Cellular and Molecular Biophysics

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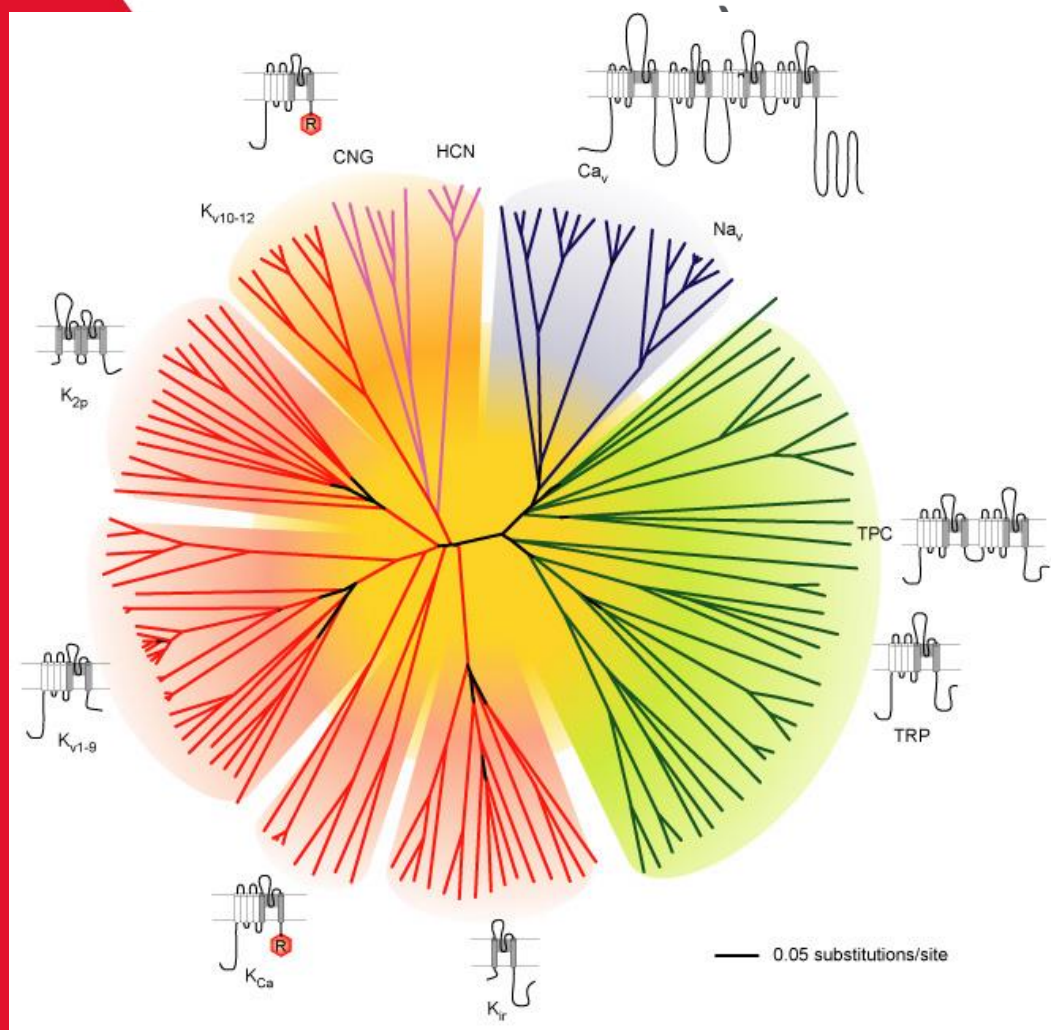
CFU 5 LM Biotecnologie Industriali- 6 LM Fisica - A.A. 2024/25

Corso di laurea in LM Biotecnologie Industriali- LM Fisica

Department of
Life Sciences
and Systems Biology

Ion Channels

STRUCTURE AND FUNCTION: SELECTIVITY



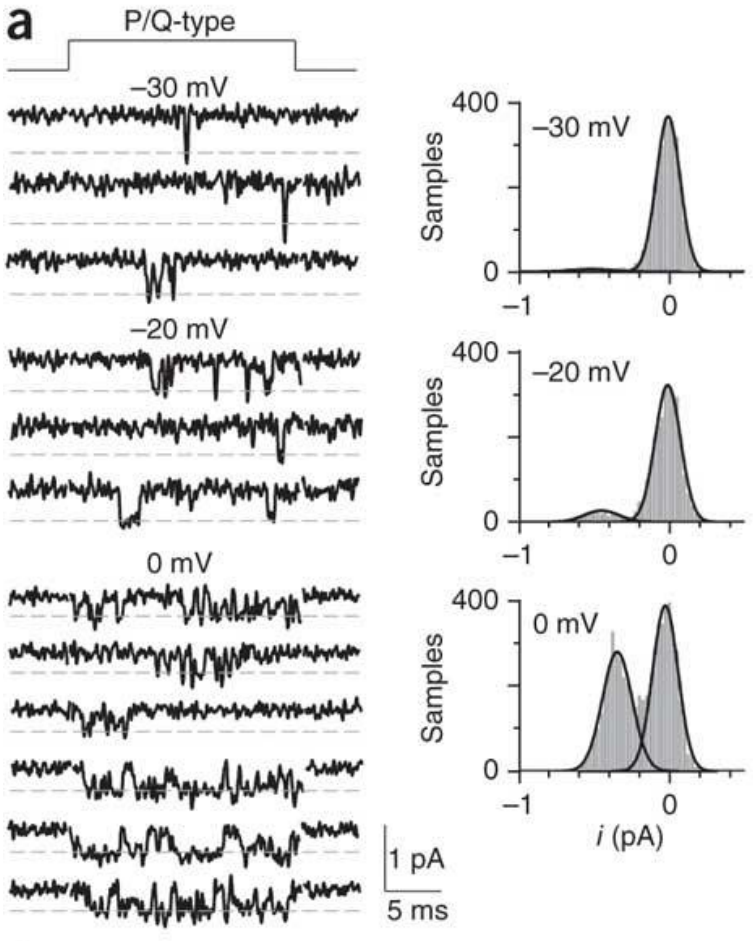
Common properties of ION CHANNELS:

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

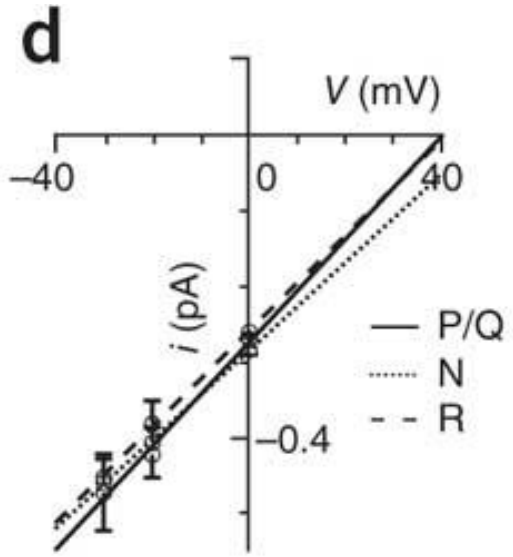
Channels can be therefore classified by the selectivity properties.

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

Common properties of ION CHANNELS: PERMEABILITY



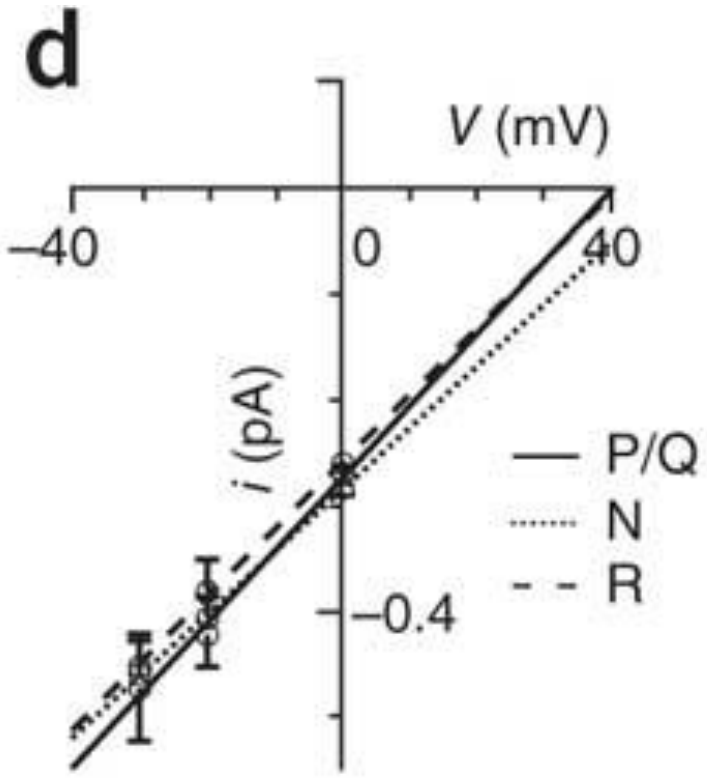
The idea of how permeable is the channel to ions can be obtained registering the current in the presence of the ion of interest at both sides at different Vm.



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

In this case is linear as expected for Ohmic conductor. This is not always the case.

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

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

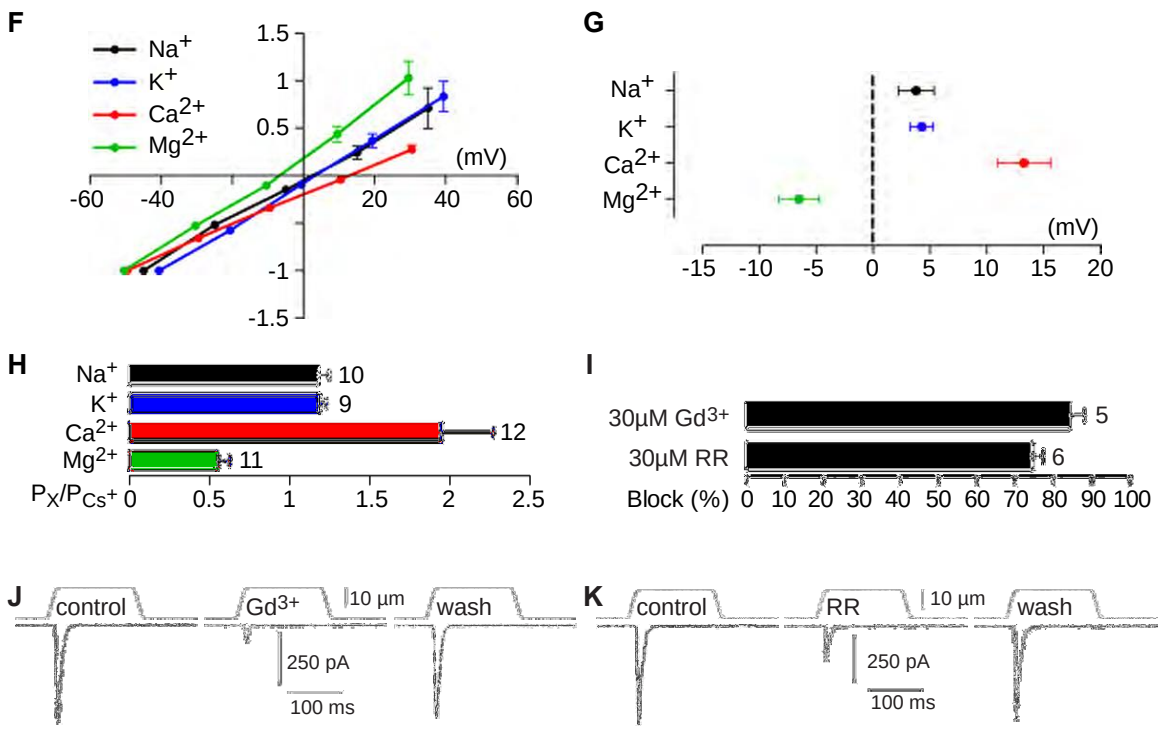
- **SELECTIVITY**: channel ability to select ion species that flows.

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.



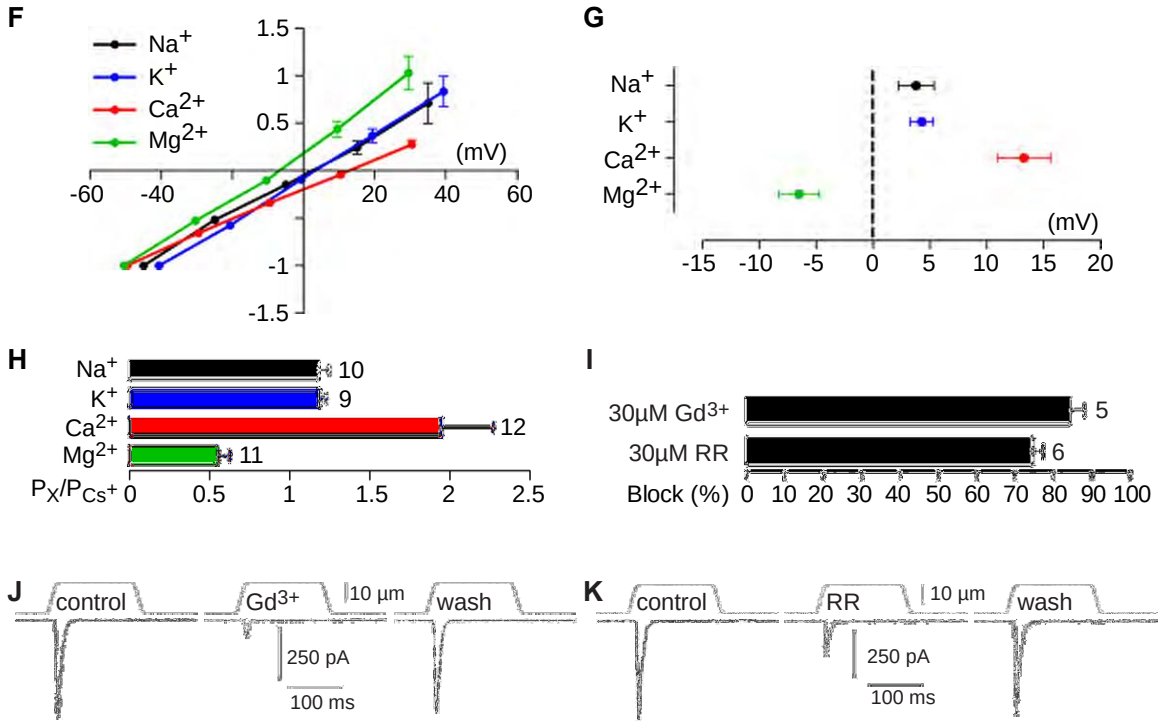
Common properties of ION CHANNELS: SELECTIVITY

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:

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

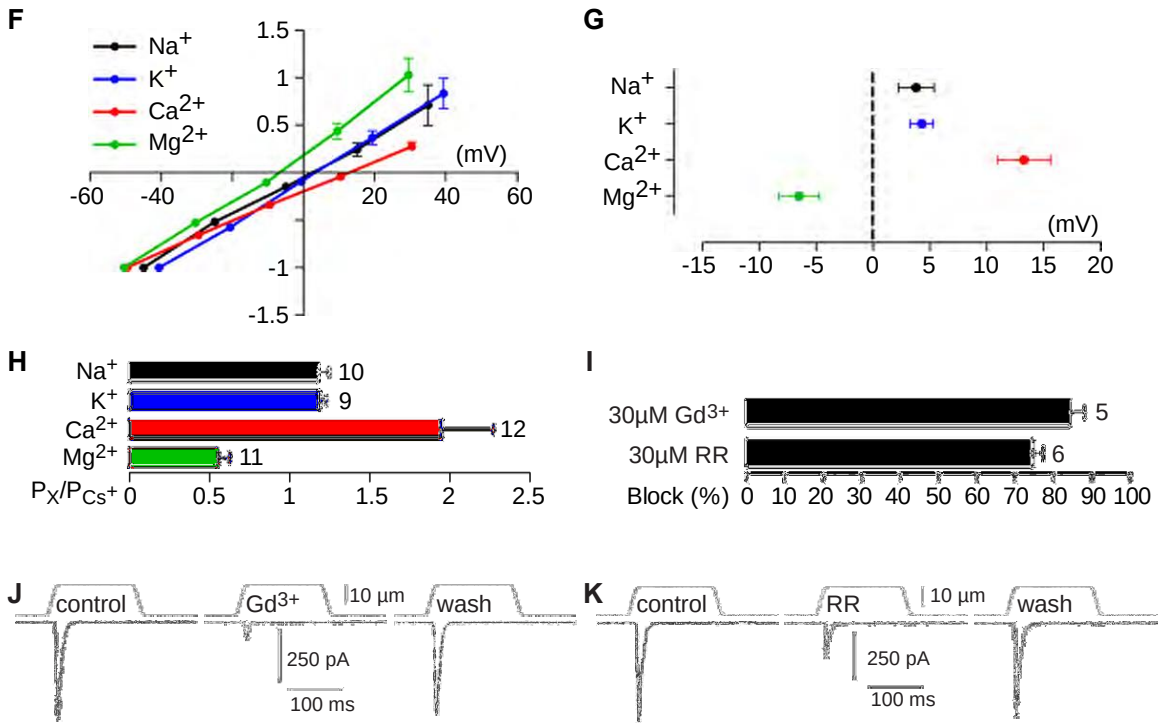


Common properties of ION CHANNELS: SELECTIVITY

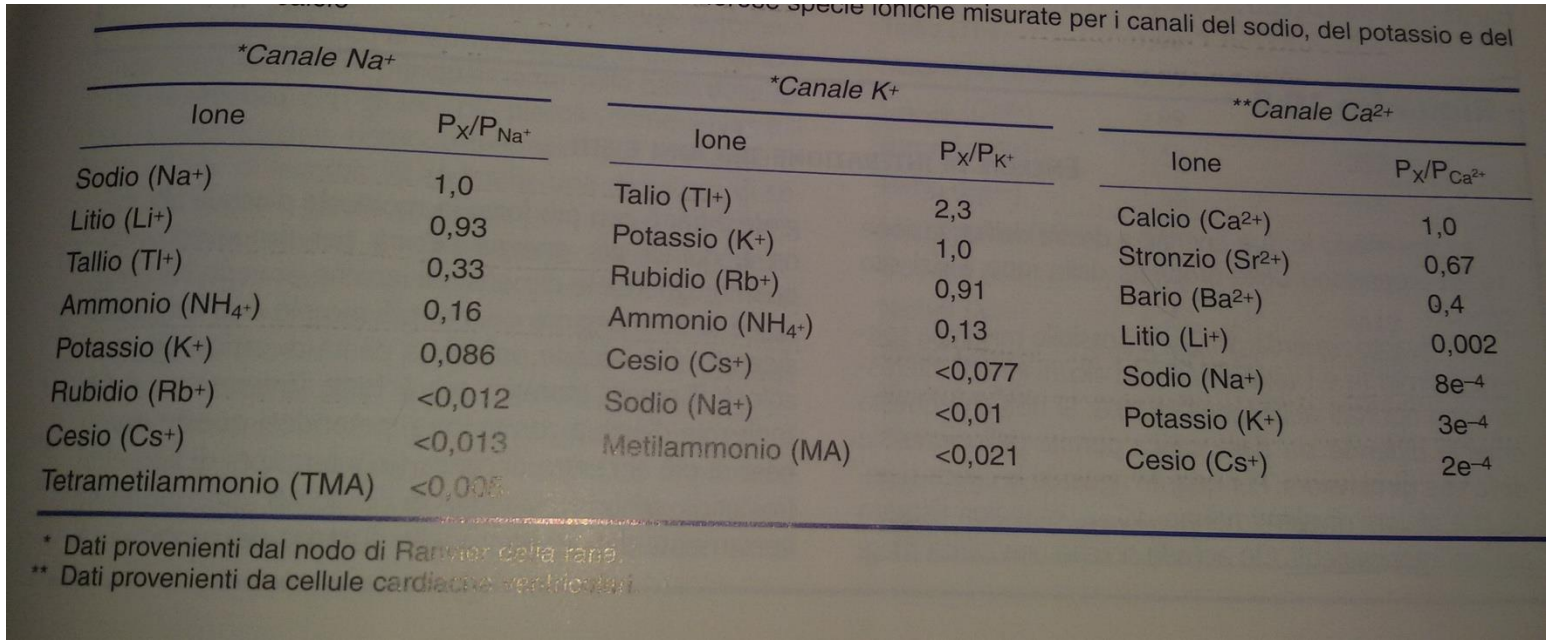
The relation between PA/PB can be obtained by measuring the Vinv (V at which I = 0) of the I-V obtained with A extracellular and B intracellular at equal concentrations :

$$\frac{P_A}{P_B} = \frac{[B]_i}{[A]_e}\, e^{\frac{zFV_{inv}}{RT}}$$

This ratio is the measure of the channels selectivity



Common properties of ION CHANNELS: SELECTIVITY



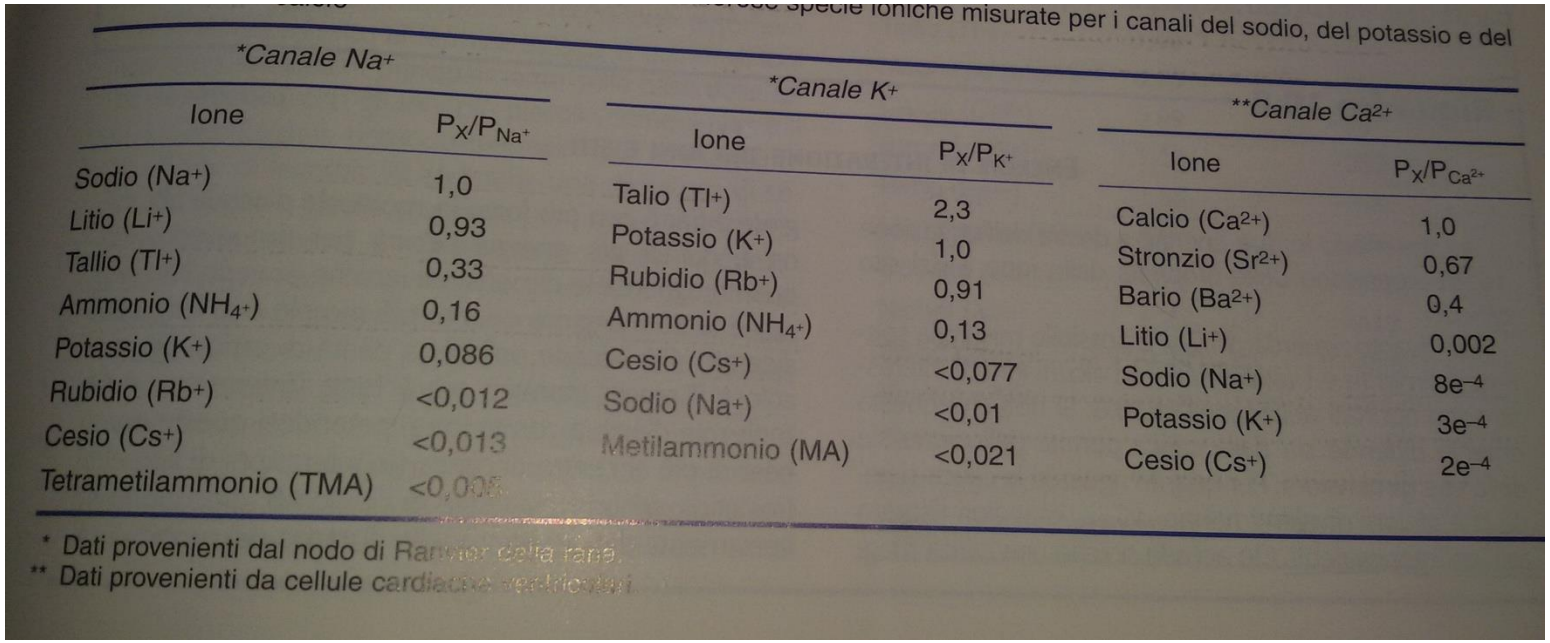
...specie ioniche misurate per i canali del sodio, del potassio e del

*Canale Na^+		*Canale K^+		**Canale Ca^{2+}	
Ione	P_X/P_{Na^+}	Ione	P_X/P_{K^+}	Ione	$P_X/P_{Ca^{2+}}$
Sodio (Na^+)	1,0	Talio (Tl^+)	2,3	Calcio (Ca^{2+})	1,0
Litio (Li^+)	0,93	Potassio (K^+)	1,0	Stronzio (Sr^{2+})	0,67
Tallio (Tl^+)	0,33	Rubidio (Rb^+)	0,91	Bario (Ba^{2+})	0,4
Ammonio (NH_4^+)	0,16	Ammonio (NH_4^+)	0,13	Litio (Li^+)	0,002
Potassio (K^+)	0,086	Cesio (Cs^+)	<0,077	Sodio (Na^+)	$8e^{-4}$
Rubidio (Rb^+)	<0,012	Sodio (Na^+)	<0,01	Potassio (K^+)	$3e^{-4}$
Cesio (Cs^+)	<0,013	Metilammonio (MA)	<0,021	Cesio (Cs^+)	$2e^{-4}$
Tetrametilammonio (TMA)	<0,00...				

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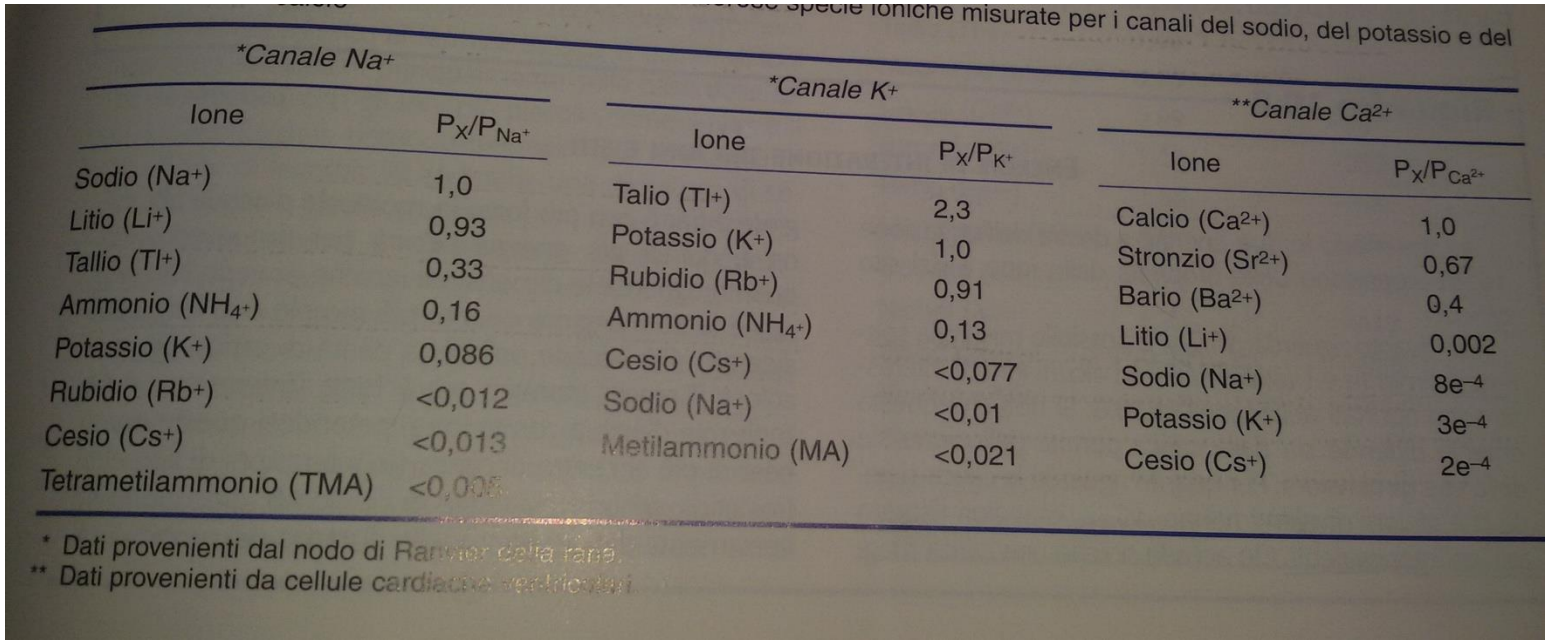
K+ channels are instead more selective and the amount of Na+ permeating the pore is not appreciable.

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.

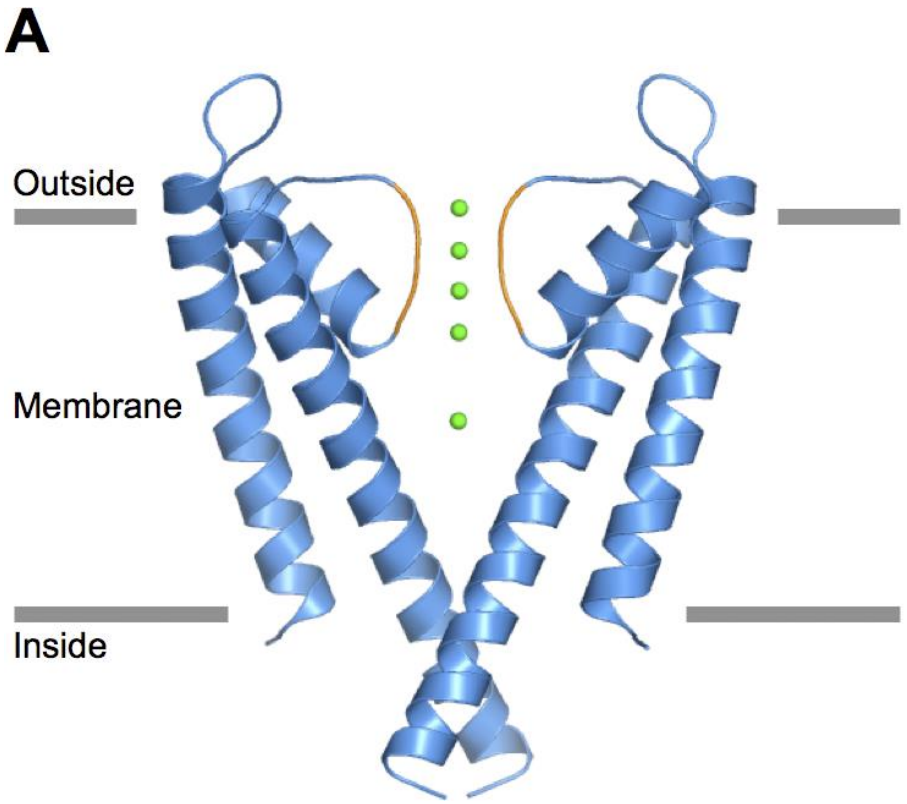
K+ channels selectivity filter

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

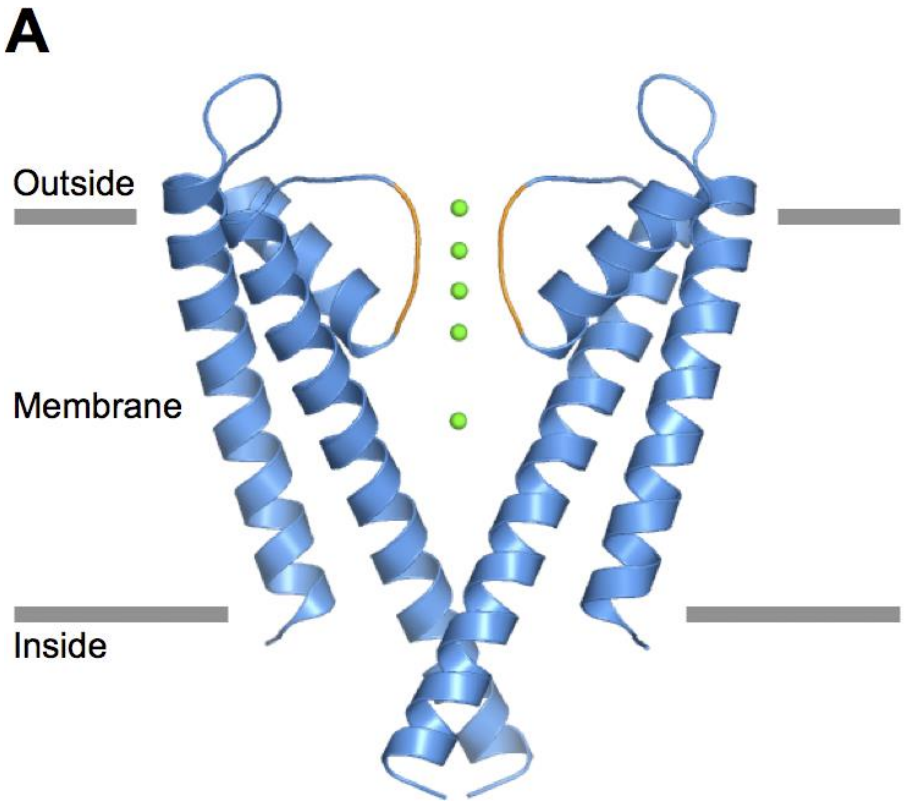


K+ channels selectivity filter

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,

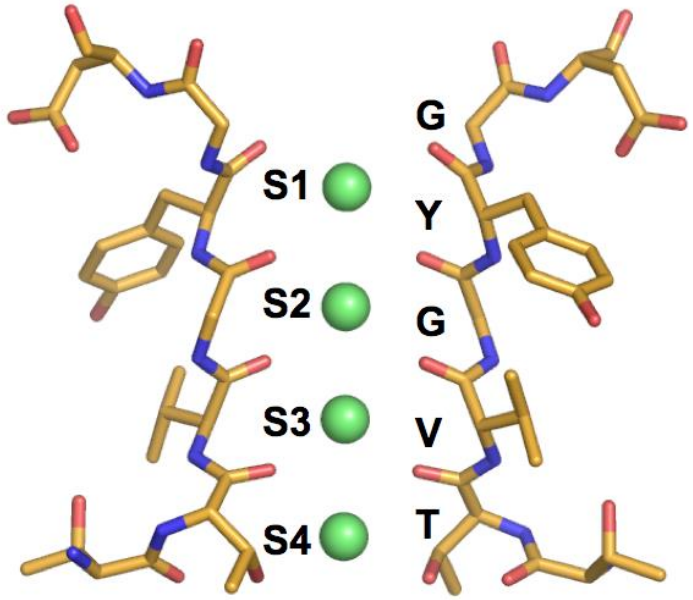
2 - The pore helixes provide a dipole whose electronegative -COOH ends point toward the inner chamber

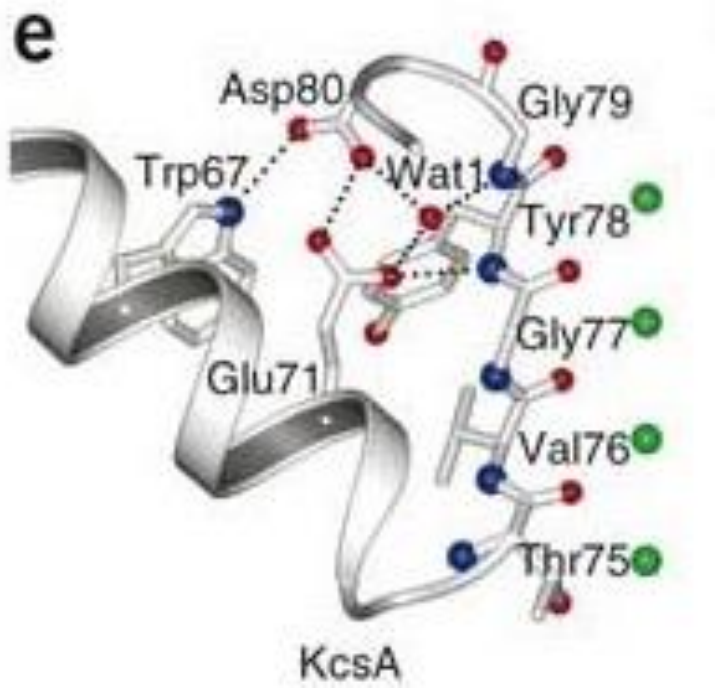


K+ channels selectivity filter

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 a total of **4 binding sites for K+ ions**.

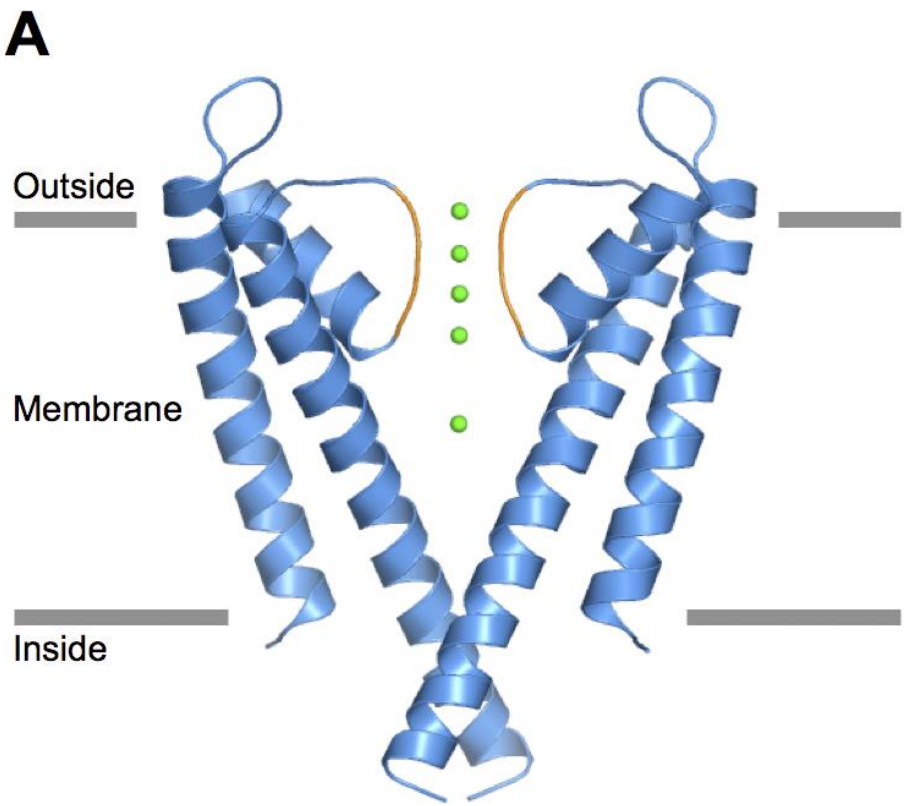


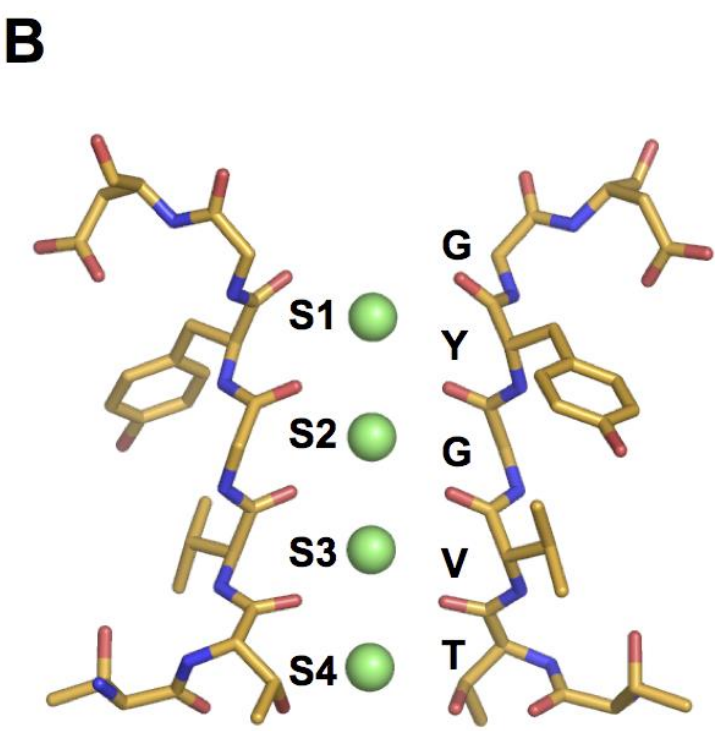


Molecular basis for SELECTIVITY

The selectivity filter in **K+ channels** is characterized by a conserved sequence, TVGYG in each P loop.

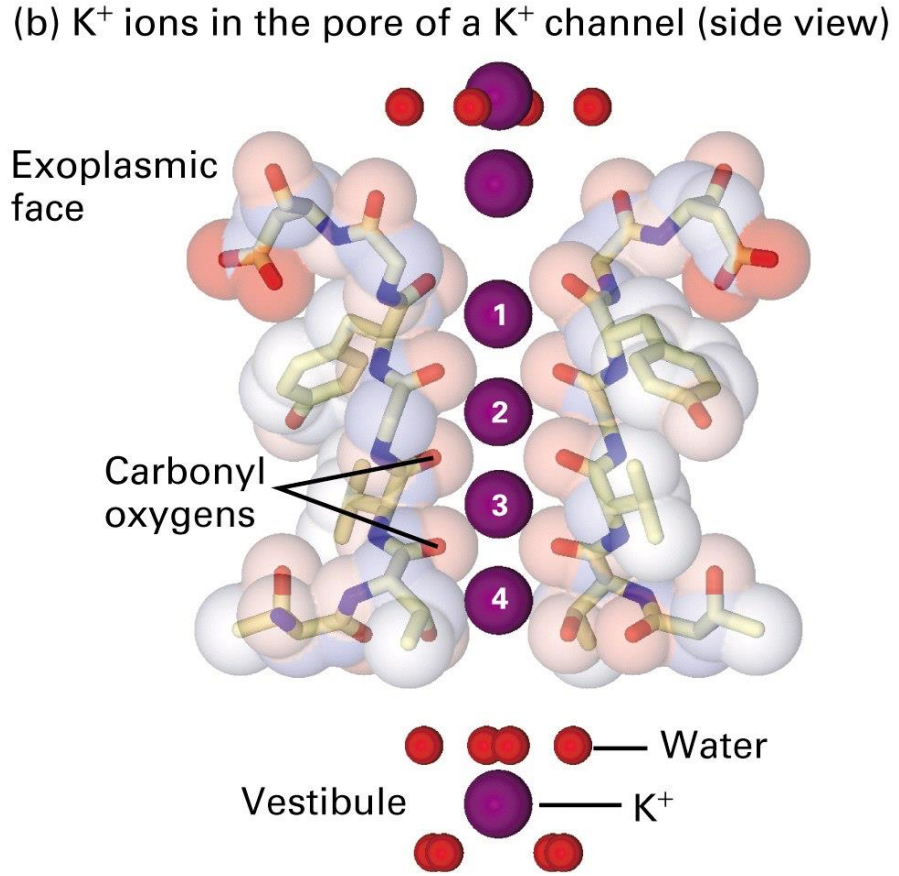
The channel present a multiionic nature with 3 K+ simultaneously present in the pore.





K+ channels selectivity filter

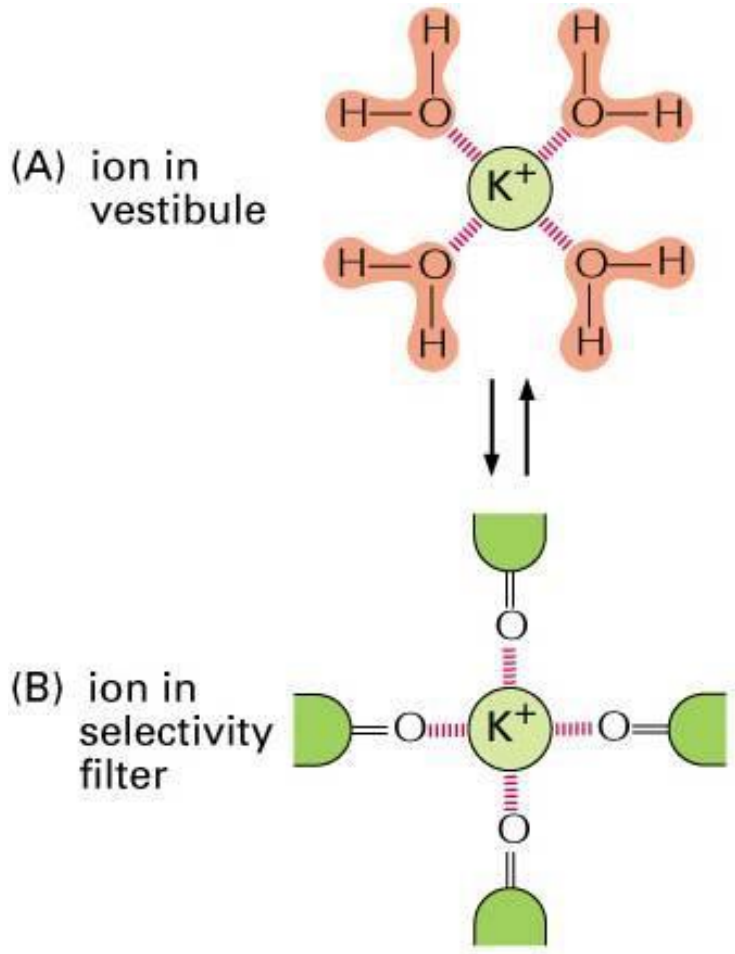
In order to interact the ion will need to be dehydrated so to be free of the molecules of water that form the shell around the ion, due to the narrow dimensions of the filter



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

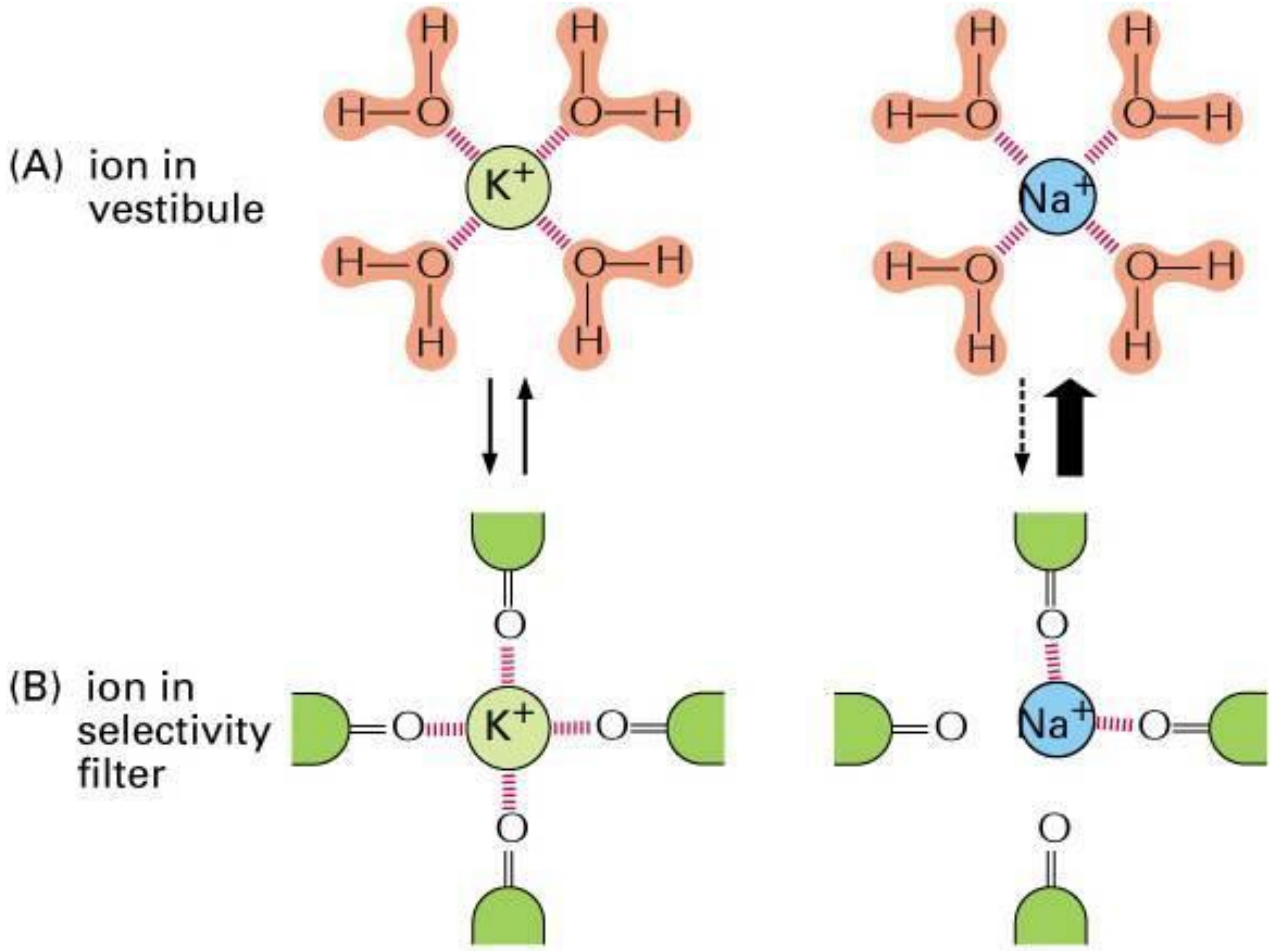
$\Delta G_{dehydration}$ = energy lost in removing water shell

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

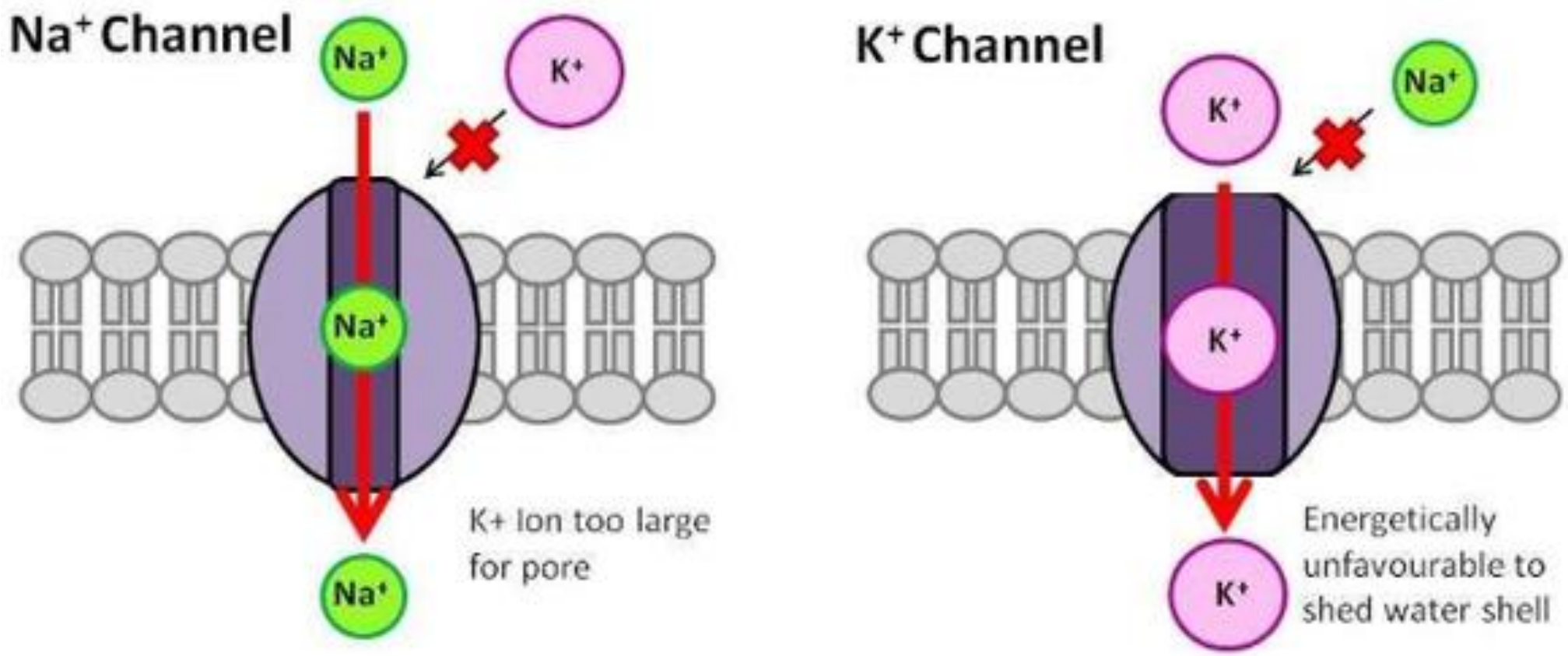
Ion	Atomic Radius	Hydration Energy (Kcal/mol)
Na+	0.95	-105
K+	1.33	-85

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



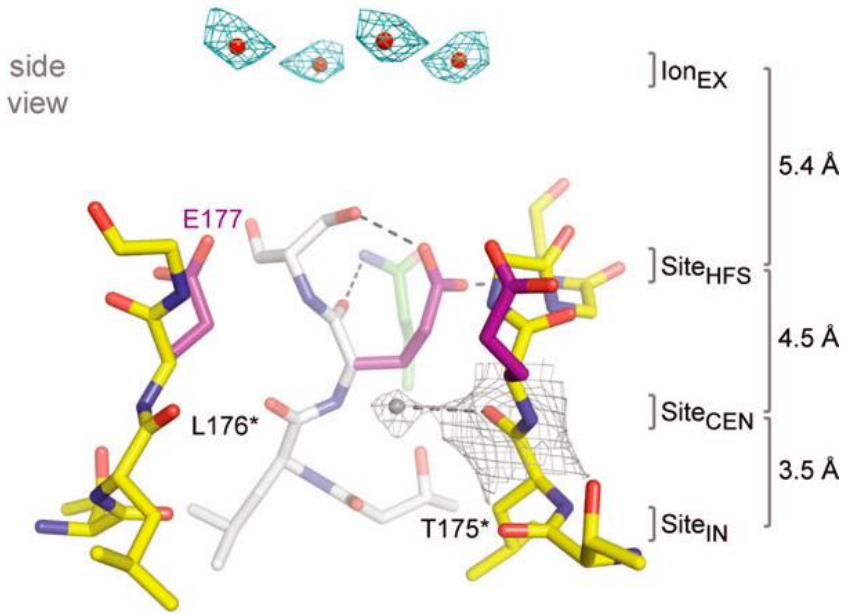
Common properties of ION CHANNELS: SELECTIVITY



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.

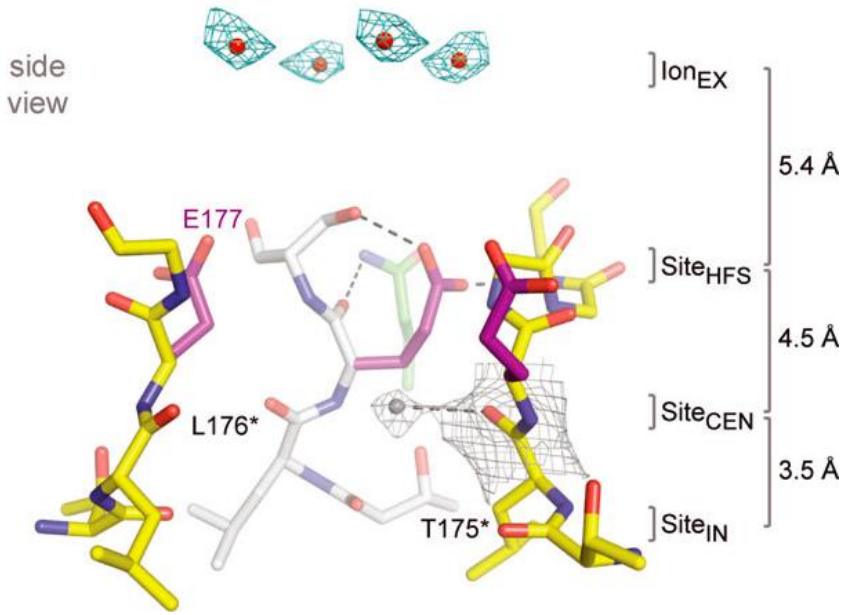


Na+ channels selectivity filter

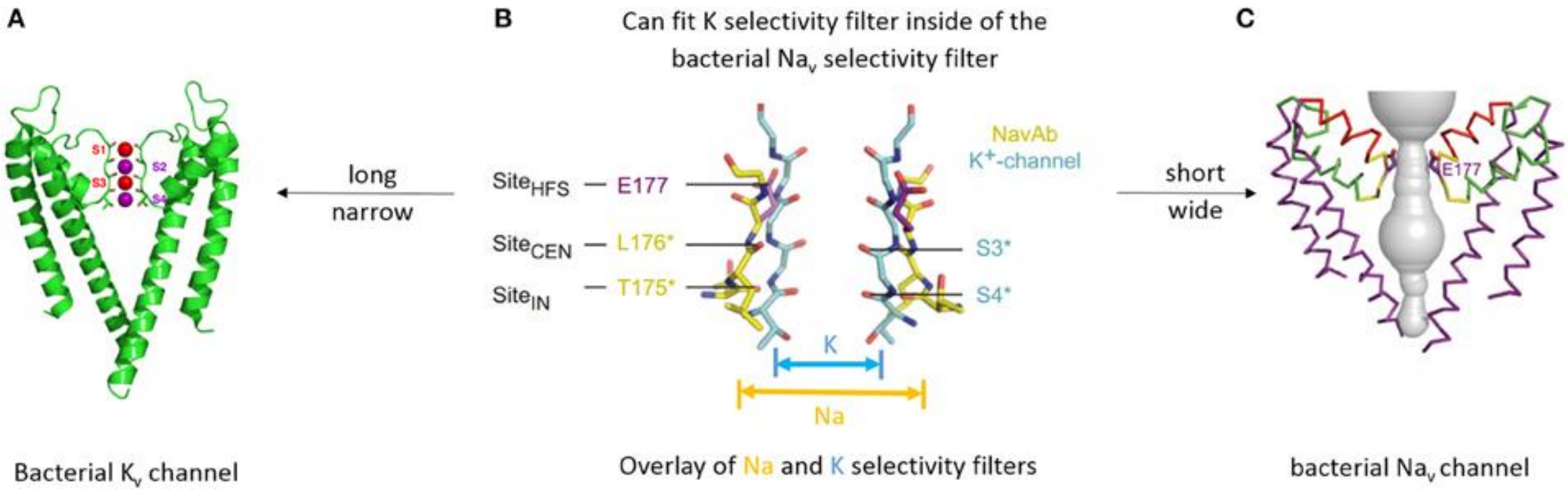
This outer site is followed by two ion coordination sites formed by backbone carbonyls.

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.**



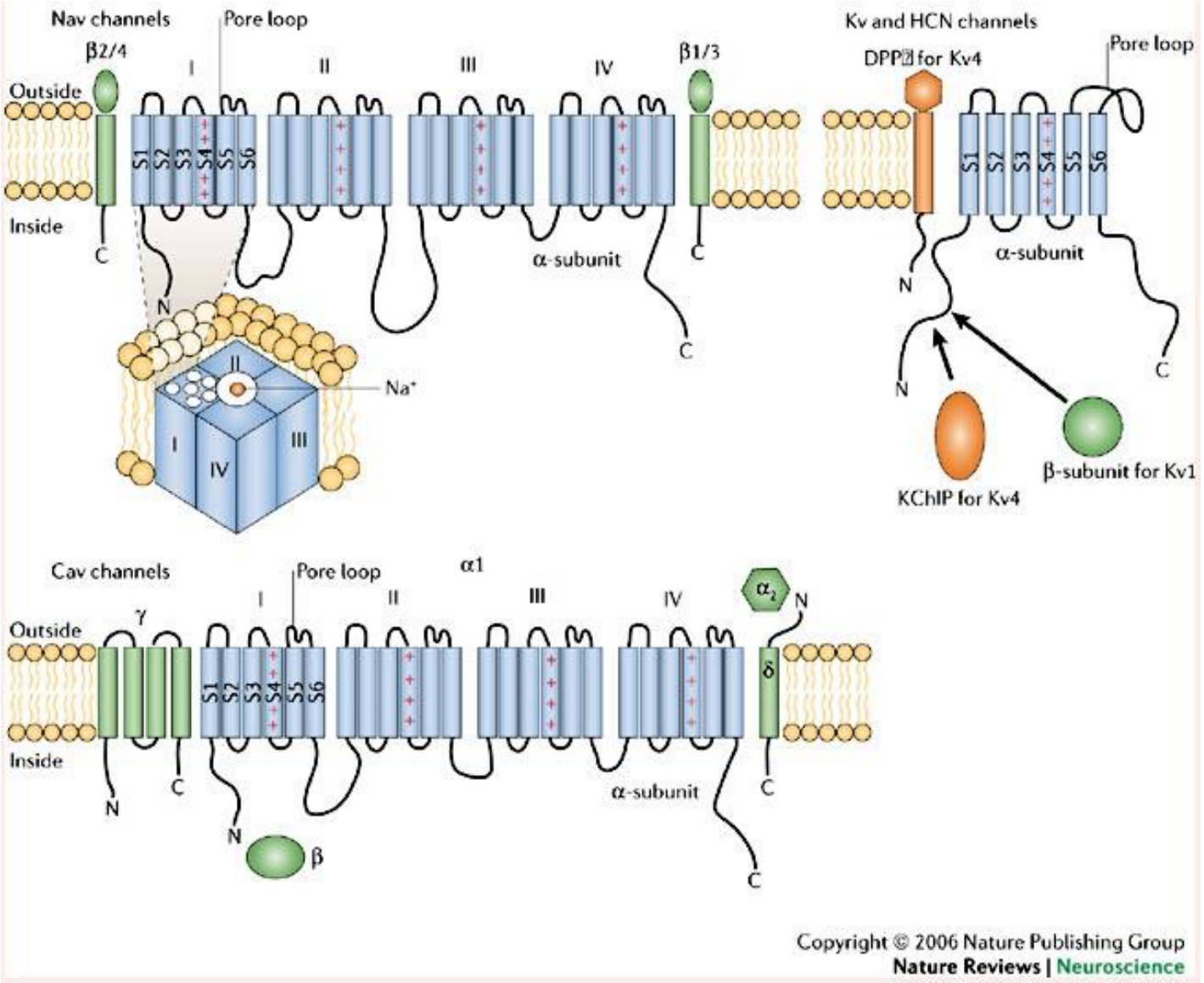
Molecular basis for SELECTIVITY



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)

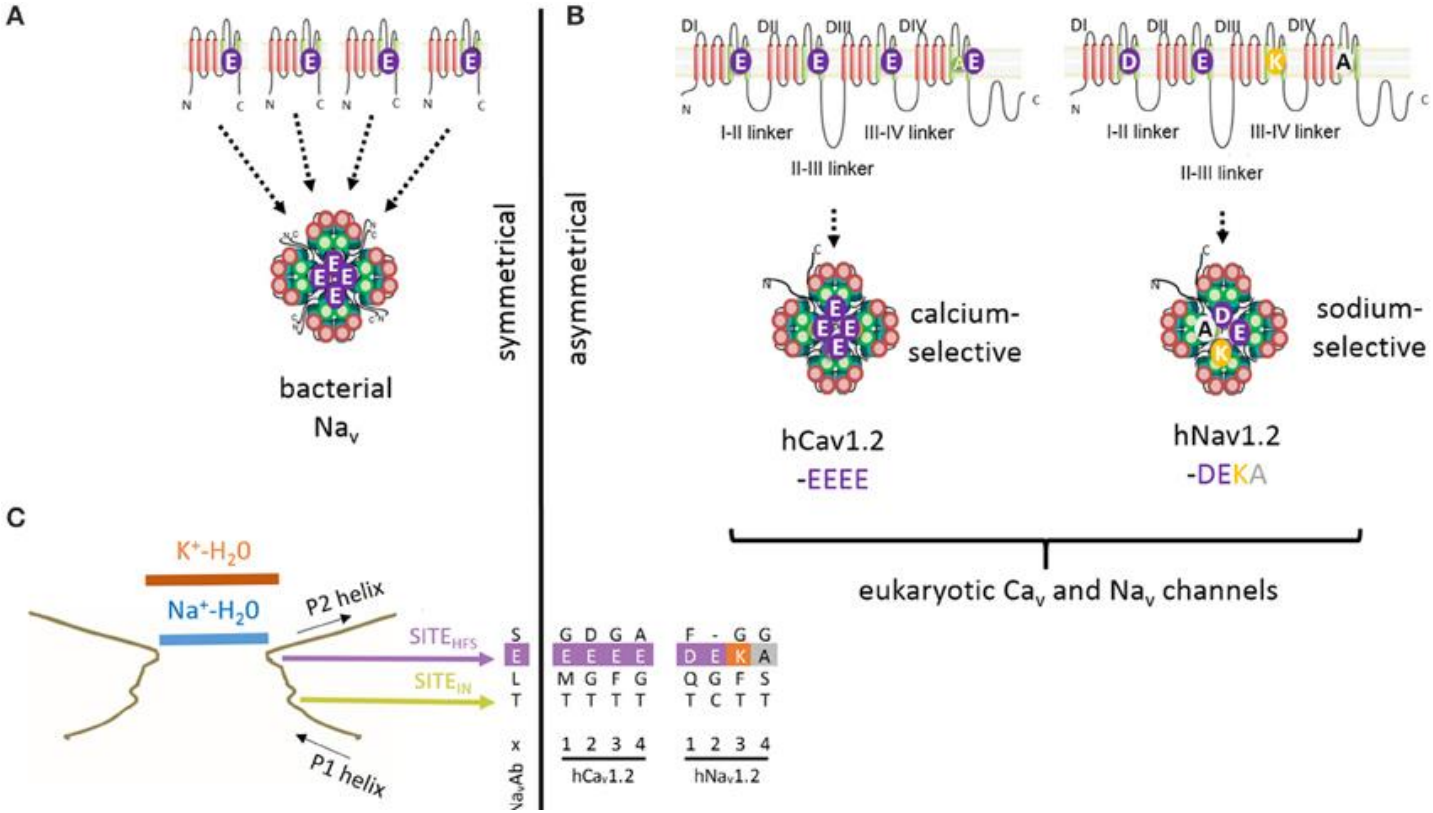


Molecular basis for SELECTIVITY

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

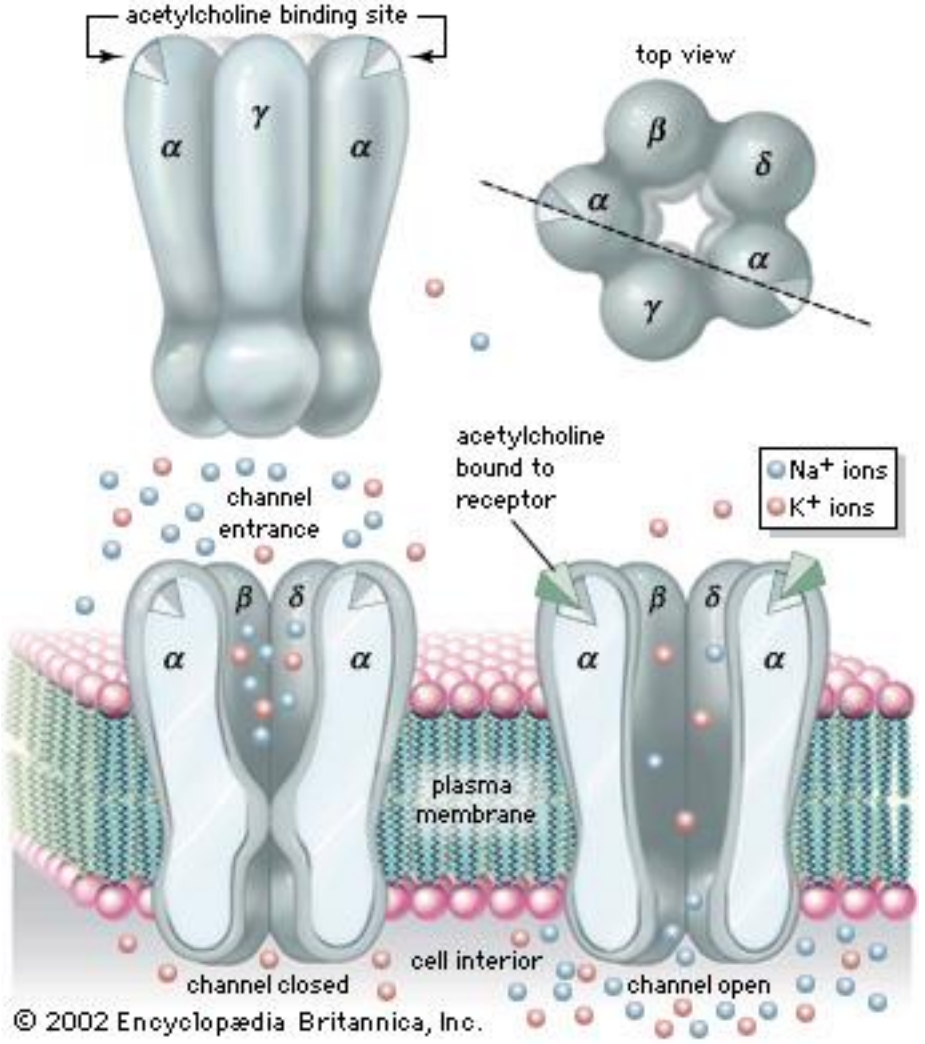
Mutations in DEKA locus with E confers the Na+ channels permeability features of Ca2+ channels



Molecular basis for SELECTIVITY

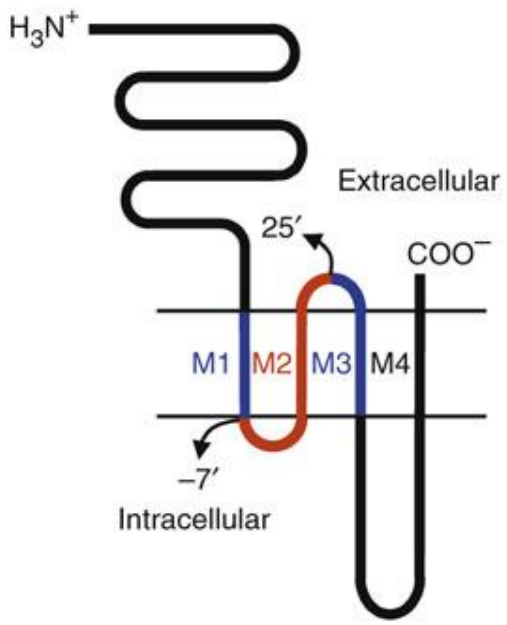
For ligand-gated ion channels the situation is different

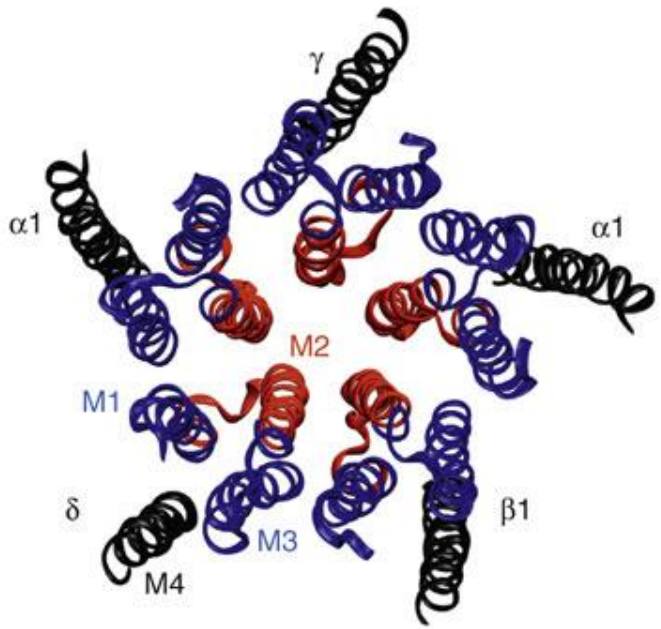
Nicotinic Receptor for Ach is formed by 5 subunits (2α, β, γ, δ).



Muscle nACHR

Each subunit present a huge extracellular N-term and 4 a helics (M1-M4).
M2 is the helic that delimits the pore.

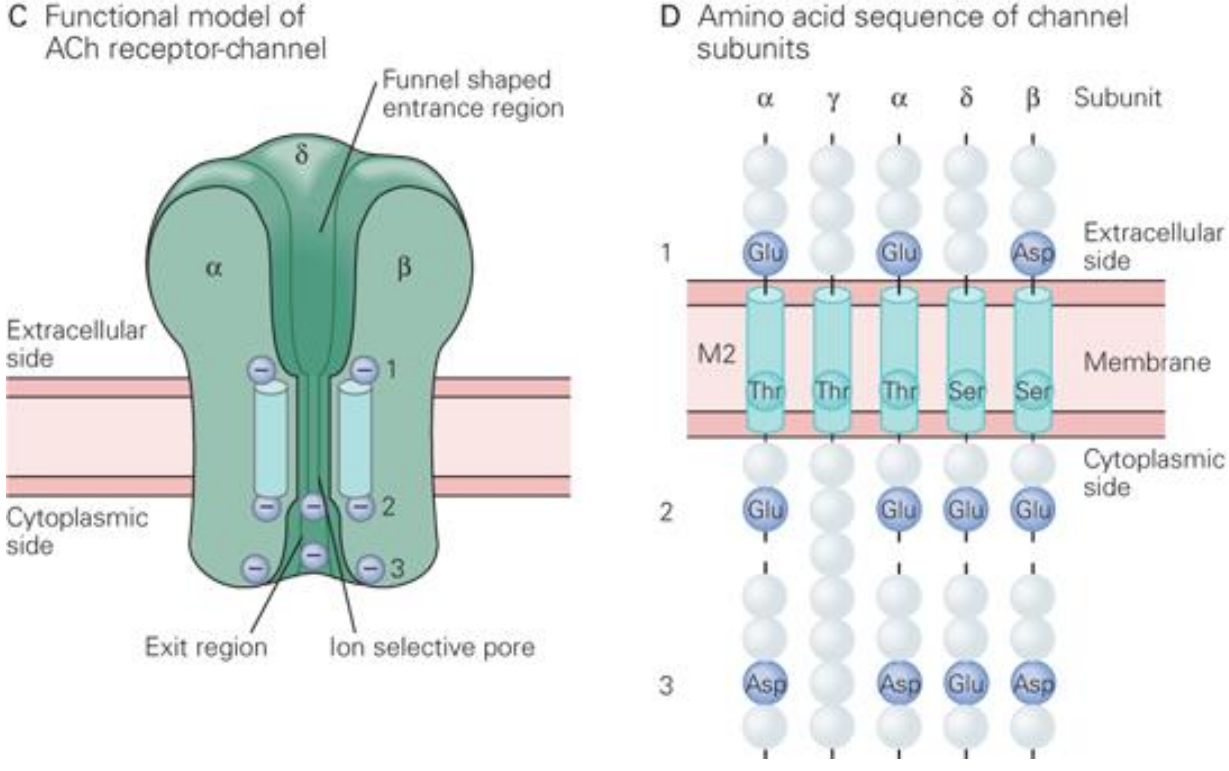




Muscle nACHR

M2 in α and β subunits presents negative charges repeated 3 times forming therefore 3 rings that play an important role in the channel's selectivity for cations. The aligned Ser and Thr residues within the M2 help forming the selectivity filter

Certain local anesthetic drugs block the channels by interacting with one ring of polar Ser residues and two rings of hydrophobic residues in the central region of the M2 helix, midway through the membrane



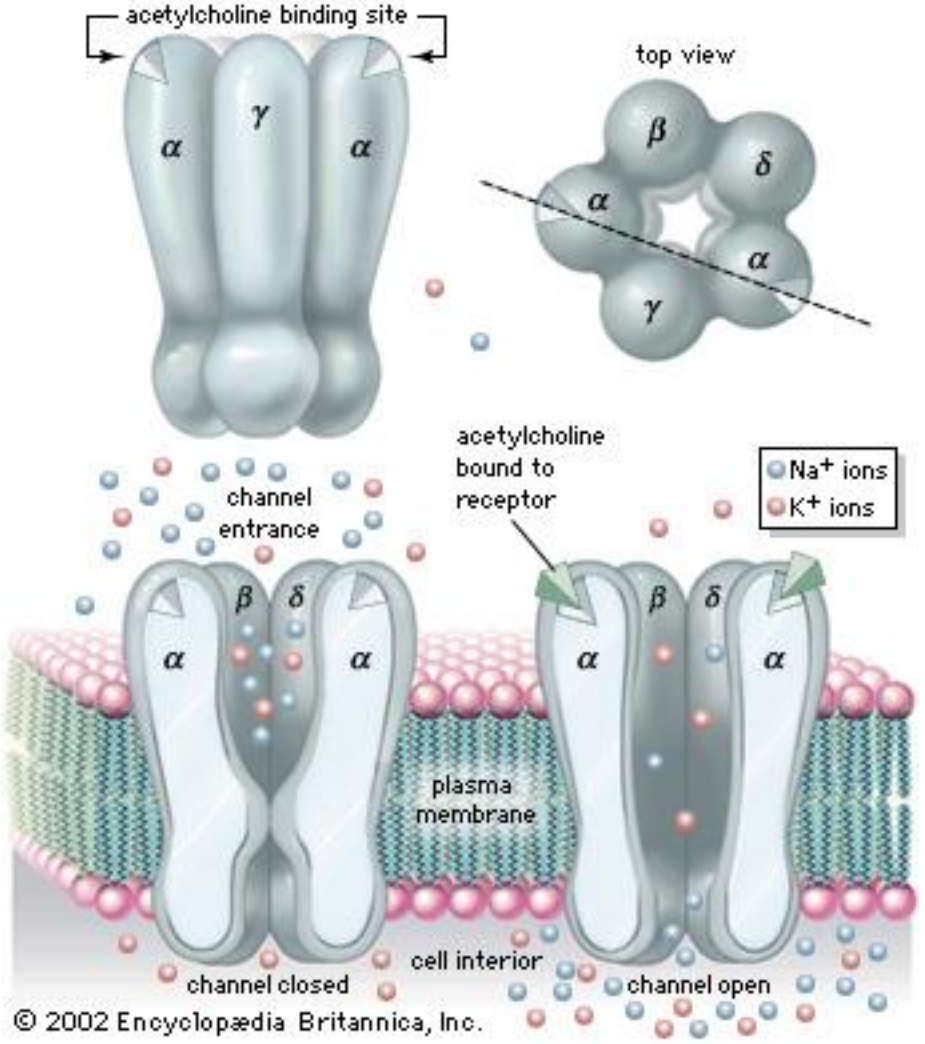
Muscle nACHR

Nicotinic acetylcholine receptors (nAChRs) belong to the superfamily of the Cys-loop ligand-gated ion channels (LGICs), which also includes the GABA, glycine, and 5-HT3 receptors.

The characteristic feature of this superfamily is a conserved sequence of 13 residues flanked by linked cysteines in the N-terminal domain of each subunit.

The first nAChR subtype was purified from the **electric organs of the fishes Torpedo and Electrophorus**, and four types of subunits, namely, α, β, γ, and δ, were identified.

ACh binding site is situated in beteween each α subunit and the neighboring γ or δ subunits.

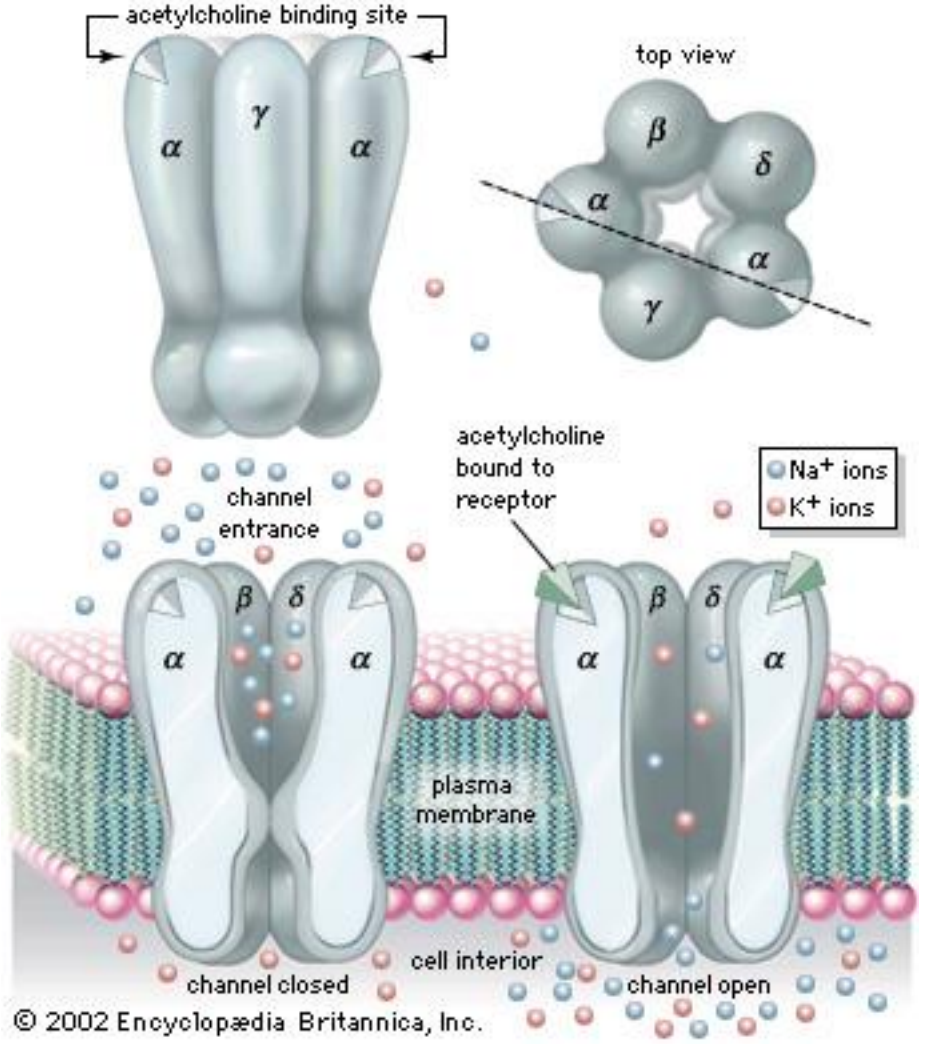


Muscle nACHR

In vertebrates, the 17 known homologous nAChR subunits ($\alpha1$–$\alpha10$, $\beta1$–$\beta4$, γ, δ, and ε) assemble into a variety of pharmacologically distinct receptor subtypes. The **muscle nAChR** is a heteropentamer, with a subunit stoichiometry of $\alpha1\ 2\beta1\ \gamma\ \delta$ in the embryo, similar to that in Torpedo ($\alpha2\beta\gamma\delta$), whereas in adults, the γ subunit is replaced by the ε subunit ($\alpha1_2\beta1\varepsilon\delta$).

Muscle and Torpedo nAChRs are often named **muscle-type nAChRs**.

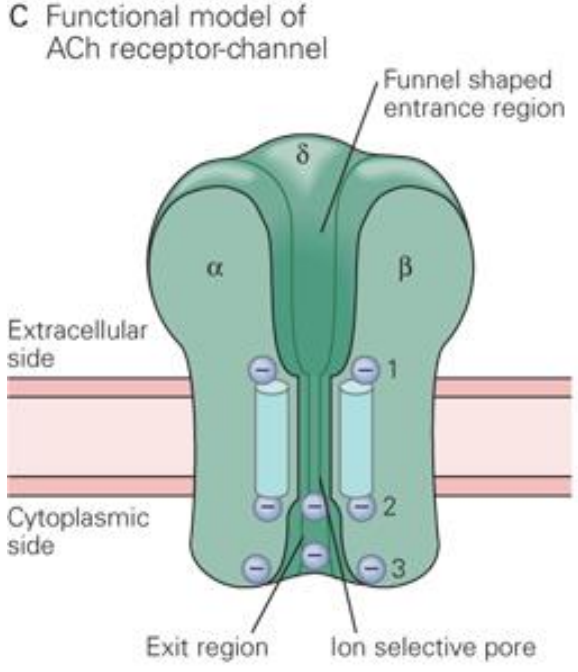
2 ACh must bind two the channels to open efficiently. The inhibitory snake venom α-bungarotoxin also bind to the ACh binding sites in the α subunit

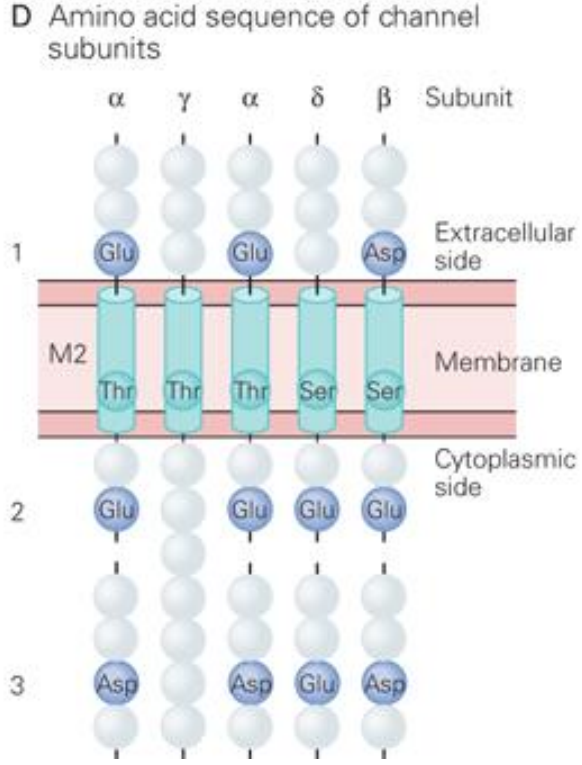


Muscle nACHR

The pore complex can be divided in three regions:
Large **extracellular portion** that contains Ach binding site
Narrow **transmembrane pore** selective for cations
Large **exit region** at the internal membrane surface

extracellular portion is surprisingly large, about 6nm in length. The extracellular end of the pore has a wide mouth approximately 2.5nm in diameter.
Within the bilayer the pore gradually narrows.





Muscle nACHR

The first milestone in understanding the structure of nAChRs in atomic detail was the elucidation of the X-ray crystal structure of the molluscan Lymnaea stagnalis AChBP (L-AChBP) at 2.7 A ° resolution (Brejc, K., et al, Nature 2001)which is homologous to the N-term of muscle nAChR:

Like the nAChRs, AChBP assembles into a homopentamer with ligand-binding characteristics that are typical for a nicotinic receptor; unlike the nAChRs, however, **it lacks the domains to form a transmembrane ion channel**.

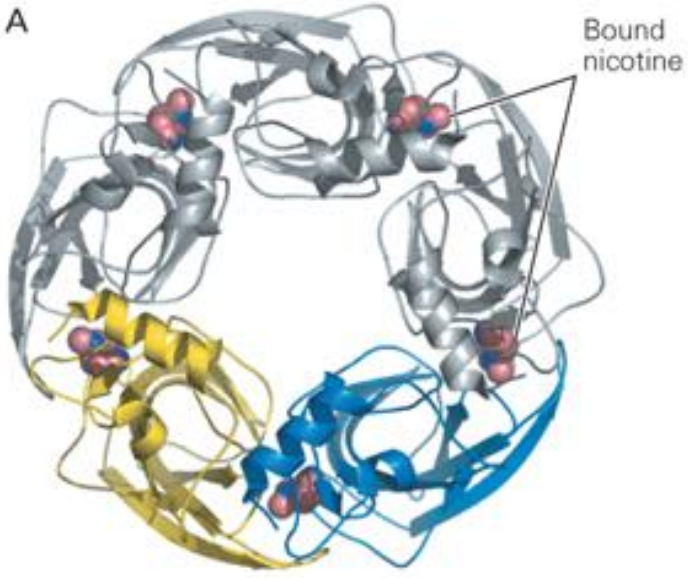
The molluscan AChR is a soluble protein secreted by glial cells into the extracellular space. At cholinerginc synapses in snails it acts to reduce the size of the excitatory postsynaptic potential.

Muscle nACHR

The resolved dimensions of the AChBP (a 62 A ° high cylinder, with a diameter of 80 A ° and a central 18 A ° diameter hole) are in good agreement with those estimated for the Torpedo nAChR-ECD by EM studies. When viewed along the fivefold axis, the AChBP resembles a toy windmill, with blade-like monomers.

The walls of the protein are seen to surround a large vestibule, which presumably funnels ions toward the narrow transmembrane domain of the receptor.

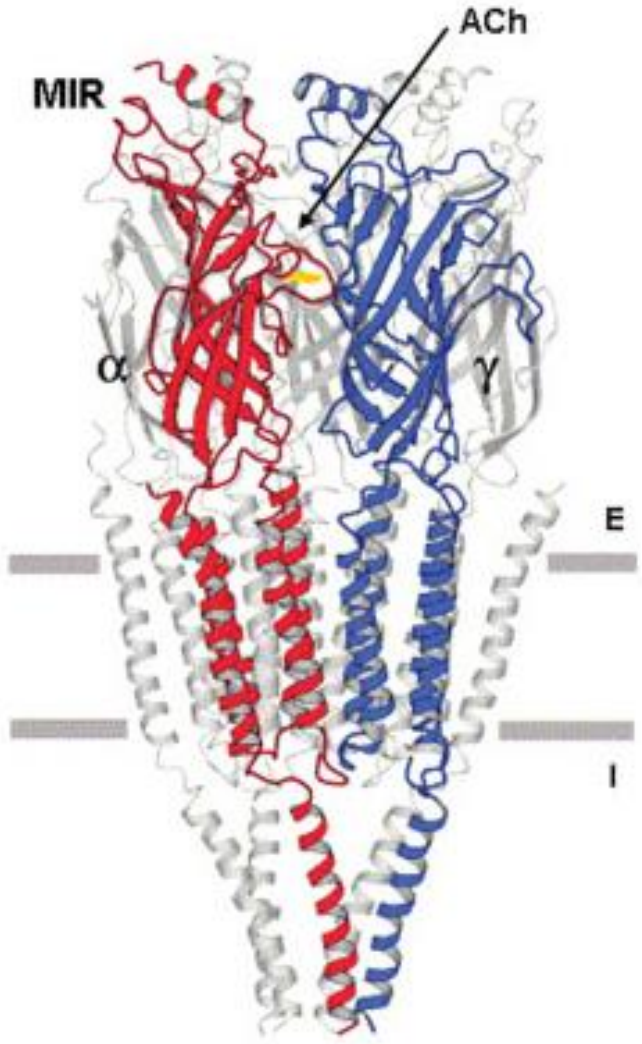
Each subunit bind one molecule of nicotine at the Ach binding site, located at the interface of two subunits = TOT 5 ACh. This is similar to some neuronal nAChR



Muscle nACHR

The structure of the AChBP and a subsequent 4 A° resolution EM study of the Torpedo nAChR (Unwin et al 1995, 2003) were used to create the refined 4 A ° model of the whole receptor in its closed state.

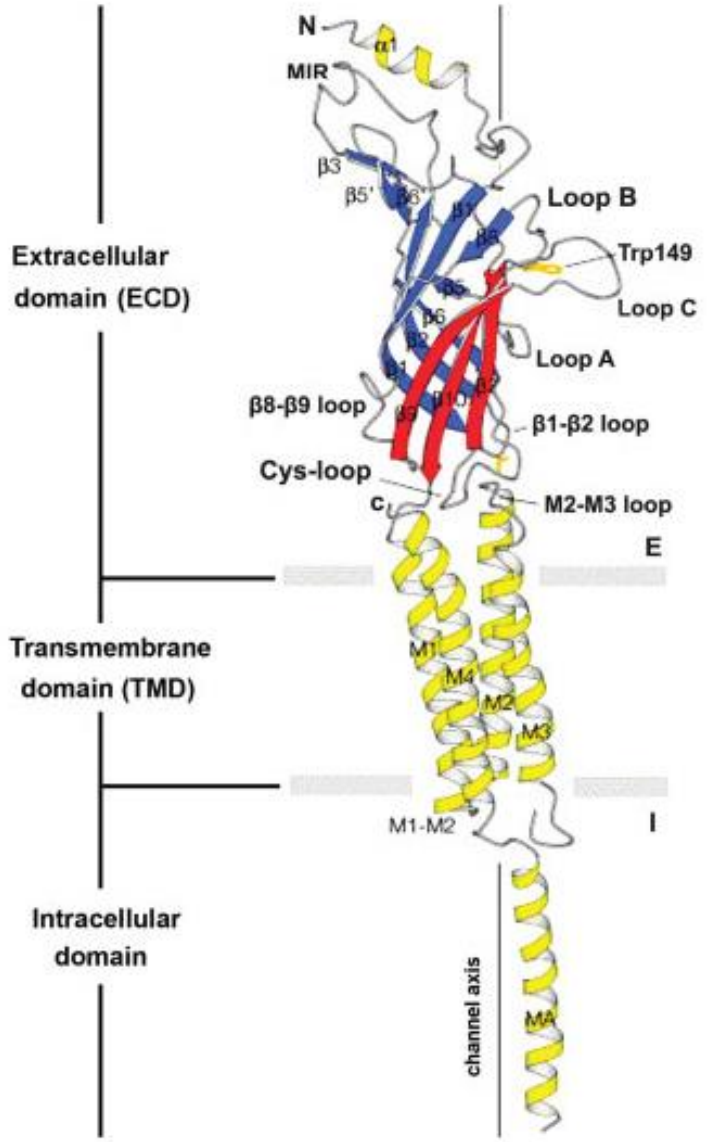
In this model, which constitutes the second milestone in our understanding of nAChR structure in atomic detail, the receptor was shown to have a total length of about 160 A ° normal to the membrane plane and to be divided in three domains:



Muscle nACHR

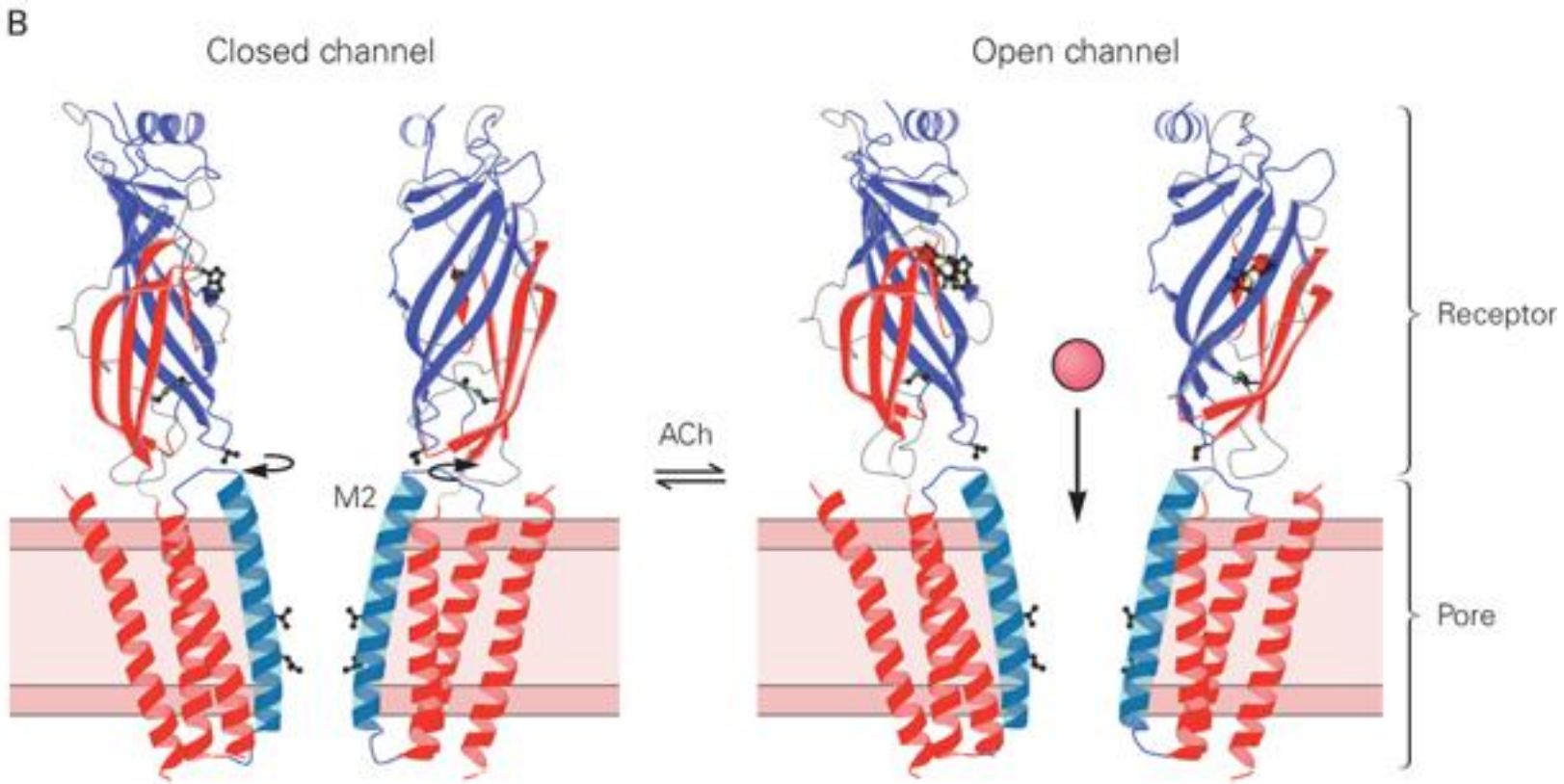
(a) an N-terminal ECD, or ligand-binding domain, which shapes a about 60 A°, a long central vestibule with a diameter of About 20 A ° , and has two binding sites for ACh,
(b) a transmembrane domain (TMD), components of which form about 40 A ° long water-filled narrow pore, containing the gate of the channel,
(c) an intracellular domain, which shapes a smaller vestibule than the extracellular one and which has narrow lateral openings for the ions, and a short C-terminal extracellular tail.

The subunits of the Torpedo nAChR all have a similar size (maximum dimensions 30 A ° x 40 A ° x 160 A °) and the same three-dimension fold.



Muscle nACHR

The pore limiting M2 segments are inclined toward the central axis of the channel, so that the pore narrows continuously from the outside of the membrane to inside.



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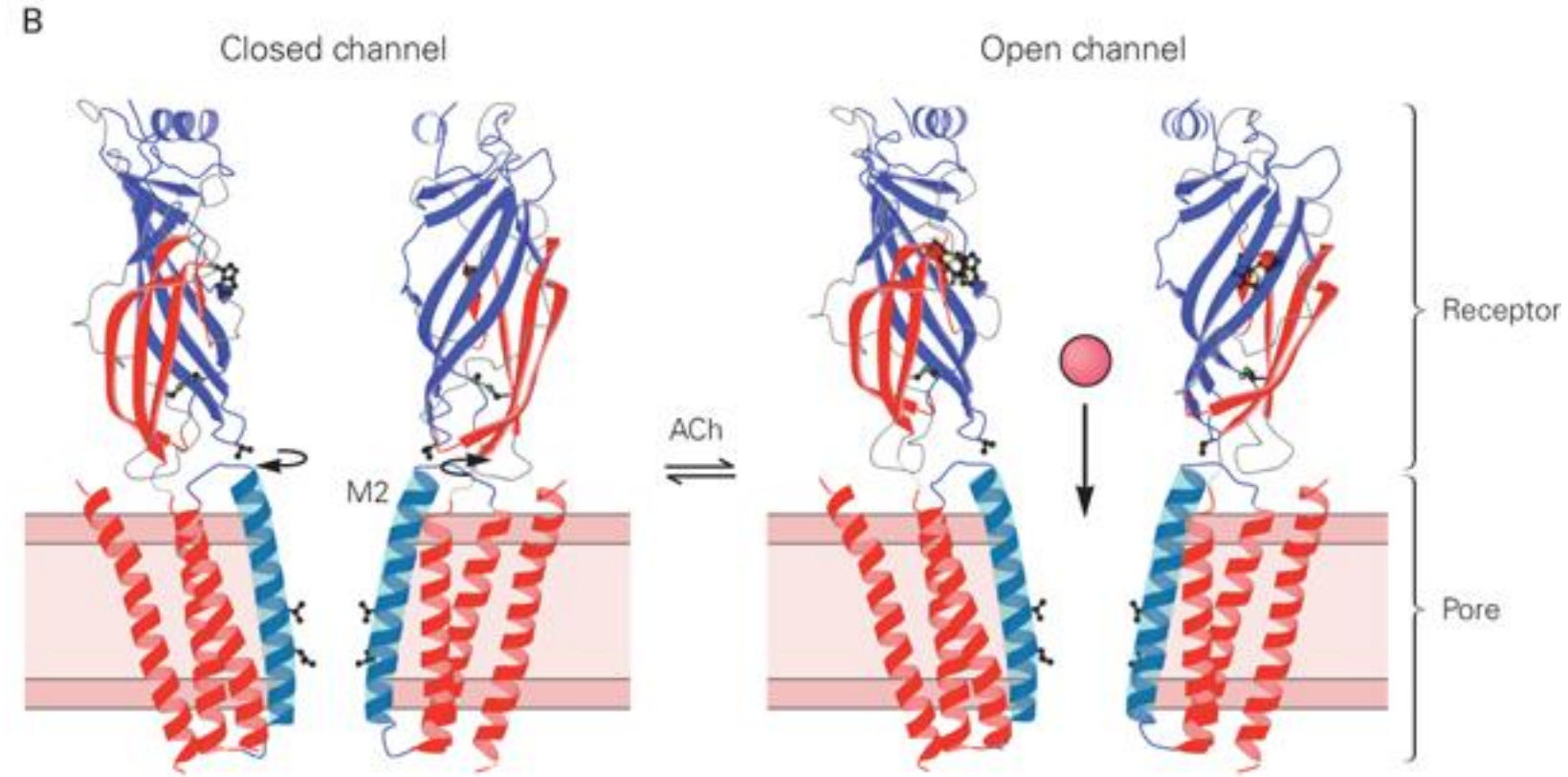
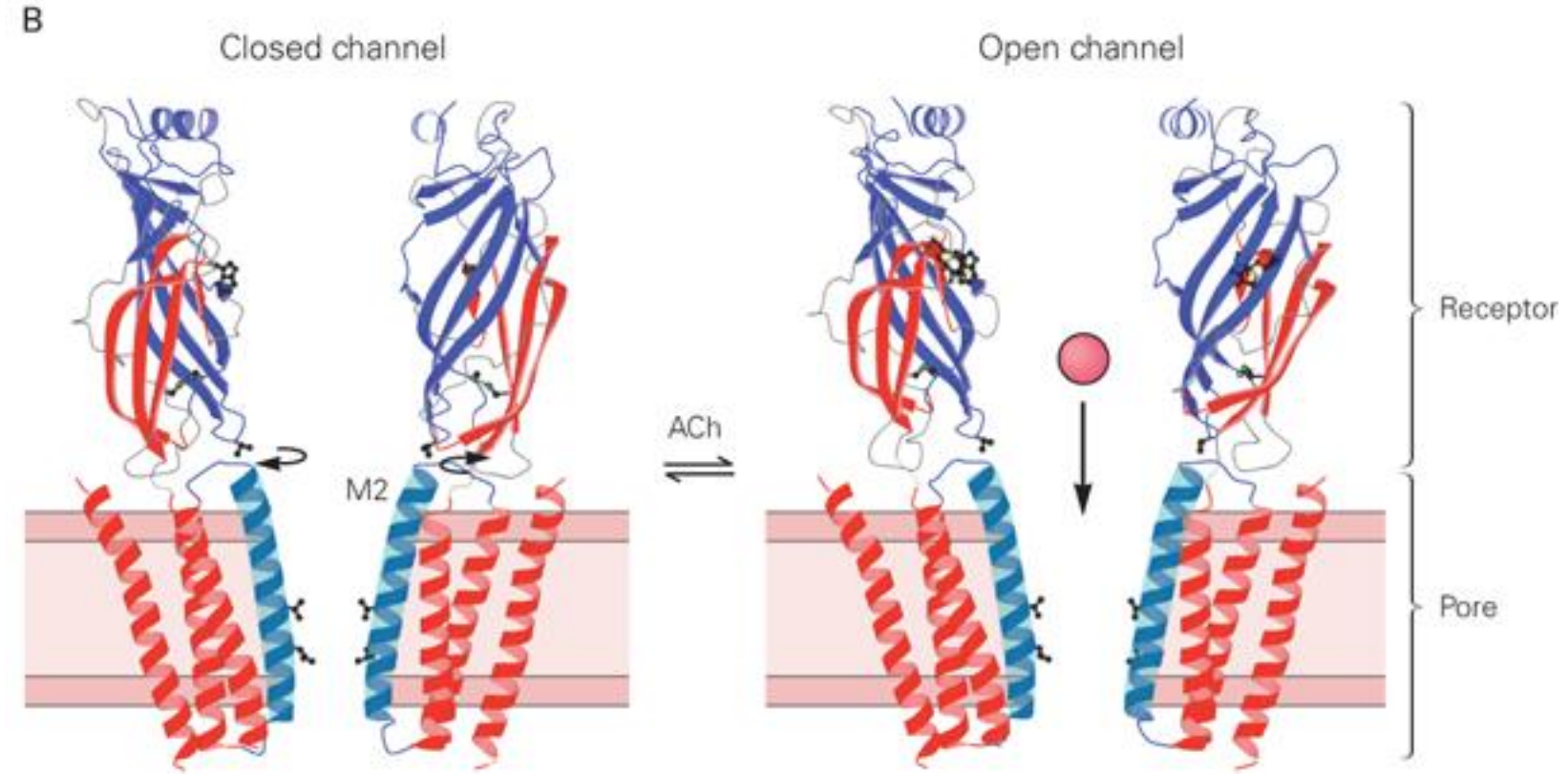
In the closed state a ring of hydrophobic residues is thought to constrict the pore in the middle of the M2 helix to a diameter of less than 0.6 nm. This hydrophobic rings may act as the channel's gate, providing a steric and energetic barrier that prevents ion conduction



Muscle nACHR

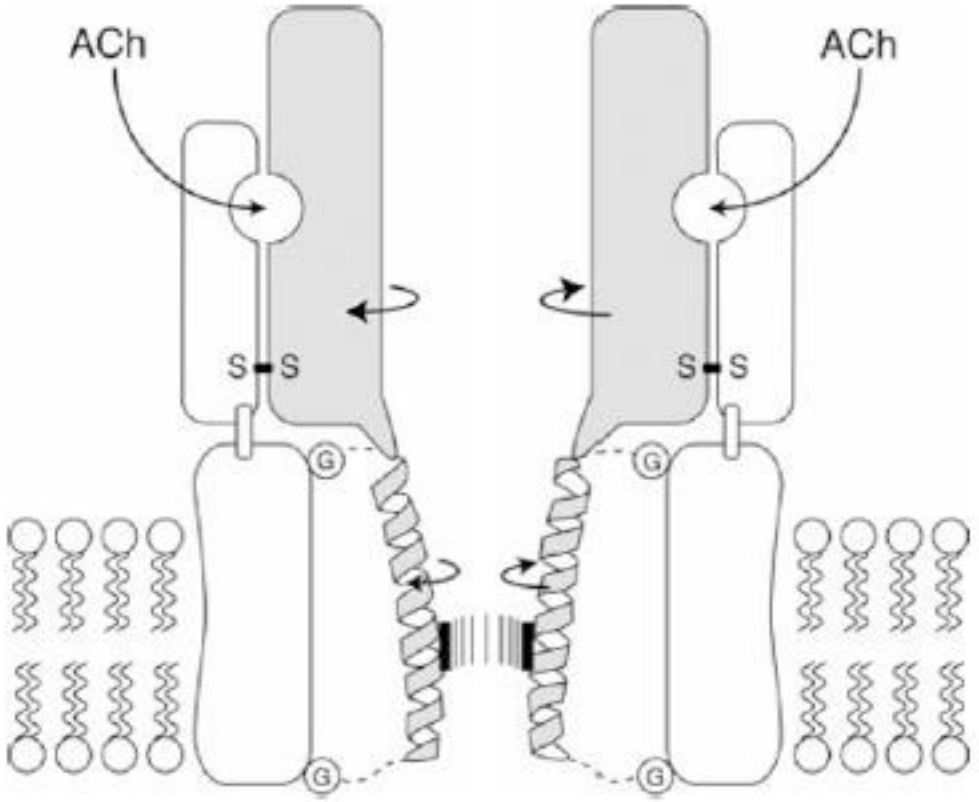
The binding of Ach is thought to trigger a rotation of the extracellular binding domain that is somehow coupled to an opposite rotation in the M2 helixes, widening the constriction in the middle of the M2 to around 0.8 to 0.9nm, enabling ion permeation



Muscle nACHR

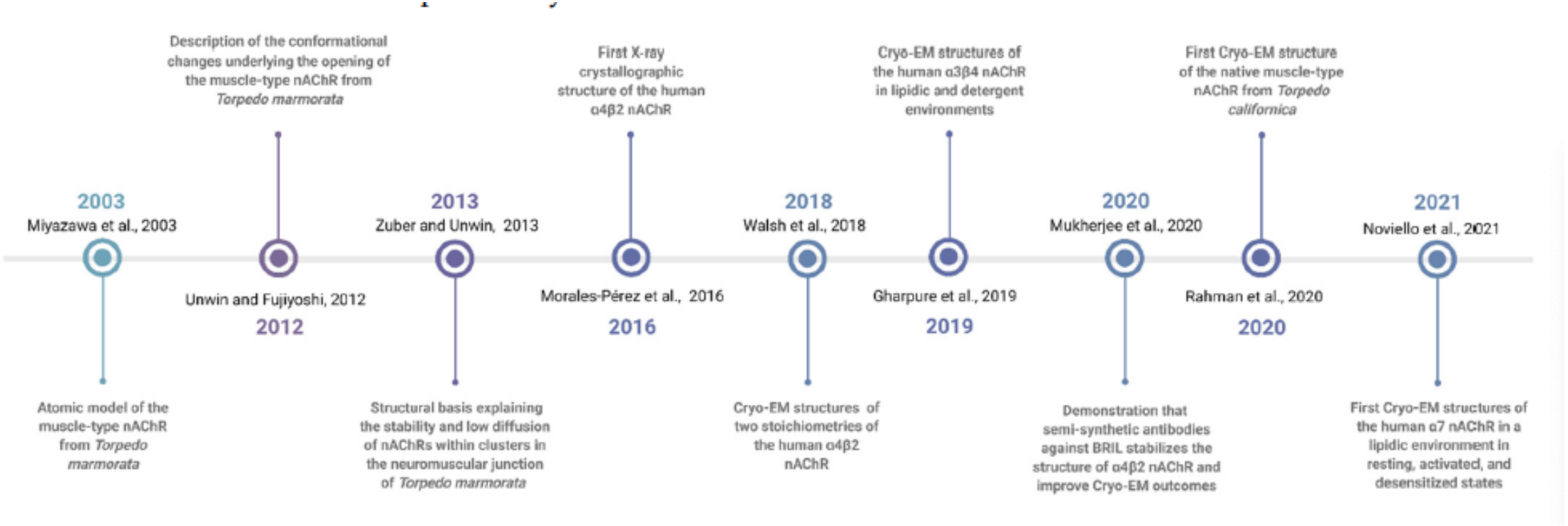
Proposed model for the gating mechanism. The ACh-induced rotations in the α subunits are transmitted to the gate—a hydrophobic barrier to ion permeation—through the M2 helices. The rotations destabilize the gate, causing the helices to adopt an alternative configuration which is permeable to the ions. The helices move freely during gating because they are mainly separated from the outer protein wall and connected to it by flexible loops, containing glycine residues (G).

S-S is the disulphide-bridge pivot in the ligand-binding domain, which is anchored to the fixed outer shell of the pore. The relevant moving parts are shaded.



Atsuo Miyazawa*, Yoshinori Fujiyoshi† & Nigel Unwin, Nature 2005

Muscle nACHR



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Thank you