Membrane transporters and ion channels

Hille, Chapters 10, 13, 17.

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

J. Abramson, I. Smirnova, et al. (2003) Structure and mechanism of the lactose permease of *Escherichia coli.* *Science* 301:610-615.

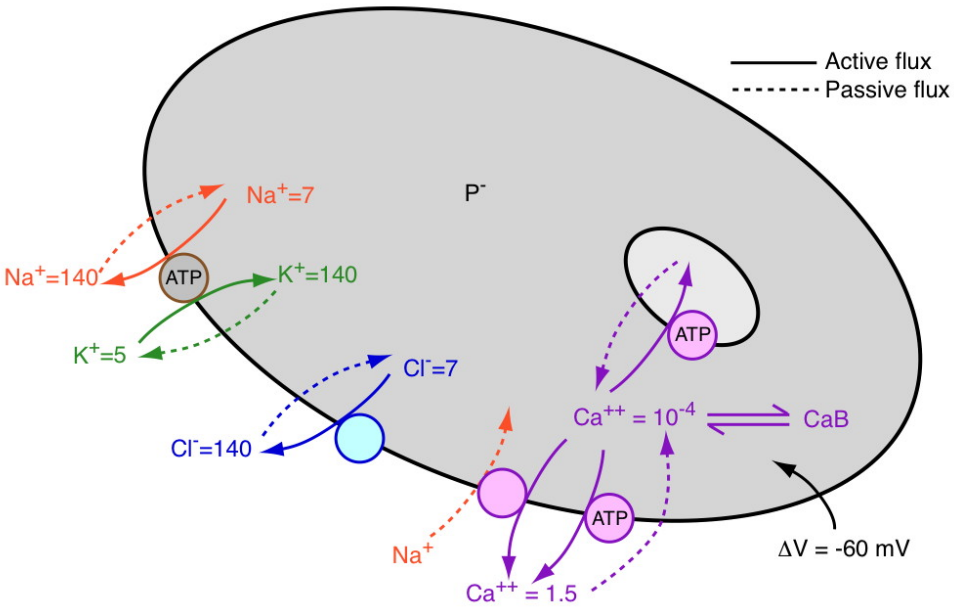
J.D. Horisberger (2004) Recent insights into the structure and mechanism of the sodium pump. *Physiology* 19:377-387.

T. Shinoda, H. Ogawa, et al. (2009) Crystal structure of the sodium-potassium pump at 2.4 Å resolution. *Nature* 459:446-450.

Doyle et al. (1998) The structure of the potassium channel: Molecular basis of K^+ conduction and selectivity. *Science* 280:70-77.

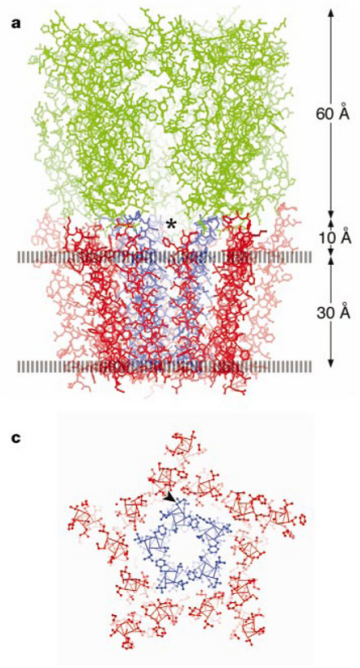
Swartz, K.J. (2008) Sensing voltage across lipid membranes. *Nature* 456:18-25.

The cellular steady state:

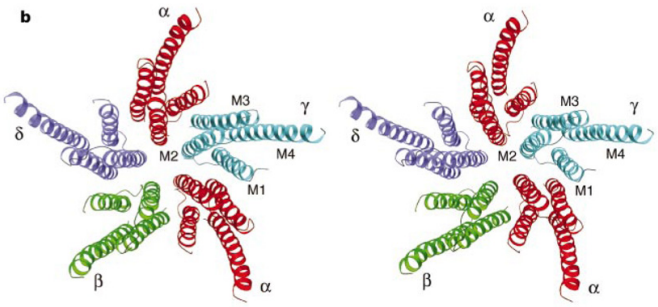


In the steady state, each ion's active and passive fluxes balance, so that there is no net transport of the ion through the membrane.

A channel with a simple pore structure is the nicotinic acetylcholine receptor. Its structure is shown below from 4 Å resolution electron diffraction. 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).



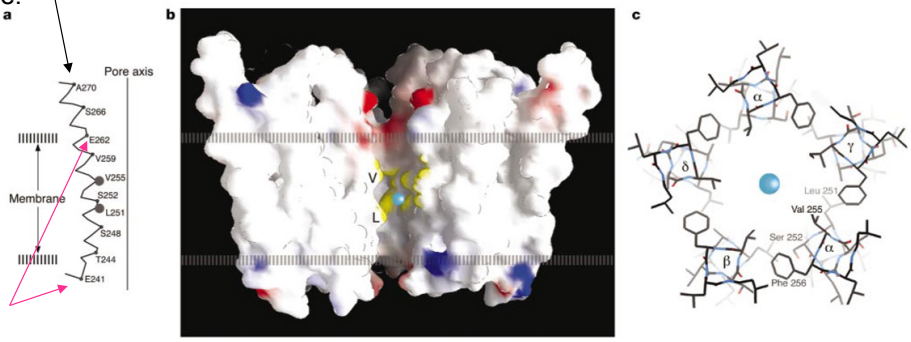
The channel consists of five protein subunits, which associate in the membrane. The transmembrane part of each contains alpha helices called M1-M4, but the extracellular domain is mostly beta sheets.



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.

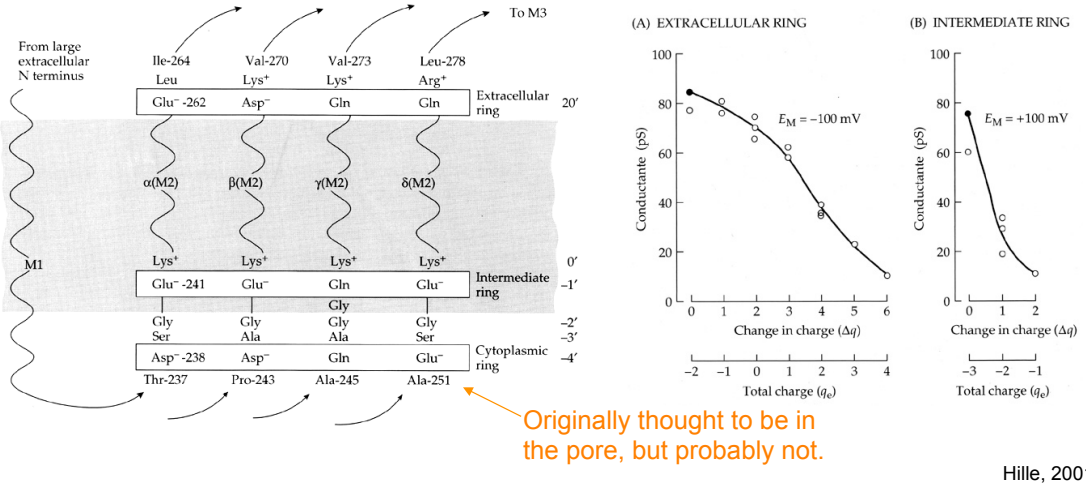
Three rings of negative charge in the pore control its permeation characteristics (non-selective cation channel).

1. The extracellular ring contains 2 charges; it attracts cations to the extracellular channel mouth.

2. An intermediate ring near the intracellular end of the pore contains 3 charges. It is more important in determining channel conductance than the external ring.

3. A cytoplasmic ring was originally believed to be in the pore, but is not in the actual structure.

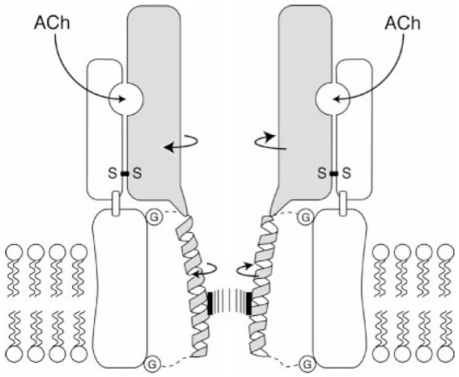
(Note the rings of positively charged residues; these are rotated on the M2 α helix out of the channel pore.)



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

Hille, 2001

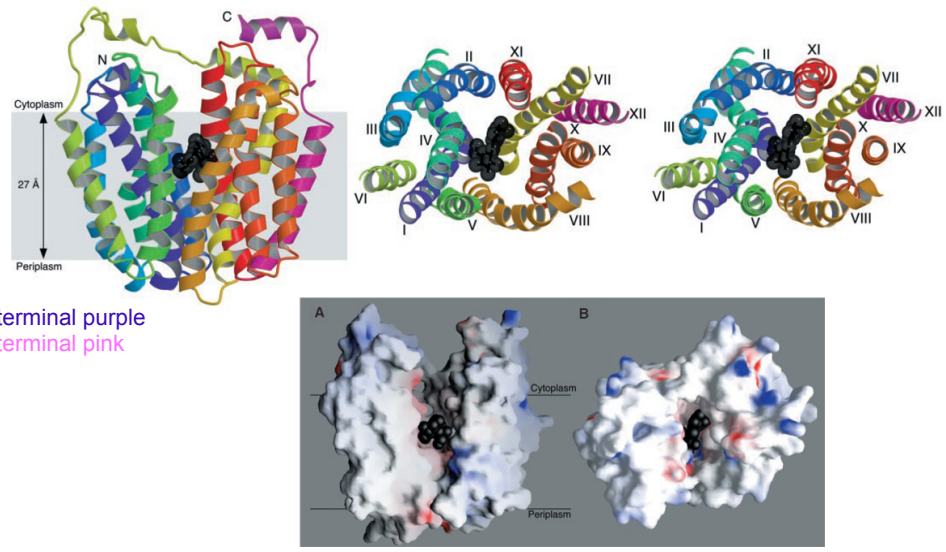
Model for gating of the NAChR. Binding of ACh causes the gray part of the extracellular domain to rotate as drawn, producing a rotation and realignment of the M2 segments increasing or decreasing the size of the pore in the vicinity of V255 and L251.



Miyazawa et al. 2003

Structure of a bacterial transporter, LacY, which transports lactose into the cell using energy stored in the gradient of H^+ ions.

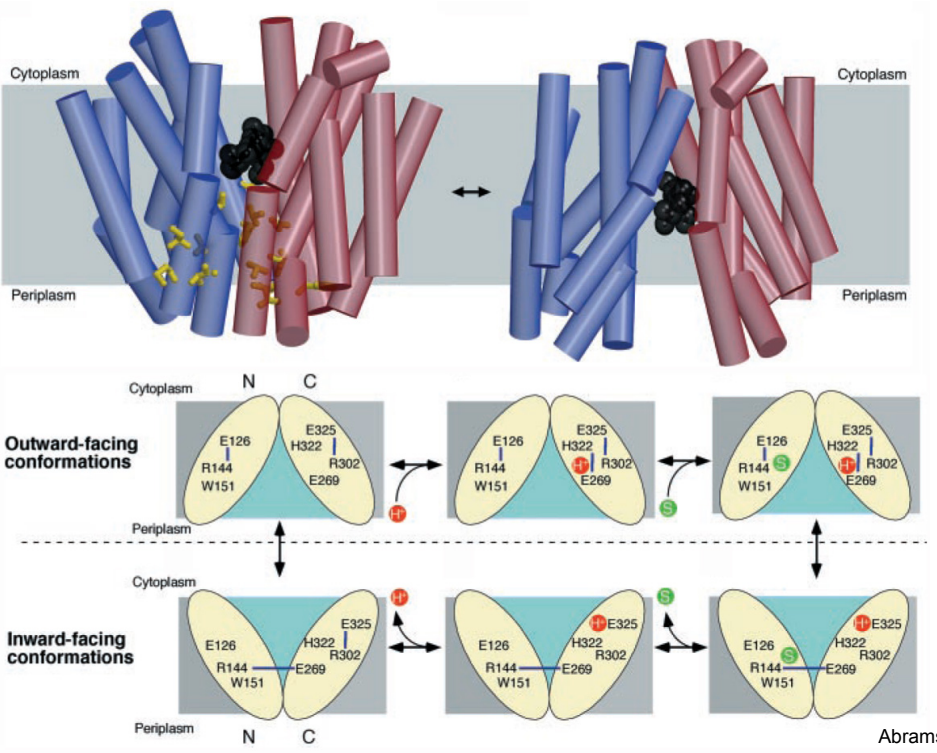
The molecule has 12 transmembrane α helices forming a cavity. The solved version was a mutated form of the molecule that is thought to be trapped in the structure with the cavity open to the cytoplasm. It is shown with a high-affinity substrate in the transport cavity.



N-terminal purple
C-terminal pink

Abramson et al. 2003

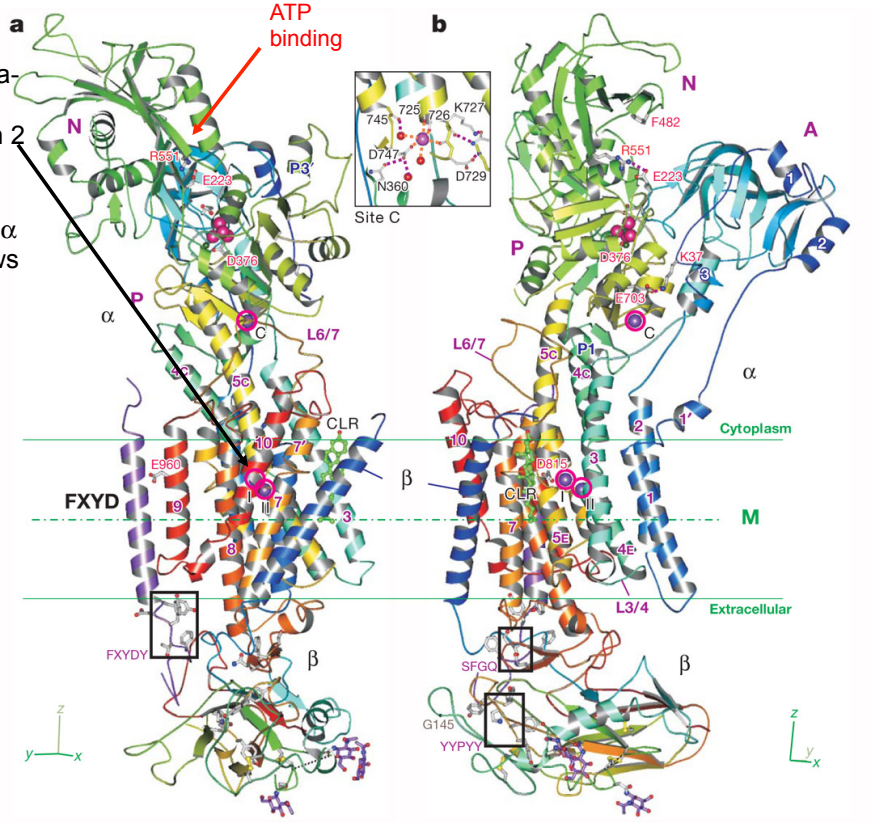
Presumed transport mechanism. A cycle involving H^+ binding, lactose binding, translocation, unbinding, and reverse-translocation.



The blue lines are H bonds.

Abramson et al. 2003

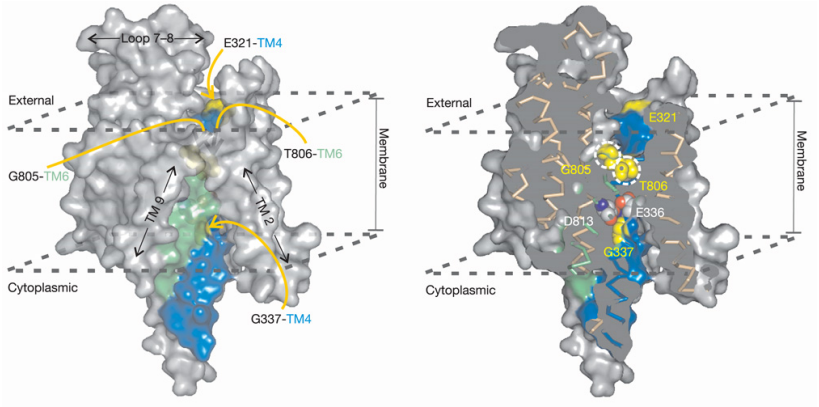
The structure of a Na-K ATPase with the molecule frozen with 2 K^+ ions bound. Two subunits of the enzyme are shown (α and β) from two views about 90˚ apart.



N-terminal blue
C-terminal red

Shinoda et al. 2009

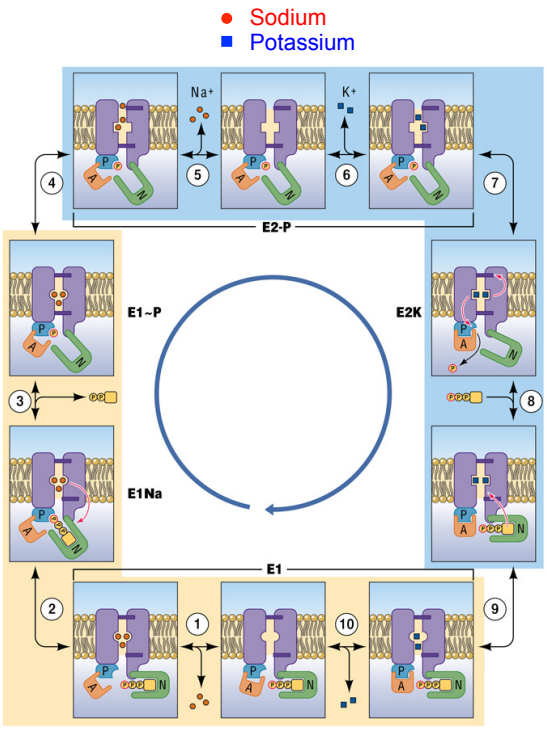
The structure contains an internal-facing and external-facing vestibule. Presumably, these are the binding sites for Na and K, but the nature of occlusion is not clear.



Reyes and Gadsby 2006

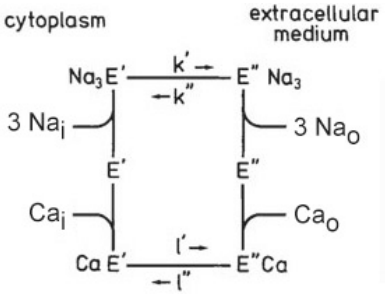
The sequence of steps in the Na-K ATPase is complex, involving separate transport of Na out, K in, and ATP cleavage.

Note the gates (black) that occlude the Na and K during the transport step.



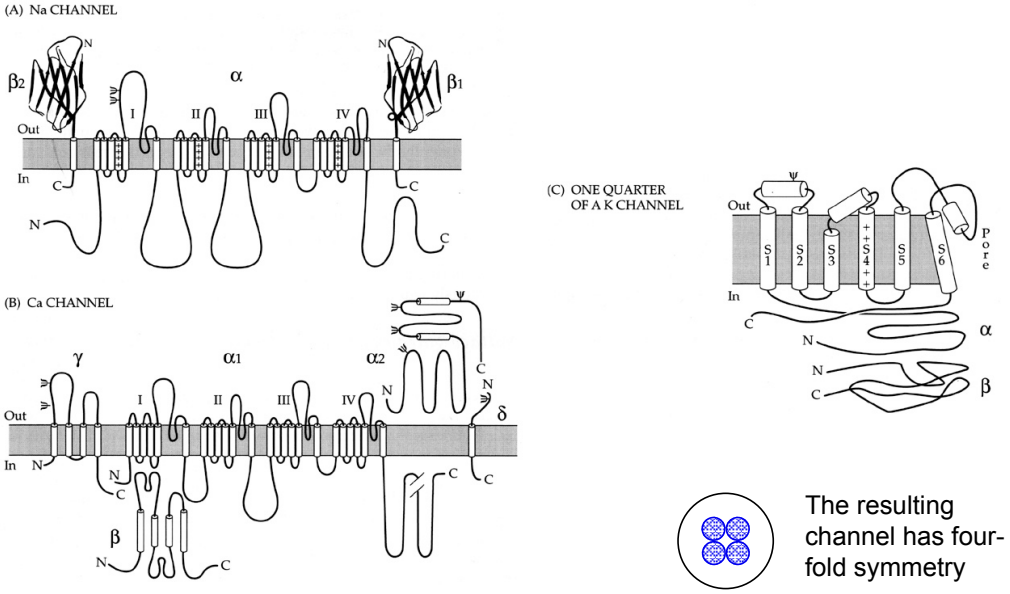
Horisberger, 2004

Motivated by the model for Na-K ATPase in the previous slide, Läuger and others have analyzed a slightly simpler transporter, the Na-Ca cotransporter with a similar scheme (discussed later).



P. Läuger 1987

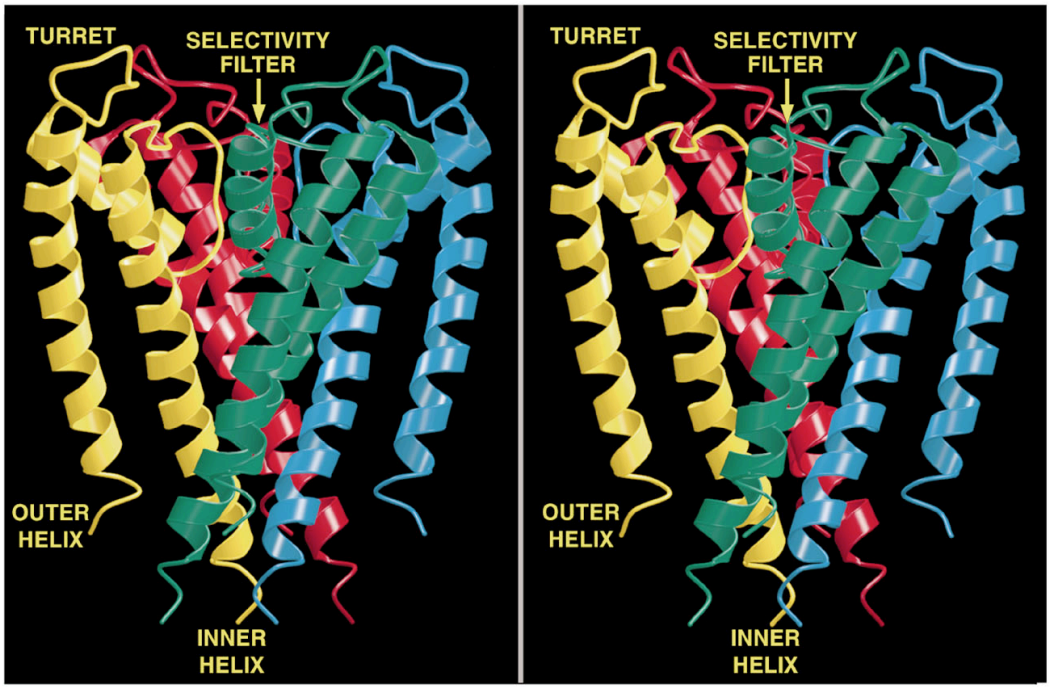
Voltage-gated cation channels consist of four subunits, each of which has 6 transmembrane segments and a **pore loop**. In sodium and calcium channels, the four subunits are part of the same molecule. In potassium channels, they are different molecules. Also shown are additional subunits, separate molecules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



The resulting channel has four-fold symmetry

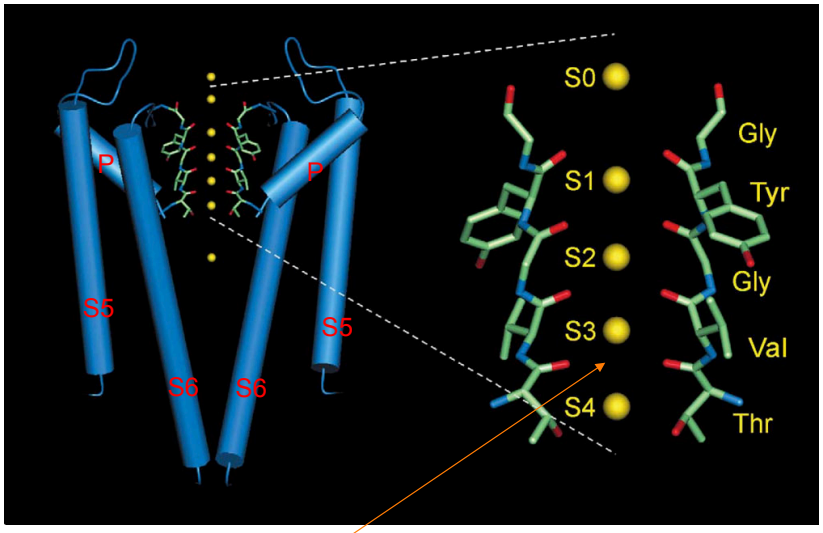
(Hille, 2001)

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, 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

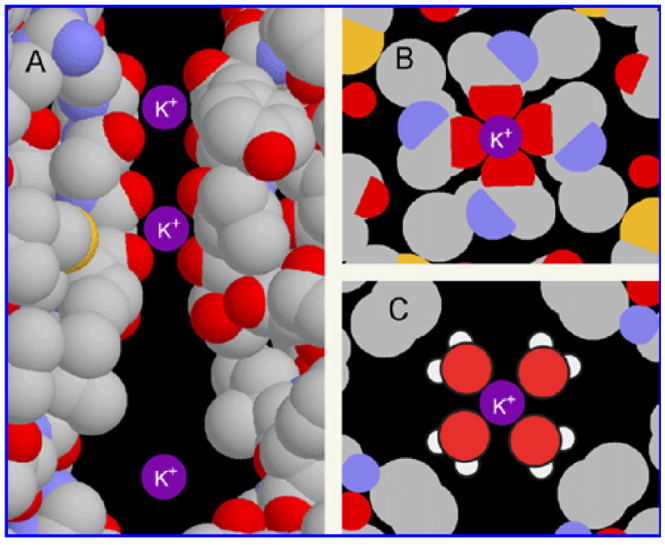
Sansom et al. 2002

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 aqueous H-bonding.

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

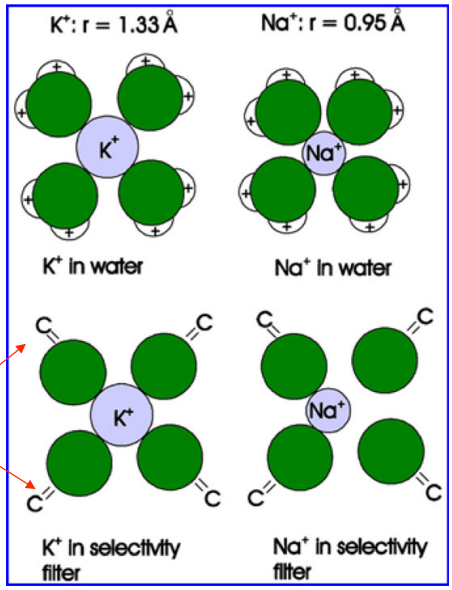
In the aqueous cavity

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.

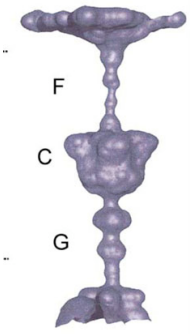
Because the binding energy varies inversely with the distance between charges, Na is less stabilized in the selectivity filter than K, and is less likely to escape from an aqueous hydration shell into the pore.

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

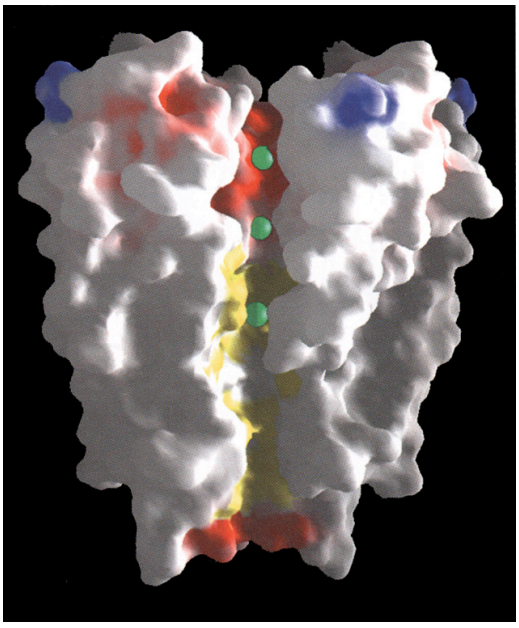


Armstrong, 2003

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**.



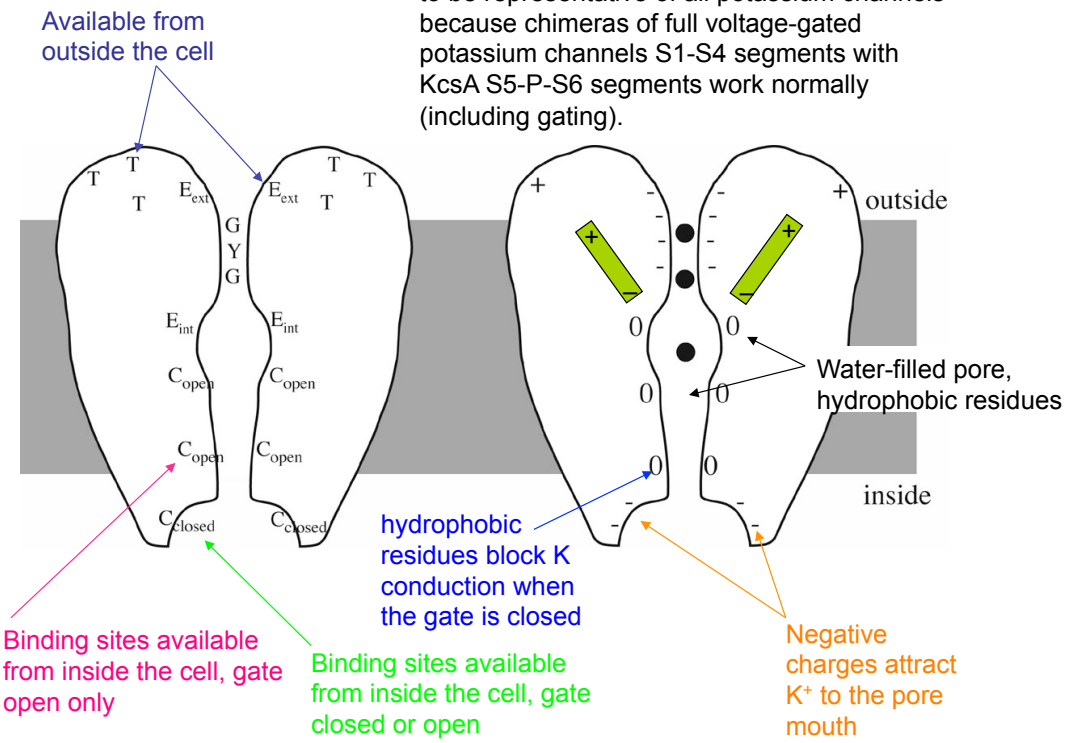
Sansom et al. 2002)



red – charge; blue + charge; yellow hydrophobic

Doyle et al, 1998

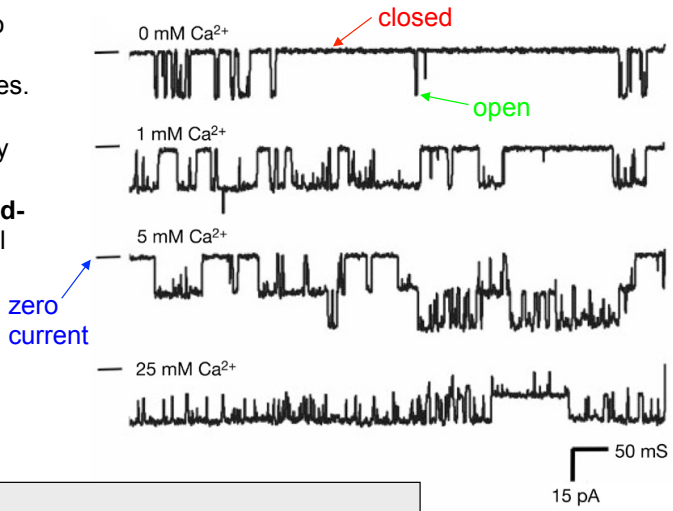
The structure of the KcsA channel is believed to be representative of all potassium channels because chimeras of full voltage-gated potassium channels S1-S4 segments with KcsA S5-P-S6 segments work normally (including gating).

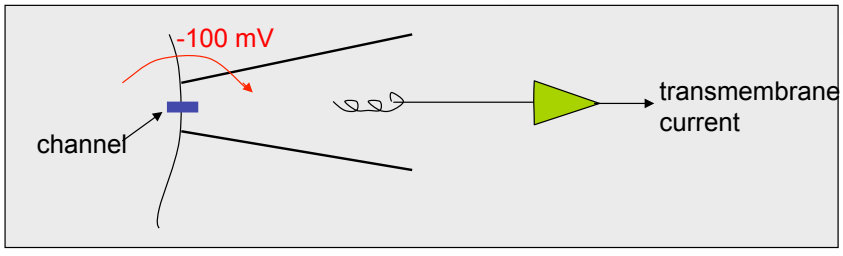


Gating refers to the fact that channels change their conductance state, from open to closed. The transitions occur sharply, over very fast time scales.

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.

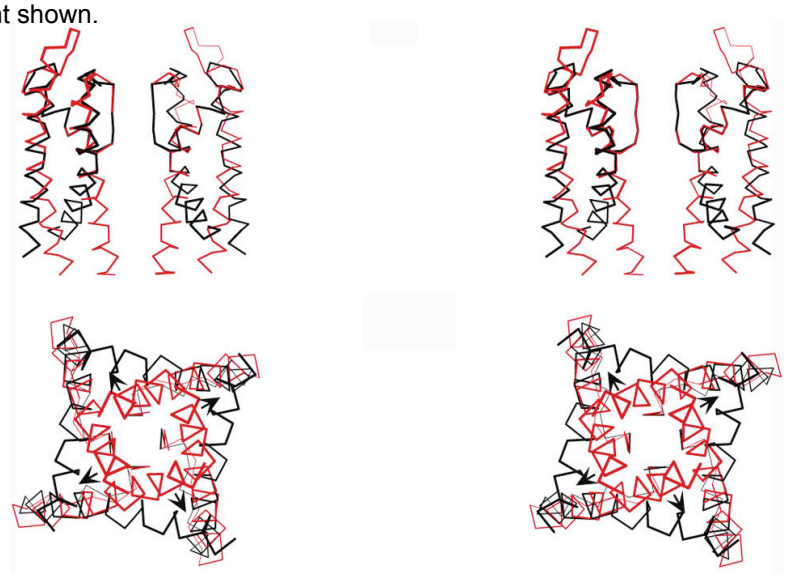
These data are from another bacterial channel, the MthK channel, that has the same structure as the KscA channel.





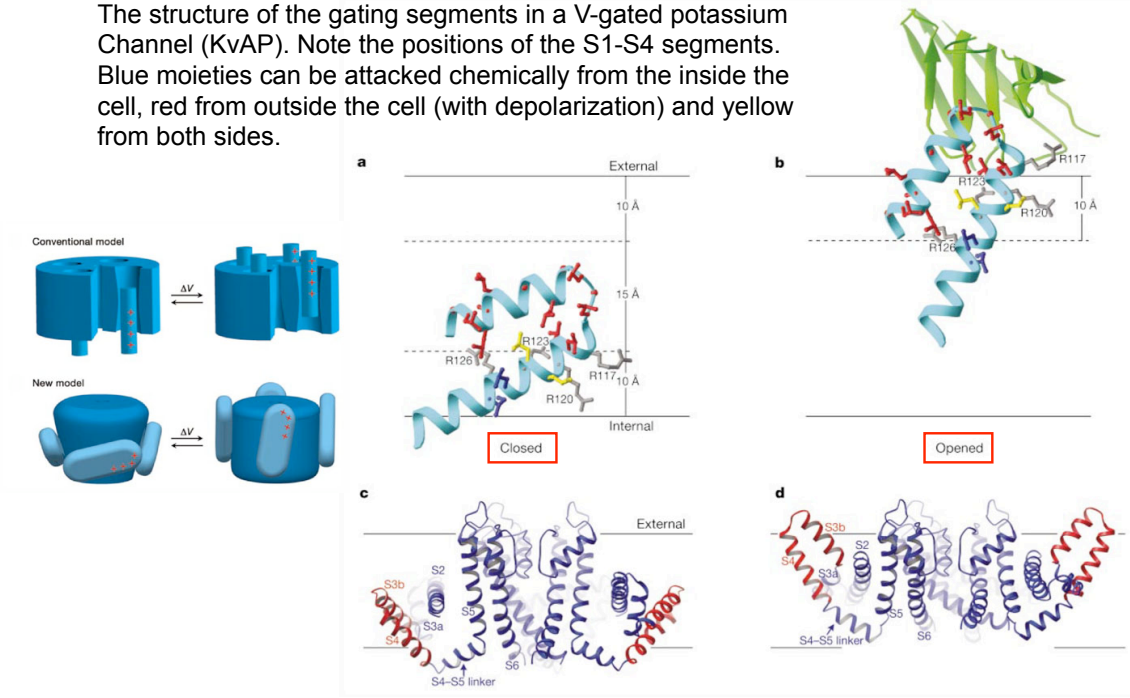
Jiang et al., 2002

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., 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.



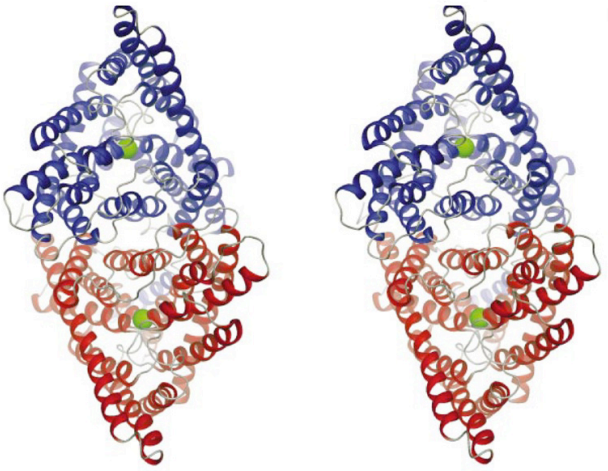
Jiang, 2003

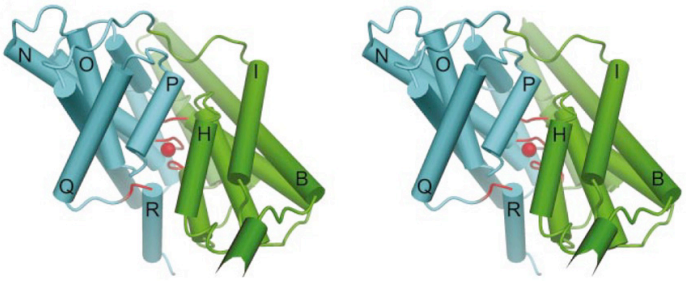
The structure of other channels can be quite different.

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

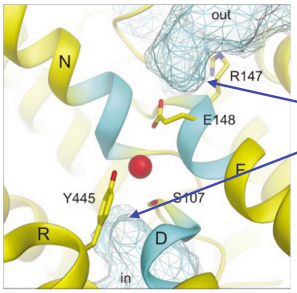
Note that the structure is completely different from the voltage-gated cation channels discussed previously.

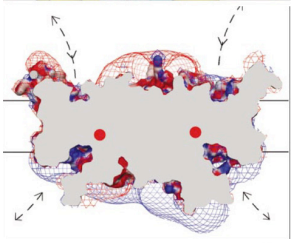
The channel is voltage-gated, presumably by electrostatic attraction of Cl^- ions into the channel where they displace a glu residue that blocks the extracellular access to the pore.





Dutzler et al. Nature 415:287 (2002)

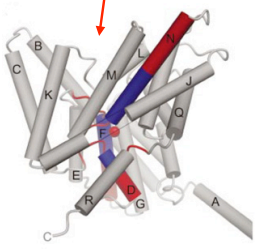


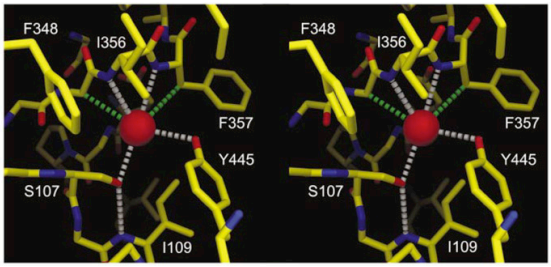


Cl^- moves between two pool of water that extend into the channel. E148 is the glutamate that forms the channels gate.

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

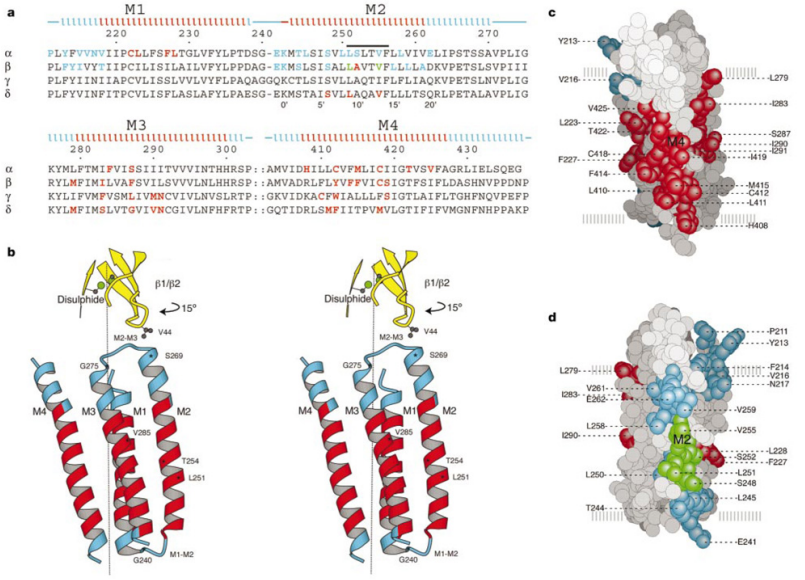
. . . and by electrostatic interactions with polar residues and the peptide bonds of the protein backbone. The stereo pair shows a Cl^- ion (red ball) making electrostatic interactions (dashed white lines) with several parts of the molecule.





Dutzler et al. Nature 415:287 (2002)

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.



Miyazawa et al. 2(

Na-K ATPase is a member of a gene family containing various cation transporters (including SERCA pumps in heart cells), Ca ATPase (PMCA), and others.

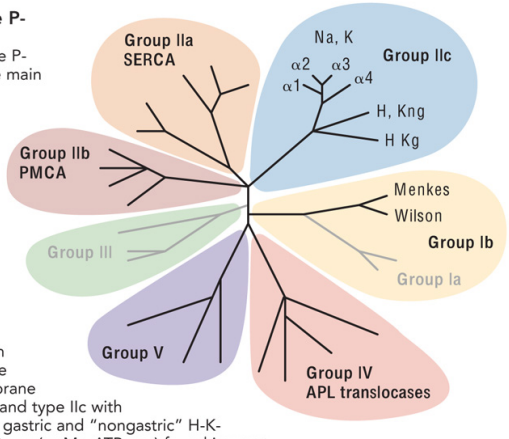


FIGURE 1. **Schematic phylogenetic tree of the P-ATPase family**
This simplified and schematic phylogenetic tree of the P-ATPase family illustrates the relationship between the main branches of this family. A more complete version of this tree can be found in the P-type ATPase Database internet site (http://biobase.dk/~axe/Patbase.html) maintained by K. B. Axelsen. The branches represent the genes present in the human genome (black lines) and, in addition, groups Ib and III (gray lines), which are not found in animal cells. Group I includes the B subunit of the bacterial KDP system (Ia) and a very large family (Ib) of cation pumps able to transport various metal ions (Cu^{2+}, Ag^{+}, Cd^{2+}, etc.). The human genome contains 2 group Ib ATPases that are both known to transport copper (the Menkes and Wilson proteins). Group II includes the sarcoplasmic-endoplasmic reticulum calcium pumps (SERCA; group IIa; in human 3 SERCA genes + 2 other genes corresponding to the secretory pathway calcium pumps), the plasma membrane calcium pump (PMCA; group IIb, 4 genes in human), and type IIc with the 4 isoforms of the Na-K-ATPase α-subunit and the gastric and "nongastric" H-K-ATPase α-subunits. Type III P-ATPases are proton ATPases (or Mg-ATPases) found in yeast, plant, and protozoa but not in multicellular animals. Up to 14 group IV genes have been found in the human genome (although some of them might be pseudogenes), and one of these genes has been characterized as an aminophospholipid (APL) transporter or "flippase" in protozoa and mammals, but very little is known about their function. No functional data are available concerning the group V P-ATPases.

Horisberger, 2004