electric organs.
- a single peptide of almost 2000 amino acids in length (with internal repeats).
- However, in other tissues, it can be found as subunits : more subunits an ion channel is composed of, the less selective it is for its respective ions.
- The channel from electric eel was found to have 30% of its weight in carbohydrates and 6% as attached fatty acids.
- Some sodium voltage-gated channels may have as many as 6 different kinds of neurotoxins which bind and inhibit them to various degrees and each toxin appears to bind at a different site, which is unusual. Some of these toxins are classified as peptides, while others are alkaloids, cyclic polyethers, esters, and heterocycles.
- Most peptide neurotoxins are 60-100 amino acids in length,
- the peptide toxins made from cone shells are often only between 10 and 30 amino acids long. They accomplish their inhibitory task by forming disulfide bonds with each other. Usually 2 or 3 come together and form these larger structures.
- Voltage-gated Sodium channels are responsible for the action potential of neurons while the voltage-gated potassium channels help to re-establish the membrane potential back to normal.
- Pore sizes are estimated to be ~3x5A for the selectivity filter region. Sodium channels deactivate quickly compared to calcium channels. This is the reason calcium ions are used by the cell for more of a sustained response to external stimuli. Some other members of this family: **mH1, mH2, SCN4A** (skeletal


= polypeptide chain
= transmembrane alpha-helix


alpha-helix
channel
6 alpha-helix domain

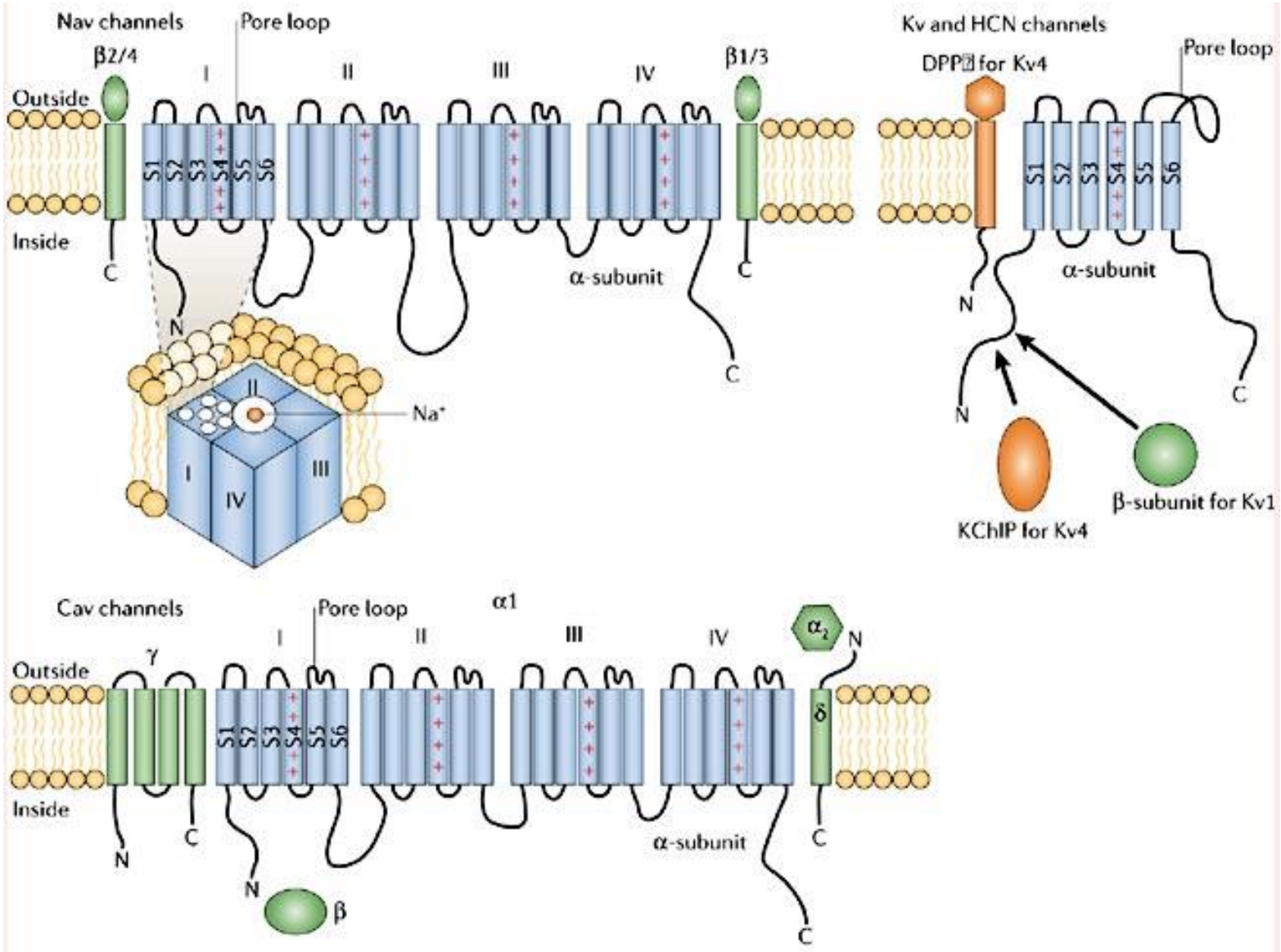
TOP VIEW

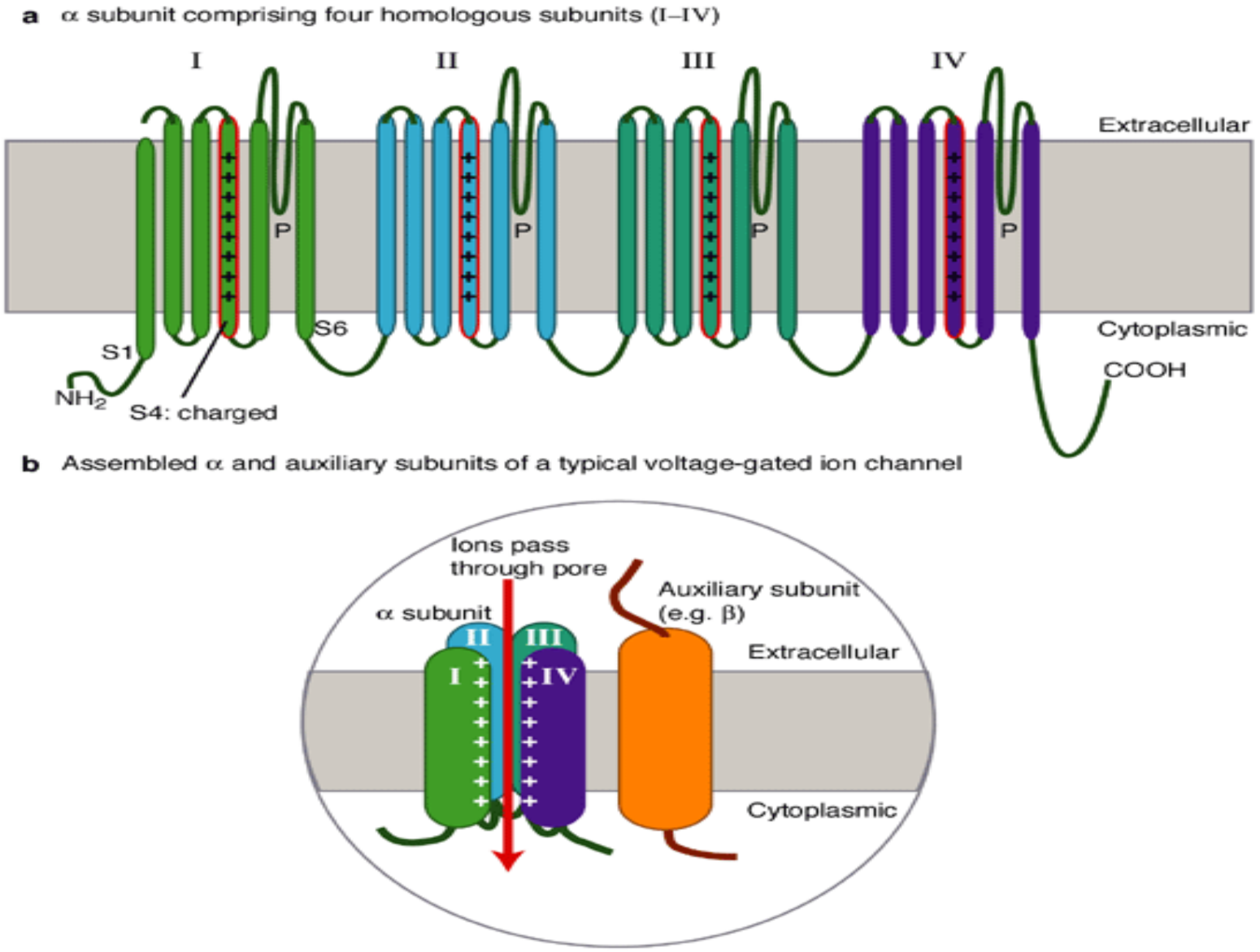

Outside
Selectivity Filter
Voltage Sensor
Gate
Inactivation Gate
Inside

- Voltage gated Na channels (Na_v)
  - Both carboxyl and amino ends are inside the cell
  - It has 4 domains similar to K , but in one chain – each domain contains 6 TM
  - All domains combine to form ion channel wall
  - The half-ring P-segments come across and form the ion selectivity filter

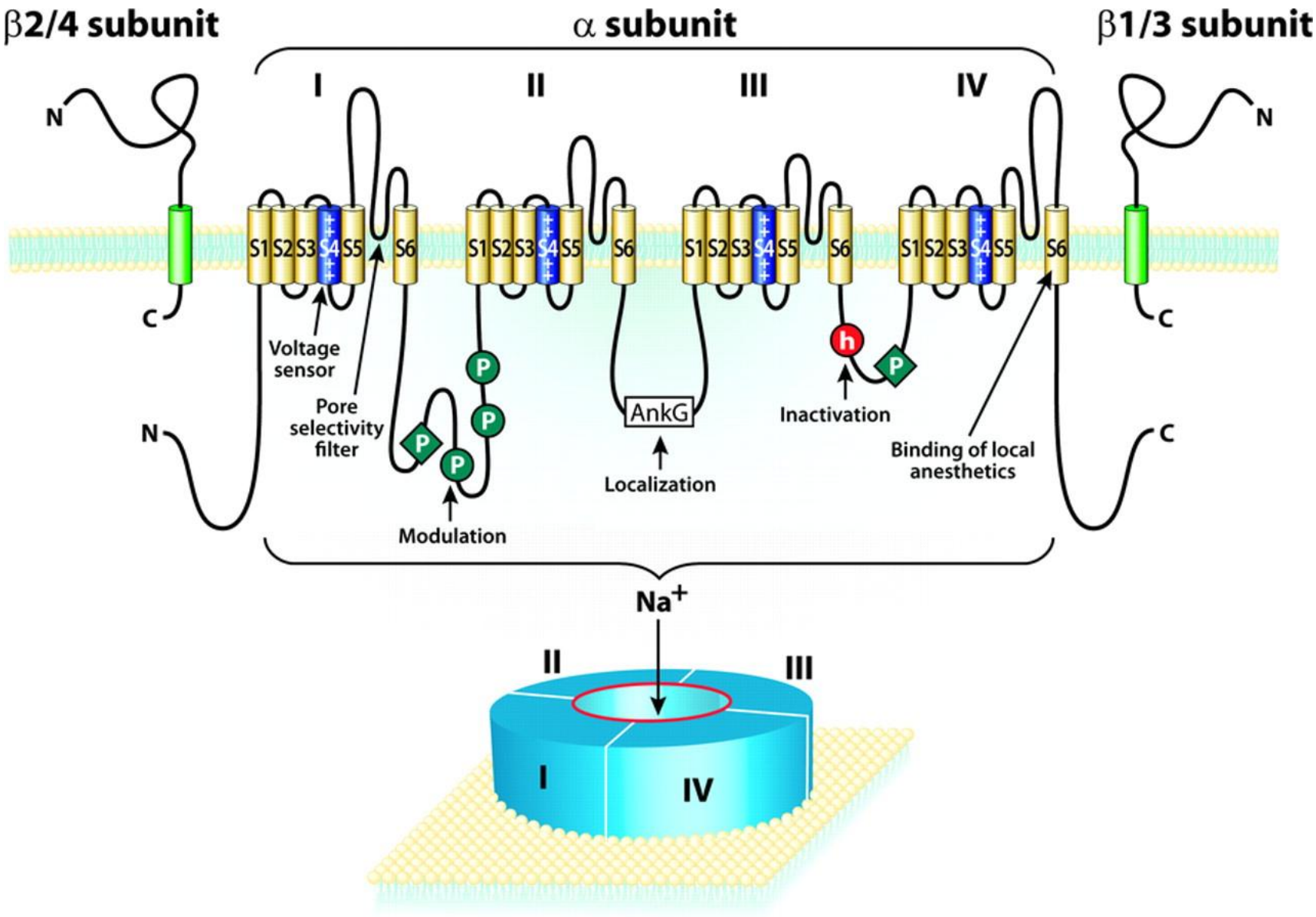
- Opening and closing mechanism are regulated by voltage-sensor units which is sensitive to membrane potential
- One alpha helix in each domain acts as a sensor
- When membrane is depolarized, sensor shift toward out and the channel opens

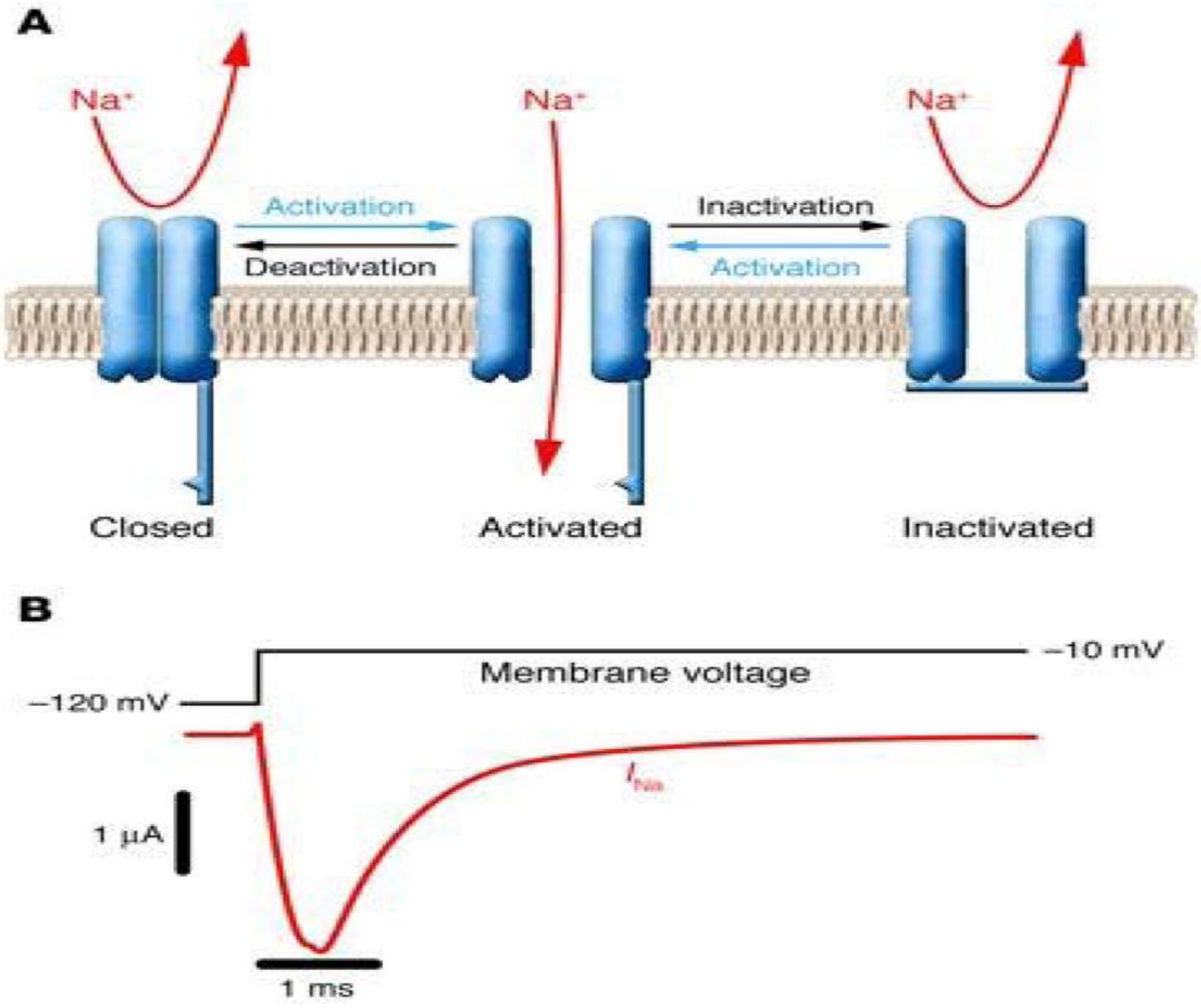
- They have automatic inactivation mechanism
- This provides quick closure of channel even if depolarization continues.
- This inactivation mechanism prevents reopening until few ms after membrane returns to its negative value



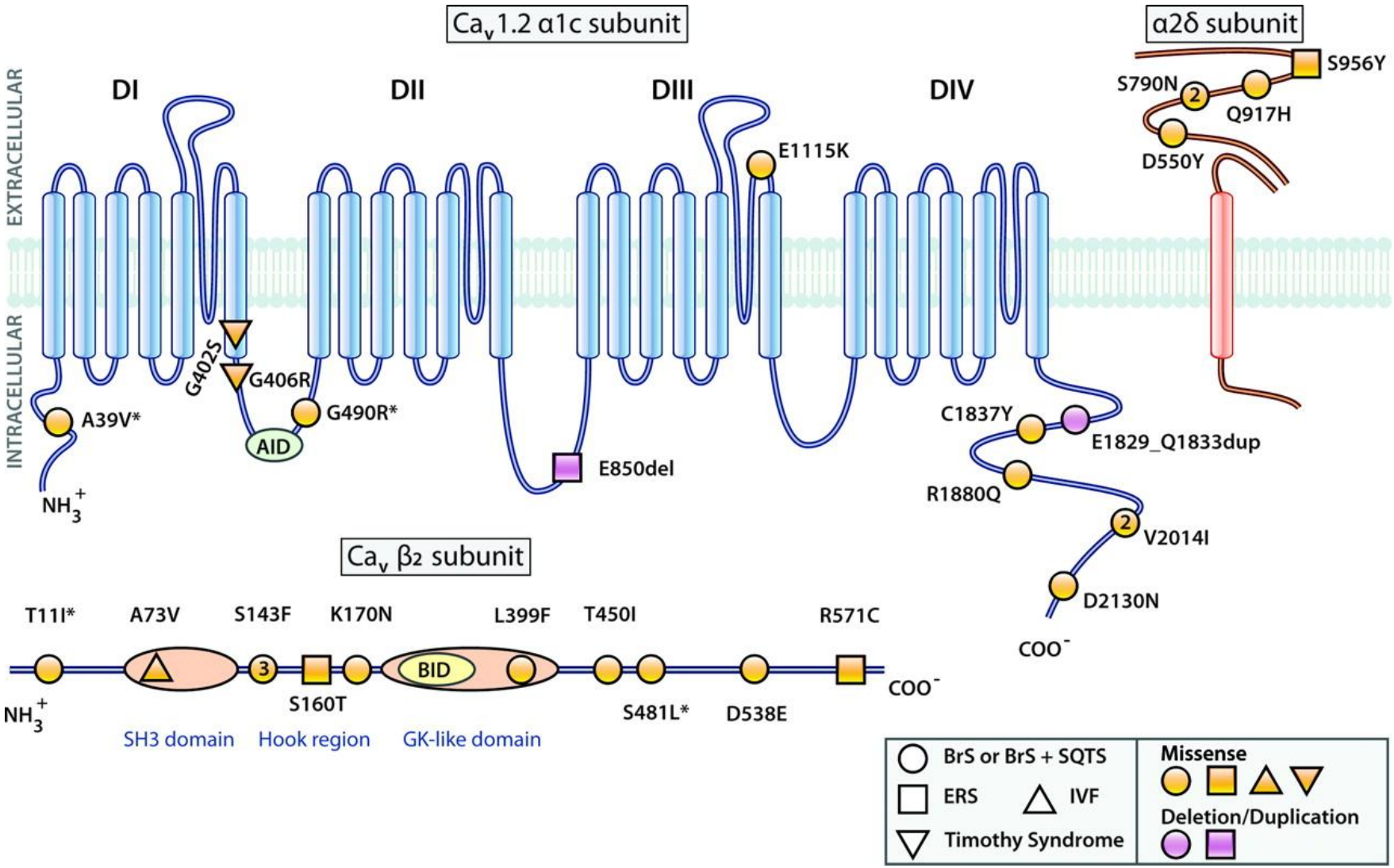


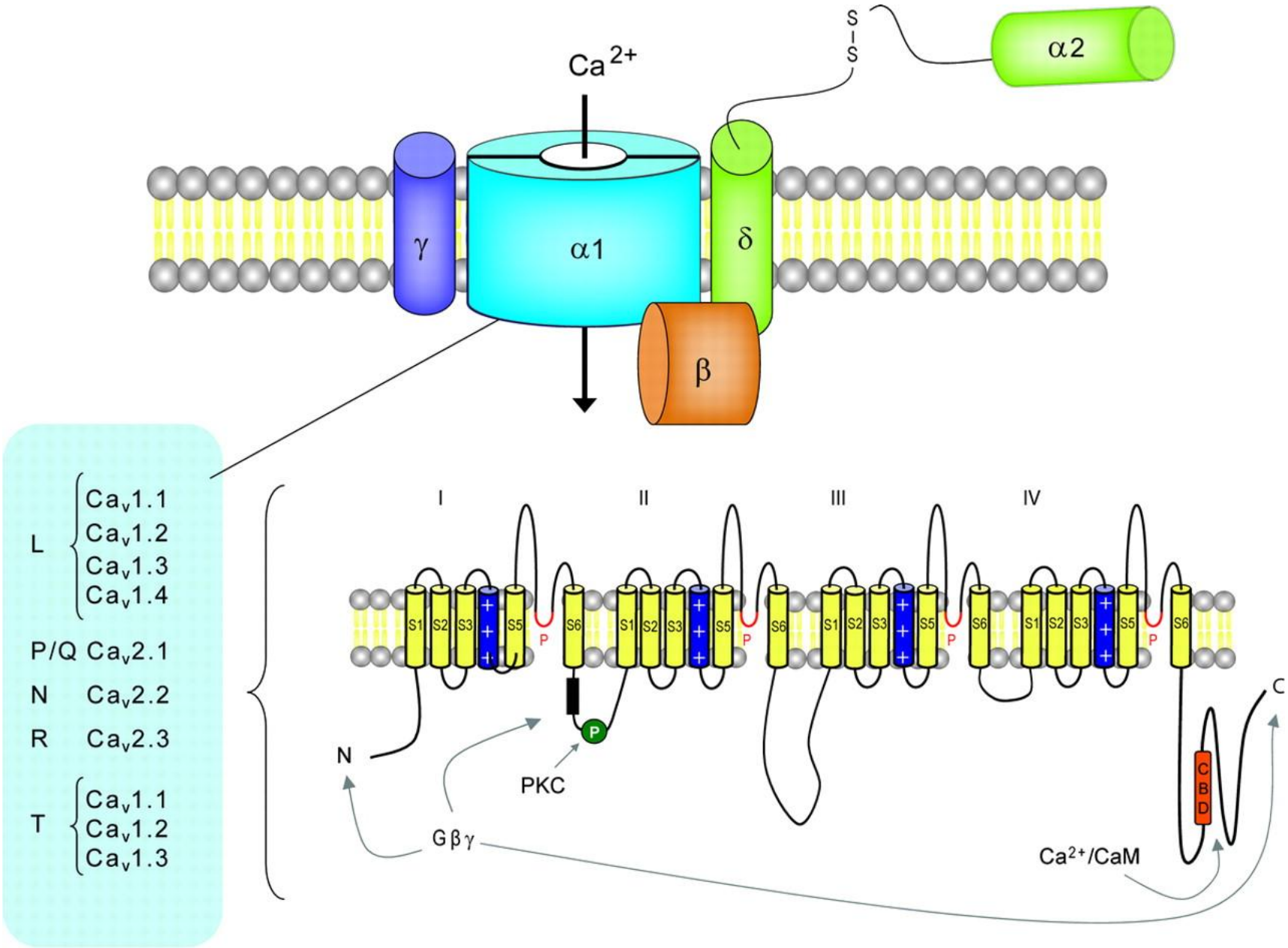
Structure of a typical voltage-gated ion channel


β2/4 subunit
α subunit
β1/3 subunit
I
II
III
IV
N
C
S1
S2
S3
S4
S5
S6
Voltage sensor
Pore selectivity filter
Modulation
AnkG
Localization
h
Inactivation
Binding of local anesthetics
P
Na+

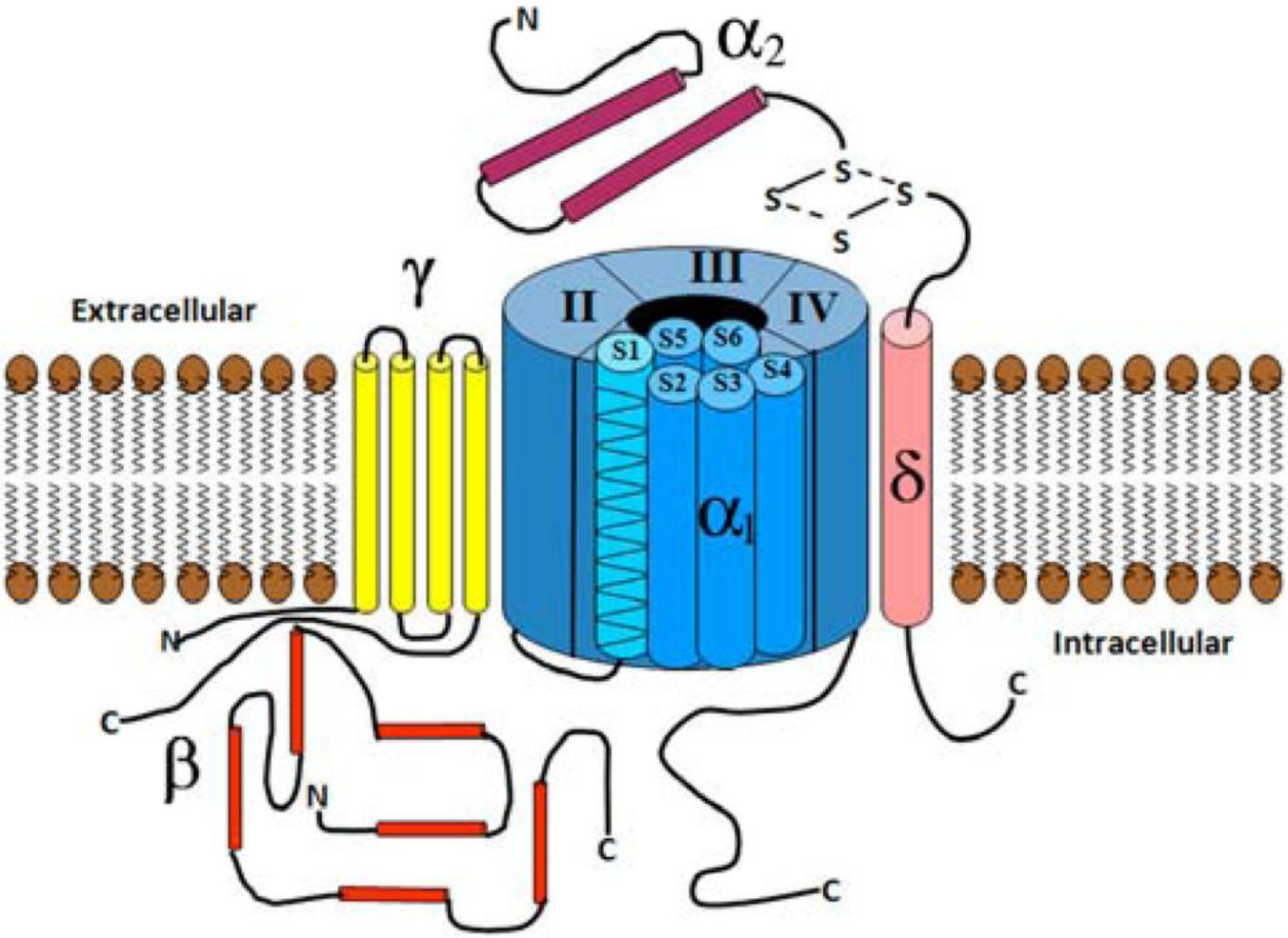

A
Na+
Na+
Na+
Activation
Deactivation
Inactivation
Activation
Closed
Activated
Inactivated
B
Membrane voltage
−10 mV
−120 mV
1 µA
1 ms

- Voltage gated Ca^{2+} channels (Ca^{2+}_V)
  - Similar to Na_V and K_V channels
  - One α poly peptide
  - 4 domains, each with a 6TM segment
  - P-loop between 5. ve 6. segments
  - There are N- and P-types
  - They show high functional variety such as in Conductance
  - Selectivity
  - Metabolic regulation
  - widely distributed in skeletal and heart muscle
  - Their conductance velocity and opening frequencies are low compared to Na and K channels


Ca$_v$1.2 α1c subunit
α2δ subunit
EXTRACELLULAR
INTRACELLULAR
DI
DII
DIII
DIV
S956Y
S790N
2
Q917H
D550Y
E1115K
G402S
G406R
A39V*
G490R*
AID
E850del
C1837Y
E1829_Q1833dup
R1880Q
2
V2014I
D2130N
NH$_3^+$
COO$^-$
Ca$_v$ β2 subunit
T11I*
A73V
S143F
K170N
L399F
T450I
R571C
3
BID
S160T
S481L*
D538E
NH$_3^+$
COO$^-$
SH3 domain
Hook region
GK-like domain
BrS or BrS + SQTS
ERS
IVF
Timothy Syndrome
Missense
Deletion/Duplication


Ca 2+
S
S
α2
γ
α1
δ
β
L
Cav1.1
Cav1.2
Cav1.3
Cav1.4
P/Q Cav2.1
N Cav2.2
R Cav2.3
T
Cav1.1
Cav1.2
Cav1.3
I
II
III
IV
S1
S2
S3
S5
S6
P
N
C
PKC
Gβγ
Ca2+/CaM
C
B
D

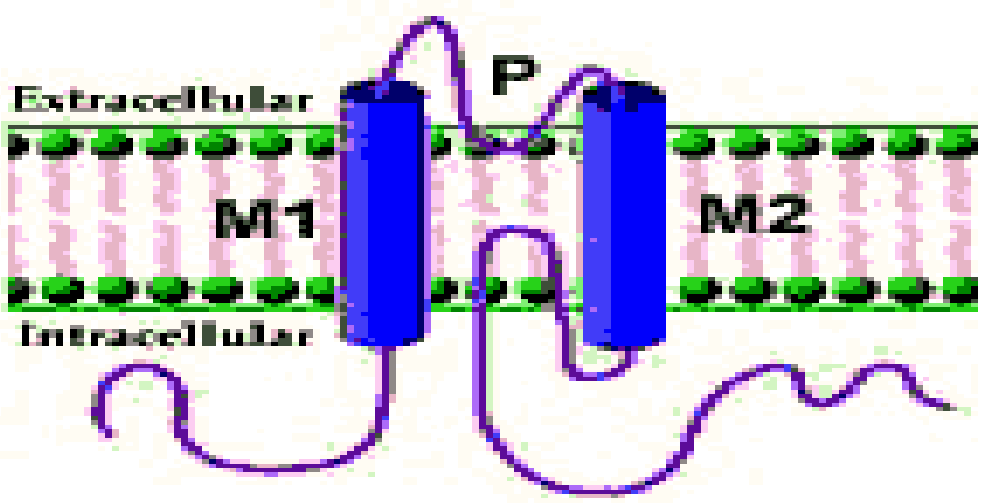
VOLTAGE-GATED CALCIUM CHANNELS

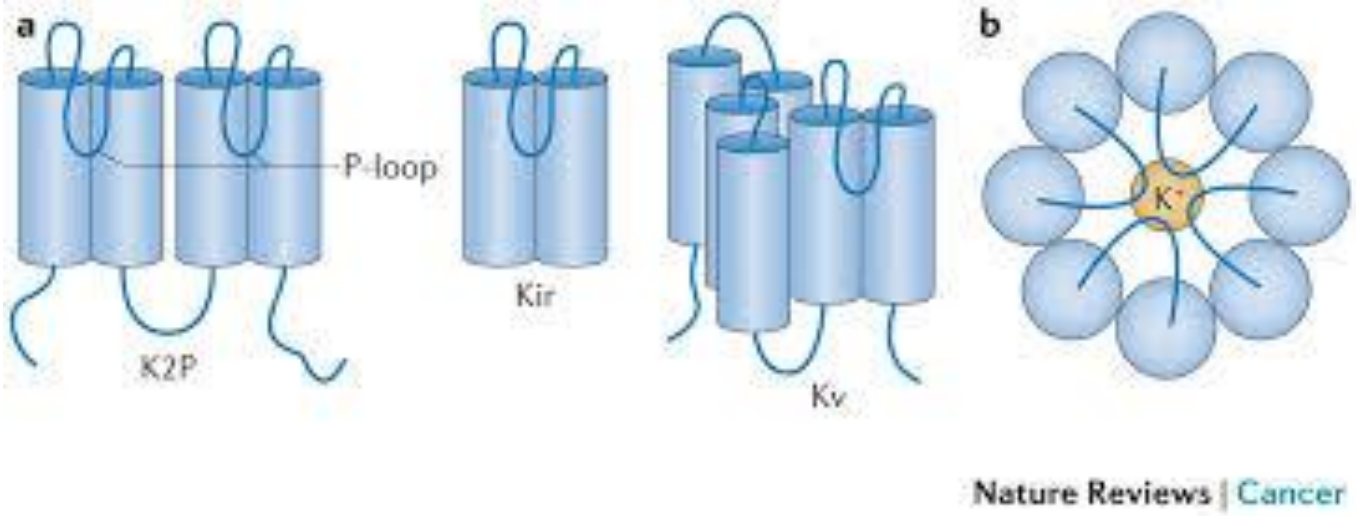


Other channels showing similar structure to K^+_V channels

- Cyclic nucleotide gated channels, CNG
- Hyperpolarization activated channels, HCN
- Transient receptor channels, TRP

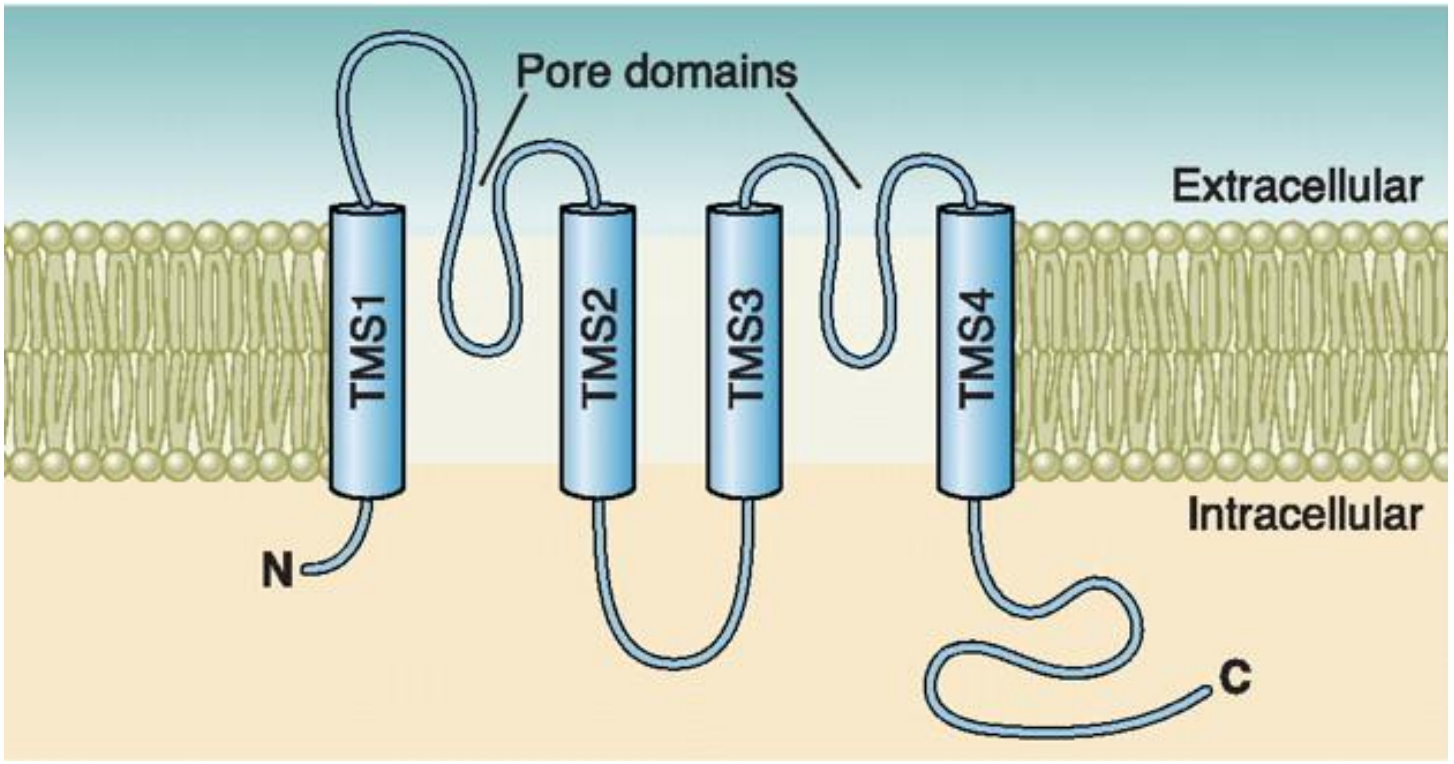
- Structure of an inwardly rectifying potassium channel.
- It is activated by hyperpolarization.
- It contains 2 TM segments and a p-loop

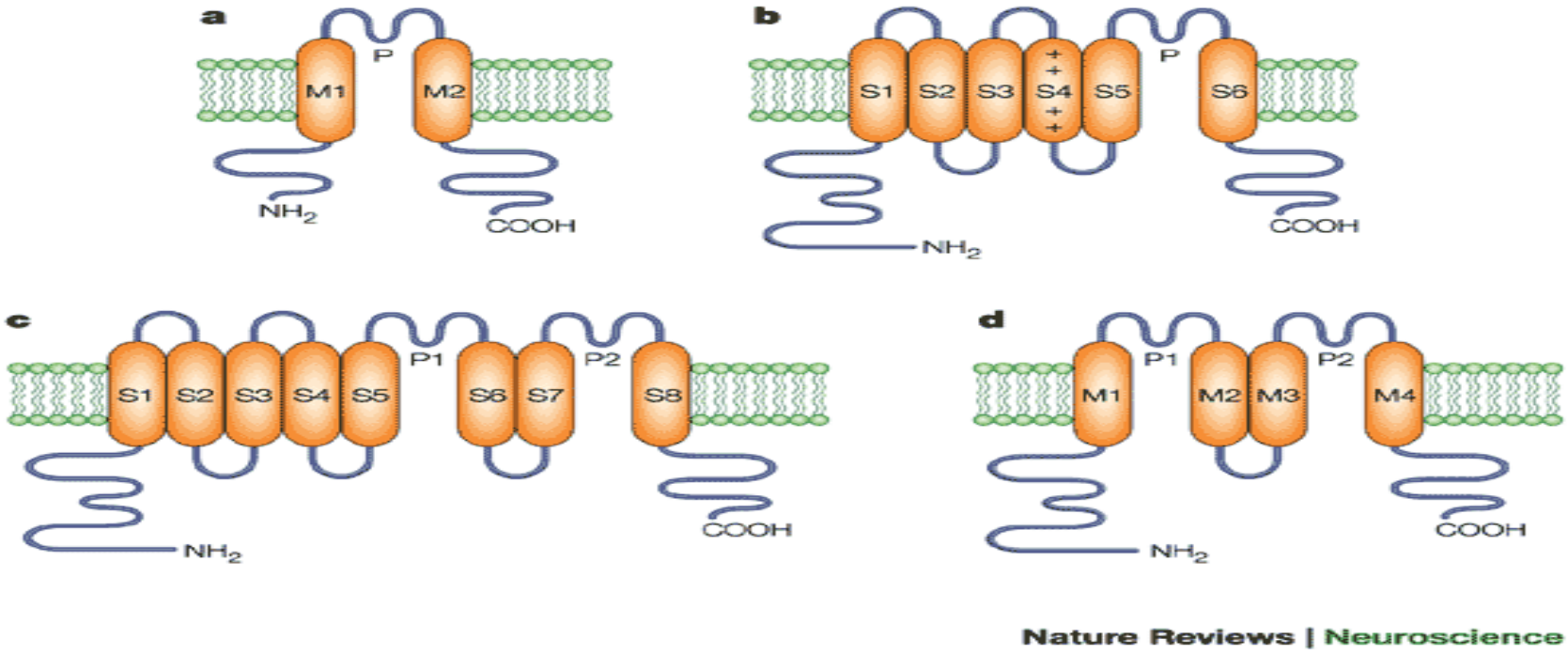




a | A lateral view of monomers of an inward rectifier potassium channel (Kir), a two-pore domain potassium channel (K2P) and a voltage-gated potassium channel (Kv). **b** | A top view of a minimal Kir or Kv channel, showing the two transmembrane segments of each of the four α-subunits and their corresponding pore-forming loops (P-loops). For K2P channels, the figure would show four transmembrane segments of each of the two α-subunits (each with two P-loops) constituting a channel.

Two pore motives K^+ channel (K_{2p})

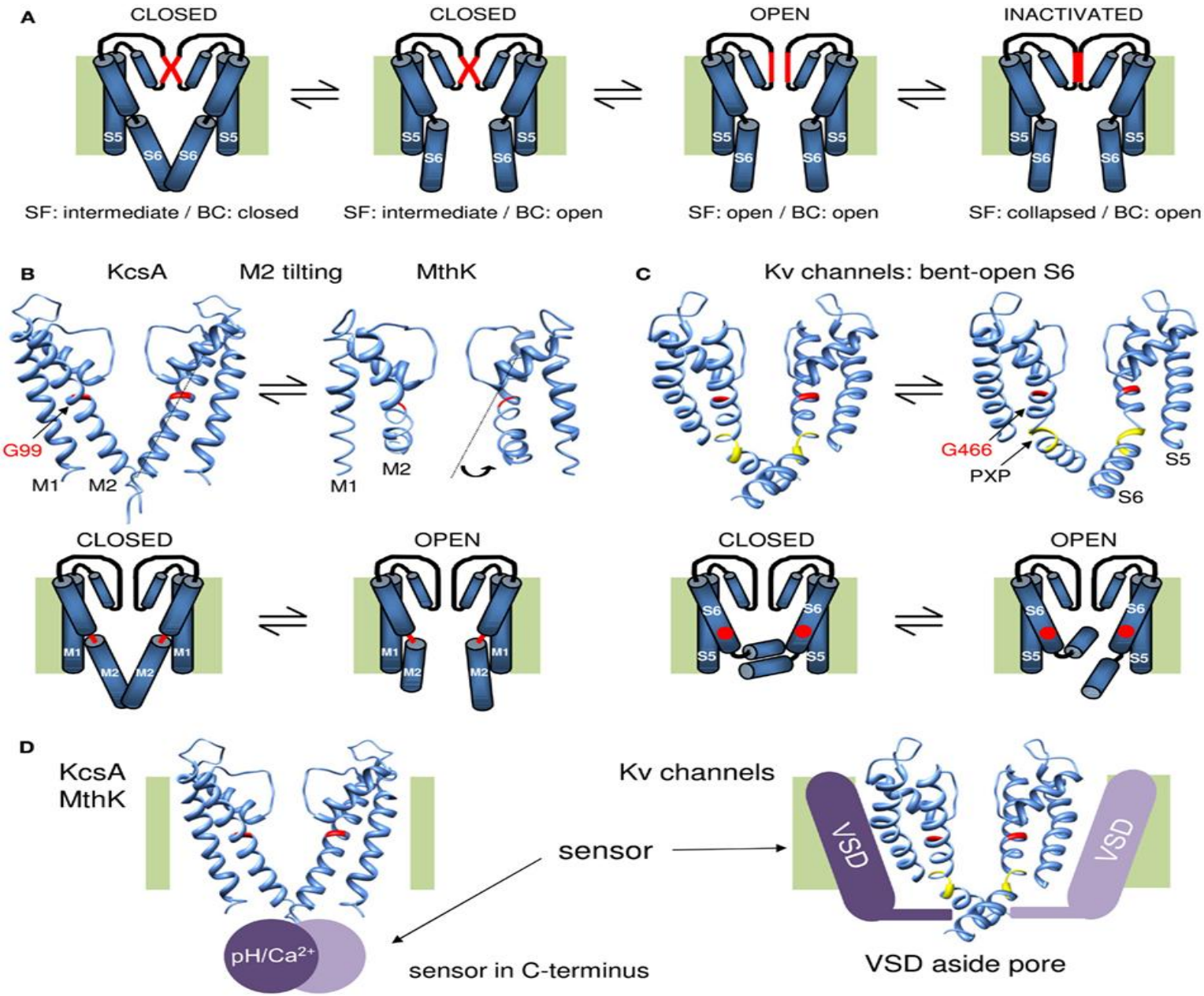


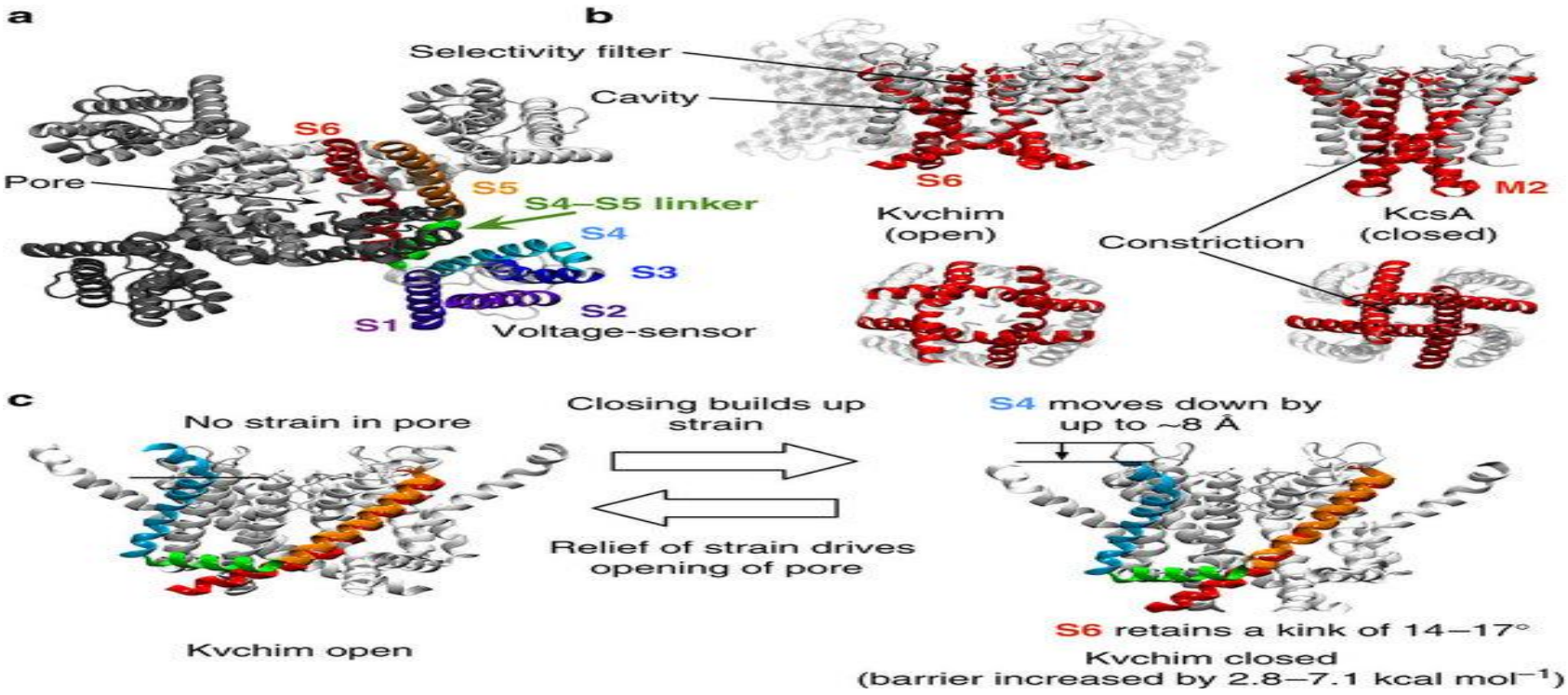


| 2TM/P channels (which consist of two transmembrane (TM) helices with a P loop between them), exemplified by inwardly rectifying K^+ channels and by bacterial K^+ channels such as KcsA. b | 6TM/P channels, predominant class among ligand-gated and voltage-gated K^+ channels. c | 8TM/2P channels, found in yeast. d | 4TM/2P channels, which consist of two repeats of 2TM/P channels These so-called 'leakage' channels are targets of numerous anaesthetics[39].

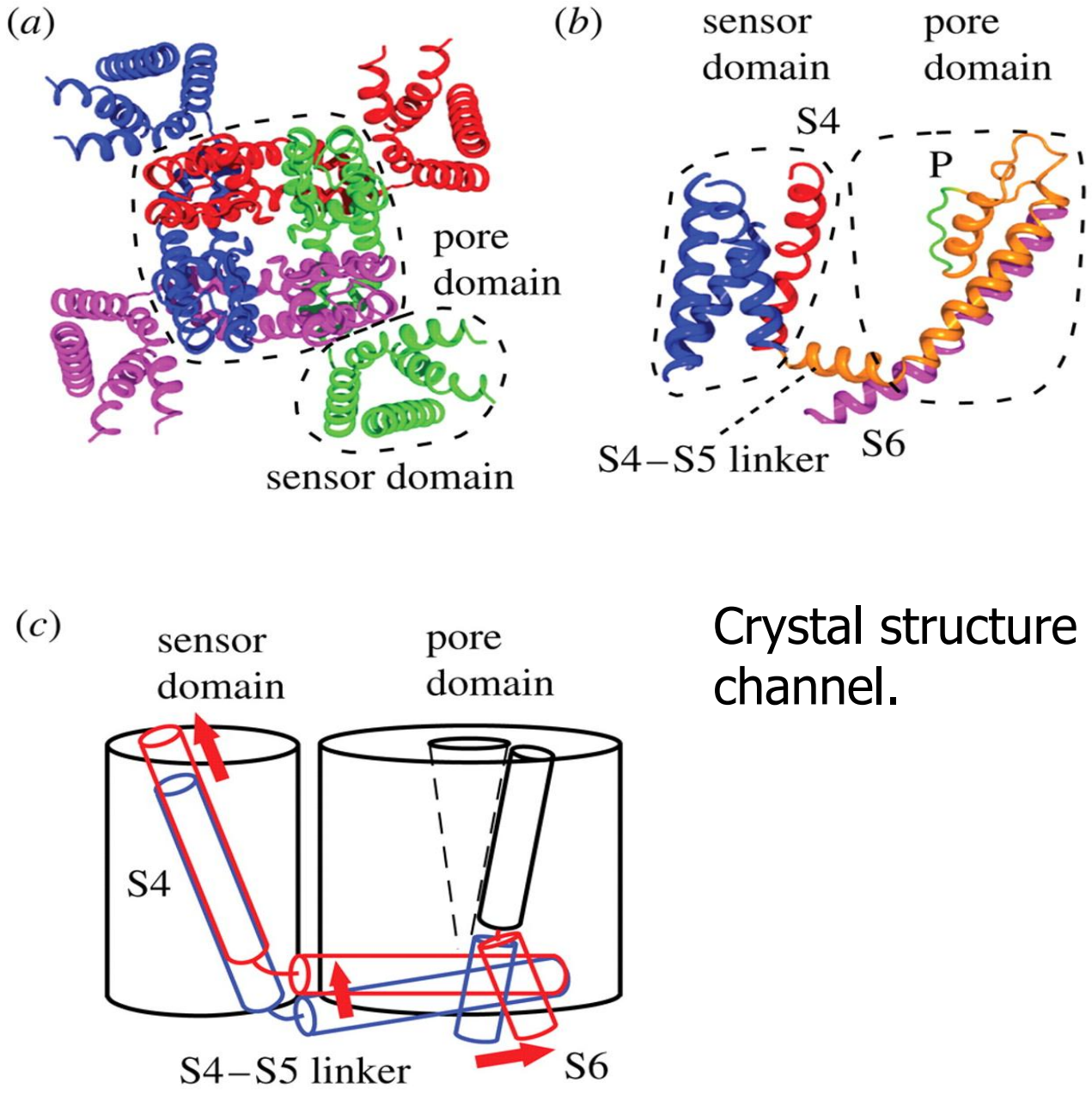
Functional classification of ion channels

- S5 & S6 segments form the wall of the channel
- S4 voltage sensitizating loops : opening and closing
  - When IC –S4 segment slides down
  - When IC + S4 segmentishifts up


A
CLOSED
CLOSED
OPEN
INACTIVATED
S5
S6
SF: intermediate / BC: closed
SF: intermediate / BC: open
SF: open / BC: open
SF: collapsed / BC: open
B
KcsA
M2 tilting
MthK
G99
M1
M2
CLOSED
OPEN
C
Kv channels: bent-open S6
G466
PXP
S5
S6
D
KcsA
MthK
pH/Ca2+
sensor in C-terminus
Kv channels
sensor
VSD
VSD aside pore



(**a**) Extracellular view of the tetrameric Kv1.2/2.1 paddle chimaera (Kvchim)[4]. The helices of one monomer are coloured and labelled. (**b**) Membrane and intracellular views of Kvchim (open) and KcsA (closed)[2], respectively. The pore domains are coloured white and the inner helices are coloured red. (**c**) Schematic illustration of how the channel closes and opens with the average motions suggested by our simulations labelled



Crystal structure of Kv1.2 K channel.

Molecular evolution of voltage gated channel family

- Many bacteria have 2 TM K_{ir} channels
- If S1-4 segments were added, they become voltage sensitive
- Some bacteria has 6 TM K channels similar to tetrameric Na channel structure.

There are additional helper subunits in channels

- Subunits forming the channel
  - Conductance
  - Gating
  - Regulation
  - Pharmacologic properties
- Helper subunits
  - Expression
  - Functional properties
  - Subcellular localization

- Na channel has one helper subunit
- $Na_V\beta 1$-4
- Ca channel has 4 helper subunits
- $Ca_V\beta 1$-3, $Ca_V\beta$, $Ca_V\gamma$, $Ca_V\delta$
- K channels show variability
- $K_V\beta 1$-3, KChIP1-4, MinK like subunit
  - K_{ir} channels : SUR subunit

Farklar

- İyon kanallarında değiim hızı (flux) çok hızlı iken değiştirici ve taşıyıcılarda çok daha yavaştır
- İyon kanallarında akım elektrokimyasal gradient yönündeyken, değiştiricilerde aksi de olabilir
- İyon kanallarında akımın oluşması için metabolik enerjiye ihtiyaç yokken, bazı değiştiriciler için vardır

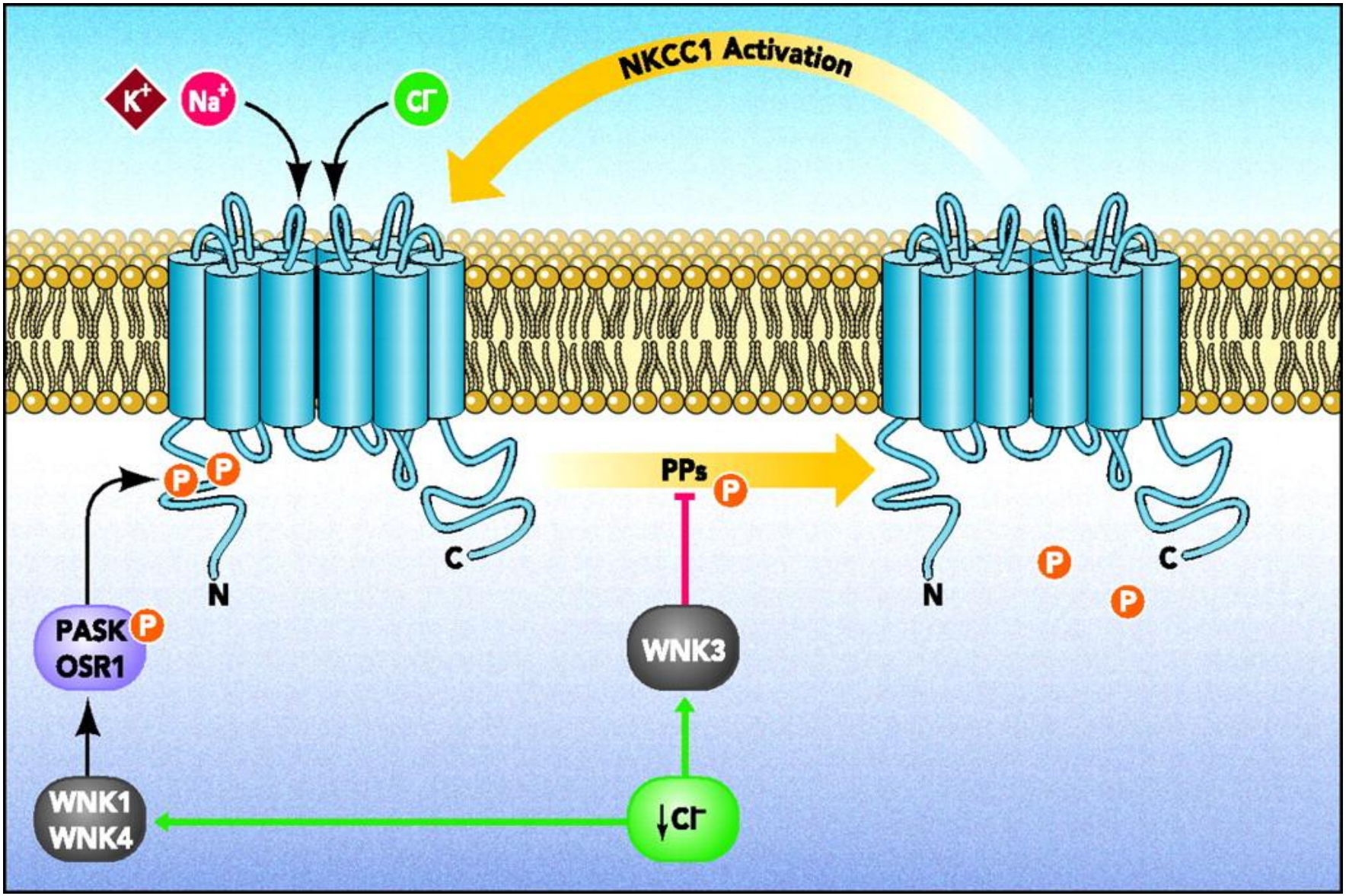
Fonksiyonları

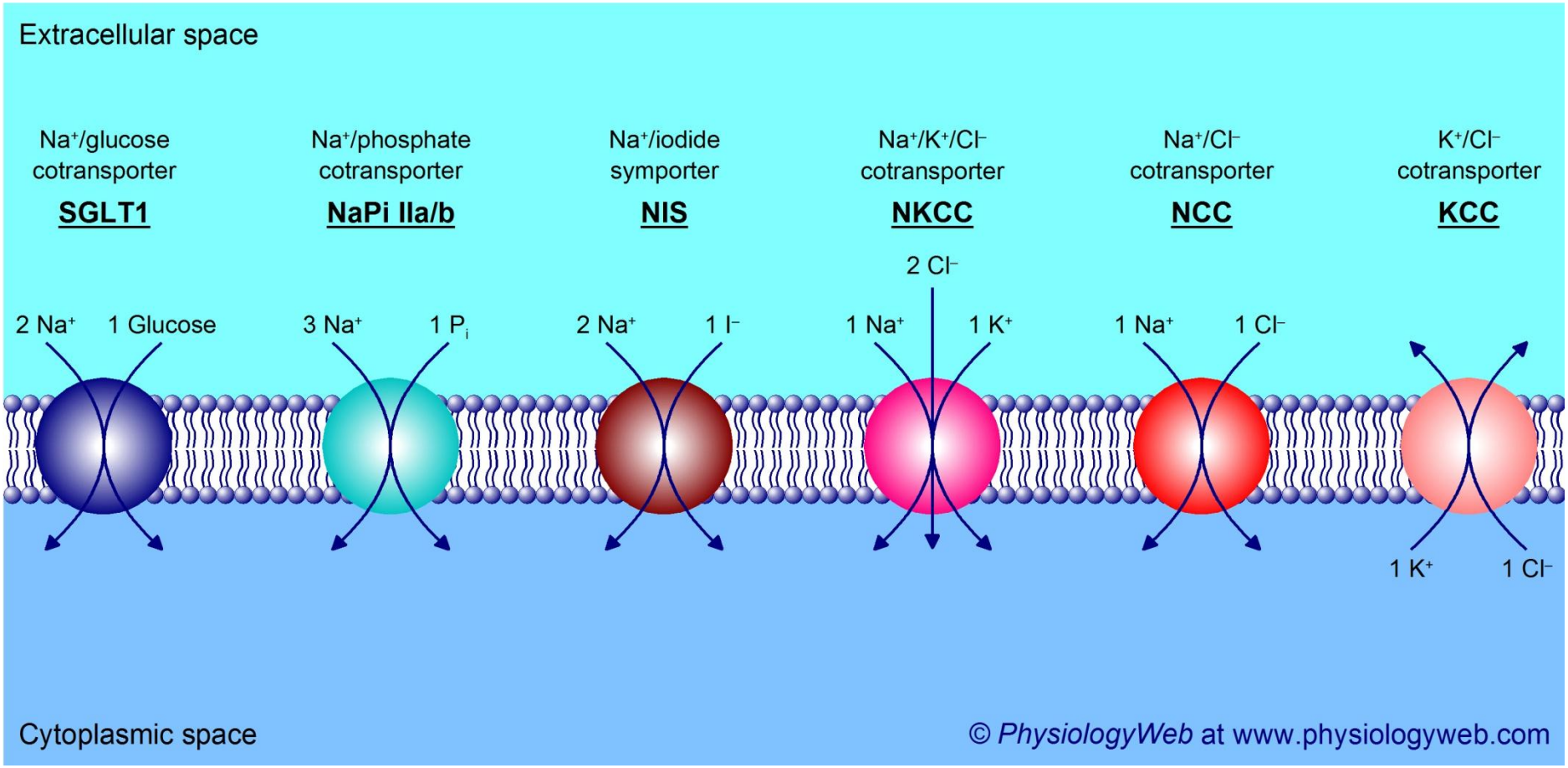
- Değiştirici ve taşıyıcıların oluşturduğu akımlar küçük ve yavaş olduğundan, hücre membranında hızlı bir elektriksel sinyal oluşturamazlar
- En temel fonksiyonları
  - Membranın iki tarafındaki yük dağılımını korumak
  - Hücrede homeostazın sağlanması
  - pHnın düzenlenmesi
  - Bazı metabolitlerin ve nörotransmitterlerin geri emilmesi sağlamak

Değiştiriciler ve Taşıyıcılar

İsmi	Yeri
Na^+ /K^+ ATPaz	Hücre membranı
Ca $^{++}$ ATPaz	Hücre membranı, Endoplazmik Retikulumda
Na^+/ Ca $^{++}$ karşı değiştirici	Hücre membranı, Mitokondri membranı
Na^+/ H^+ karşı değiştirici	Hücre membranı, Mitokondri membranı
Na^+/ Mg $^{++}$ karşı değiştirici	Hücre membranında
Na^+/ Mg $^{++}$ karşı değiştirici	Hücre membranı
Na^+ -K^+ / $2Cl^-$ karşı değiştirici	Hücre membranında
Na^+/ HCO_3^+ taşıyıcı	Hücre membranı

- Bazı değiştiriciler metabolik enerjiye ihtiyaç duymadan membranın iki tarafı arasında oluşan elektrokimyasal gücü kullanır
  - Na^{+} -K^{+} / $2Cl^{-}$
  - Na/ Ca: 3Na 1Ca

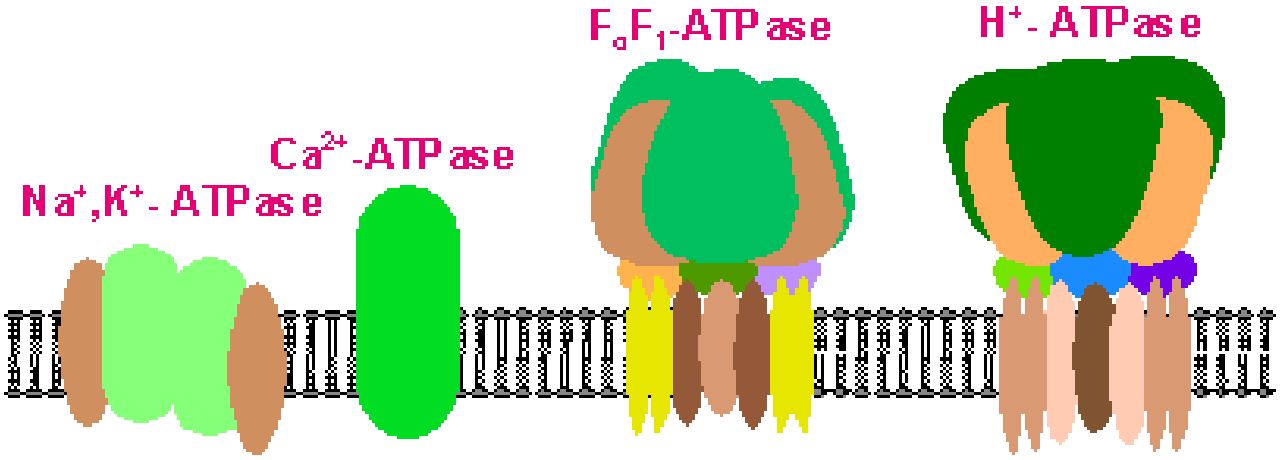

NKCC1 Activation
K+
Na+
Cl-
P
P
N
C
PPs
P
N
C
P
P
PASK
OSR1
P
WNK3
WNK1
WNK4
↓Cl-


Extracellular space
Na+/glucose cotransporter
SGLT1
Na+/phosphate cotransporter
NaPi IIa/b
Na+/iodide symporter
NIS
Na+/K+/Cl– cotransporter
NKCC
Na+/Cl– cotransporter
NCC
K+/Cl– cotransporter
KCC
2 Na+
1 Glucose
3 Na+
1 Pi
2 Na+
1 I–
2 Cl–
1 Na+
1 K+
1 Na+
1 Cl–
1 K+
1 Cl–
Cytoplasmic space
© PhysiologyWeb at www.physiologyweb.com

Na^+/K^+ ATPaz

- P ATPaz ailesinin üyesi
- 3Na karşılık 2K değiştirir
- α ve β alt birimlerinden oluşur
- Forforu bağlayıp bırakmakla konfigürasyon değişikliğine uğrar
- Konsantrasyon, membran potansiyeli etkisinde ATP kullanarak iyonları bağlar, hapseder ve taşır

ATPase: Structures



P-type ATPase

simple subunit organization

phosphorylated protein intermediate

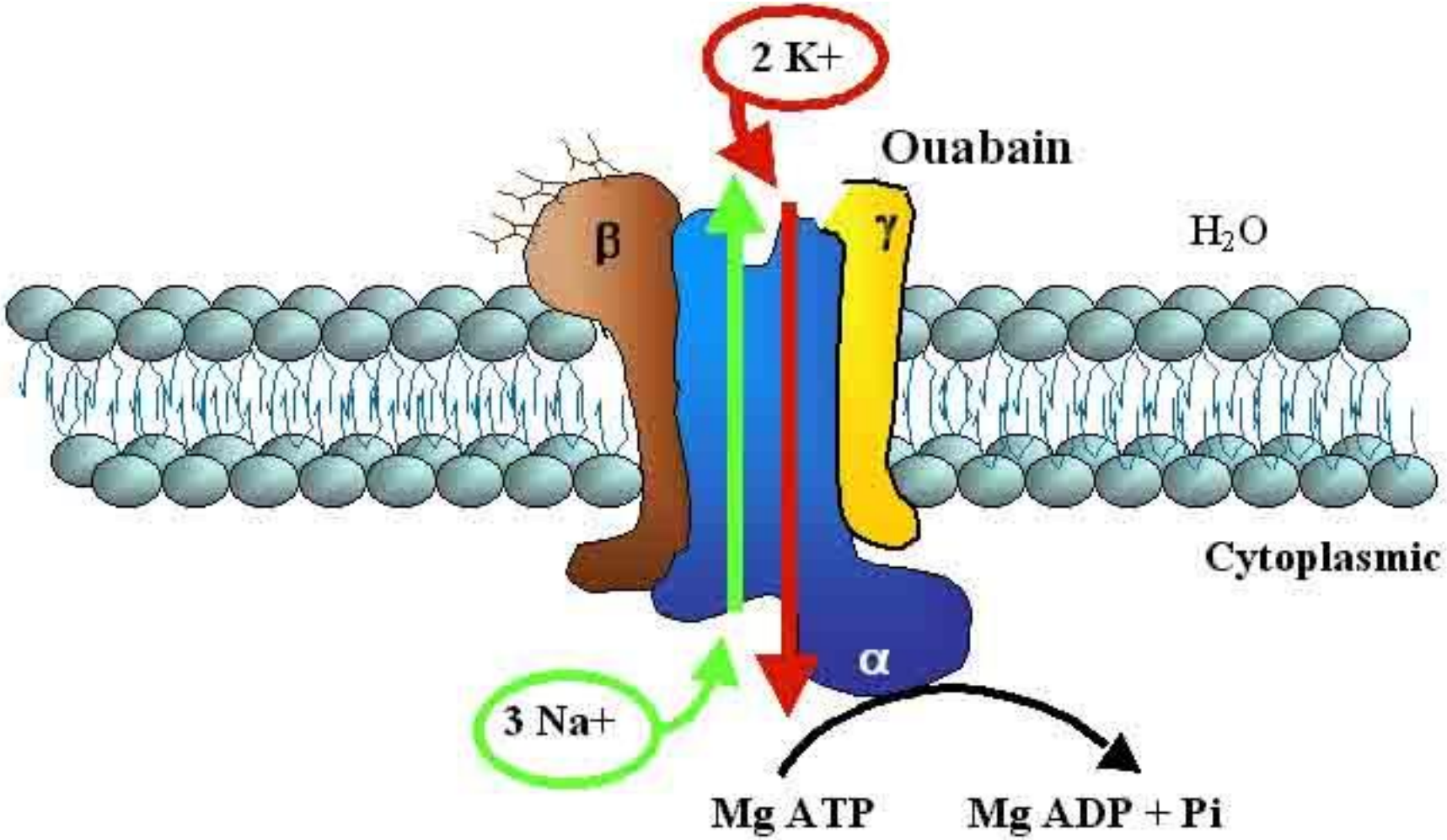
F-type ATPase

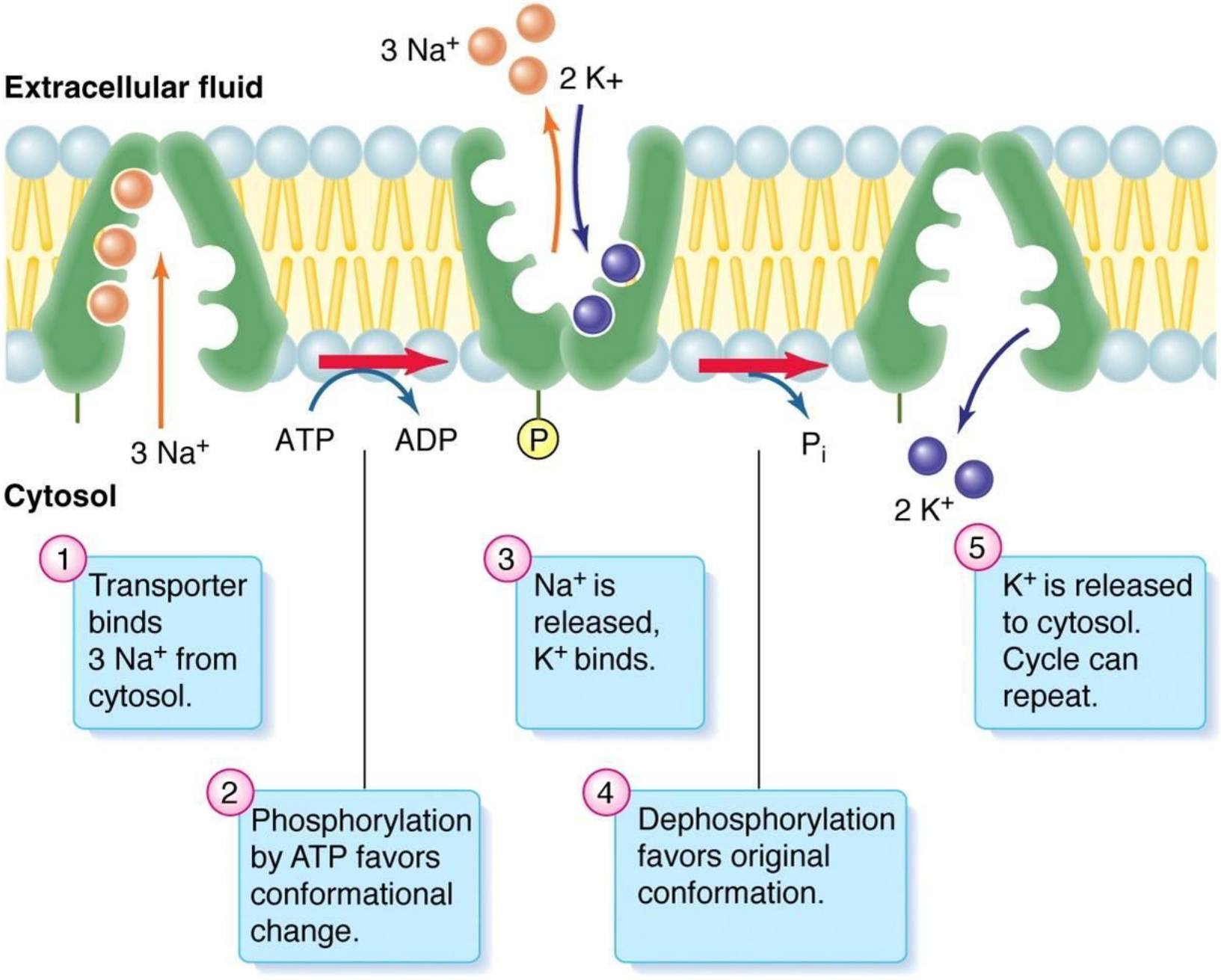
complex subunit organization

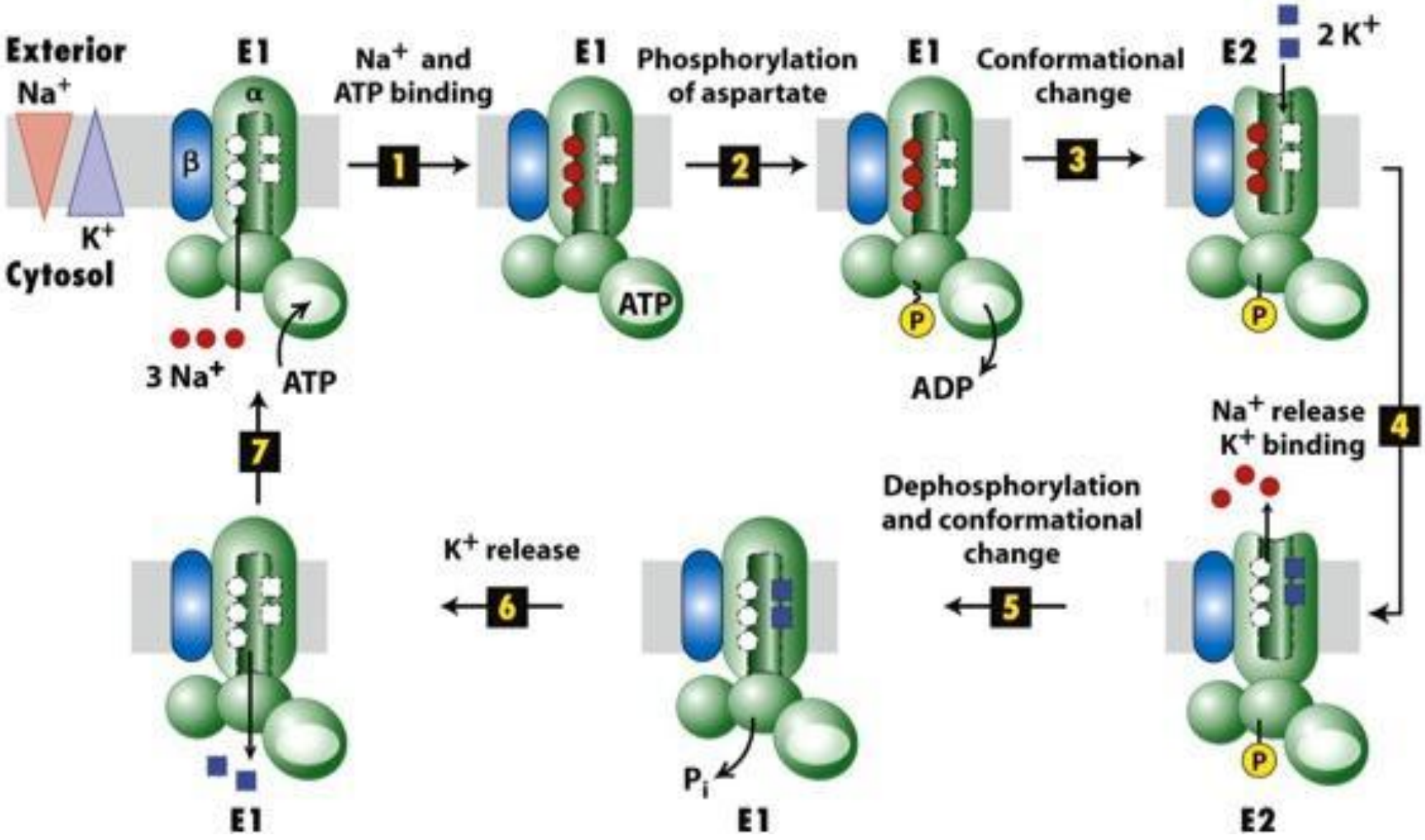
V-type ATPase

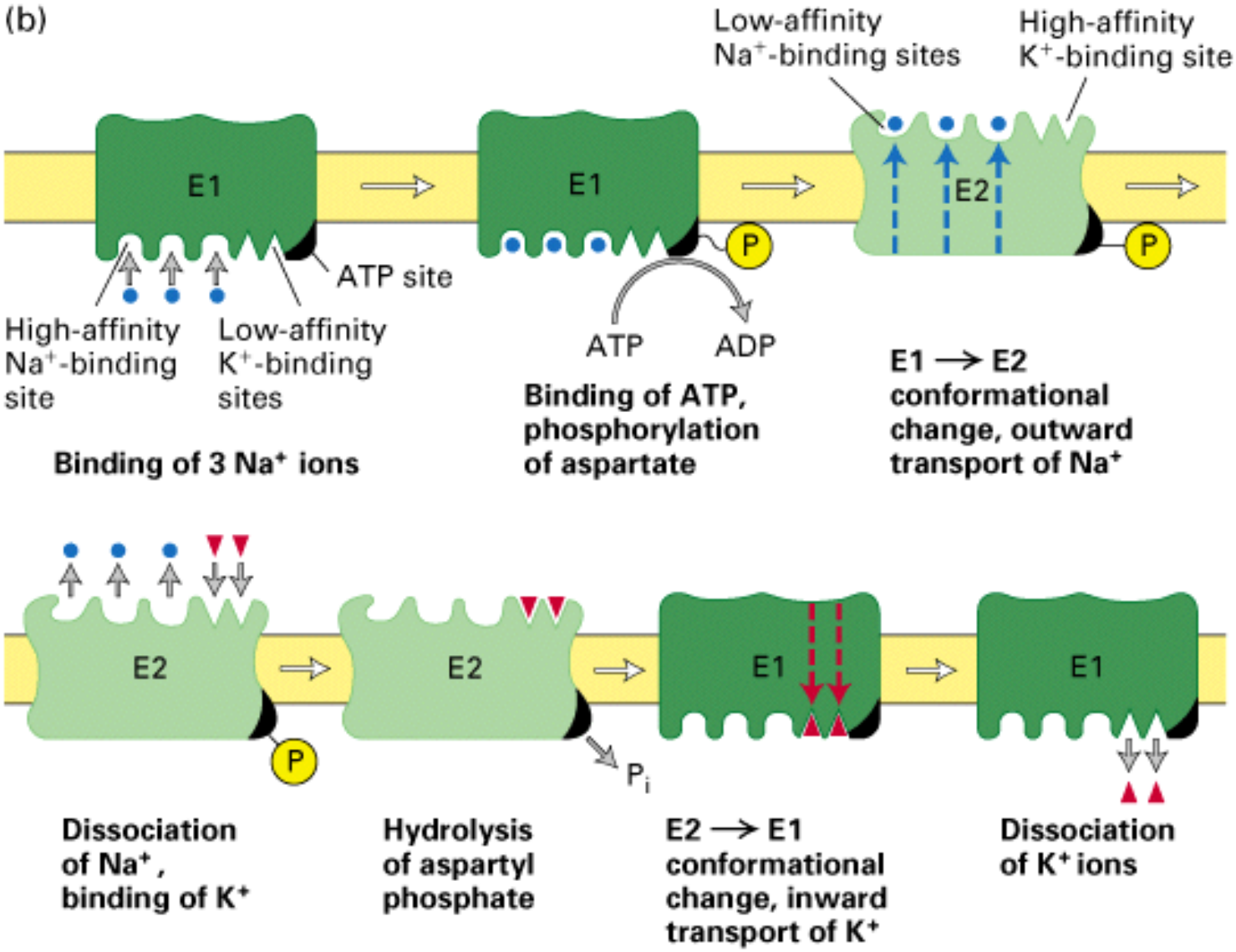
complex subunit organization

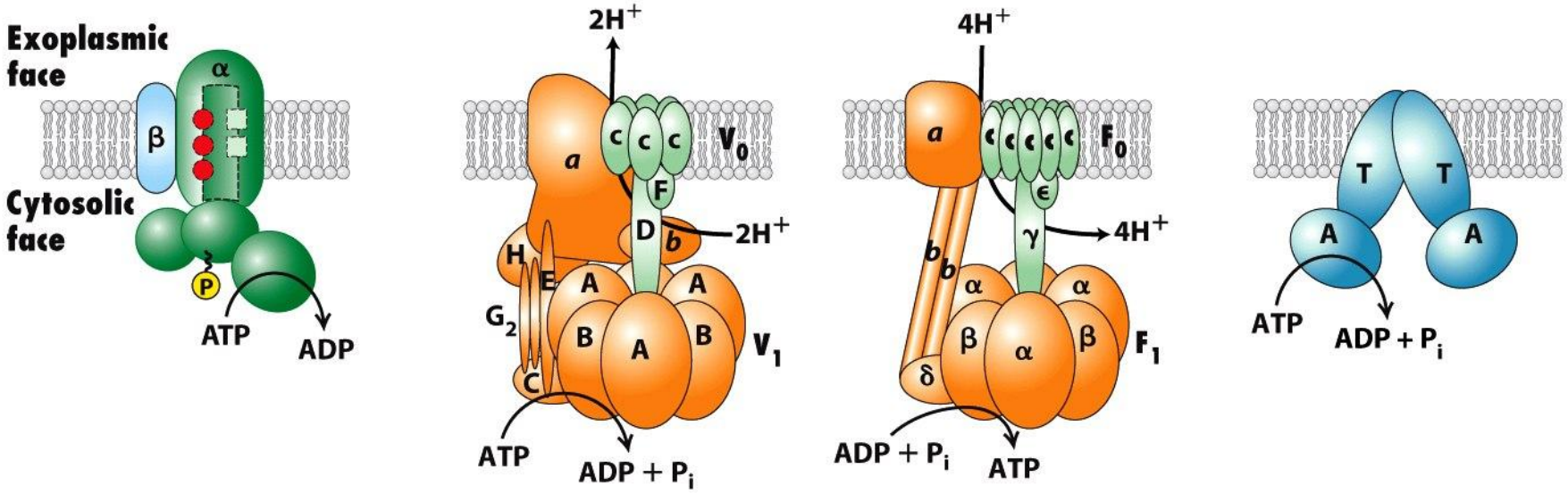
adapted from H. Lodish *et al.* (1995)


2 K+
Ouabain
β
γ
H_2O
Cytoplasmic
α
3 Na+
Mg ATP
Mg ADP + Pi


3 Na+
2 K+
Extracellular fluid
3 Na+
ATP
ADP
P
Pi
2 K+
Cytosol
1
Transporter binds 3 Na+ from cytosol.
2
Phosphorylation by ATP favors conformational change.
3
Na+ is released, K+ binds.
4
Dephosphorylation favors original conformation.
5
K+ is released to cytosol. Cycle can repeat.


Exterior
Na+
K+
Cytosol
E1
α
β
3 Na+
ATP
Na+ and
ATP binding
1
E1
ATP
Phosphorylation
of aspartate
2
E1
P
ADP
Conformational
change
3
E2
2 K+
P
Na+ release
K+ binding
4
E2
P
Dephosphorylation
and conformational
change
5
E1
Pi
K+ release
6
E1
7


(b)
Low-affinity Na+-binding sites
High-affinity K+-binding site
E1
E1
E2
P
P
ATP site
High-affinity Na+-binding site
Low-affinity K+-binding sites
ATP
ADP
Binding of 3 Na+ ions
Binding of ATP, phosphorylation of aspartate
E1 → E2 conformational change, outward transport of Na+
E2
E2
E1
E1
P
Pi
Dissociation of Na+, binding of K+
Hydrolysis of aspartyl phosphate
E2 → E1 conformational change, inward transport of K+
Dissociation of K+ ions



P-class pumps

Plasma membrane of plants, fungi, bacteria (H^+ pump)

Plasma membrane of higher eukaryotes (Na^+/K^+ pump)

Apical plasma membrane of mammalian stomach (H^+/K^+ pump)

Plasma membrane of all eukaryotic cells (Ca^{2+} pump)

Sarcoplasmic reticulum membrane in muscle cells (Ca^{2+} pump)

V-class proton pumps

Vacuolar membranes in plants, yeast, other fungi

Endosomal and lysosmal membranes in animal cells

Plasma membrane of osteoclasts and some kidney tubule cells

F-class proton pumps

Bacterial plasma membrane

Inner mitochondrial membrane

Thylakoid membrane of chloroplast

ABC superfamily

Bacterial plasma membranes (amino acid, sugar, and peptide transporters)

Mammalian plasma membranes (transporters of phospholipids, small lipophilic drugs, cholesterol, other small molecules)

Figure 11-9
Molecular Cell Biology, Sixth Edition