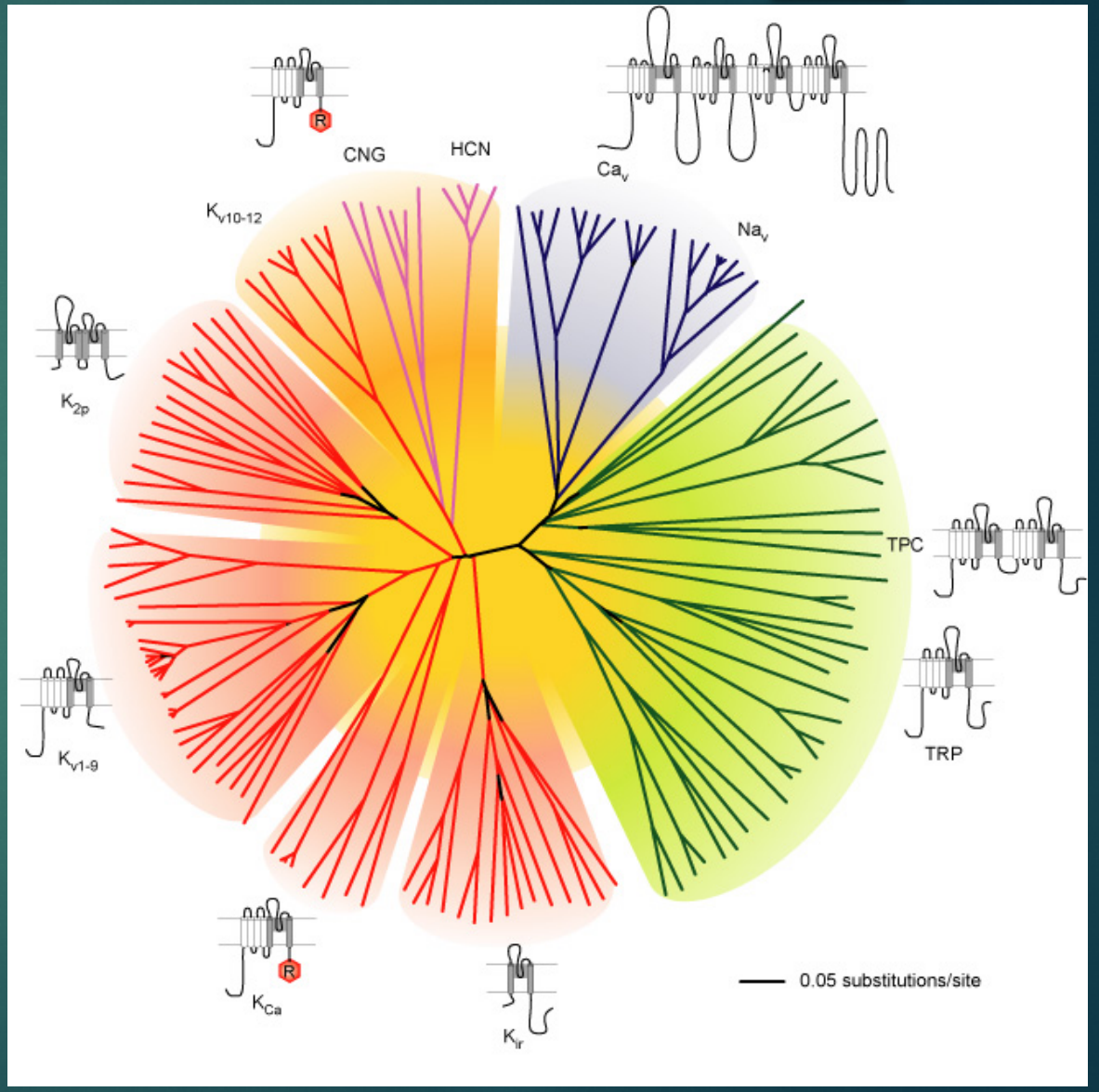
Ion Channels

STRUCTURE AND FUNCTION



Gating mechanisms : Ligand-dependent gating

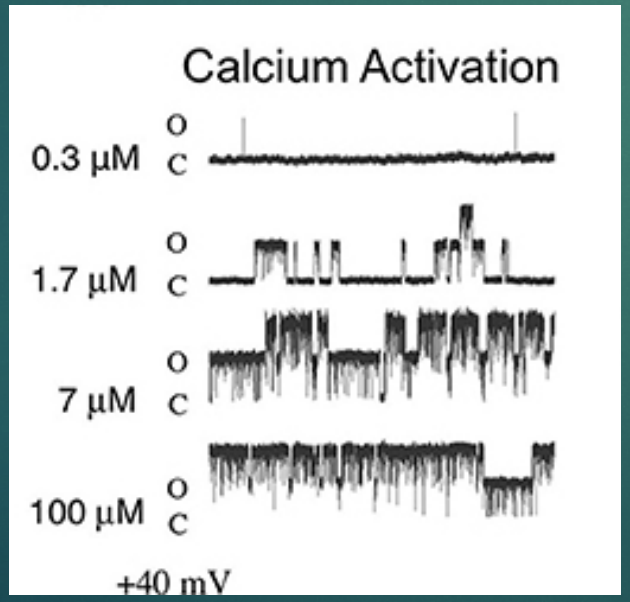
- For ligand-gated Ion channels, the conformational transitions and p_o are controlled by agonists.
- The ligand can be extracellular as in the case of channels activated by neurotransmitters
- Or intracellular as in the case of K+ channels activated by Ca2+ (K_{Ca}).

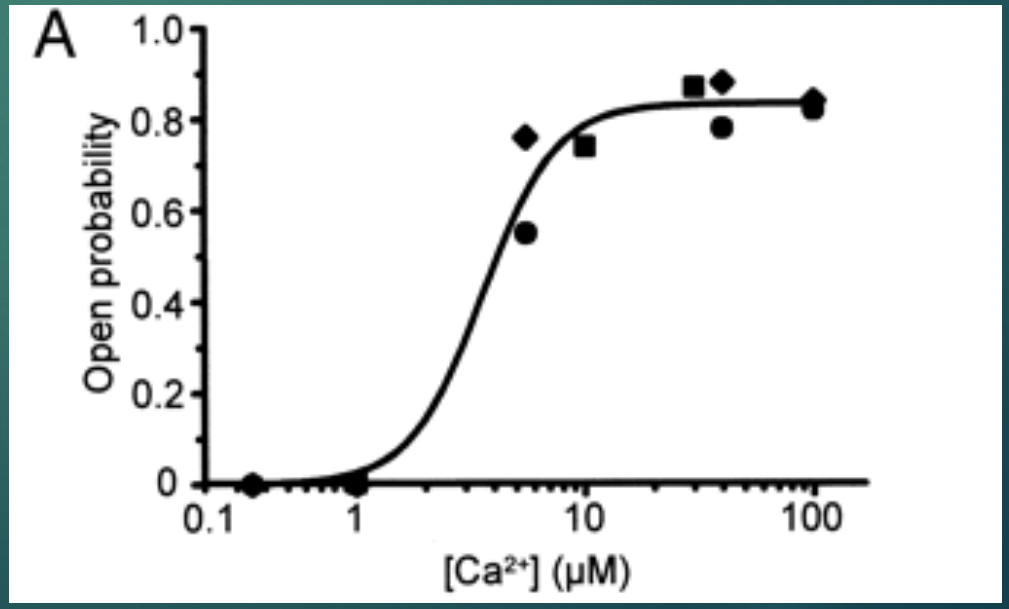
Gating mechanisms : Ligand-dependent gating

- Example:

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

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





Gating mechanisms : Ligand-dependent gating

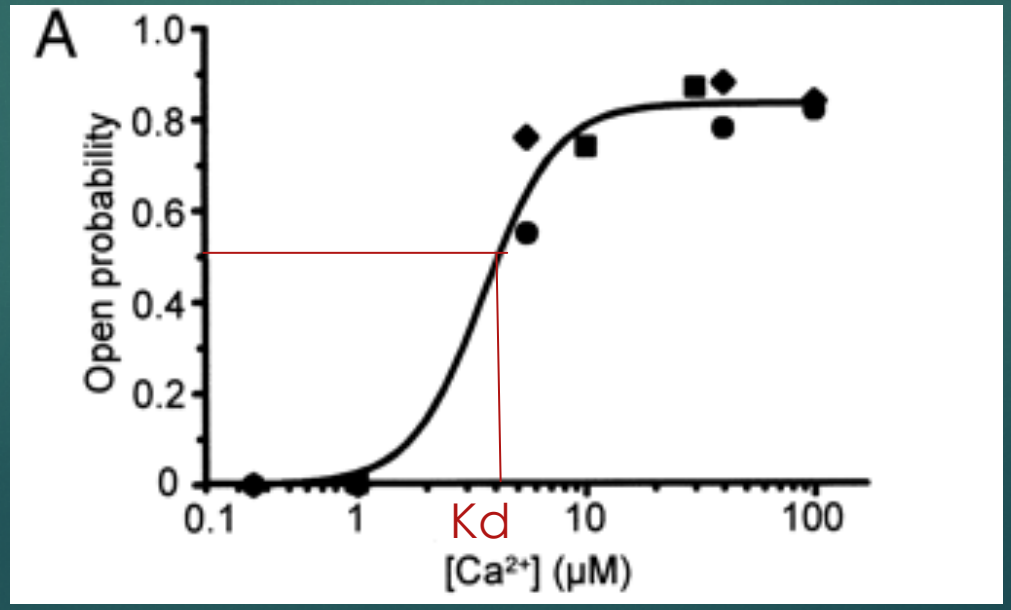
- *Po*- [Ca^{2+}] relation is well described by *Hill equation*

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

C_A = Agonist Concentration

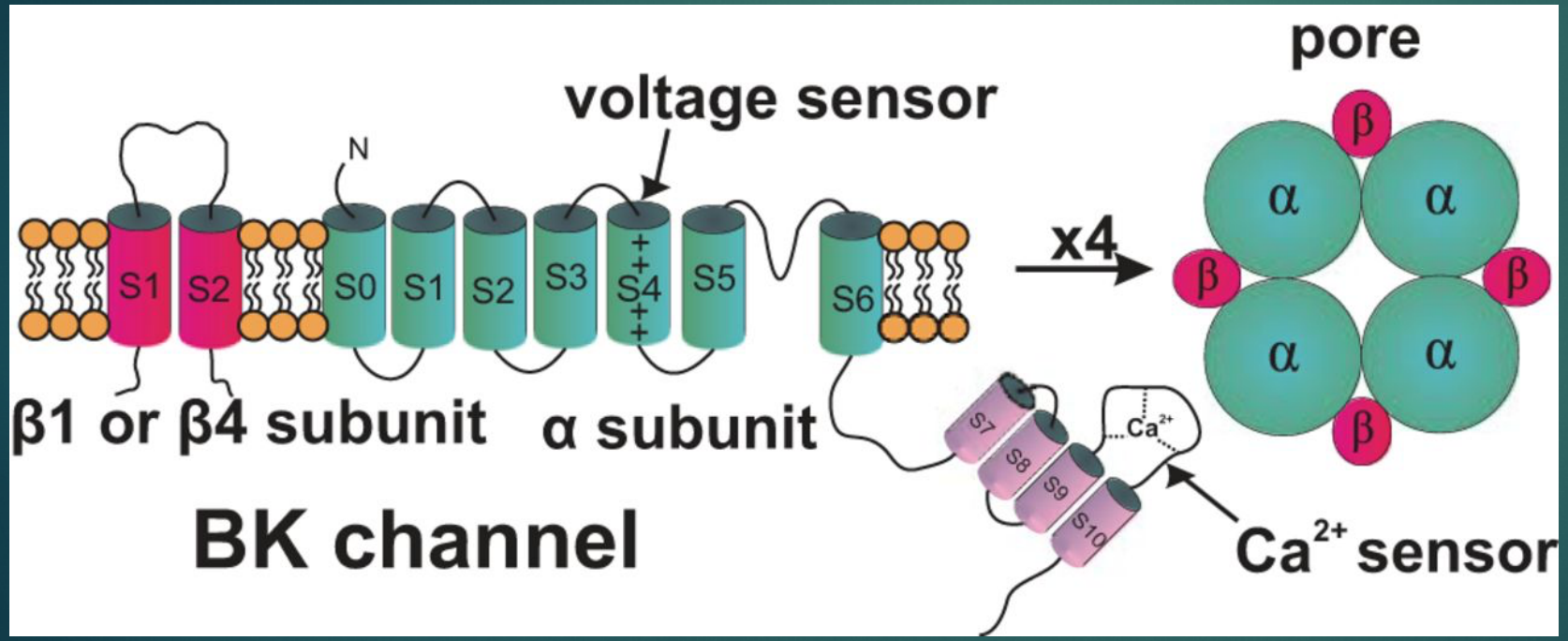
Kd = Dissociation constant

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.

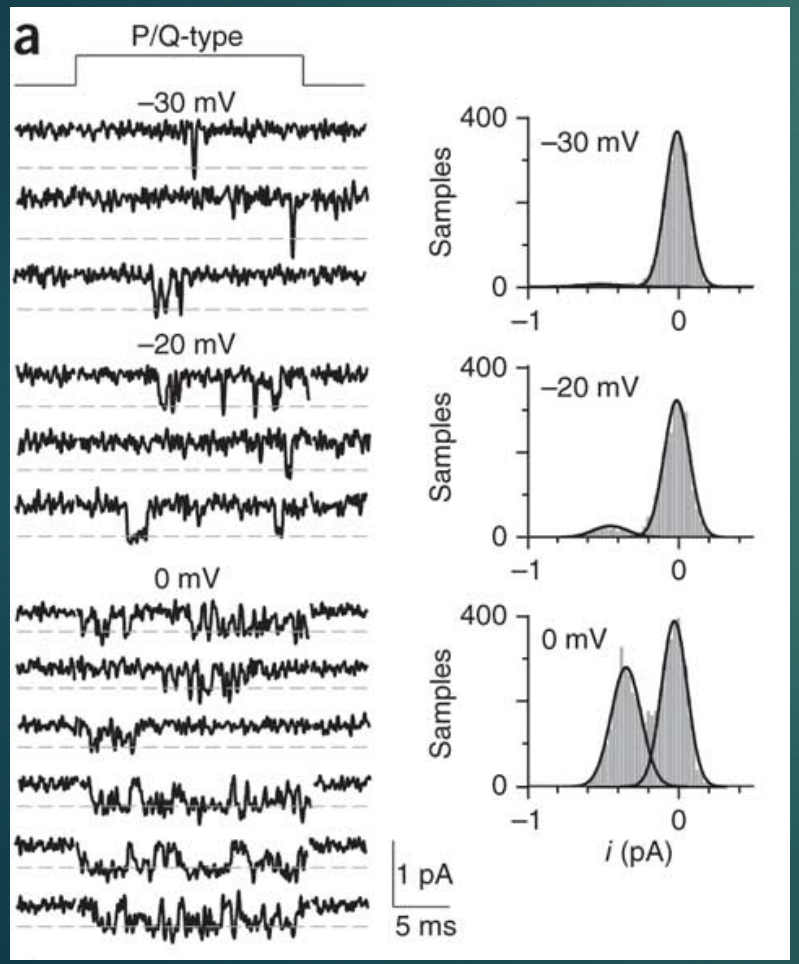
Common properties of ION CHANNELS:

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

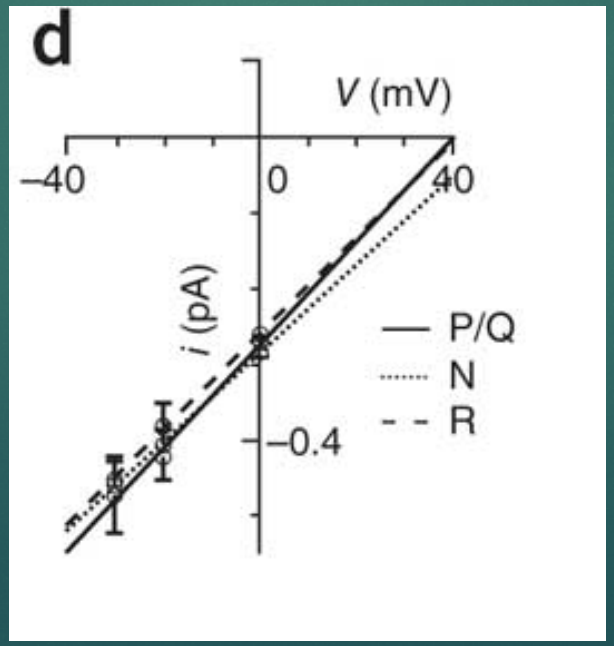
Channels can be therefore classified by the selectivity properties.

- **PERMEABILITY**: channels ability to conduct ions along their electrochemical gradient

Common properties of ION CHANNELS: PERMEABILITY



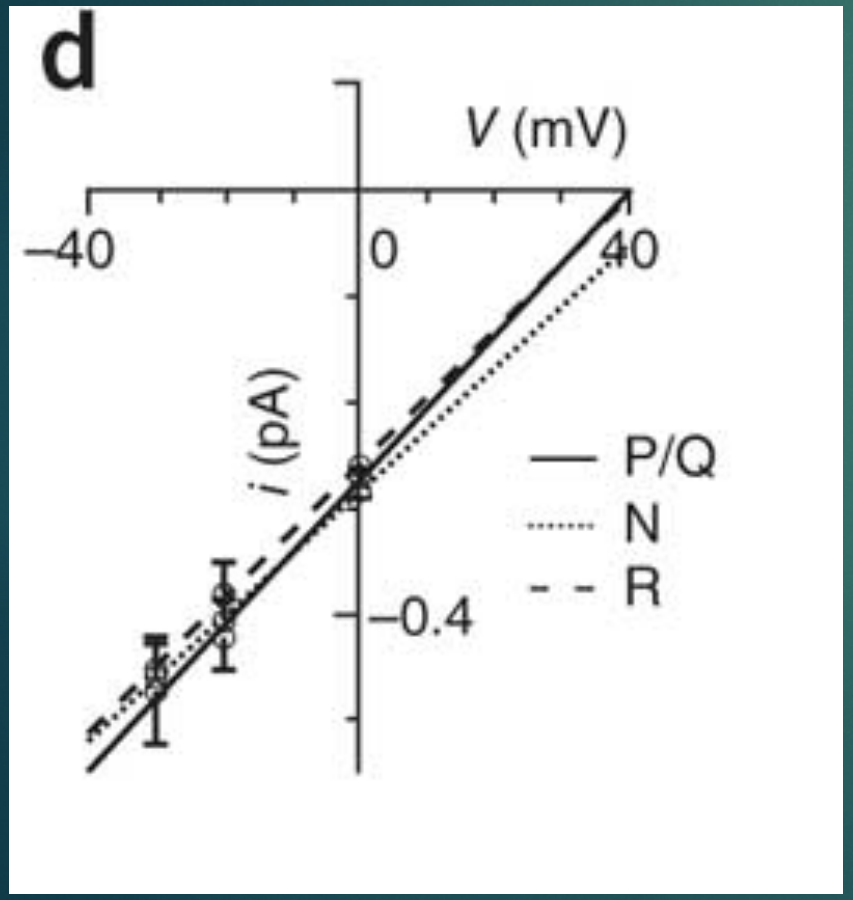
The idea of how permeable is the channel to ions can be obtained registering the current in the presence of the ion of interest at both sides at different Vm.



I-V Curves are obteined by measuring I amplitude at different V.

In this case is linear as expected for Ohmic conductor. More often the I-V are not linear.

Common properties of ION CHANNELS: PERMEABILITY



The slope of the curve is the **CONDUCTANCE** = γ and is expressed in pS

Since I-V curves are non linear in most cases, is important to specify the V range and the ion concentration (in and out) used to calculate γ.

There is a huge variability for γ and the range can go fro 1pS to 200pS

Selective permeability

Cell permeability to any ion changes with opening/closing of ion channels

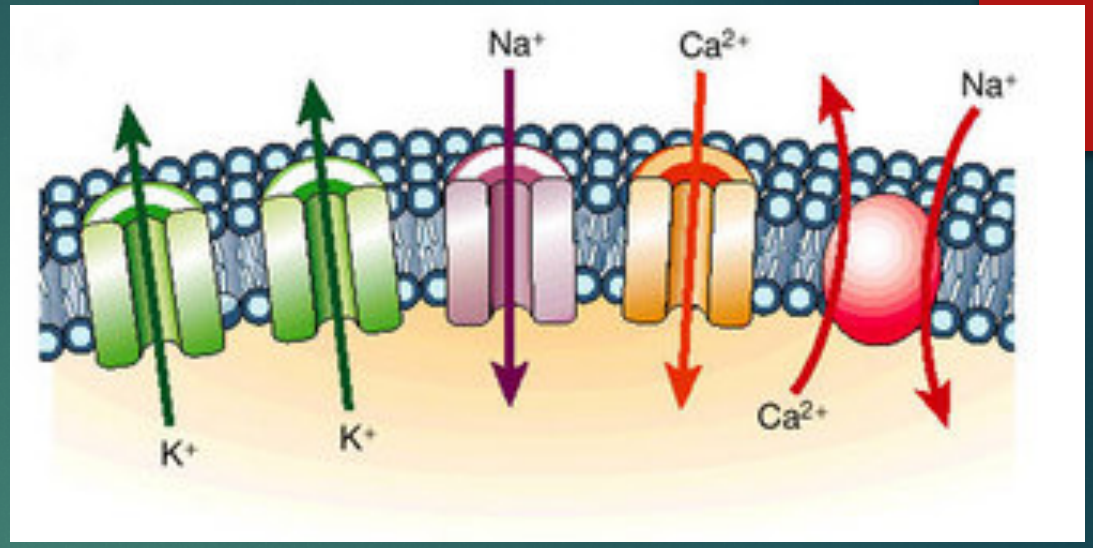
Direction of movement of one ion is dictated by the electrochemical driving force:

(V_m - V_{ion})

Membrane potential

Nernst potential

Driving force



GHK equation

$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_e + P_{Na}[Na^+]_e + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_e}$$

NERNST equation

$$V_{eq} = \frac{RT}{zF} \log \frac{C_{out}}{C_{in}}$$

Common properties of ION CHANNELS:

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

Channels can be therefore classified by the selectivity properties.

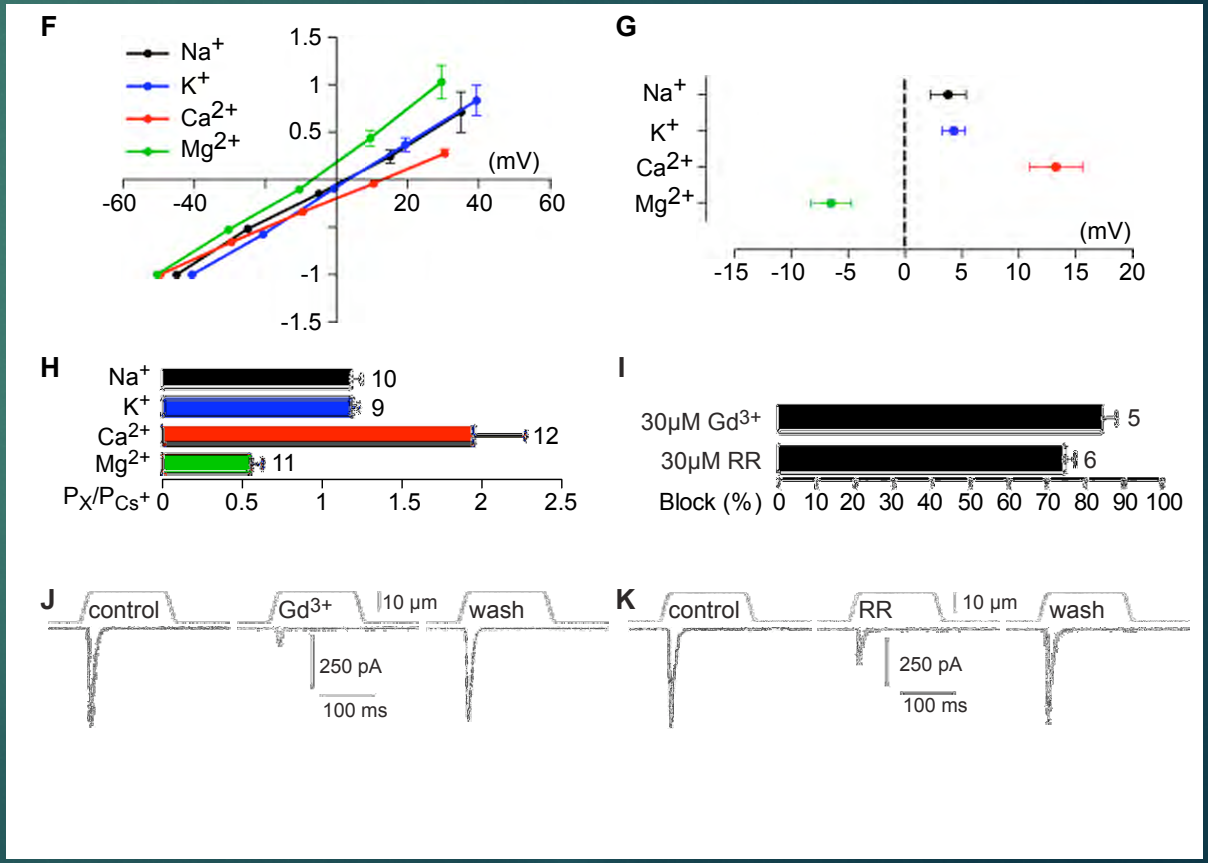
Common properties of ION CHANNELS: SELECTIVITY

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

Channels can be therefore classified by the selectivity properties. However the selectivity is not absolute: different ion species can permeate within the channels but in a more or less efficient way.

Common properties of ION CHANNELS: SELECTIVITY

To quantify the selectivity for different ions I-V curves can be performed using different ion concentrations.
From V_{inv} for each curve we can calculate the Permeability ratio between ions.



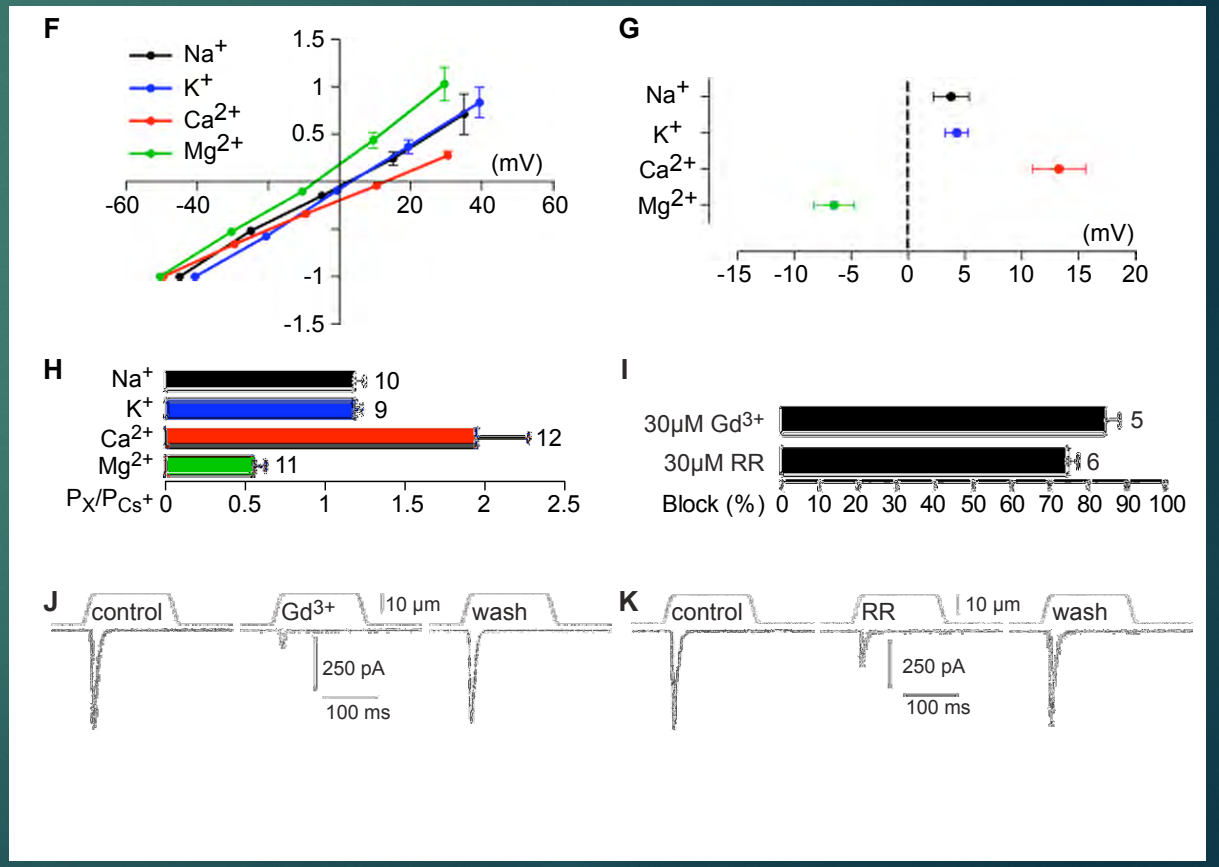
Common properties of ION CHANNELS: SELECTIVITY

From GHK equation

$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_e + P_{Na}[Na^+]_e + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_e}$$

The relation between PA/PB can be obtained by the **biionic potential** equation:

$$V_{inv} = \frac{RT}{zF} \ln \frac{[A]_o}{[B]_i} \frac{P_A}{P_B}$$

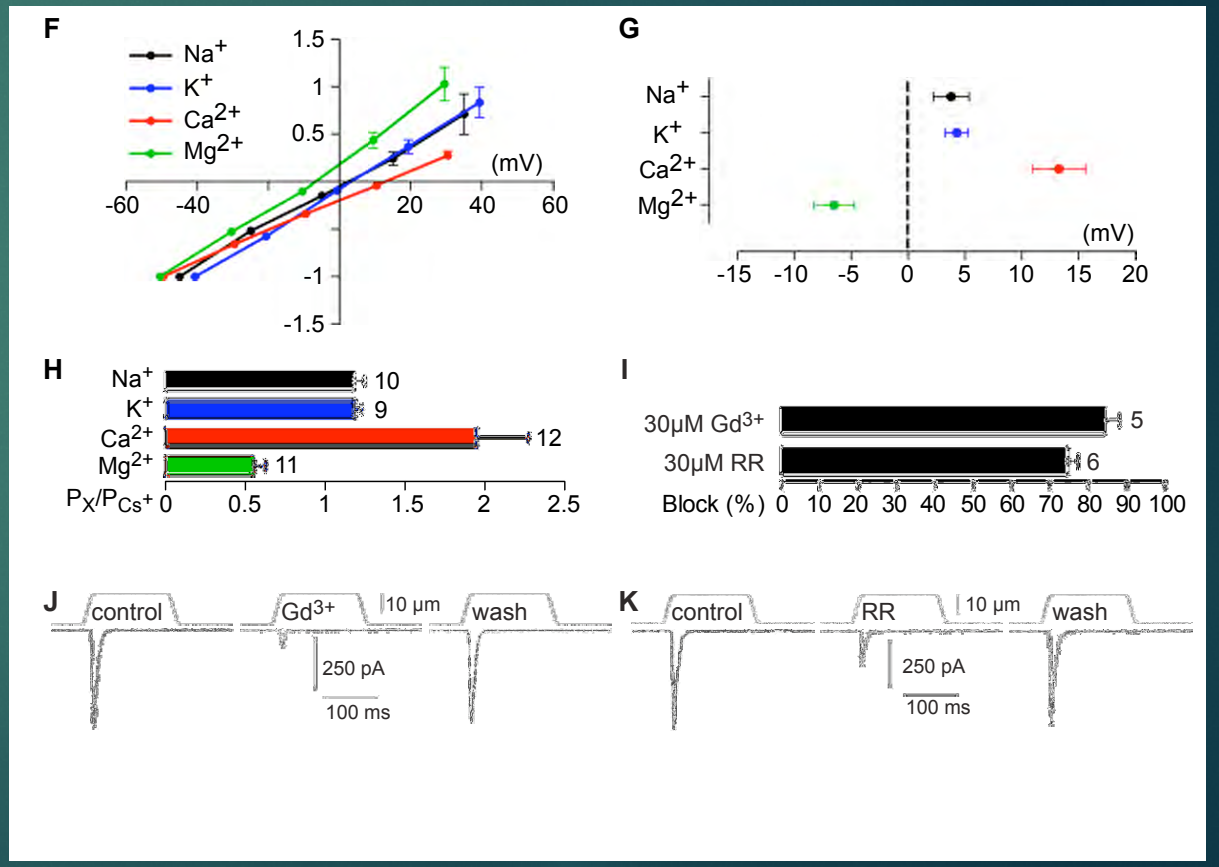


Common properties of ION CHANNELS: SELECTIVITY

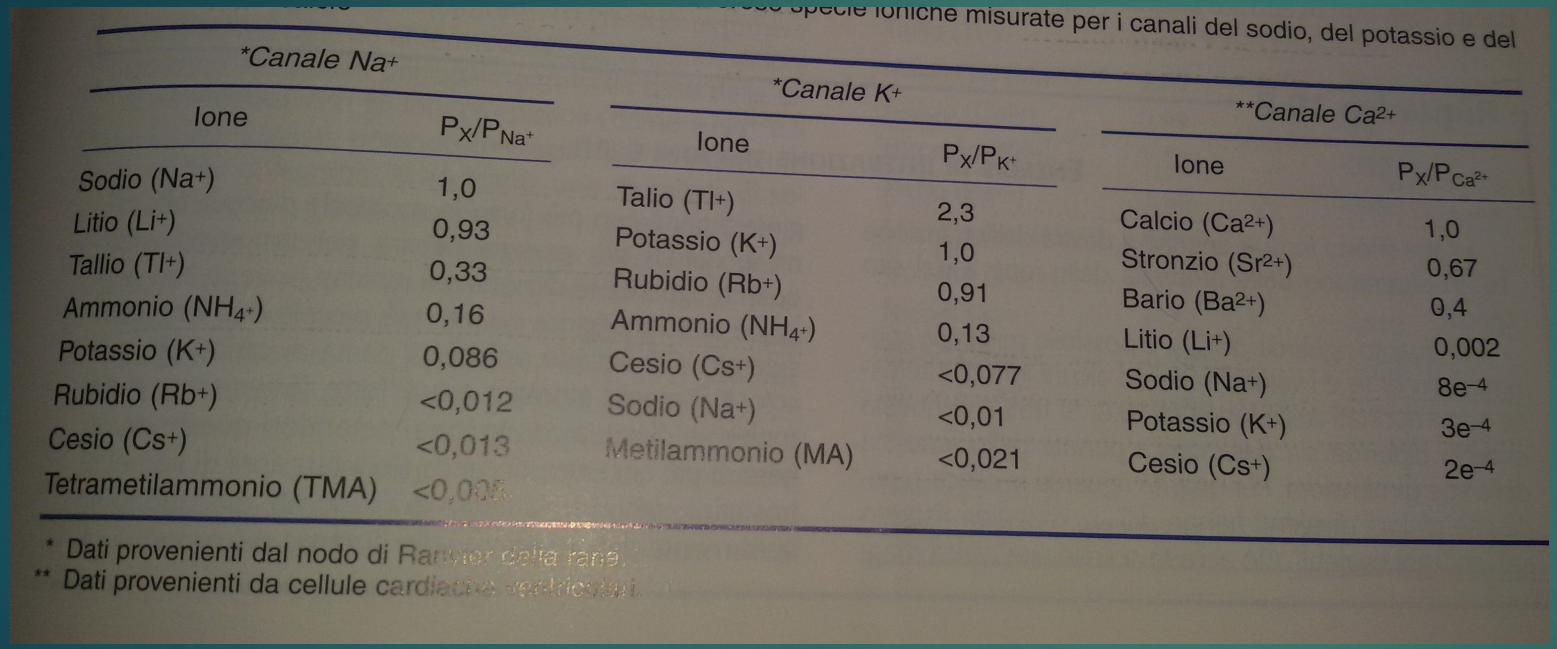
The relation between PA/PB can be obtained by measuring the Vinv (V at which I = 0) of the I-V obtained with A extracellular and B intracellular at equal concentrations :

$$\frac{P_A}{P_B} = \frac{[B]_i}{[A]_o} \exp\frac{zF\,V_{inv}}{RT}$$

This ratio is the measure of the channels selectivity



Common properties of ION CHANNELS: SELECTIVITY



...specie ioniche misurate per i canali del sodio, del potassio e del

*Canale Na^+	
Ione	P_X/P_{Na^+}
Sodio (Na^+)	1,0
Litio (Li^+)	0,93
Tallio (Tl^+)	0,33
Ammonio (NH_4^+)	0,16
Potassio (K^+)	0,086
Rubidio (Rb^+)	<0,012
Cesio (Cs^+)	<0,013
Tetrametilammonio (TMA)	<0,005

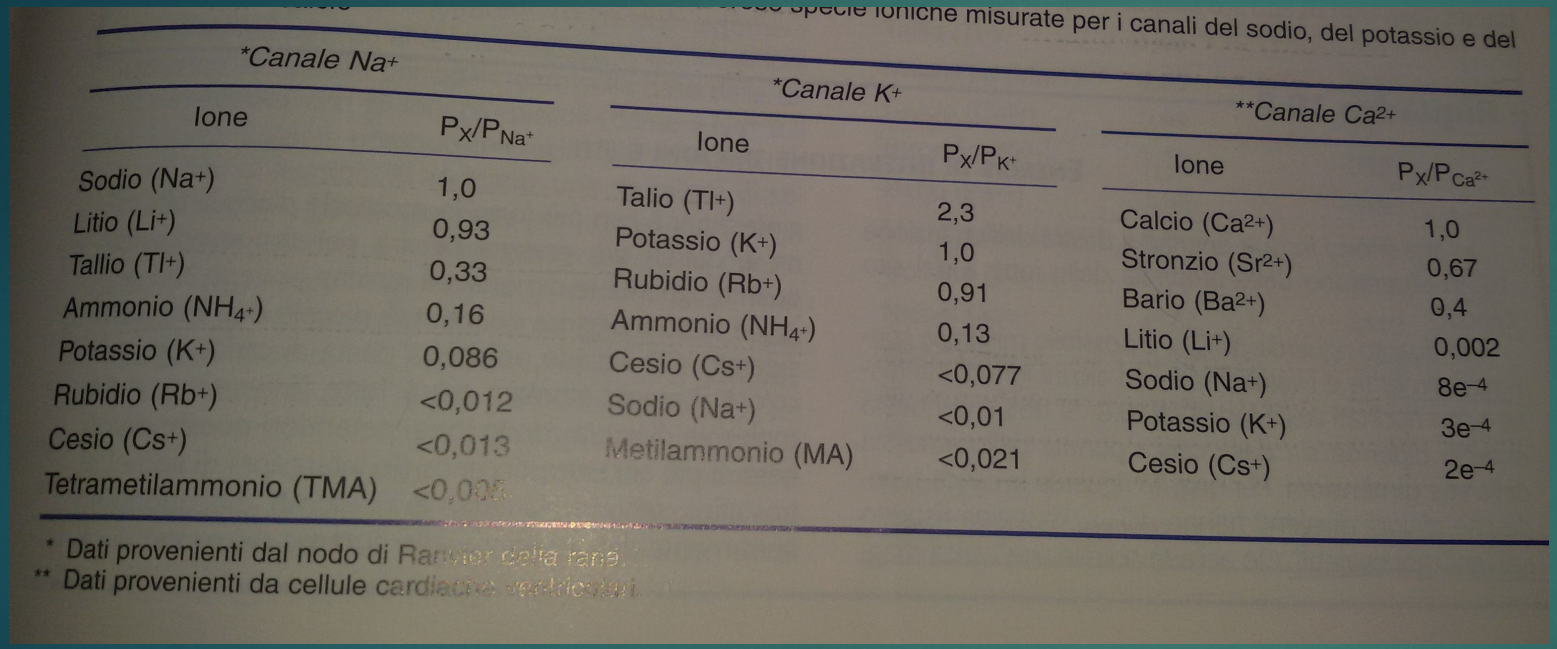
*Canale K^+	
Ione	P_X/P_{K^+}
Talio (Tl^+)	2,3
Potassio (K^+)	1,0
Rubidio (Rb^+)	0,91
Ammonio (NH_4^+)	0,13
Cesio (Cs^+)	<0,077
Sodio (Na^+)	<0,01
Metilammonio (MA)	<0,021

**Canale Ca^{2+}	
Ione	$P_X/P_{Ca^{2+}}$
Calcio (Ca^{2+})	1,0
Stronzio (Sr^{2+})	0,67
Bario (Ba^{2+})	0,4
Litio (Li^+)	0,002
Sodio (Na^+)	$8e^{-4}$
Potassio (K^+)	$3e^{-4}$
Cesio (Cs^+)	$2e^{-4}$

* Dati provenienti dal nodo di Ranvier della rana.
** Dati provenienti da cellule cardiache ventricolari.

Na+ channels can permeate K+ (although in about 12 less than Na+). For these reasons Vinv for Na+ cahnnels is always more negative than expected.

Common properties of ION CHANNELS: SELECTIVITY



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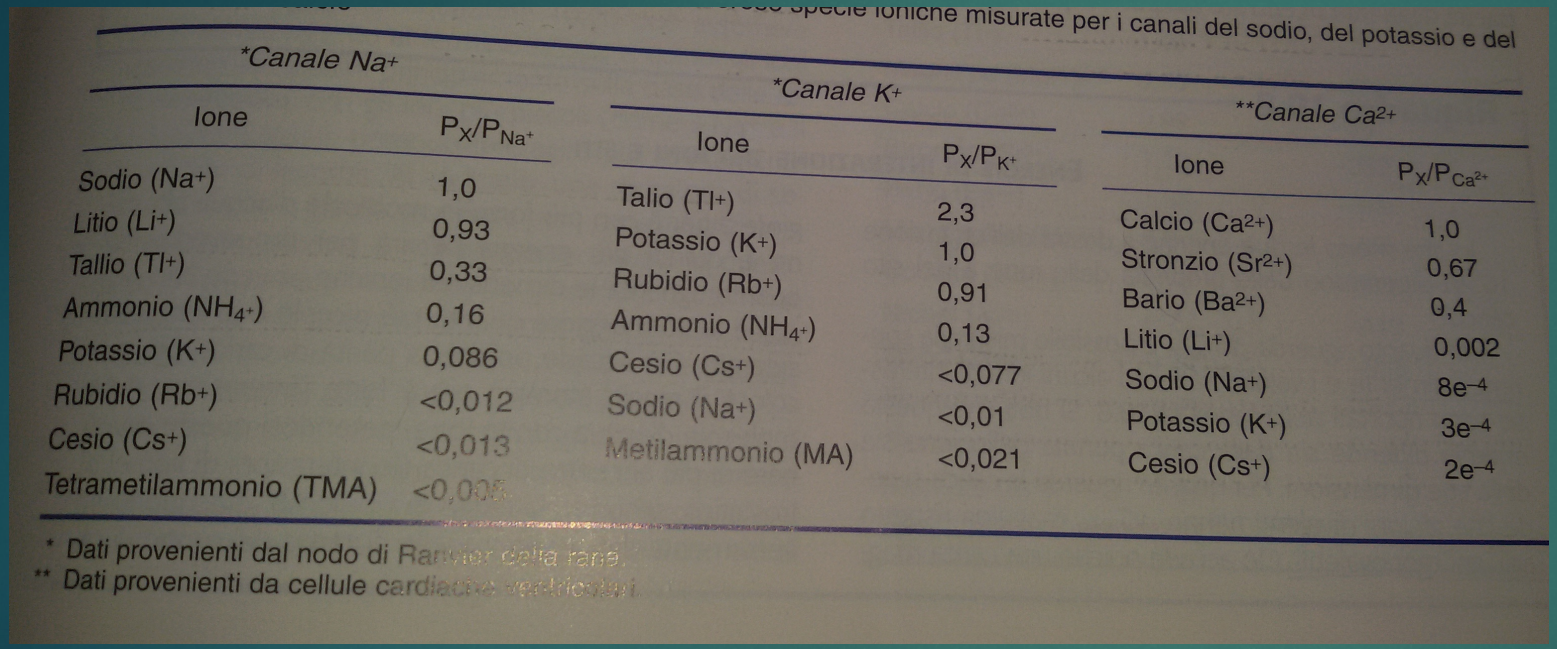
K+ channels are instead more selective and the amount of Na+ permeating the pore is not appreciable.

Common properties of ION CHANNELS: SELECTIVITY



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Ca2+ channels are very selective for divalent ions. This property is physiologically very important since the [Ca2+] is very low as compared with other monovalent ions.

Common properties of ION CHANNELS: SELECTIVITY

The mechanism on which the selectivity of the channels is based is a selectivity filter = inner region within the channel pore that act as a filter.

For K+ channels that are the most selective, this region has a diameter of about 3Å. Only smaller ions can permeate the channel. The selectivity filter for Ca2+ have bigger dimension, around 5Å.

The dimension of the selectivity filter acts therefore as a **MOLECULAR SIEVE**.

Common properties of ION CHANNELS: SELECTIVITY

The **MOLECULAR SIEVE** does not explain all the selectivity features of an ion channels.

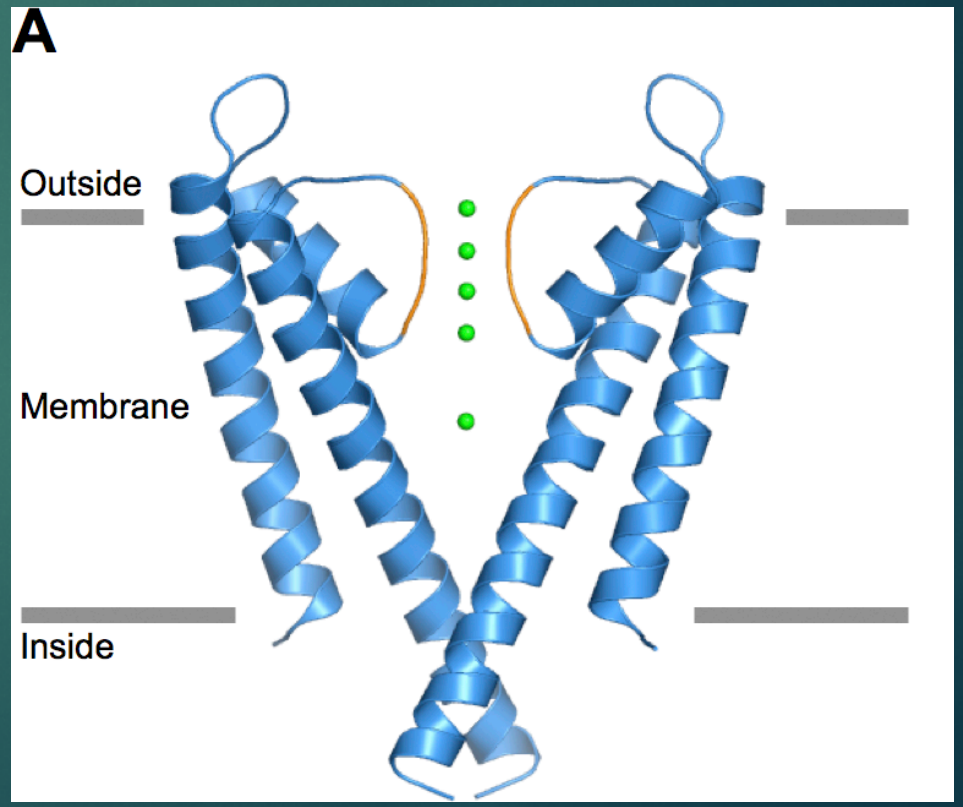
K+ channels selectivity filter

As an example it doesn't explain why Na+ is excluded from K+ channel although its atomic ray is smaller than K+.

K+ channels selectivity filter

Going from inside to outside, the pore consists of a medium wide tunnel (18Å length) leading to a wider spherical inner chamber (10Å diameter). This chamber is lined by side chains of hydrophobic aa. These regions are followed by the SELECTIVITY FILTER (12Å length)

A high ion throughput rate is ensured by the fact that the inner 28Å of the pore (from cytoplasmic entrance to the selectivity filter, lacks polar groups that could delay ion passage by binding or unbinding the ion

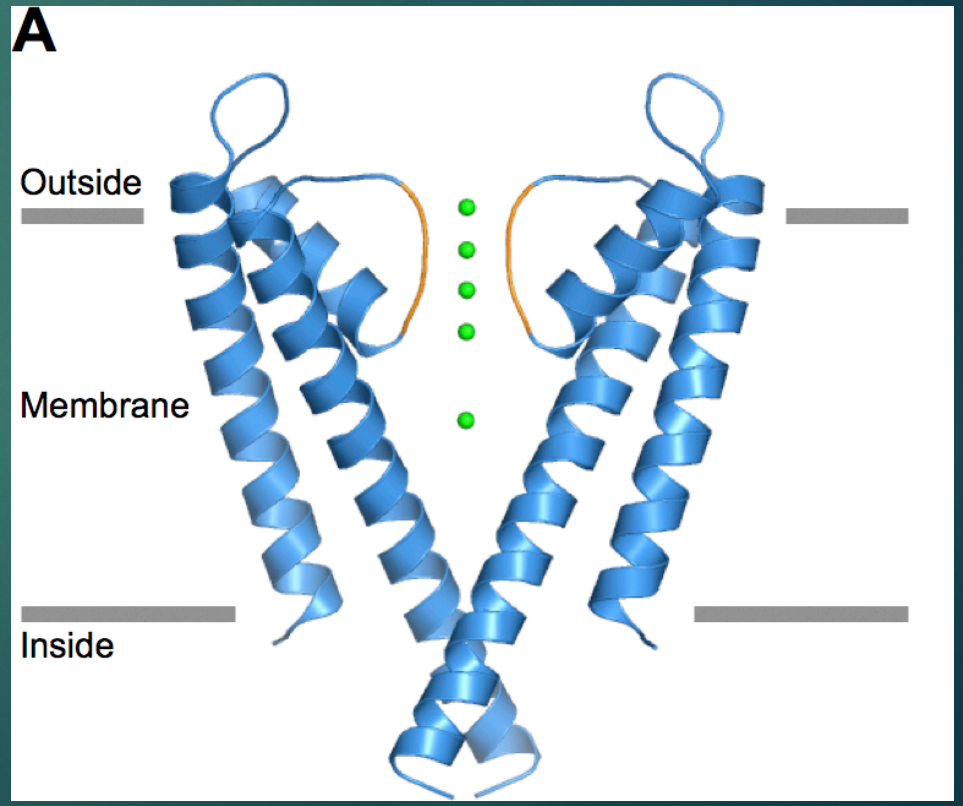


K+ channels selectivity filter

An ion passing from polar solution through the non polar lipid bilayer encounters the least energetically favorable region in the middle of the bilayer. The high energetic cost for K^+ to enter this regions minimized by two details in the channels structure:

1- The inner chamber is filled with water, highly polar environment,

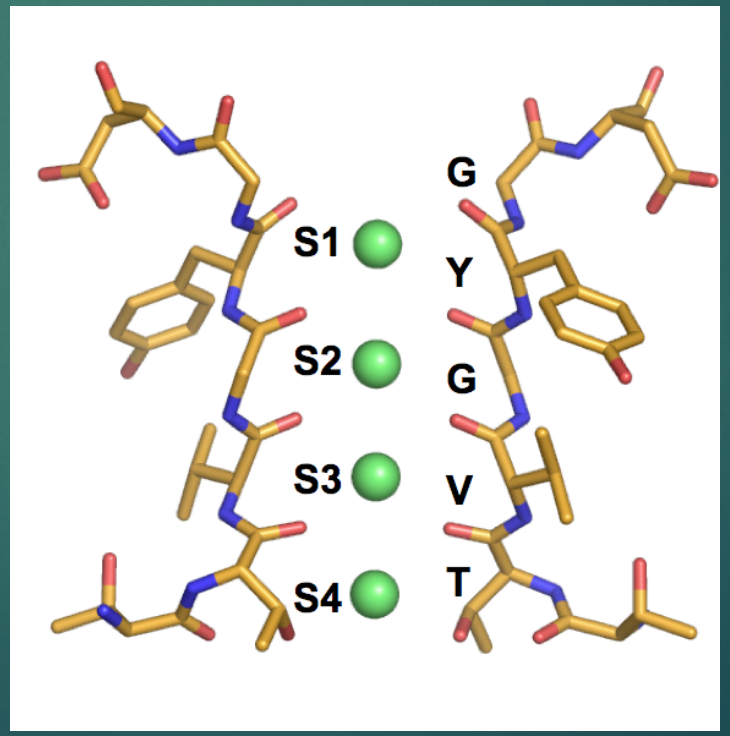
2 - The pore helixes provide a dipole whose electronegative -COOH ends point toward the inner chamber

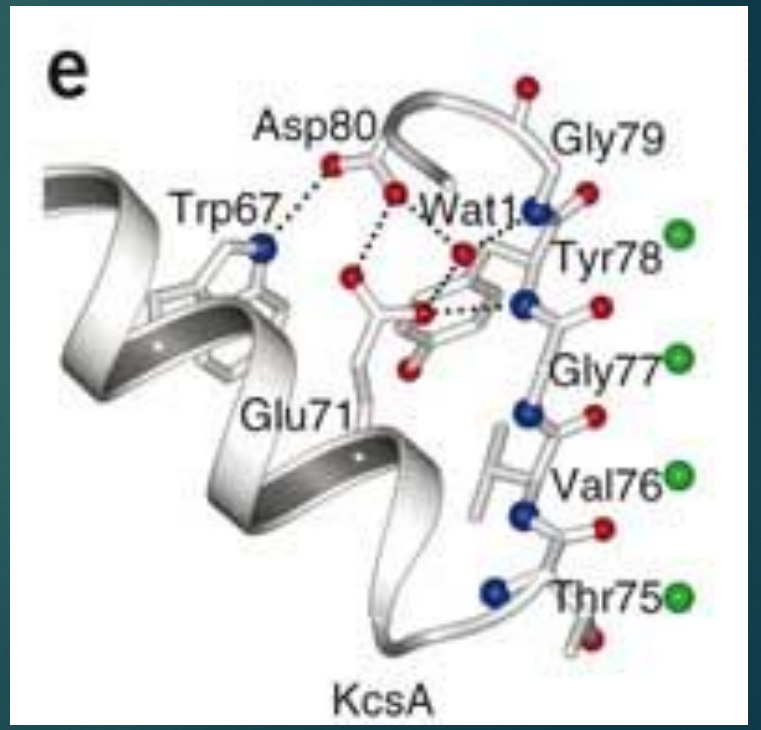


K+ channels selectivity filter

Ions permeate the pore by interacting with 20 oxygen atoms that line the walls of the selectivity filter and form favorable electrostatic interactions with the permeant ion.

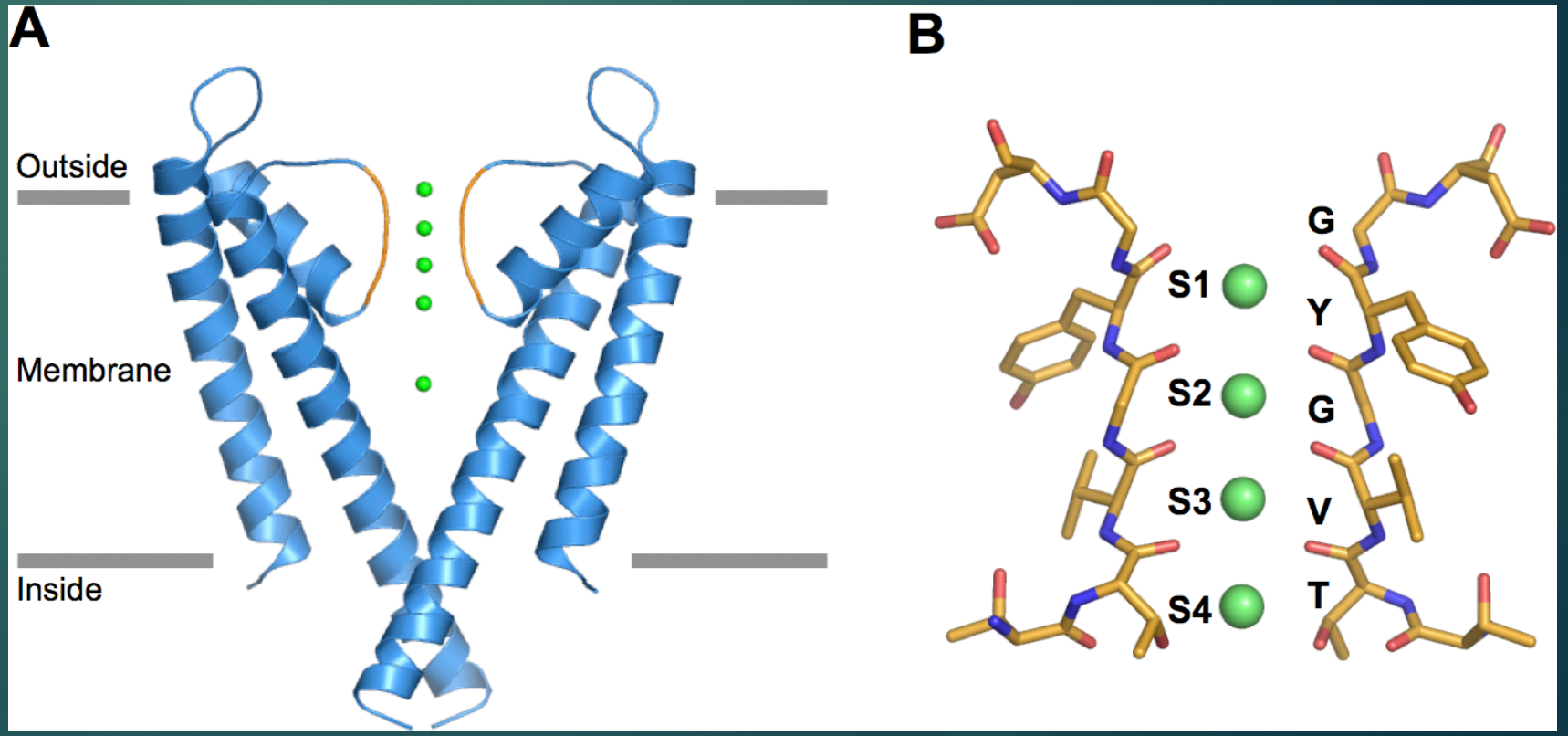
The selectivity filter in **potassium channels** is characterized by a conserved sequence, **TVGYG**.
Each subunit contributes with **4 main chain carboxyl oxygen** atoms from the protein back bone and **1 side-chain hydroxyl oxygen** atom to form to form a total of **4 binding sites for K+ ions**.





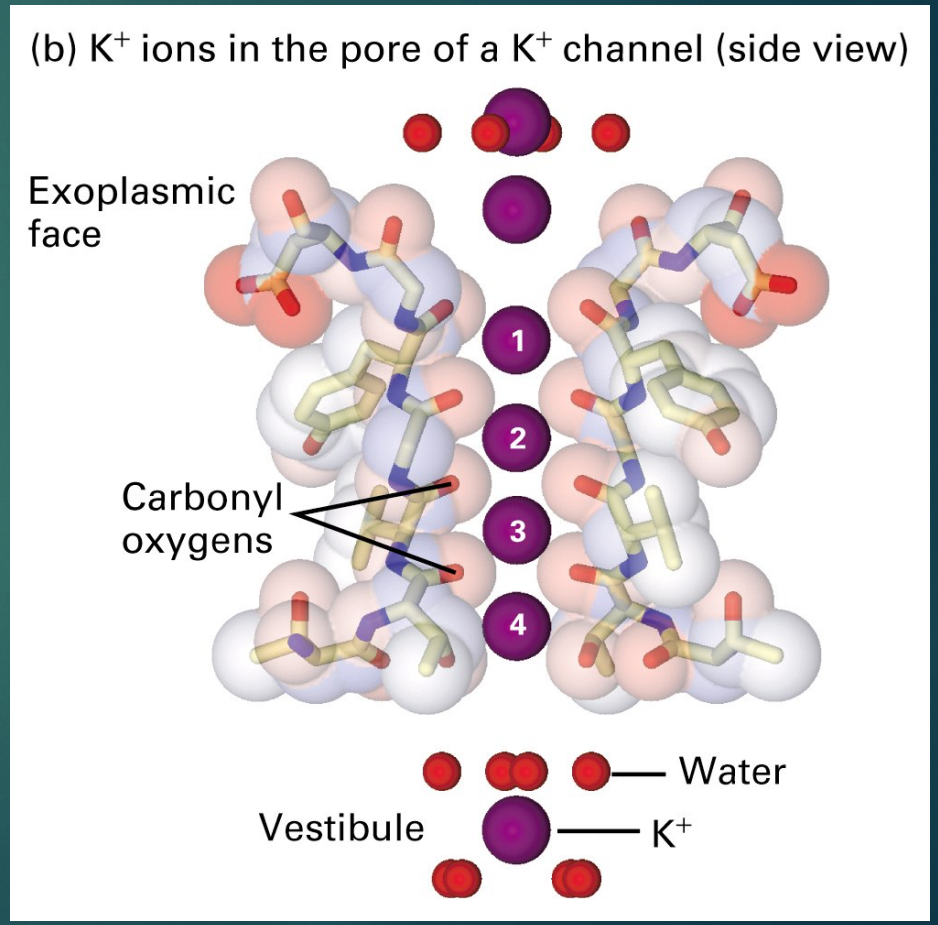
Molecular basis for SELECTIVITY

The selectivity filter in **K+ channels** is characterized by a conserved sequence, TVGYG in each P loop.
The channel present a multiionic nature with 3 K+ simultaneously present in the pore.



K+ channels selectivity filter

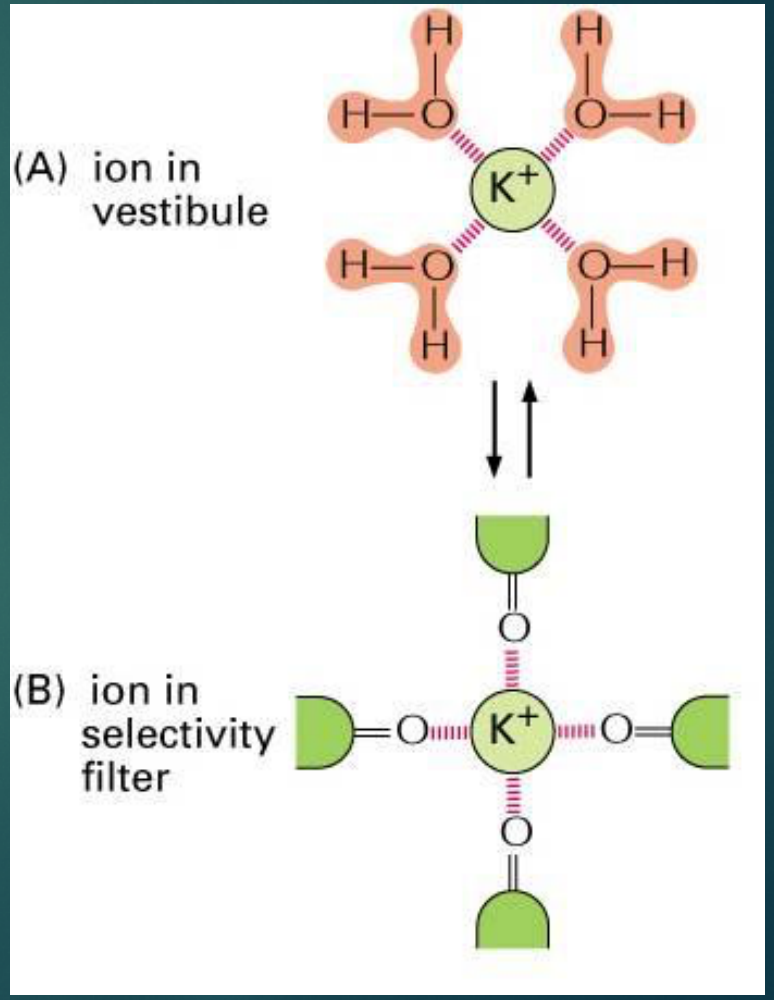
In order to interact the ion will need to be dehydrated so to be free of the molecules of water that form the shell around the ion, due to the narrow dimensions of the filter



K+ channels selectivity filter

In the channel, the residues of the selectivity filter replace water molecules with polar oxygen atoms

The energy involved in these two processes (interaction with charges and dehydration) will change depending on the ion and on the channel structure and will therefore explain the selectivity. The more negative is the difference in energy involved in these processes (ΔG_{total}), the more the channel will be selective for the considered ion



K+ channels selectivity filter

$\Delta G_{total} = \Delta G_{binding} - \Delta G_{dehydration}$

$\Delta G_{binding}$ = energy gained by the system during the binding ion-charged site on the pore

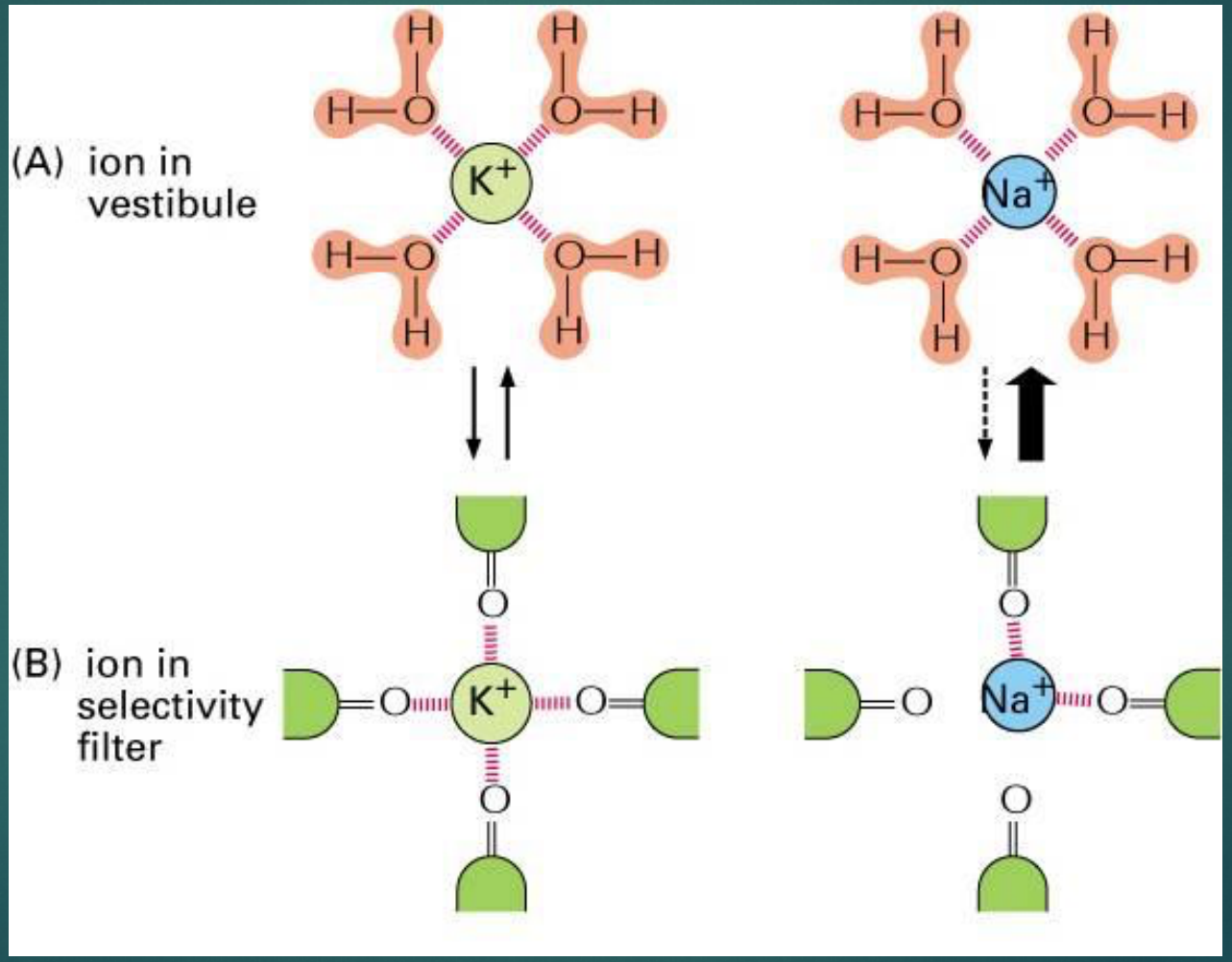
$\Delta G_{dehydration}$ = energy lost in removing water shell

Sodium, is a smaller atom, but has a **higher energy of hydration**, i.e it is harder to pull the waters out of sodium:

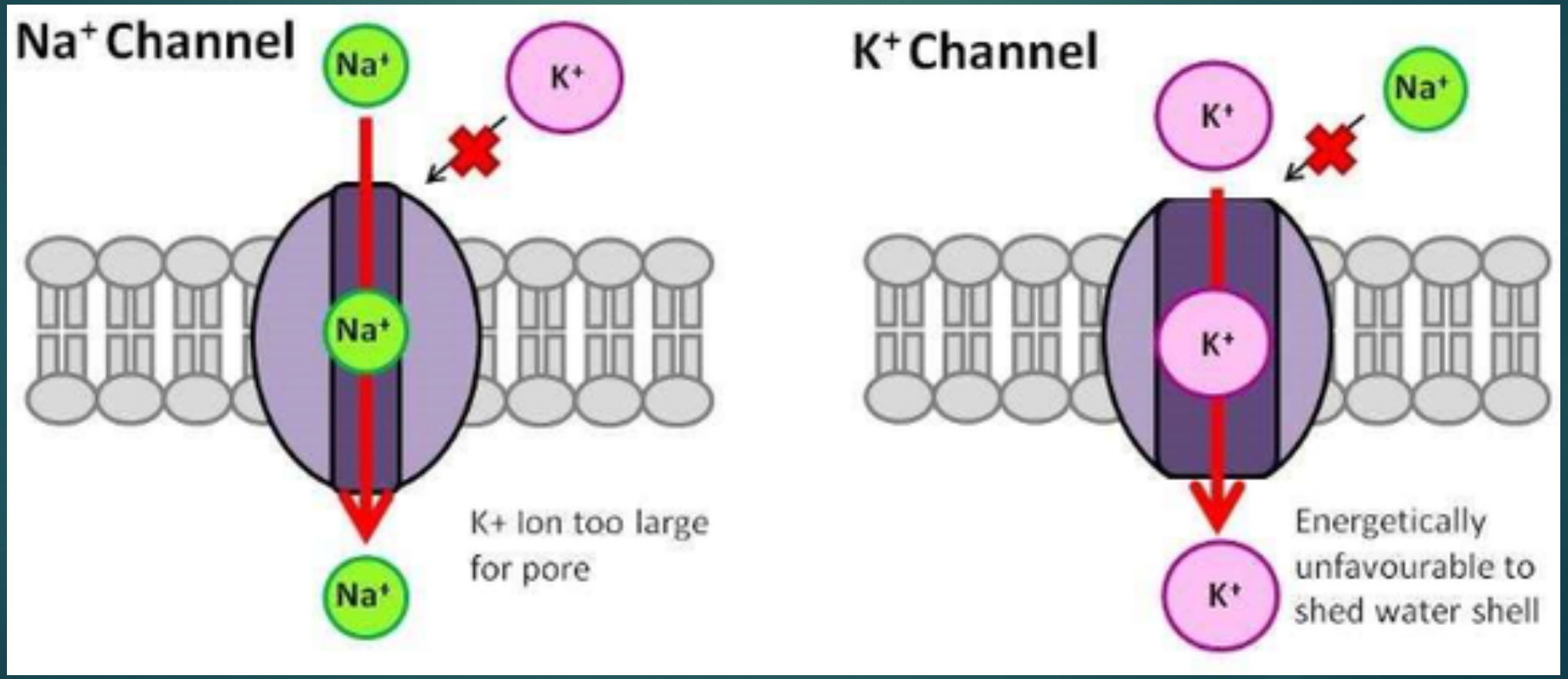
Ion	Atomic Radius	Hydration Energy (Kcal/mol)
Na+	0.95	-105
K+	1.33	-85

Thus the channel is lined by carbonyl atoms of the selectivity filter, and it is geometrically constrained so that only a dehydrated K+ fits with appropriate coordination but the Na+ is too small. Also, Na+ binding is thermodynamically unfavorable.

K+ channels selectivity filter



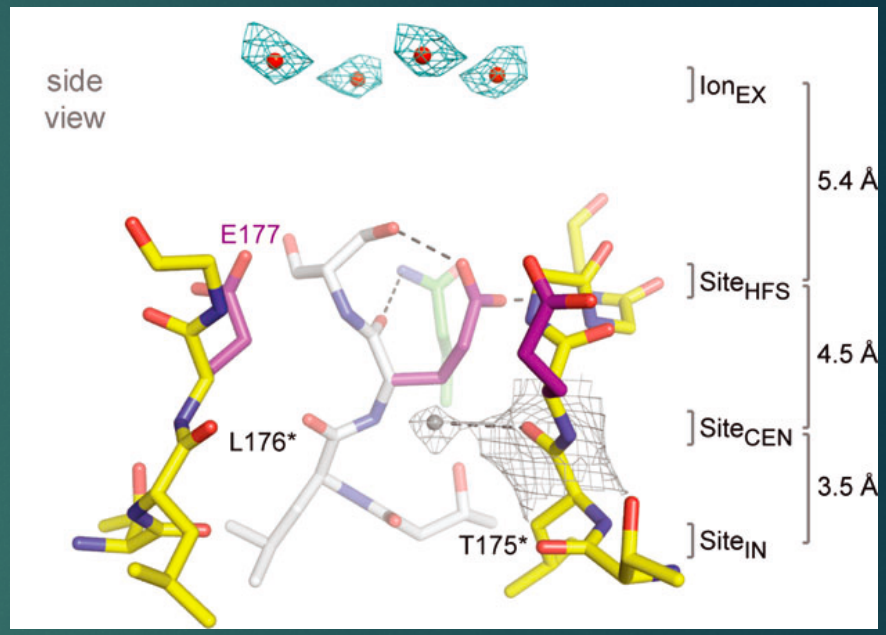
Common properties of ION CHANNELS: SELECTIVITY



Na+ channels selectivity filter

In contrast to K+ channels, the **NavAb ion selectivity filter** has a high-field-strength site at its extracellular end, formed by the side chains of four glutamate residues, which are highly conserved and are key determinants of ion selectivity in vertebrate sodium and calcium channels.

Considering its dimensions of approximately 4.6 °A square, **Na+ with two planar waters of hydration could fit in this high-field-strength sit**e.

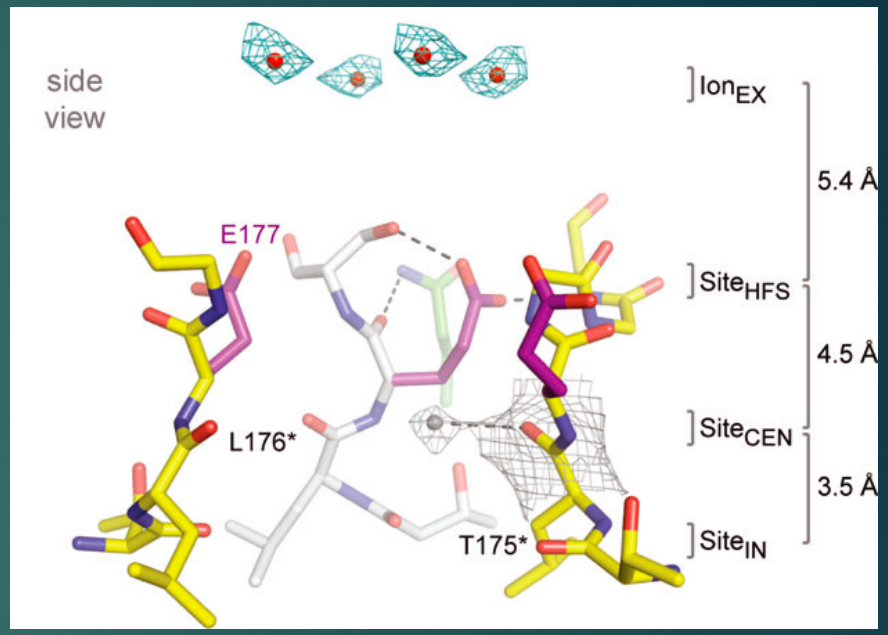


Na+ channels selectivity filter

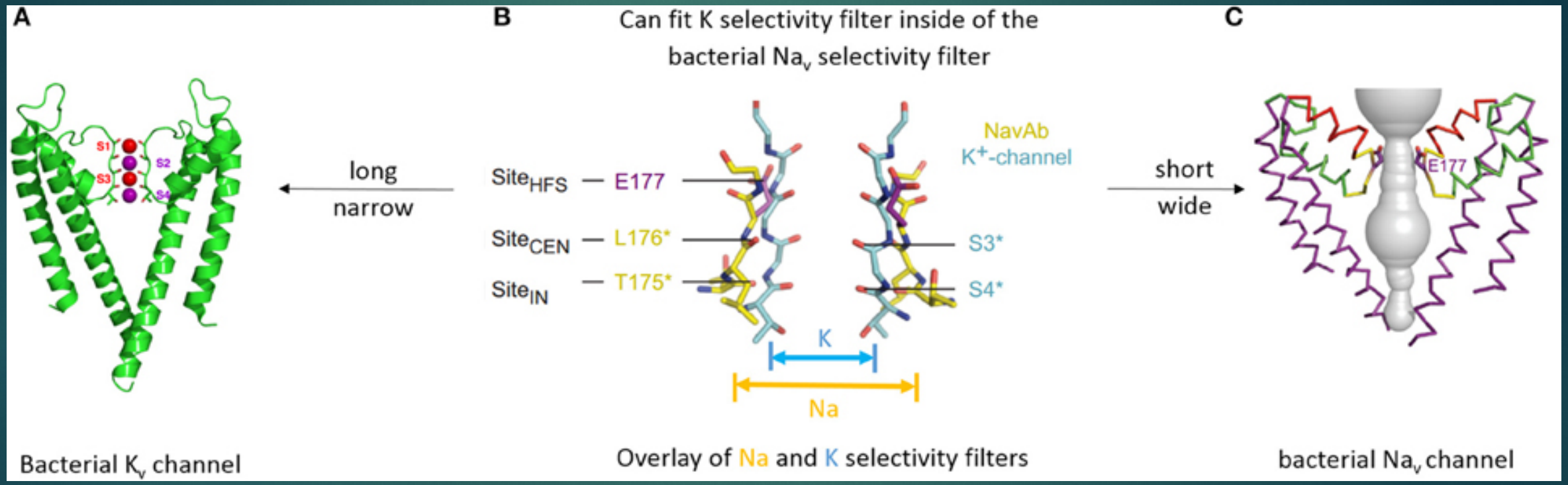
This outer site is followed by two ion coordination sites formed by backbone carbonyls.

These two carbonyl sites are perfectly designed to bind Na+ with four planar waters of hydration but would be much too large to bind Na+ directly. In fact, the NavAb selectivity filter is large enough to fit the entire potassium channel ion selectivity filter inside it.

Thus, the chemistry of **Na+ selectivity and conductance is opposite to that of K+:** negatively charged residues interact with Na+ to remove most (but not all) of its waters of hydration, **and Na+ is conducted as a hydrated ion interacting with the pore through its inner shell of bound waters.**



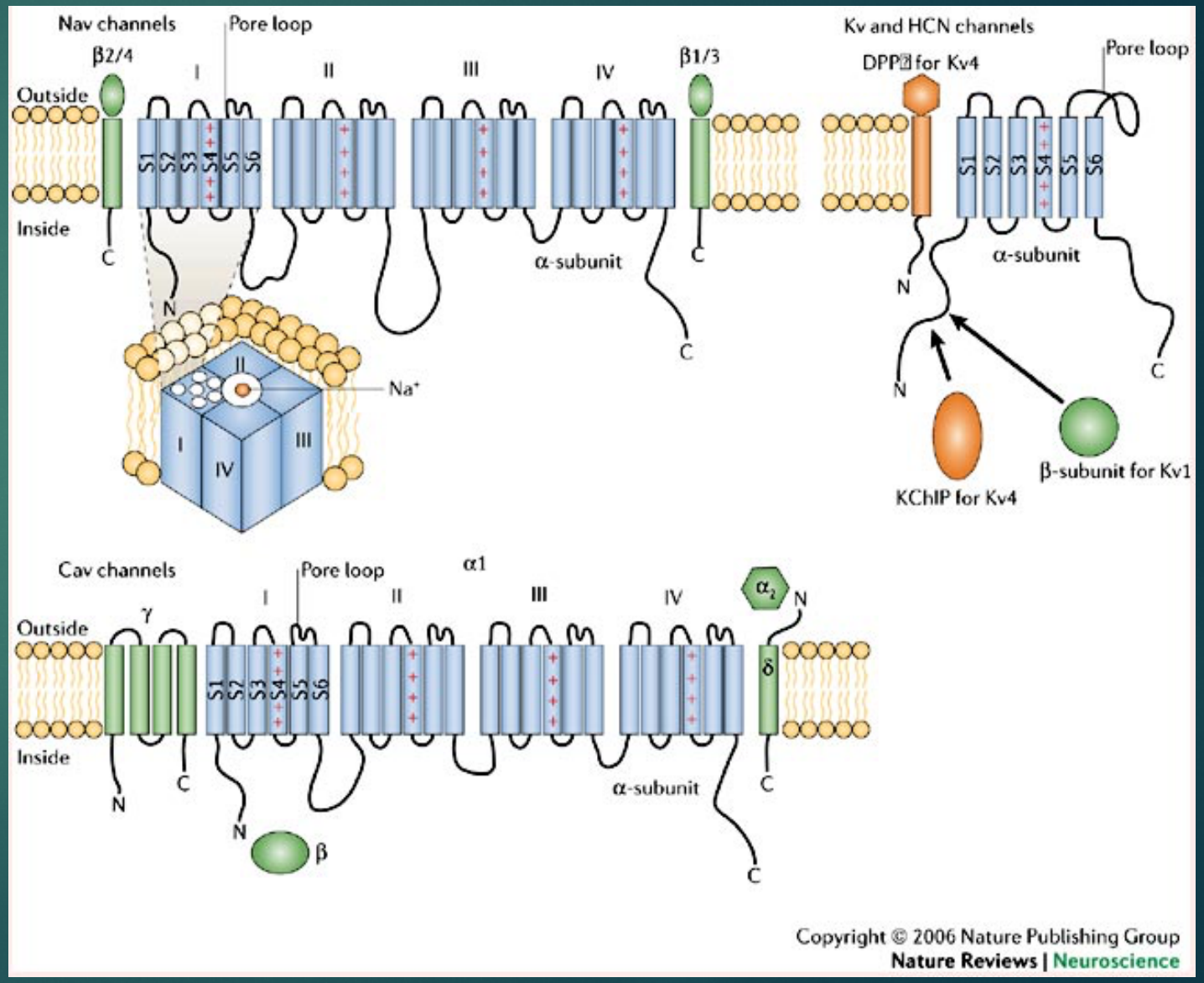
Molecular basis for SELECTIVITY



Molecular basis for SELECTIVITY

The "P loop" connecting S5 and S6 represent the region involved in the permeability and selectivity of Voltage-gated ion channels. The P loops forms a U facing the inner part of the pore.

The pore is made by 4 P loops form different subunits (K+ channels) or different domains (Na+ or Ca2+ channels)

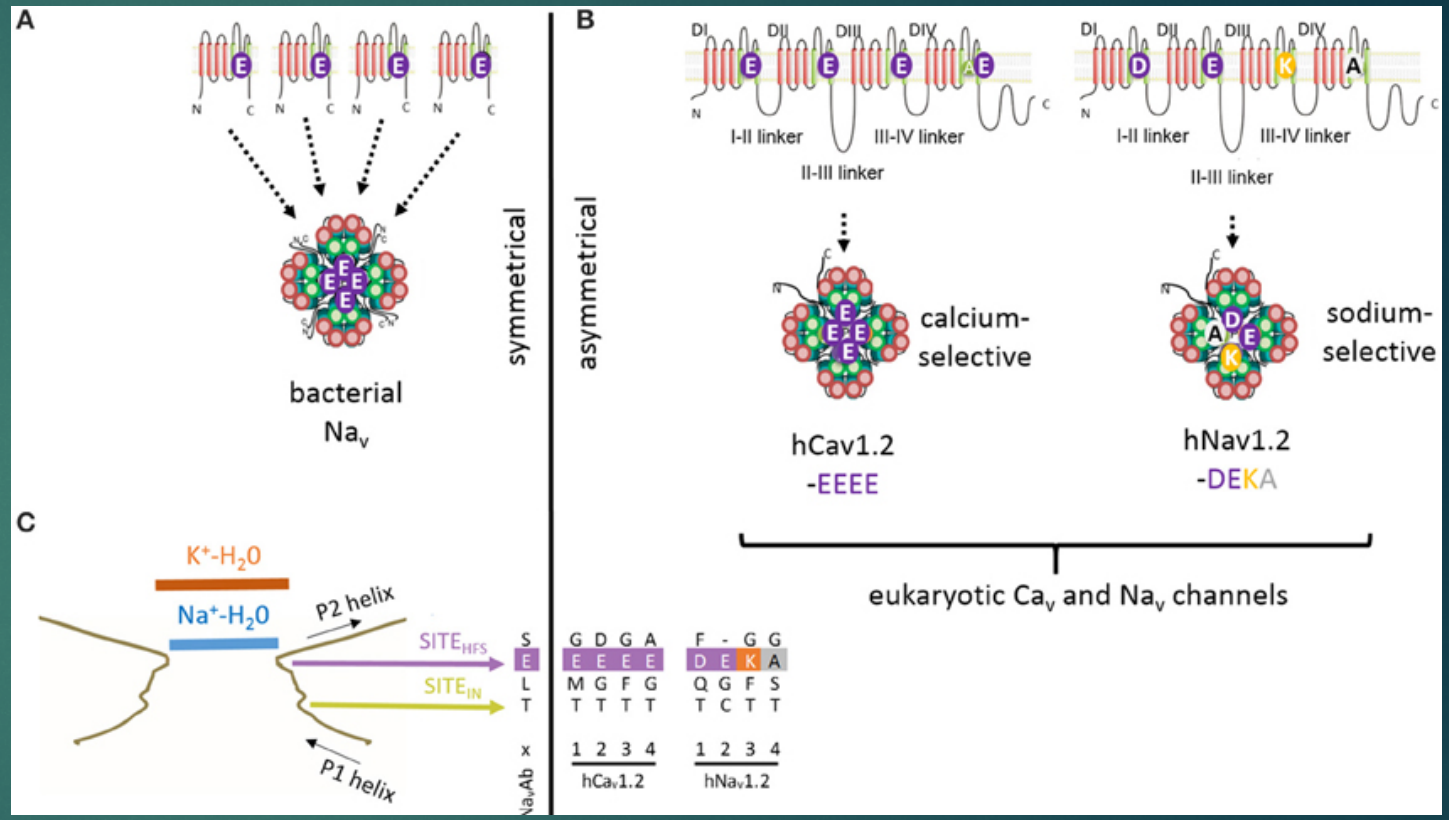


Molecular basis for SELECTIVITY

In **Na+ channels** The filter is due to the **DEKA locus** = presence in each P loop of Asp(D), Glu (E), Lys (K) and Ala (A) highly conserved

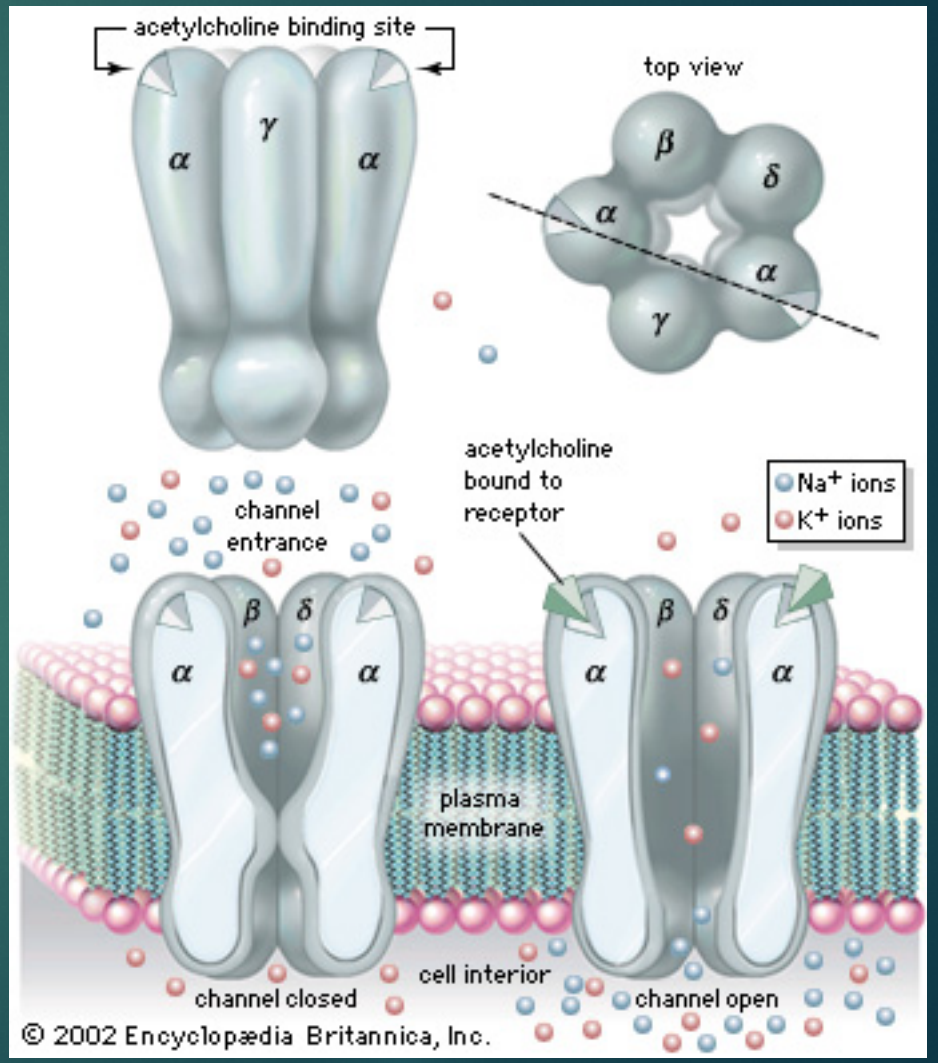
In **Ca+ channels** The filter is due to the **EEEE locus** = presence in each P loop of Glu (E) highly conserved

Mutations in DEKA locus with E confers the Na+ channels permeability features of Ca2+ channels



Molecular basis for SELECTIVITY

For ligand-gated ion channels the situation could be different
Nicotinic Receptor for Ach is formed by 5 subunits (2α, β, γ, δ).



Molecular basis for SELECTIVITY

Each subunit present a huge extracellular N-term and 4 a helics (M1-M4).
M2 is the helics that delimites the pore.
M2 in a and b subunits presents negative charges repeated 3 times forming therefore 3 rings