Biological membranes and ion channels

Reading: Hille (3rd ed.) chapts 10, **13**, 17

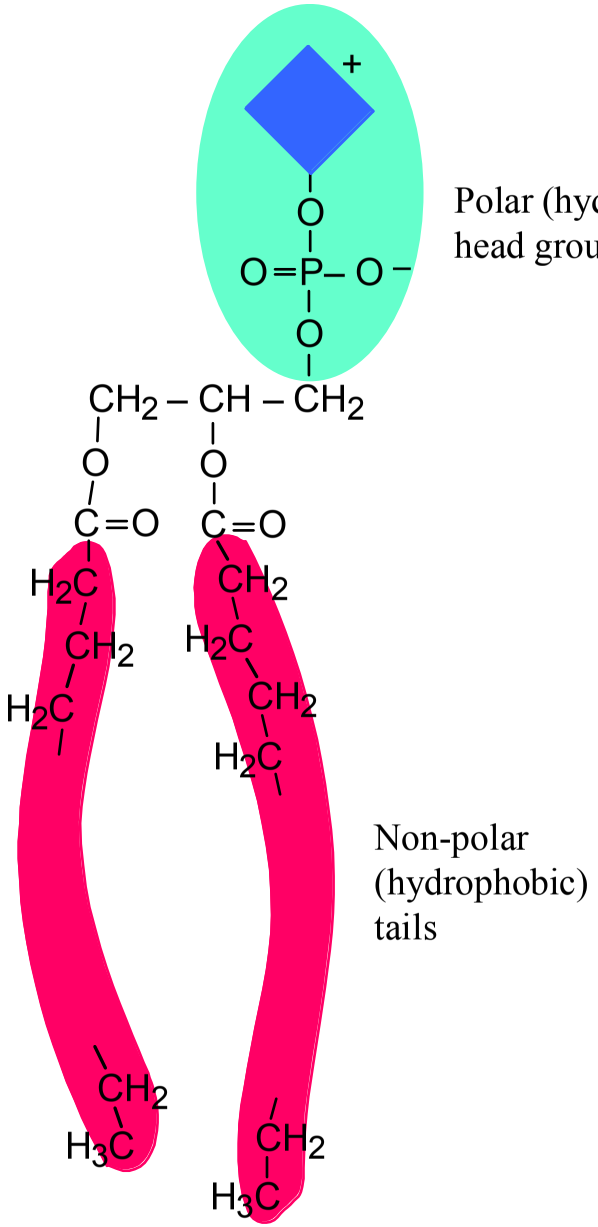
Koch , Chapt 8, 9

G. Yellen *The voltage-gated potassium channels and their relatives*. Nature 419:35-42 (2002).

Doyle et al. *The Structure of the Potassium Channel: Molecular Basis of K1 Conduction and Selectivity.* Science 280:70-77 (1998).

Miyazawa et al. *Structure and gating mechanism of the NACh receptor pore* Nature 423:949-955 (2003).

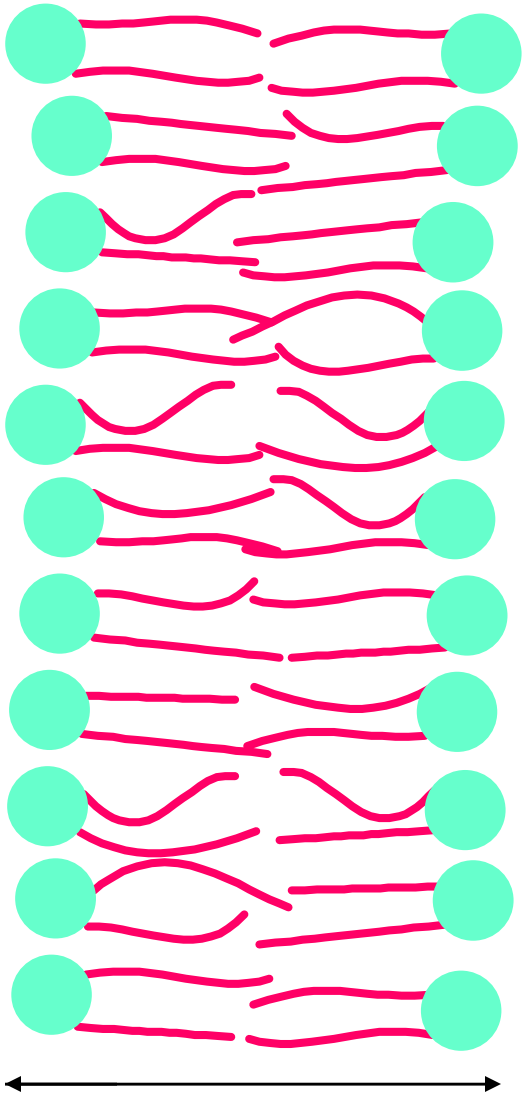
Swartz, K.J. *Sensing voltage across lipid membranes.* Nature 456:18-25 (2008).



Polar (hydrophilic) head group

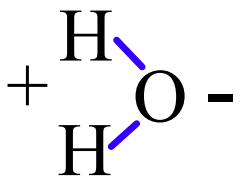
Non-polar (hydrophobic) tails

The lipid bilayer structure of membrane:

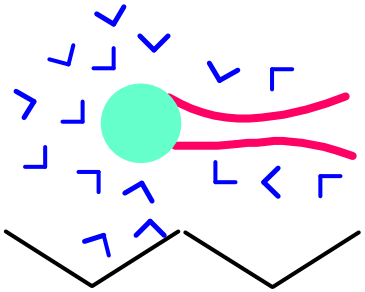


3-4 nm 30-40 Å

Membranes are stabilized and ions are unable to permeate membranes in significant numbers because of **hydrogen bonding** in aqueous solutions.

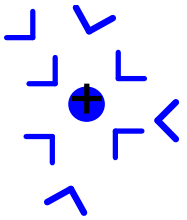


Water molecule dipole

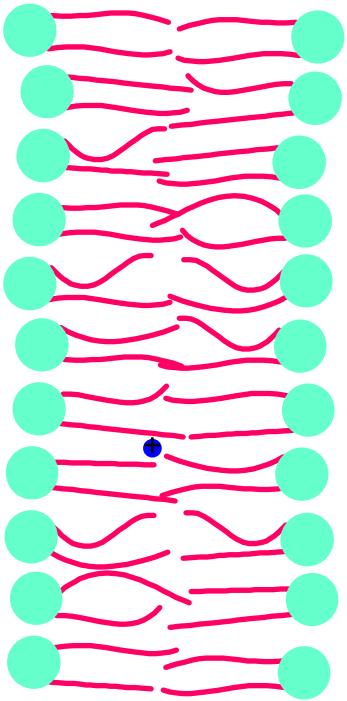


H-bonded head region

Tail region not H-bonded, **energetically unfavorable!**

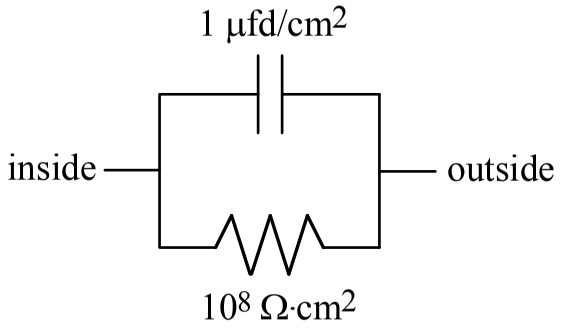


Sodium ion stabilized in solution by H-bonds (176 RT bond energy/ mole)

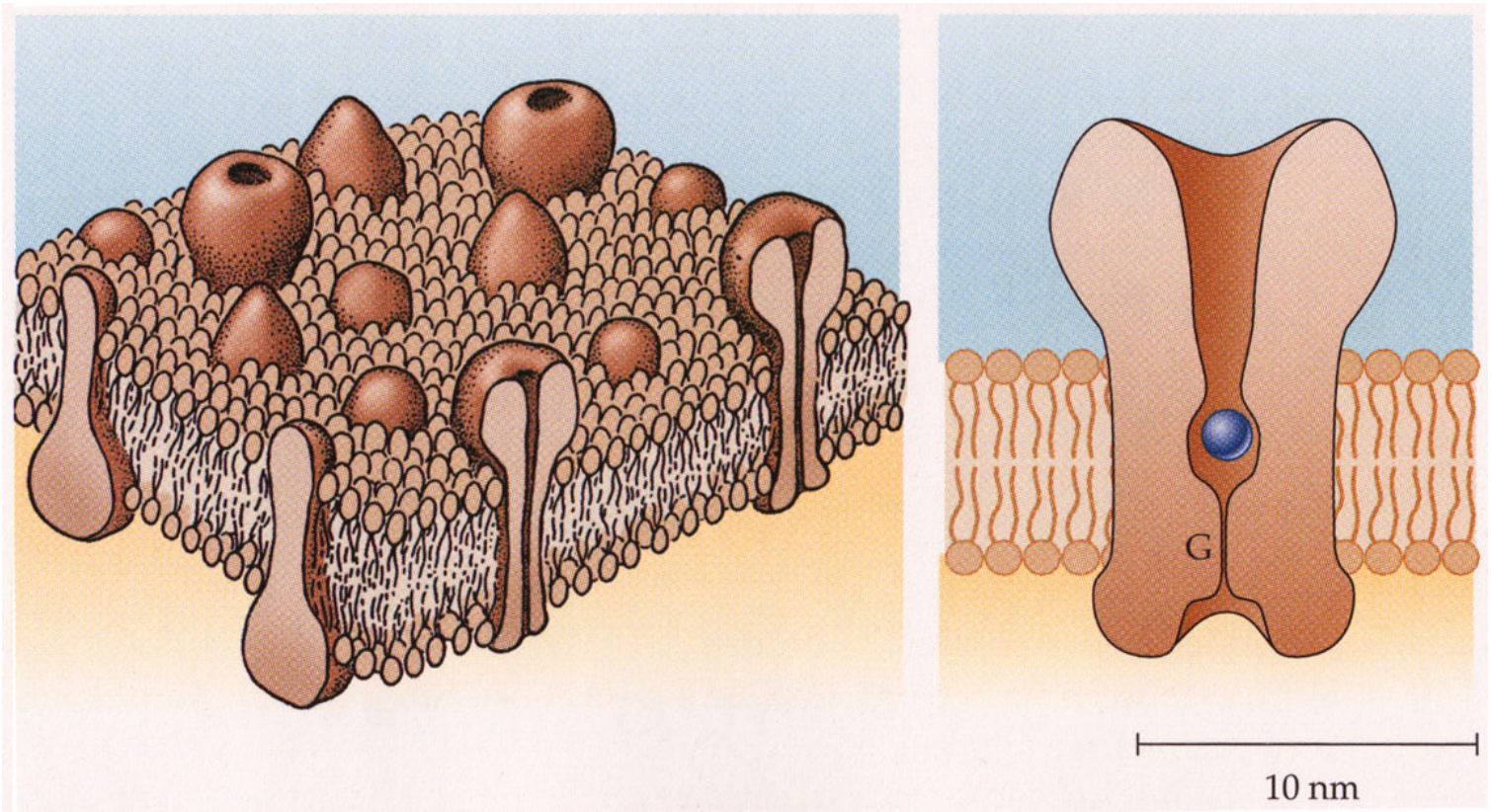


Same ion in membrane interior, no H-bonds. A high-energy state.

Pure lipid bilayers can be created artificially and have electrical characteristics like the circuit at right. The capacitance is about the same as for real nerve membrane, but real nerve membrane has a resistance several orders of magnitude smaller, about 10^3 - 10^5 $\Omega \cdot cm^2$



The reason for the difference, of course, is that membrane contains **ion channels**, **transporters** and other proteins that provide specialized ionic conduction pathways through the membrane.

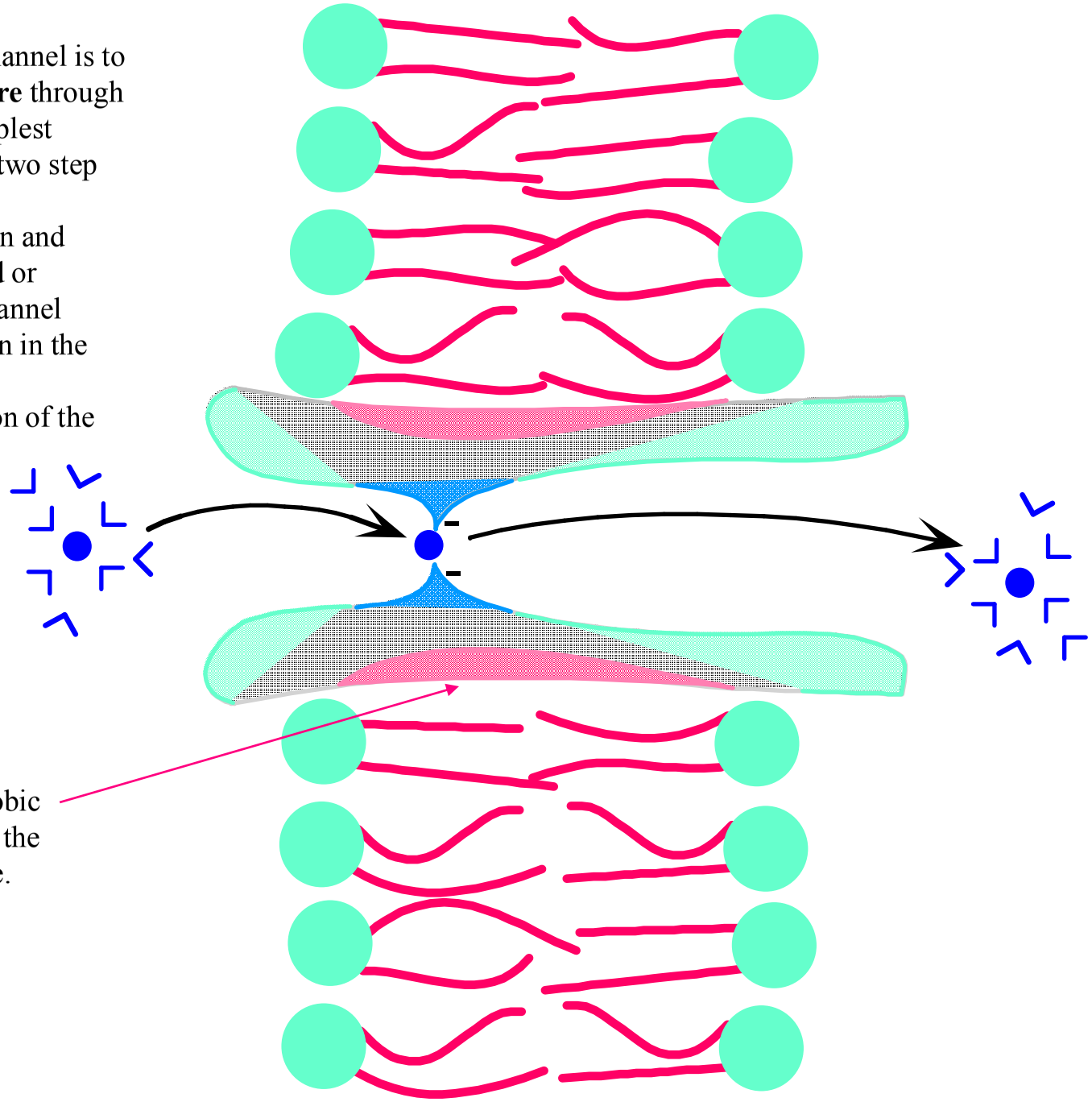


Nicholls et al., 2001

The function of the ion channel is to provide a **hydrophilic pore** through the membrane. In the simplest model, we can imagine a two step process:

1. Dehydration of the ion and binding to a polarized or charged site in the channel
2. Rehydration of the ion in the opposite solution

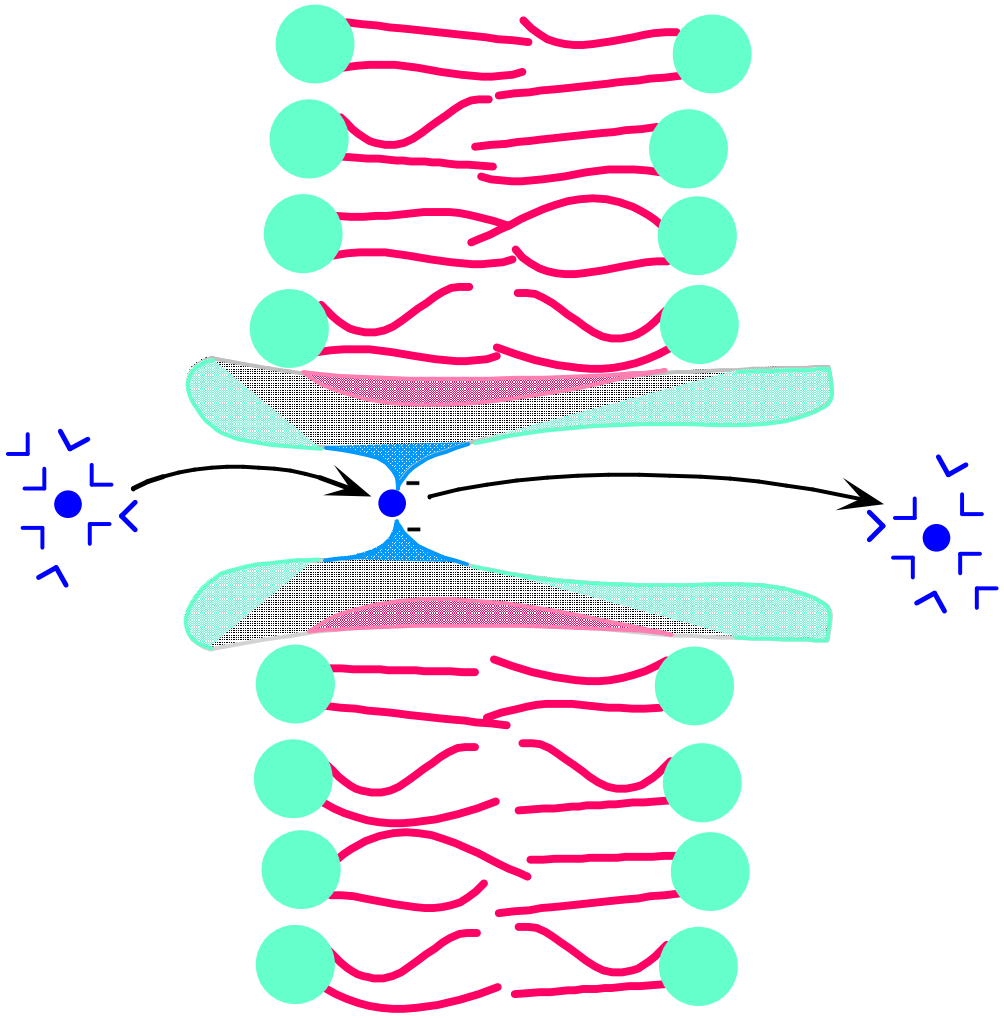
The energy for dehydration of the ion is provided by the binding energy to the site in the channel.



Note that the channel molecule has a hydrophobic exterior which stabilizes the channel in the membrane.

The channel is shown to have a narrow spot where an ion might be found with high probability, almost a binding site. This spot serves as the **selectivity filter**, determining the selectivity of the channel for particular ions.

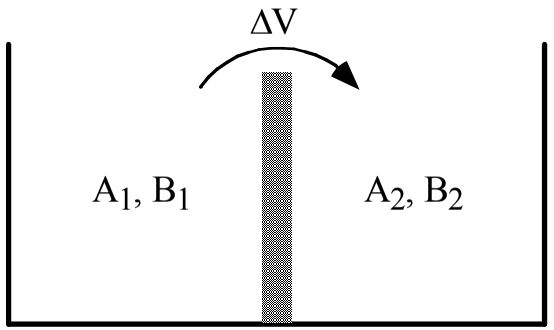
Note that the ion cannot really *bind* to the selectivity filter, because that would slow propagation through the channel. Conductance through an open channel is about the same as through an aqueous pore of similar size (see Hille, p. 294).



Selectivity refers to the relative permeability of a membrane for different ions. Selectivity can be measured in various ways, but a simple (in principle) measurement is to use the GHC diffusion potential equation, applied to a situation in which only two permeable ions are present. Suppose the ions are both monovalent cations. Then in steady state,

$$\Delta V = \frac{RT}{F} \ln \frac{P_A A_1 + P_B B_1}{P_A A_2 + P_B B_2}$$

$$= \frac{RT}{F} \ln \frac{A_1 + \frac{P_B}{P_A} B_1}{A_2 + \frac{P_B}{P_A} B_2}$$



The diffusion potential equation can be solved for the relative permeability P_B/P_A as below. Then, by measuring the membrane potential with various concentrations of ions, the relative permeability can be calculated.

$$\frac{P_B}{P_A} = \frac{B_1 - e^{F\Delta V/RT} B_2}{e^{F\Delta V/RT} A_2 - A_1}$$

Voltage gated ion channels show considerable selectivity, inferred from membrane potential experiments.

TABLE 14.2 Permeability Ratios, P_X/P_{Na}, for Na Channels[a]

Ion	Frog node[1]	Frog muscle[2]	Squid axon[3]	*Myxicola* axon[4]
H^+	252[5]	—	>2[6]	—
Na^+	1.0	1.0	1.0	1.0
Li^+	0.93	0.96	1.1	0.94[9]
Ca^{2+}	<0.11	<0.093	0.1[8]	0.1
K^+	0.086	0.048	0.083	0.076[9]
Rb^+	<0.012	—	0.025	—
Cs^+	<0.013	—	0.016	—
TMA	<0.005	<0.008	—	—

TABLE 14.4 Permeability Ratios, P_X/P_{Ca}, for L-Type Ca Channels[a]

Ion	P_X/P_{Ca}	Ion	P_X/P_{Ca}
Ca^{2+}	1.0	Li^+	1/424
Sr^{2+}	0.67	Na^+	1/1170
Ba^{2+}	0.40	K^+	1/3000
		Cs^+	1/4200

TABLE 14.3 Permeability Ratios, P_X/P_K, for Several Types of K Channels[a]

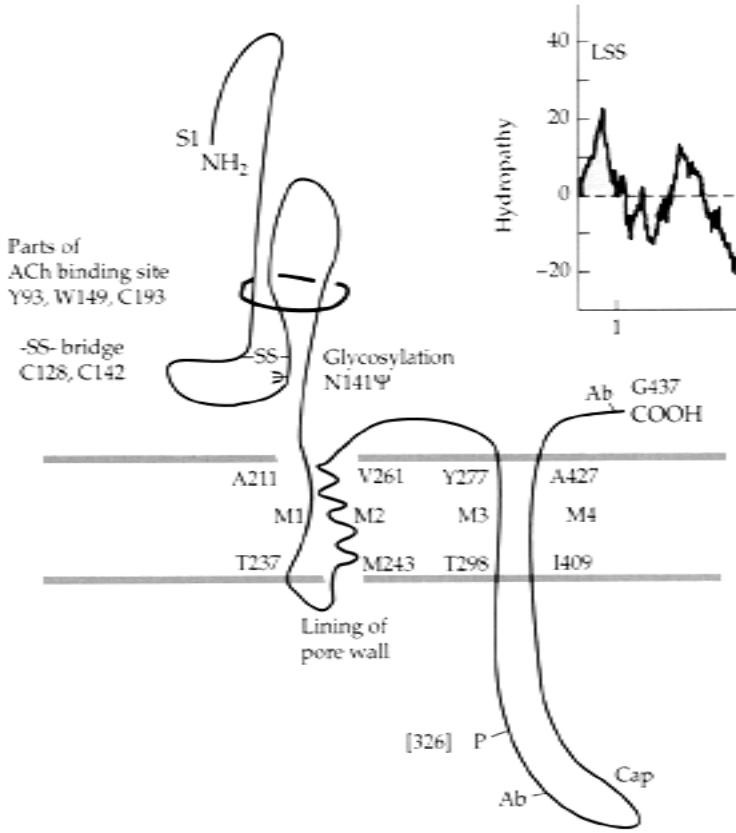
	Delayed rectifier			Inward rectifier	M current	BK K(Ca)	SK K(Ca)
Ion	Frog node[1]	Frog muscle[2]	Snail neuron[3]	Starfish egg[4]	Frog neuron[5]	Rat muscle[6]	Rat chromaffin[7]
K^+	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Rb^+	0.91	0.95	0.74	0.35	0.94	0.67	0.81
Cs^+	<0.077	<0.11	0.18	<0.03	0.10	<0.05	0.16
Li^+	<0.018	<0.02	0.09	—	<0.004	<0.02	<0.005
Na^+	<0.010	<0.03	0.07	<0.03	<0.004	<0.01	<0.005

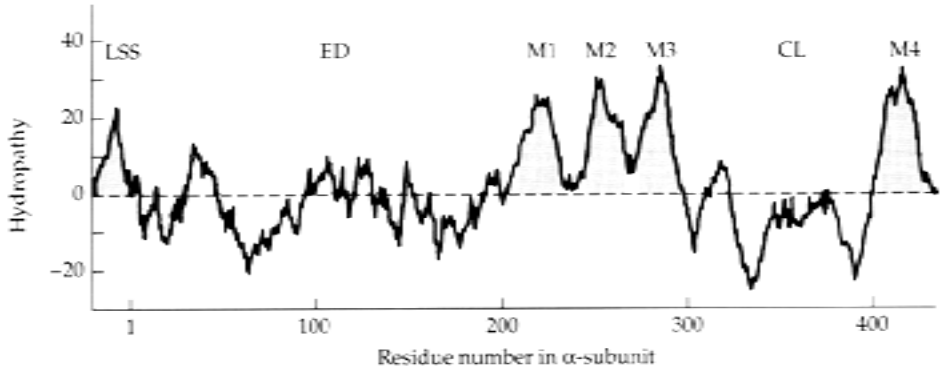
Tables from Hille, 2001

Ion channels are protein molecules with extracellular and intracellular domains and transmembrane domains. The **nicotinic acetylcholine receptor channel** subunit is diagrammed below. Note the **transmembrane segments**, denoted M1 - M4.

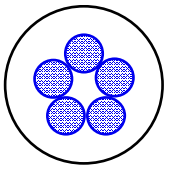
Evidence for channel structure:

1. Hydropathy plots
2. Binding sites for various ligands



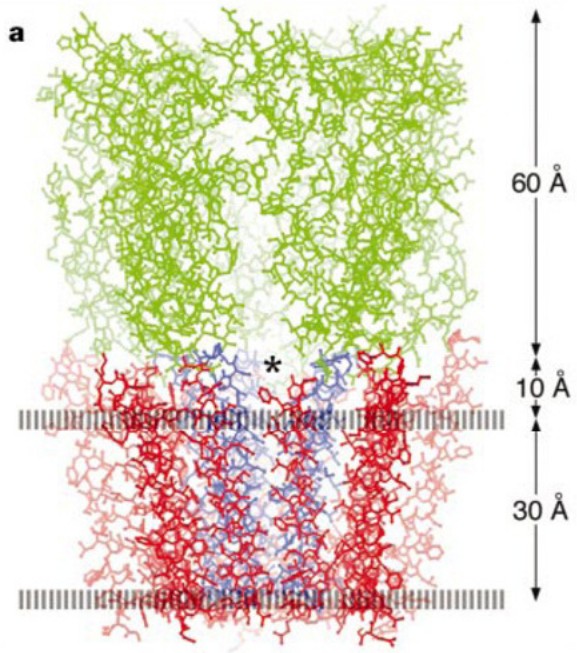


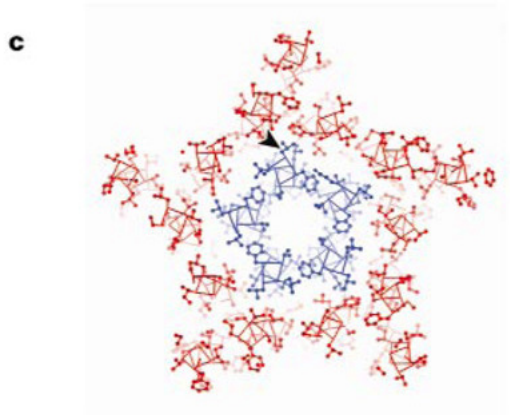
Subunits combine with five-fold symmetry, with M2s forming the pore. (True also of GABA and gly receptors.)



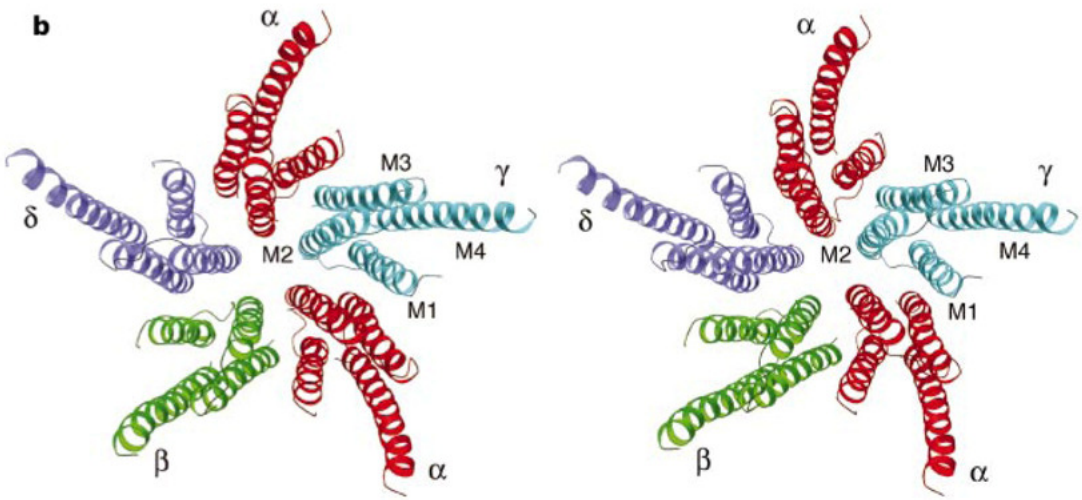
Hille, 2001

Using electron diffraction the structure of the NAChR was determined at 4 Å resolution. The M2 segments form a loose pore (blue) with substantial aqueous space in the membrane (between blue and red). The large extracellular domain contains the ACh binding site (green).





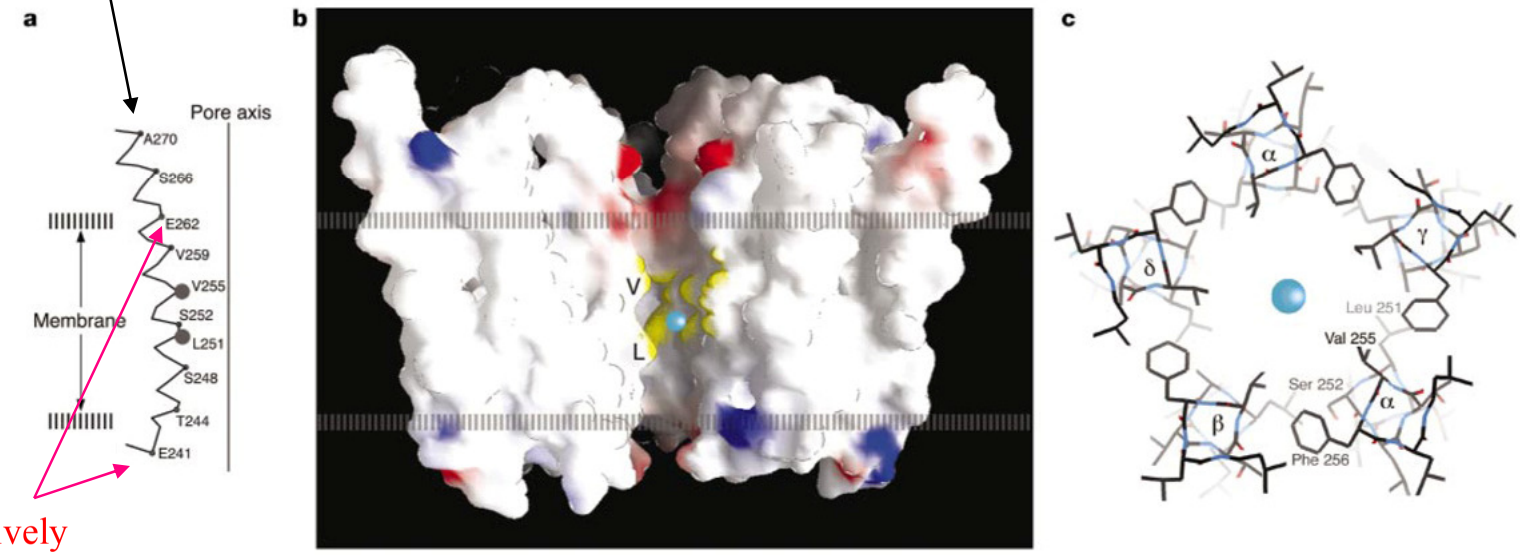
The M1-M4 domains are alpha helices, but the extracelular domain is mostly beta sheets.



Miyazawa et al. 2003

A space-filling model of the transmembrane part of the channel shows the pore, which is large. Red regions are negatively charged or polarized, to attract ions to the channel. The yellow region around the valine and leucine at positions 255 and 251 is non-polar and is the gate, which closes or opens in response to ACh binding.

The amino acids making up the pore.



Note negatively charged glutamates

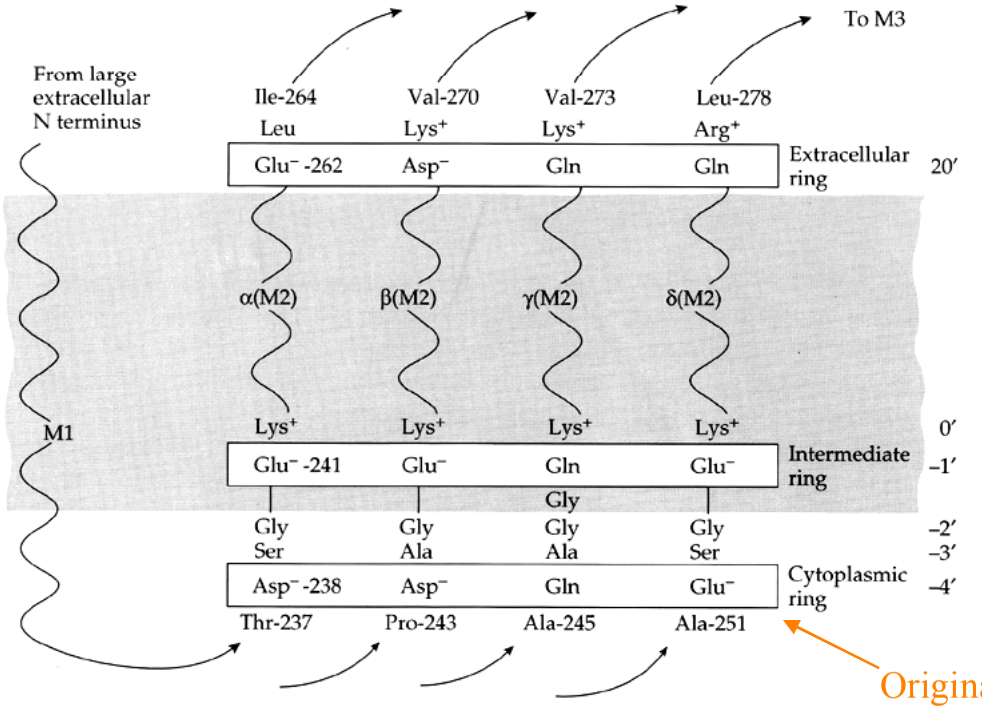
Miyazawa et al. 2003

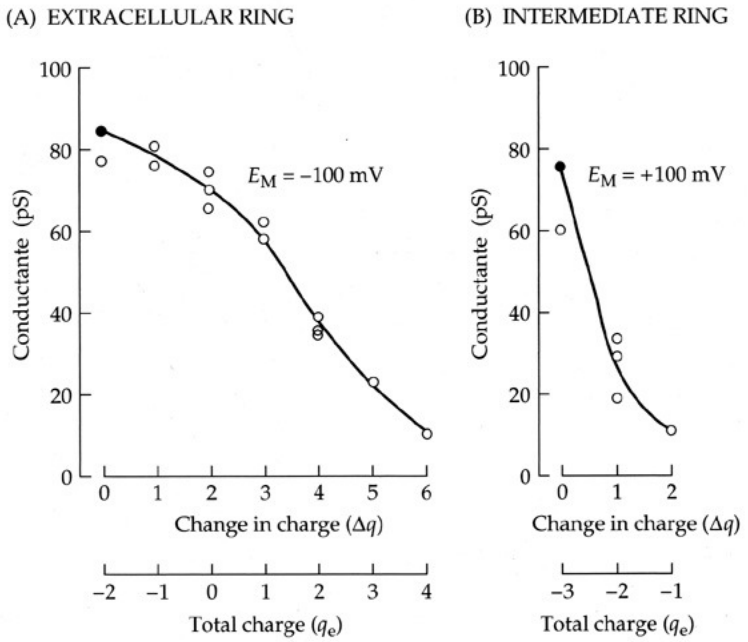
The channel of the NAChR is formed by M2 transmembrane segments.

Three rings of negative charge in the pore control its permeation characteristics.

1. Extracellular and cytoplasmic rings contain 2 and 3 charges; they appear to attract cations to the channel mouth.
2. An intermediate ring near the narrow spot in the pore contains 3 charges. It is more important in determining channel conductance than the external rings.

(Note the rings of positively charged residues; presumably these are rotated on the M2 α helix out of the channel pore.)



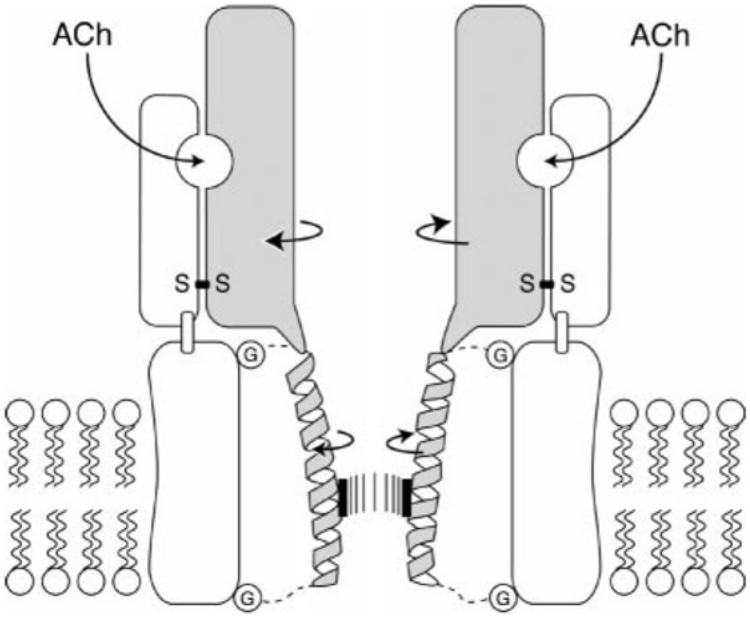


Originally thought to be in the pore, but probably not.

(Hille, 2001)

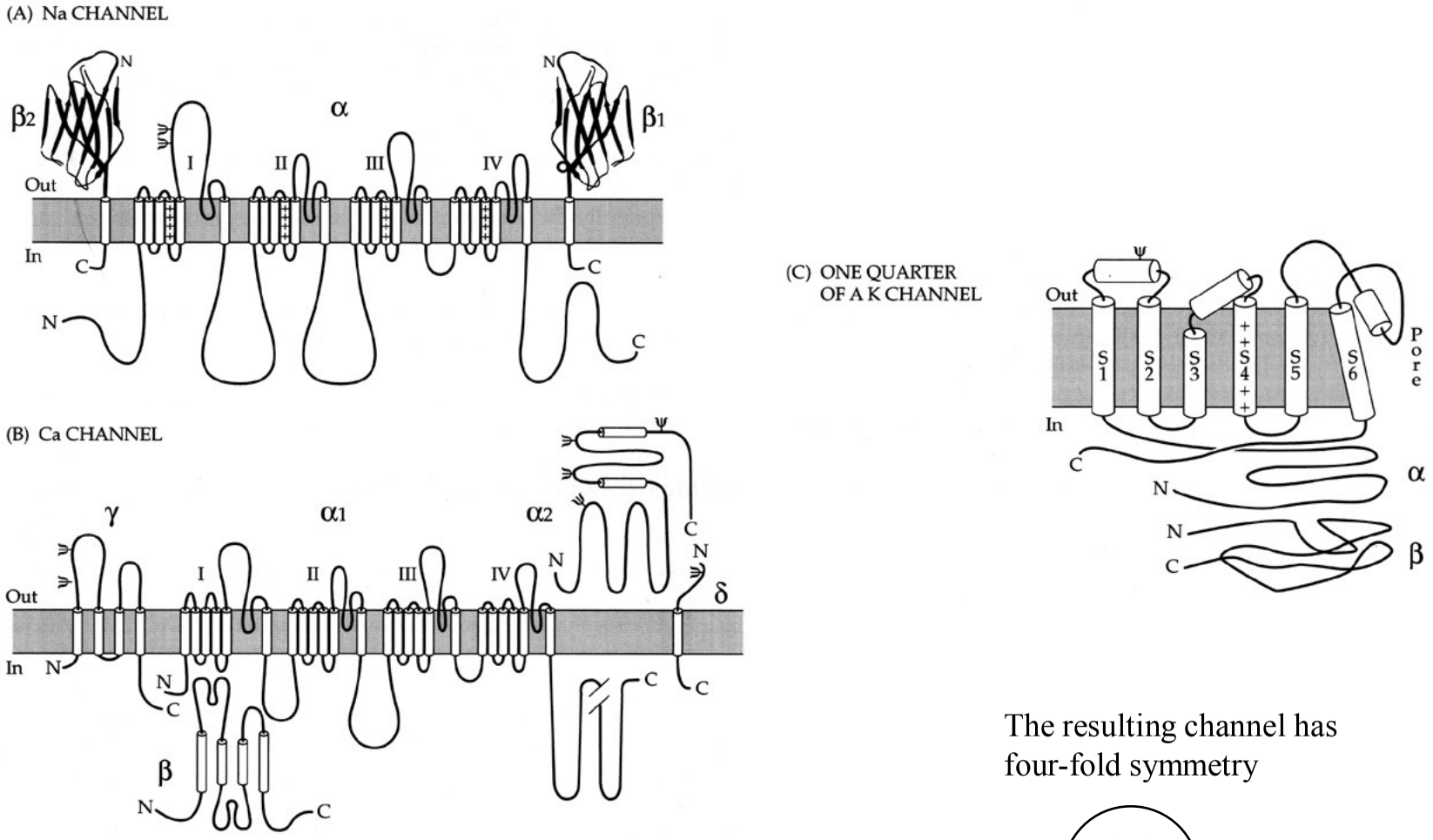
Model for gating of the NAChR. Binding of ACh causes the gray part of the extracellular domain to rotate as drawn, producing a rotation and realignment of the M2 segments increasing or decreasing the size of the pore in the vicinity of V255 and L251.

2010, lect #5



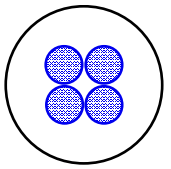
Miyazawa et al. 2003

Voltage-gated cation channels consist of four subunits, each of which has 6 transmembrane segments and a **pore loop**. In sodium and calcium channels, the four subunits are part of the same molecule. In potassium channels, they are different molecules. Also shown are β and γ subunits, separate molecues that bind to the channel and change its properties.

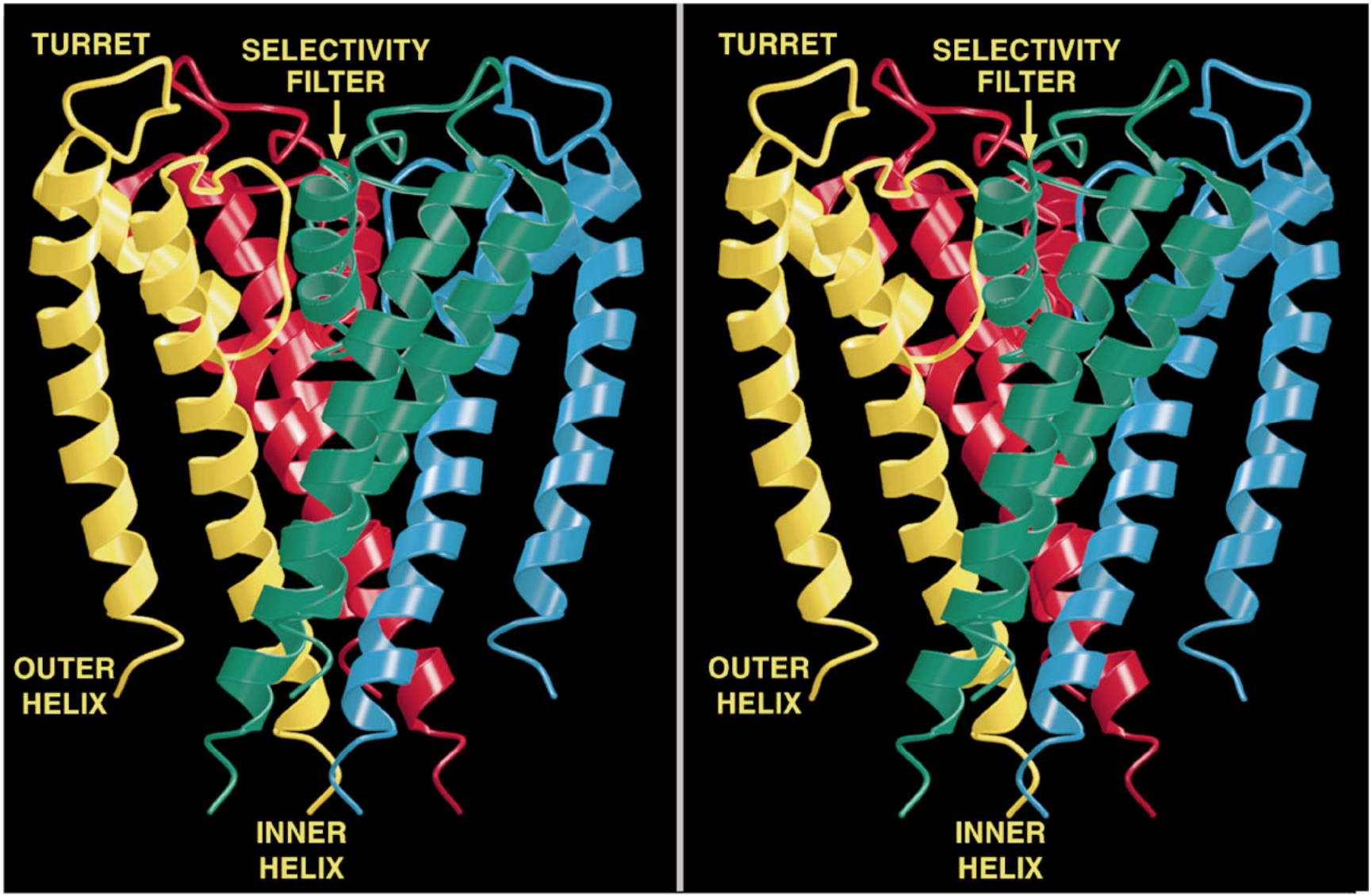


(Hille, 2001)

The resulting channel has four-fold symmetry

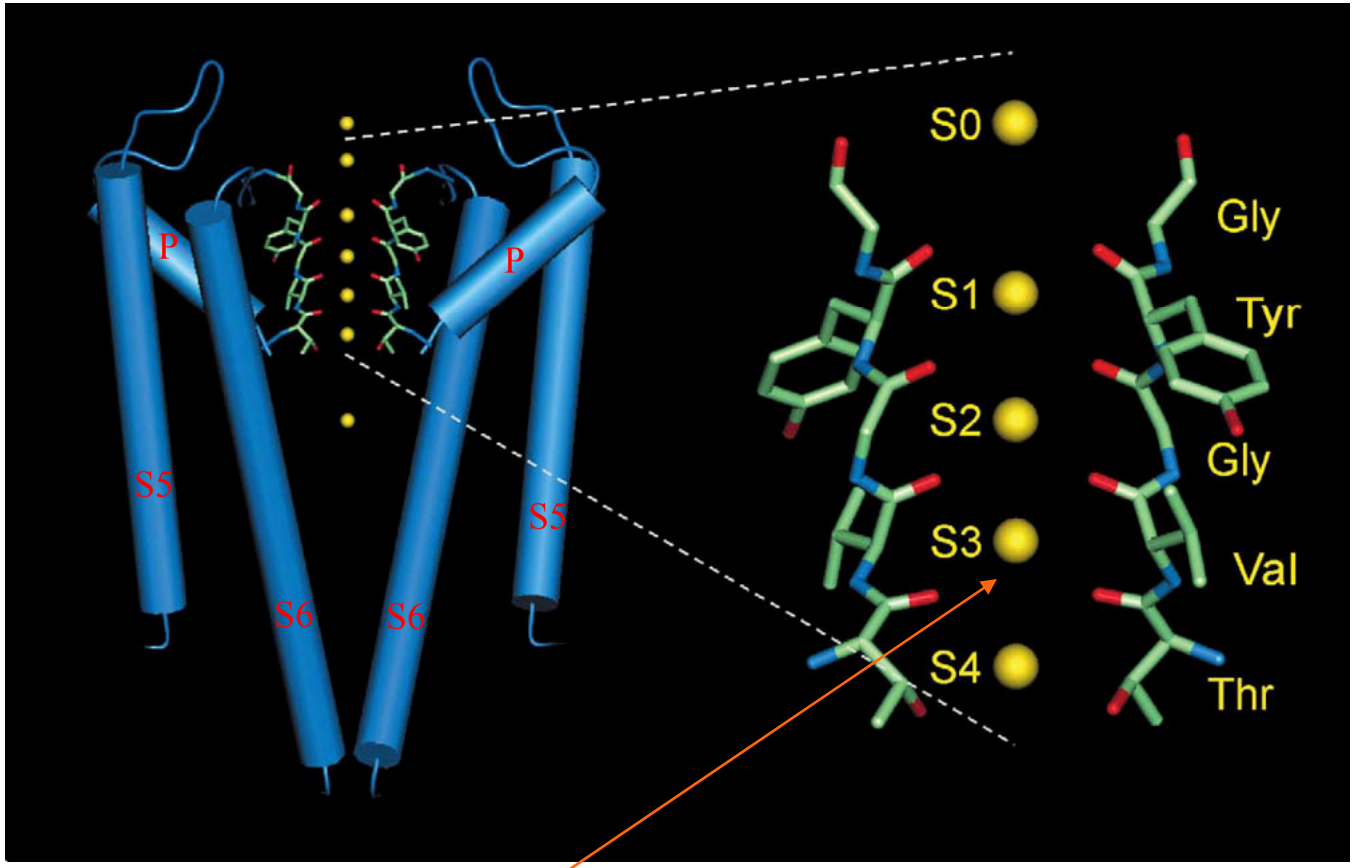


The structure of potassium channels has been inferred from the KcsA channel, a bacterial K channel having only the S5, S6, and P domains. The pore is formed by the S6 and P domains.



(Doyle et al, *Science* 280:69, 1998)

The **KcsA selectivity filter** consists of five amino acids (TVGYG) on the S6-P connector. The potassium ions (yellow balls) interact with the peptide backbone carbonyl oxygens (small red balls), not the side chains. There are four stable positions for K^+ ions (S1-S4), plus a fifth, entry, position. Only two are occupied at a time, because of electrostatic interaction among the K^+s. **Selectivity** is determined by the properties of K^+ ion interaction with this structure.



Note that the ions are in **single file.** This fact is consistent with a number of previous biophysical observations

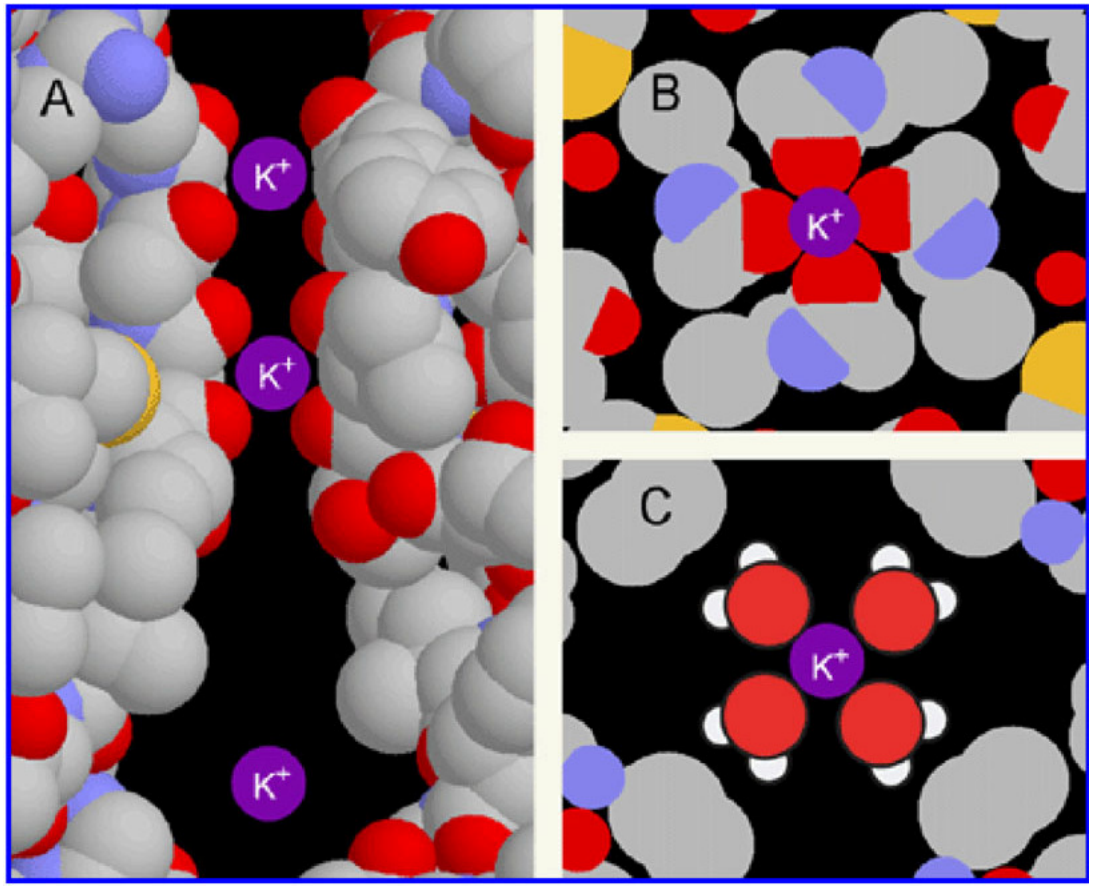
Sansom et al. 2002)

How might the KcsA selectivity filter work?

Potassium ions (dehydrated) are stabilized in the pore region (A) by negatively charged carbonyl groups from the protein making up the wall of the selectivity filter.

Presumably, the K^+ ions "just fit" into the cross section of the pore (B). The electrostatic binding between the K^+ and the carbonyls replaces the H-bonding in the aqueous environment, facilitating entry of K^+ into the channel.

In the cavity of the KcsA channel, there is room for the potassium ions to carry a hydration shell, facilitating transport of ions in and out of the channel on the cytoplasmic side.



At the level of thr 75

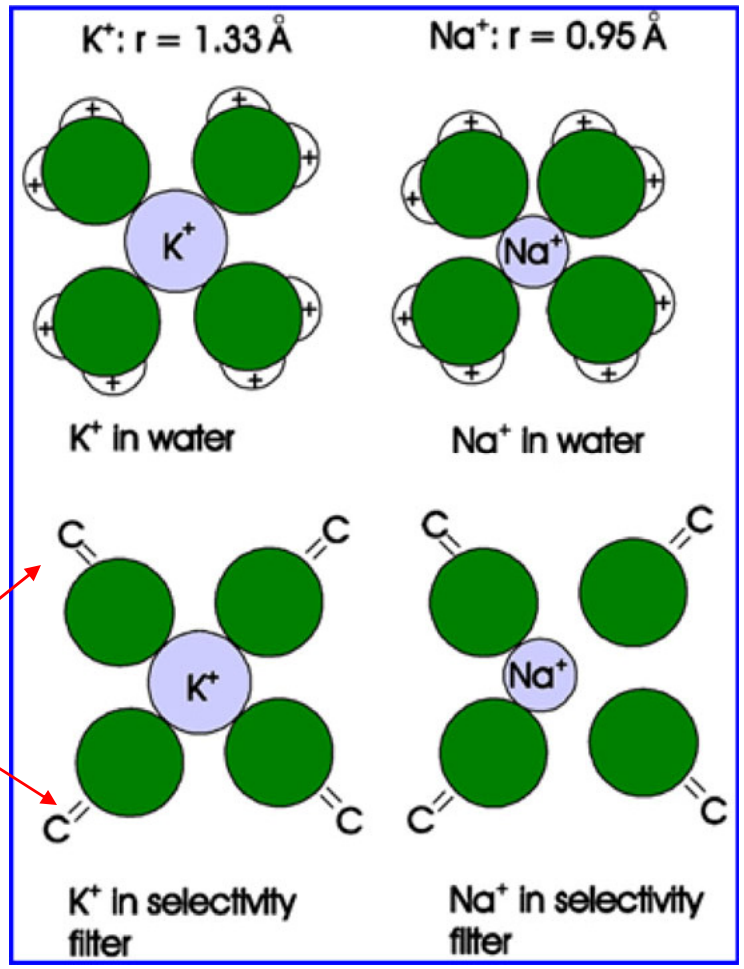
In the aqueous cavity

Armstrong, 2003

By contrast, Na^+ ions, which have a smaller ionic radius, do not bind efficiently to all four carbonyls, as shown in the schematic cross sections at right.

Because the binding energy varies inversely with the distance between charges, Na is less stabilized in the selectivity filter than K, and is less likely to escape from an aqueous hydration shell into the pore.

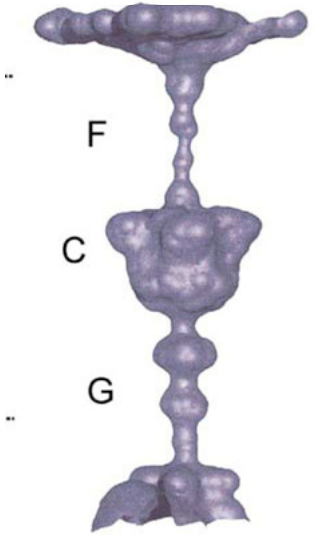
Interactions with surrounding moieties stiffen the selectivity filter so it can't collapse on the Na^+ ion



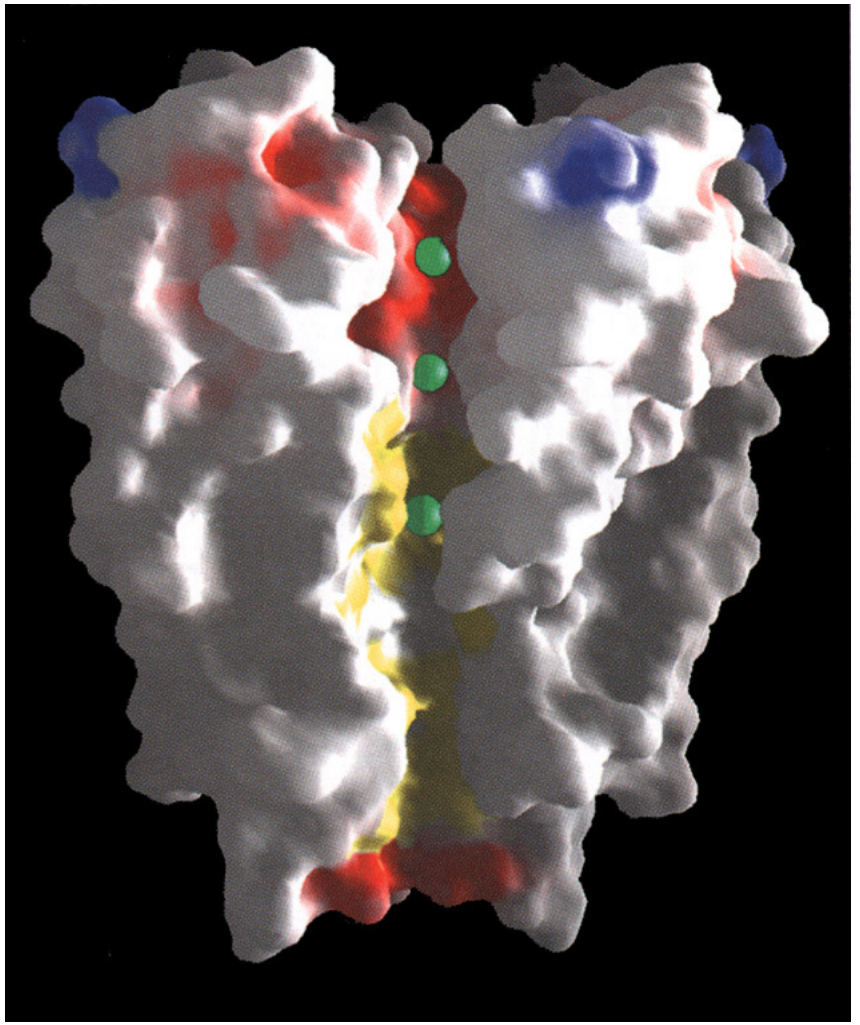
Armstrong, 2003

(Doyle et al, *Science* 280:69, 1998)

At right is a space-filling model of the KcsA channel, showing the pore. Ions (green balls) tend to occupy three sites in the channel, two in the **selectivity filter** and one in a **pool of water** in the center of the channel. Note the negative charges (red) at the two ends of the pore, which attract ions to the channel's entrance. Subsequently, it has been shown that the hydrophobic (yellow) narrow spot on the cytoplasmic side of the channel is the **gate**.

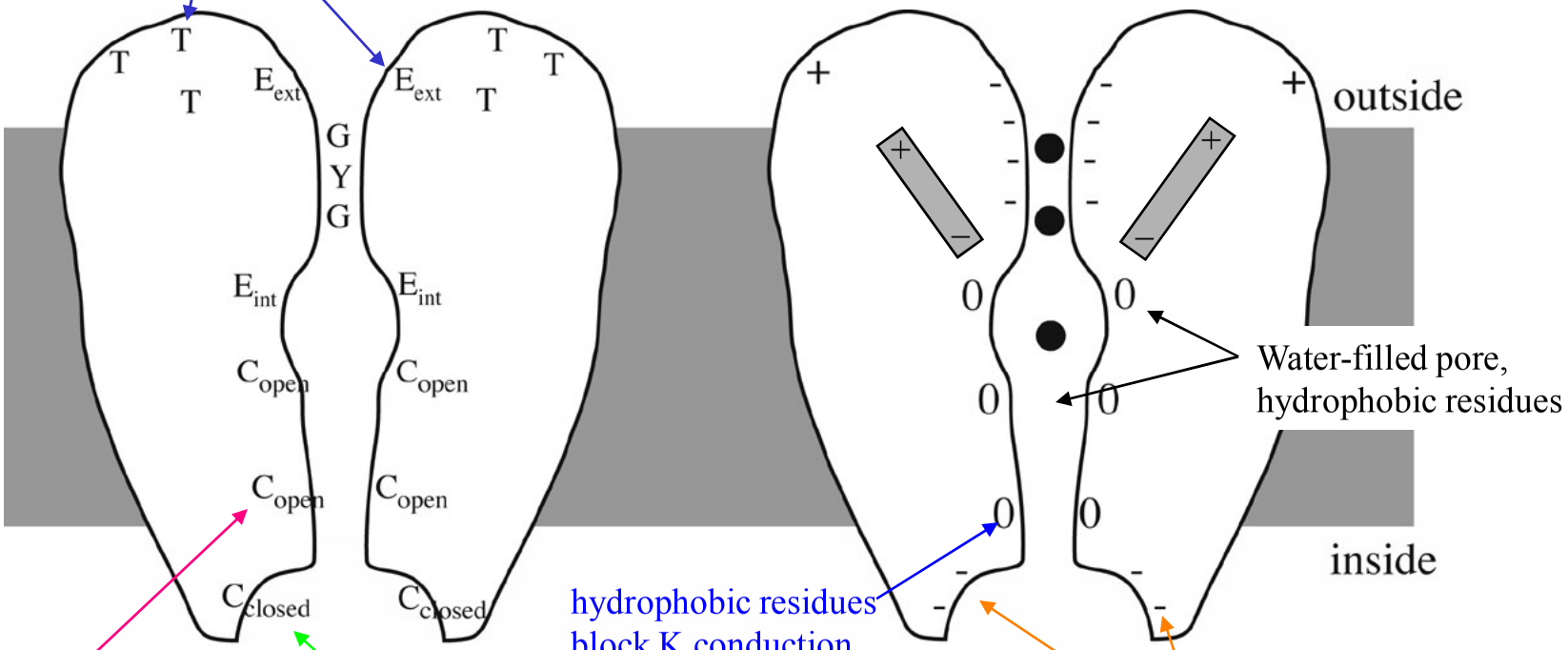


Sansom et al. 2002)



red – charge; blue + charge; yellow hydrophobic

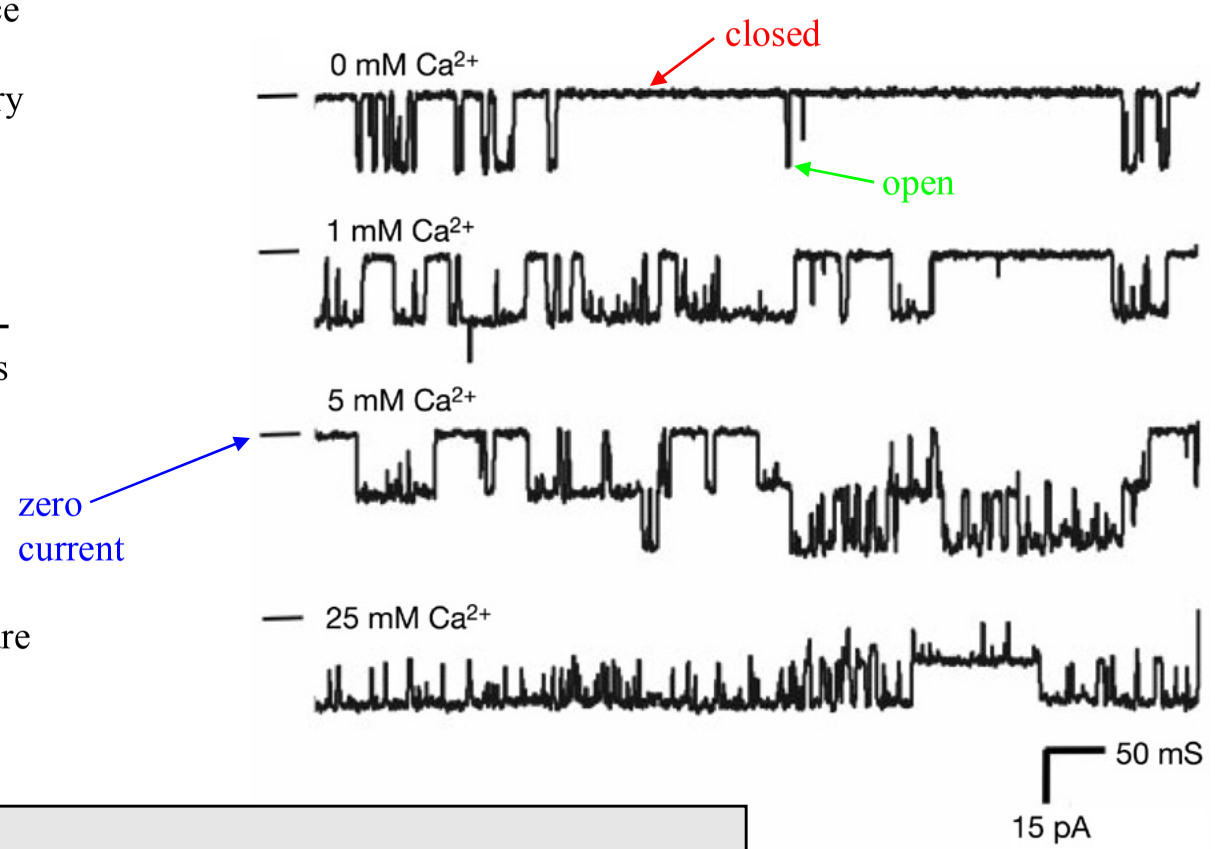
The structure of the KcsA channel is believed to be representative of all potassium channels because chimeras of full voltage-gated potassium channels S1-S4 segments with KcsA S5-P-S6 segments work normally (including gating).

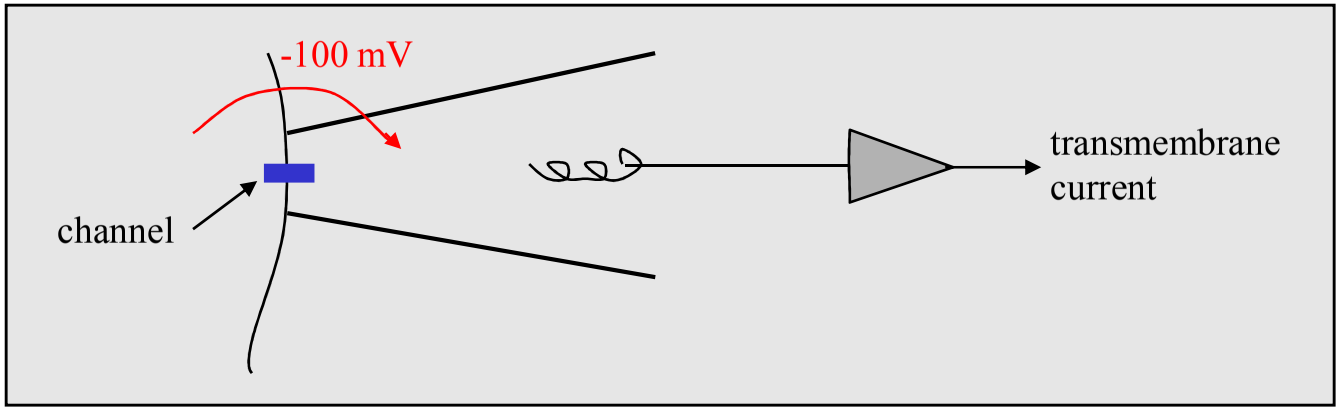


Gating refers to the fact that channels change their conductance state, from open to closed. The transitions occur sharply, over very fast time scales.

Generally gating is influenced by membrane potential (**voltage-gated**) or by some ligand (**ligand-gated**). In this case, the channel is more likely open as the Ca^{++} concentration increases.

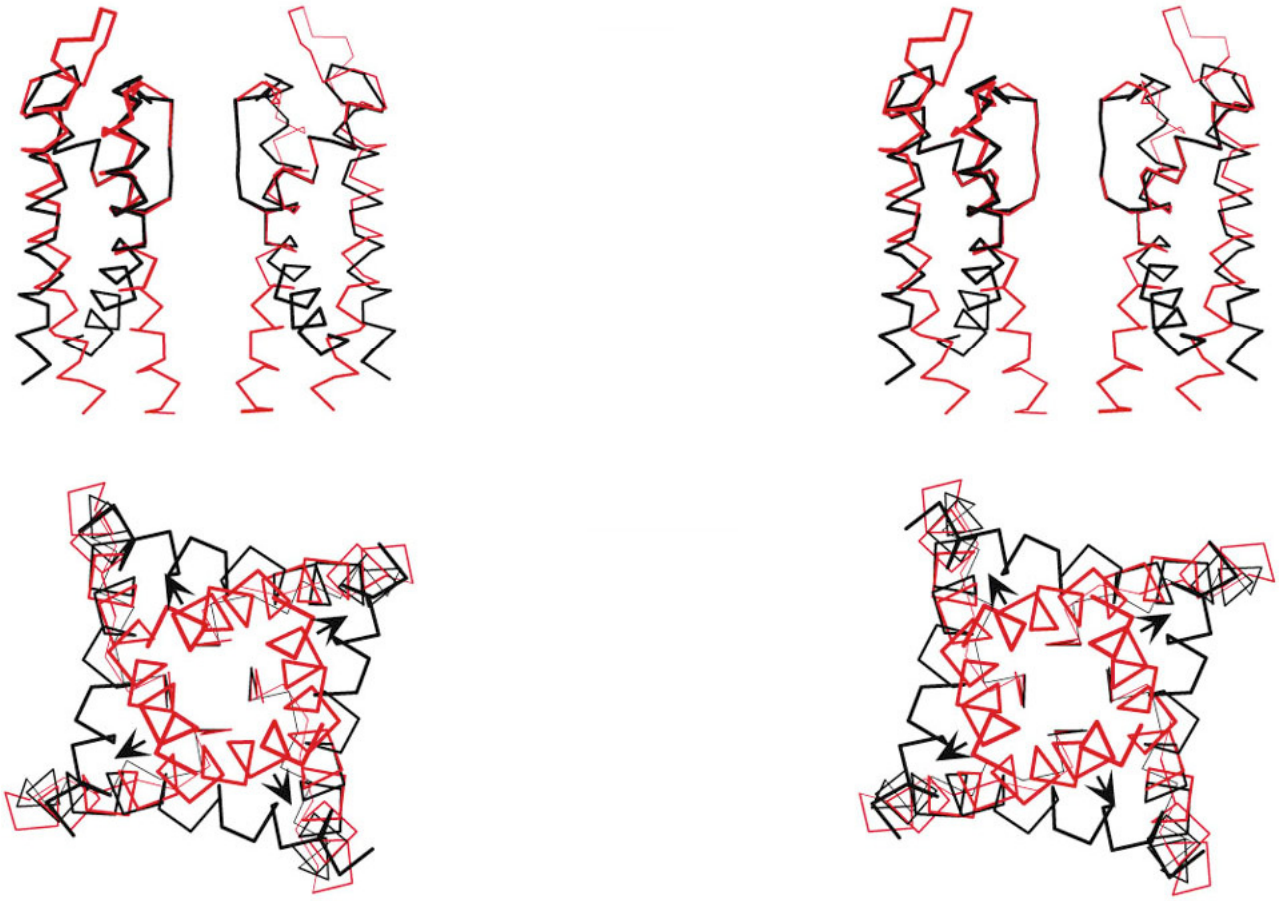
These data are from another bacterial channel, the MthK channel, that has the same structure as the KscA channel.





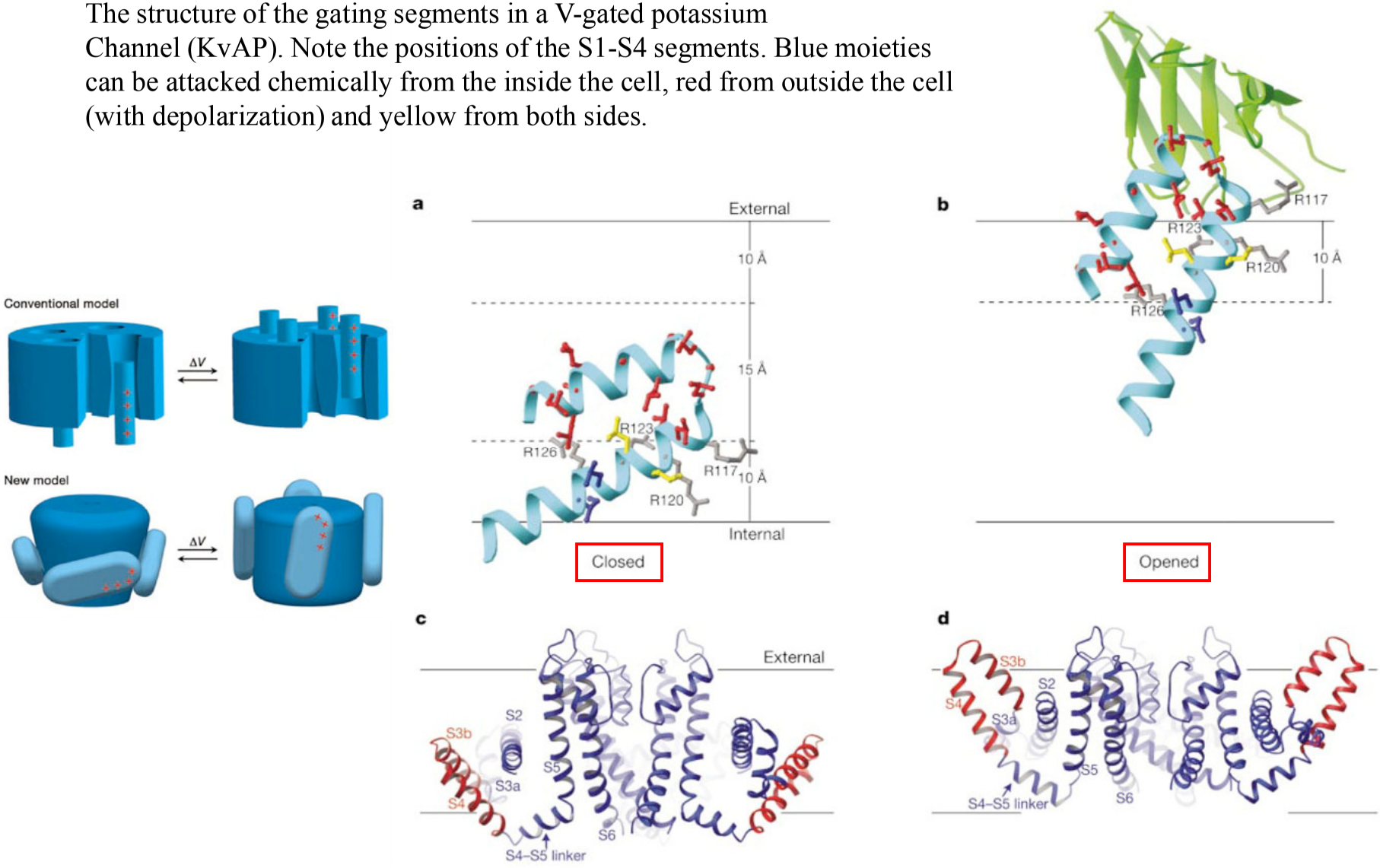
Jiang et al., Nature 417:515 (2002).

For the KcsA-like potassium channels, the gate opens by a splaying of the S5 and S6 domains, thus opening the pore at the inner side of the membrane. Below the KcsA channel (red) is shown in comparison to a similar bacterial channel, MthK, which is gated open by calcium. The S6 domain hinges at a glycine residue at the point shown.



Jiang et al., Nature 417:523, 2002.

The structure of the gating segments in a V-gated potassium Channel (KvAP). Note the positions of the S1-S4 segments. Blue moieties can be attacked chemically from the inside the cell, red from outside the cell (with depolarization) and yellow from both sides.



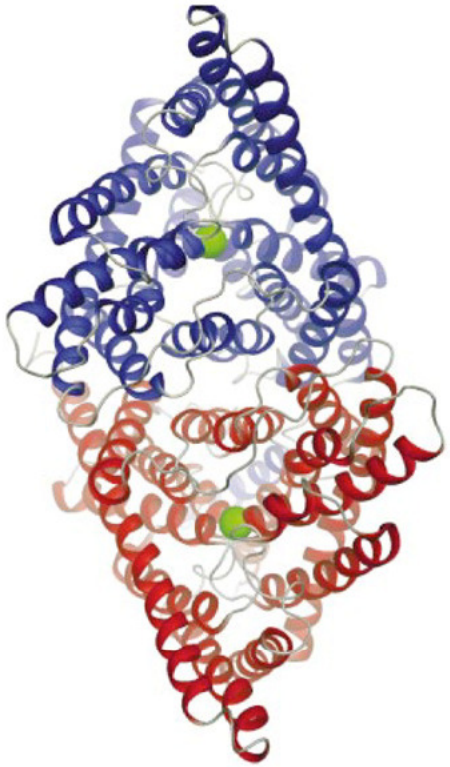
Jiang, Nature, 2003a,b

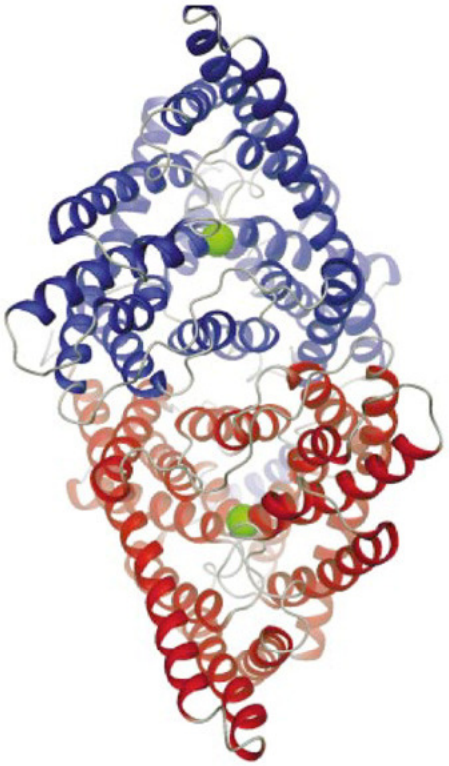
The structure of other channels can be quite different.

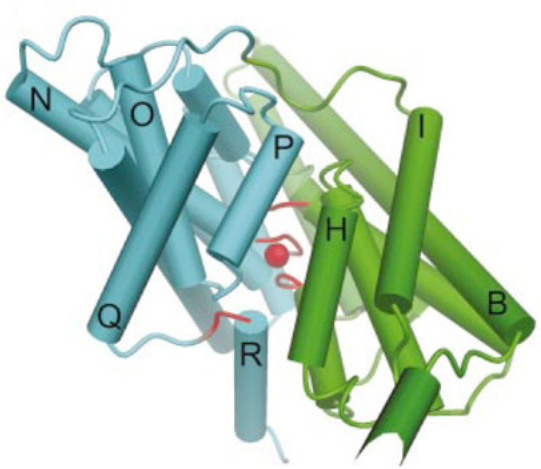
The stereo pairs at right show a type of **chloride channel** which consists of two channels in parallel.

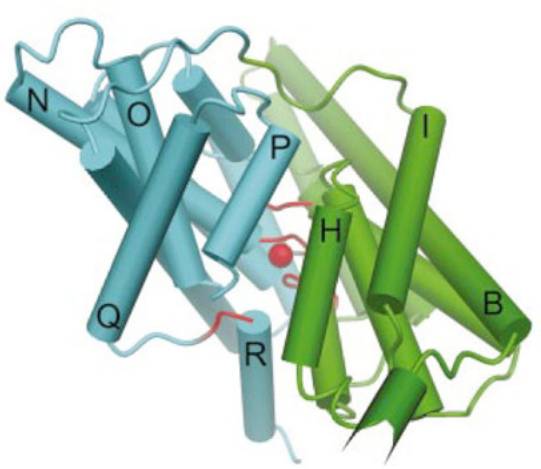
Note that the structure is completely different from the voltage-gated cation channels discussed previously.

The channel is voltage-gated, presumably by electrostatic attraction of Cl^- ions into the channel where they displace a glu residue that blocks the extracellular access to the pore.

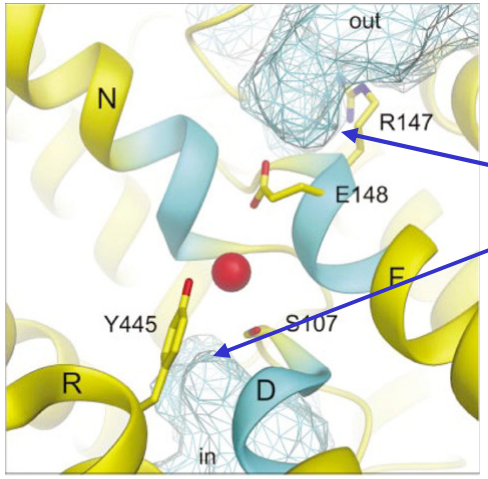


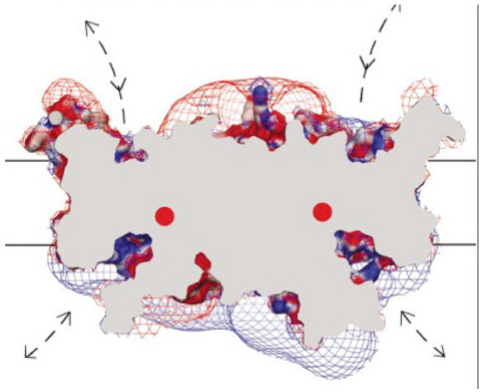






Dutzler et al. Nature 415:287 (2002)

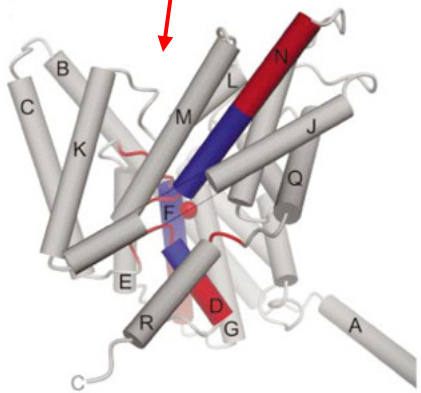


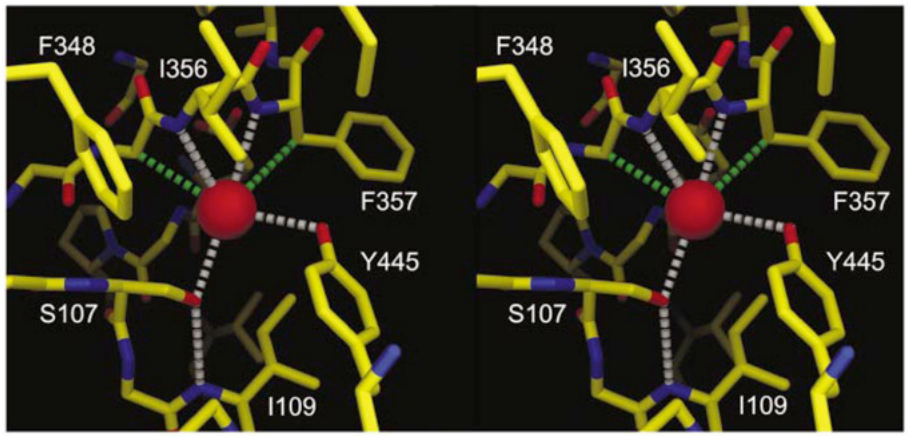


Cl^- moves between two pool of water that extend into the channel. E148 is the glutamate that forms the channels gate.

In between, the ion is stabilized by the dipole fields of three alpha helices . . .

. . . and by electrostatic interactions with polar residues and the peptide bonds of the protein backbone. The stereo pair shows a Cl^- ion (red ball) making electrostatic interactions (dashed white lines) with several parts of the molecule.





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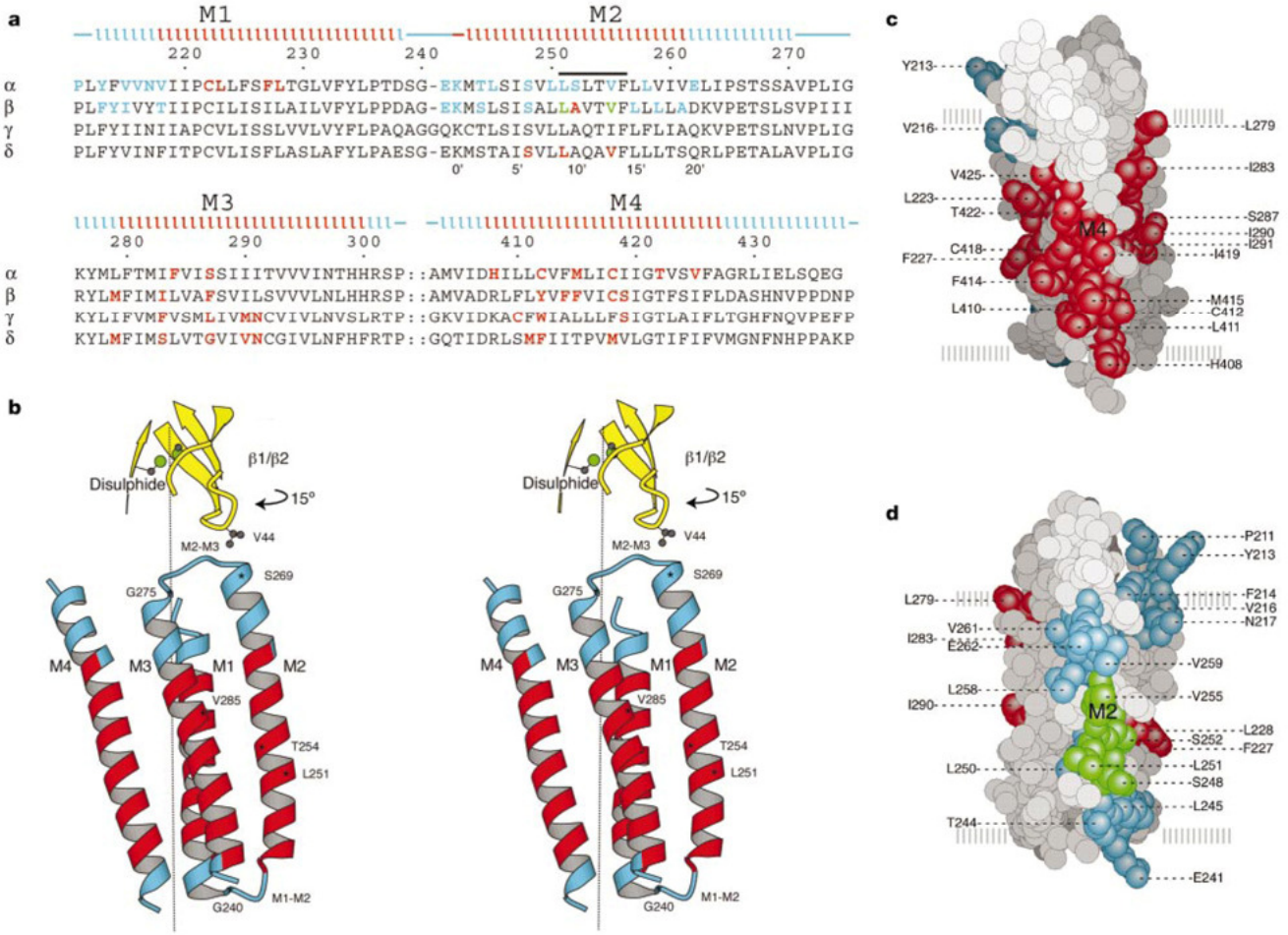
Details of the arrangement of one M1-M4 sector. Red are hydrophobic sites that interact with the membrane. Blue are hydrophilic sites and green are neutral sites in the pore.



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