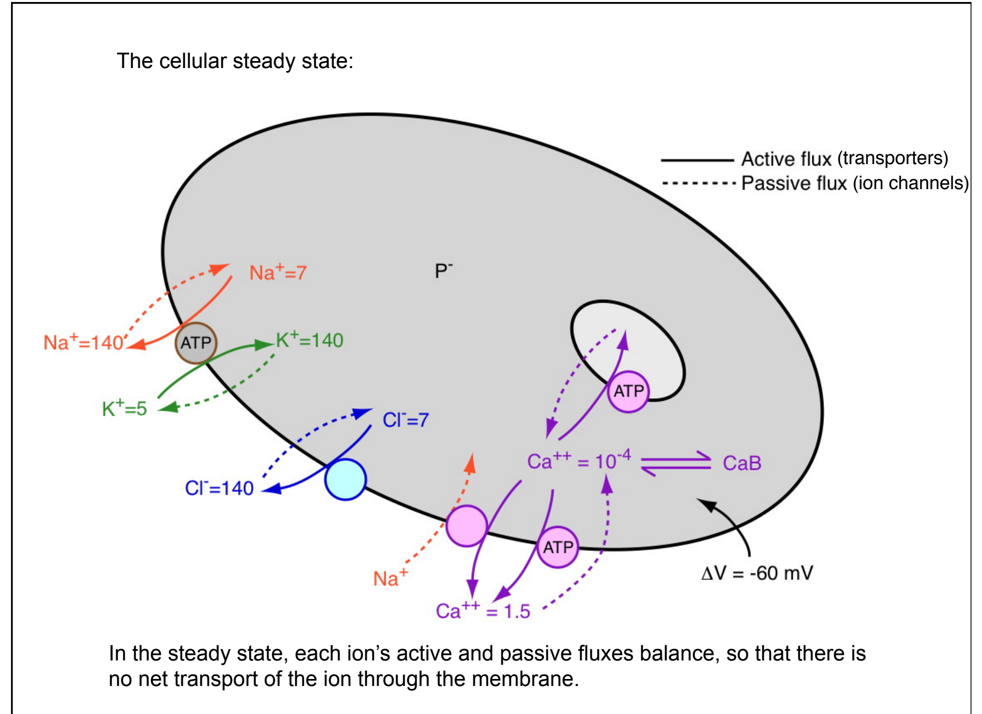
The steady state, membrane properties and the nicotinic acetylcholine channel

Hille, Chapters 10, 13, 17.

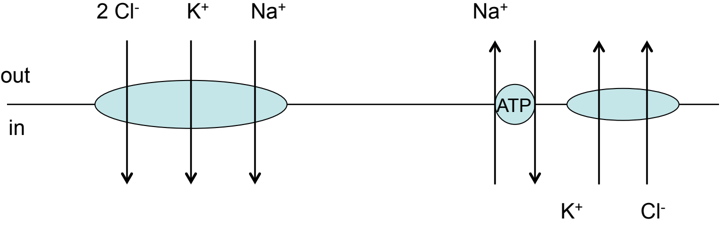
Miyazawa et al. Structure and gating mechanism of the NACh receptor pore *Nature* 423:949-955 (2003).

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.

Chloride transporters are often co-transporters, using the energy stored in the Na^+ or K^+ gradients to transport Cl^-. These take two forms, sketched below, which vary during development in neurons.



NKCC

KCC

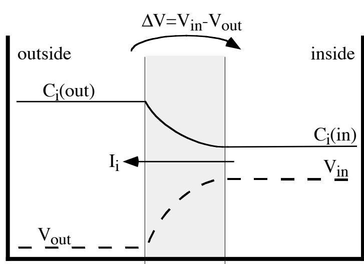
$$E_{Cl} = -\frac{RT}{F}\ln\frac{Cl_{out}}{Cl_{in}} = \frac{RT}{F}\ln\frac{Cl_{in}}{Cl_{out}}$$

In immature neurons, Cl^- is transported <u>into</u> cells, making E_{Cl} more positive.

In mature neurons, Cl^- is transported <u>out of</u> cells, making E_{Cl} more negative.

To illustrate a simple form of steady state, begin with the current-voltage equation derived in class from the Nernst-Planck equation with the constant field assumptions:

- Steady state flux
- Independent fluxes
- Stirred bounding solutions
- Constant electric field in the membrane

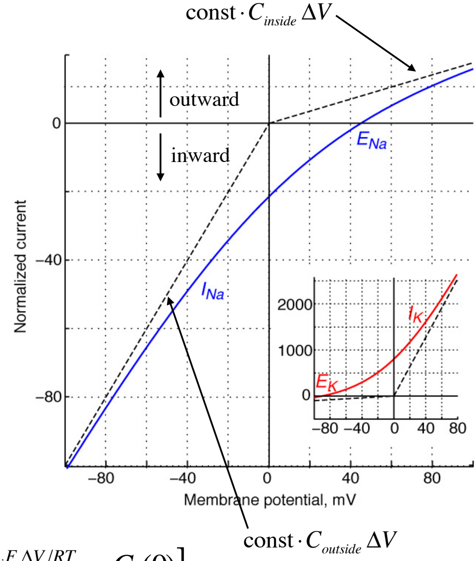


$$I_i = \frac{(z_i F)^2 u_i}{d}\,\Delta V\,\frac{\left[C_i(d)e^{z_i F\,\Delta V/RT} - C_i(0)\right]}{e^{z_i F\,\Delta V/RT} - 1}$$

This model has some properties typical of actual membrane currents:

1) The current goes to zero when $\Delta V = E_i$, the equilibrium potential.

2) The current-voltage relationship is rectified in that the flux from the side with the larger concentration (outside in the drawing) is larger.



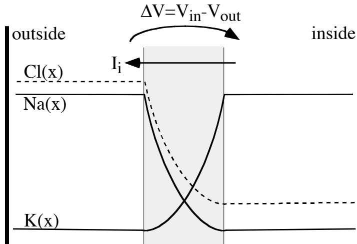
$$I_i = \frac{(z_i F)^2 u_i}{d} \Delta V \frac{\left[C_i(d) e^{z_i F \Delta V/RT} - C_i(0) \right]}{e^{z_i F \Delta V/RT} - 1}$$

Diffusion potential: Suppose a membrane separates solutions containing sodium, potassium, and chloride, as at right.

There will be fluxes of all three ions, driven by the concentration gradients.

If the sum of the fluxes does not add up to zero net current, then the membrane potential will change as charge is transported through the membrane.

To obtain a constant steady-state membrane potential ($d\Delta V/dt = 0$), there must be zero total current:



$$I_{total} = I_K + I_{Na} + I_{Cl} = 0$$

Substituting three current-voltage equations from the previous slide into the equation above and rearranging gives the Goldman Hodgkin Katz (GHK) constant-field equation (for the equations here, permeability *P* is just mobility *u*).

$$\Delta V = \frac{RT}{F} \ln \frac{P_K K_O + P_{Na} Na_O + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_O}$$

Note that a diffusion potential is dissipative, meaning that the fluxes continue (and are non-zero) in the steady state. We know that this is so, because none of the ions is at equilibrium. This system releases energy as heat, represented by the battery-resistor model, where current flows through a resistor.

The potential energy stored in the concentration gradients is used to drive fluxes through the membrane.

Thus the cellular steady state can be considered to be a balance of storing energy in the concentration gradients and dissipating that energy in fluxes through ion channels. The ultimate energy source is most often ATP.

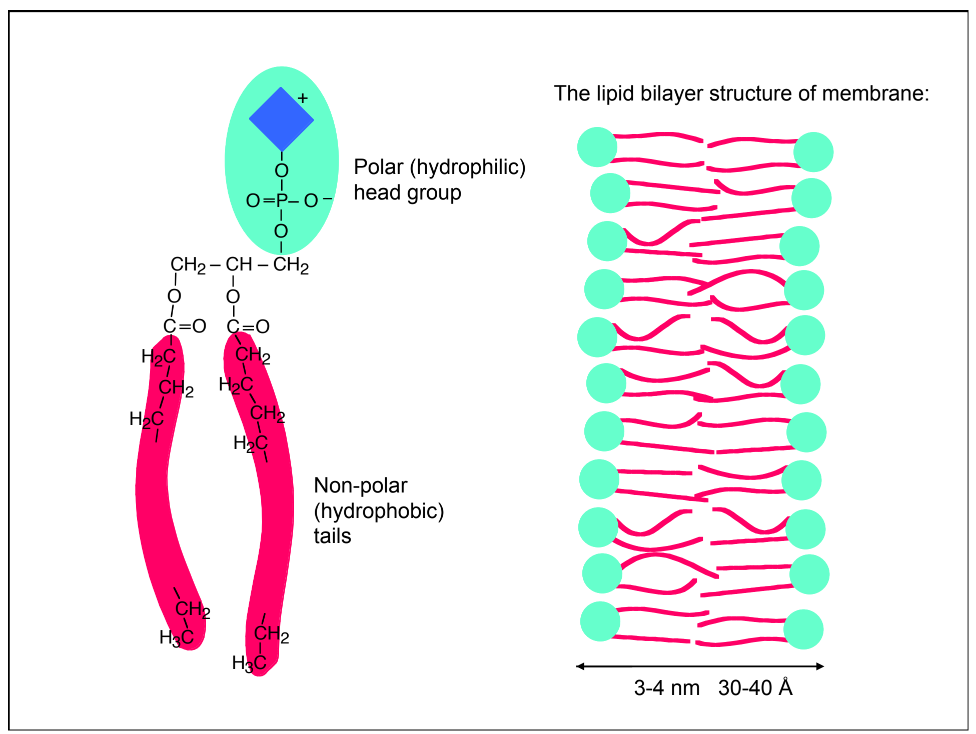
The steady-state model used to derive the diffusion potential above is greatly simplified. In a real steady-state model, there must be explicit recognition that ion concentrations as well as membrane potential are constant.
Thus, a more accurate steady-state model has to consider both active and passive transport, e.g. for potassium

$$\frac{dK_{in}}{dt} = \frac{A_{cell}}{F\,Vol_{cell}}\left[-I_{Kpassive}(\Delta V, K_j) - I_{K\,NaKATPase}(\Delta V, K_j, Na_j) - I_{K\,KCC}(\Delta V, K_j, Cl_j)\right]$$

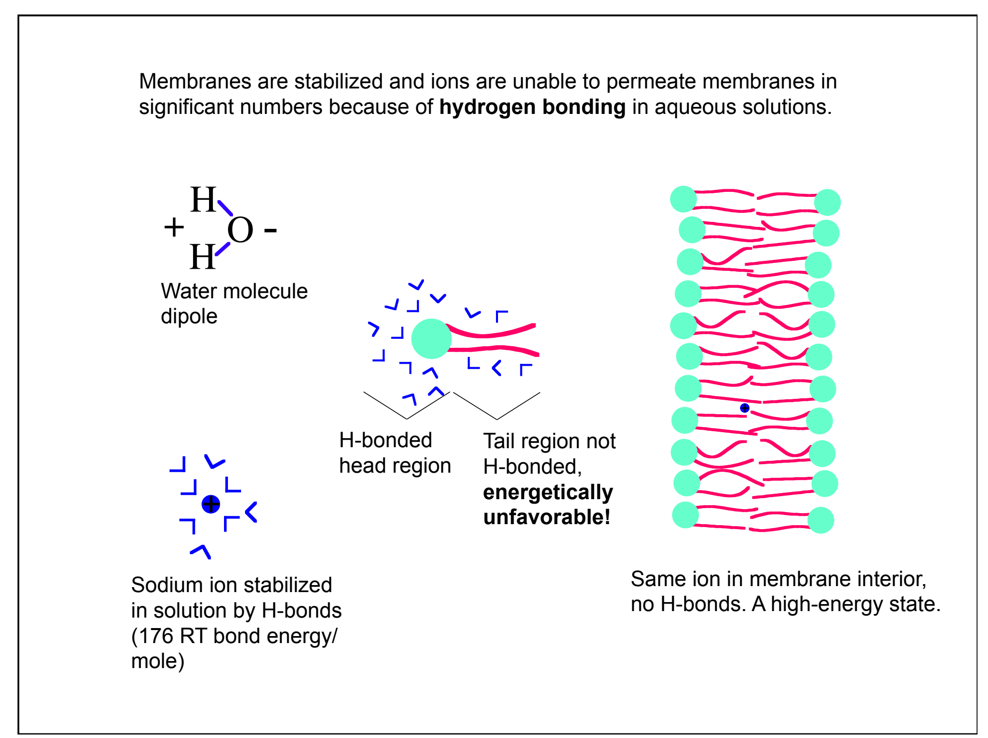
e.g. GHK equation, passive transport

active transport due to Na-KATPase and KCC cotransporter

A similar equation can be written for Na, Cl, Ca, and any other permeable ion. This gives a set of simultaneous equations, the steady-state solution, which demands that $dX/dt = 0$ for all ion concentrations X. Note that an explicit equation for the membrane potential ΔV is not necessary. Note also that the ion steady states lead to zero net charge transfer through the membrane, so the condition used on the previous slide is actually redundant! (For an example, see Problem 3, part d of the first homework set).

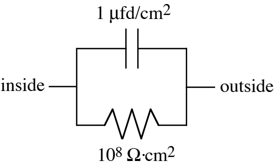


The lipid bilayer structure of membrane:
Polar (hydrophilic) head group
Non-polar (hydrophobic) tails
3-4 nm 30-40 Å

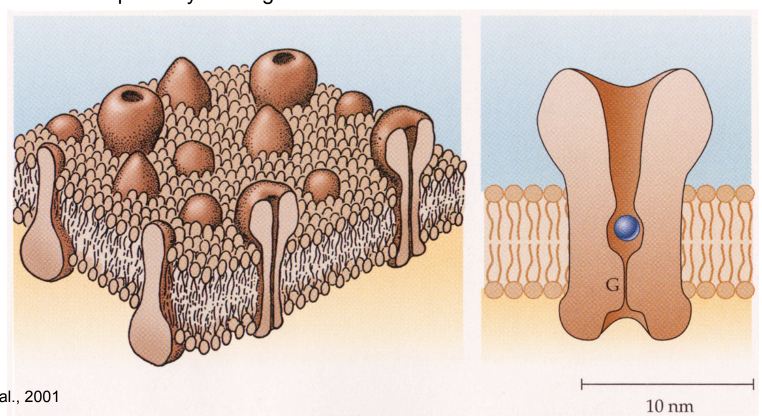


Membranes are stabilized and ions are unable to permeate membranes in significant numbers because of **hydrogen bonding** in aqueous solutions.
Water molecule dipole
H-bonded head region
Tail region not H-bonded, **energetically unfavorable!**
Sodium ion stabilized in solution by H-bonds (176 RT bond 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 Ω·cm²



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.

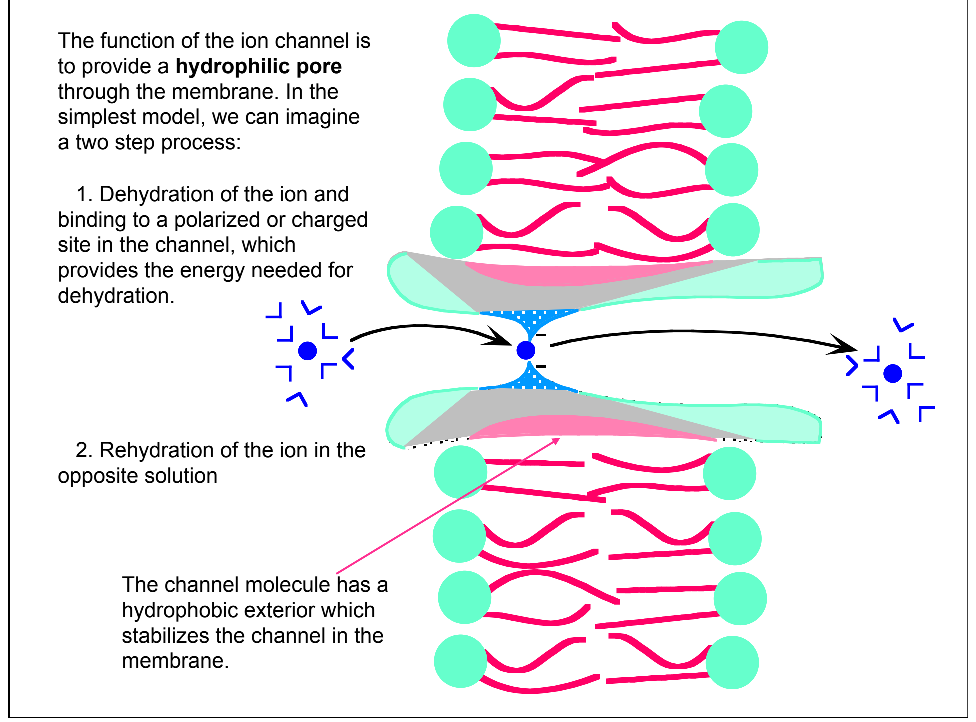


Nicholls et al., 2001

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, which provides the energy needed for dehydration.

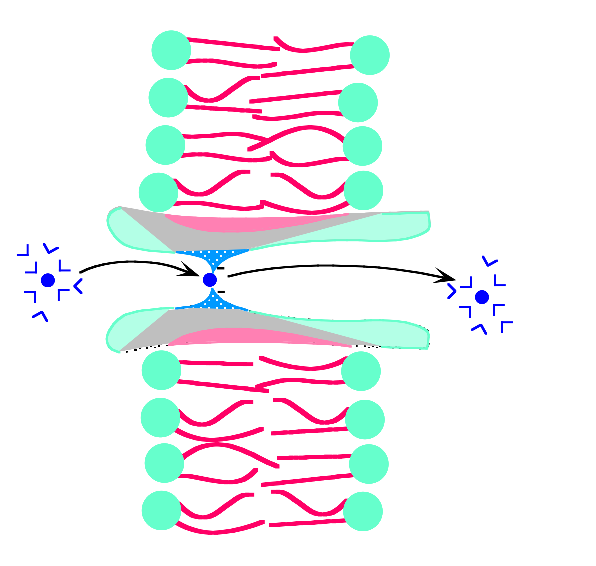
2. Rehydration of the ion in the opposite solution



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

The channel is imagined 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).



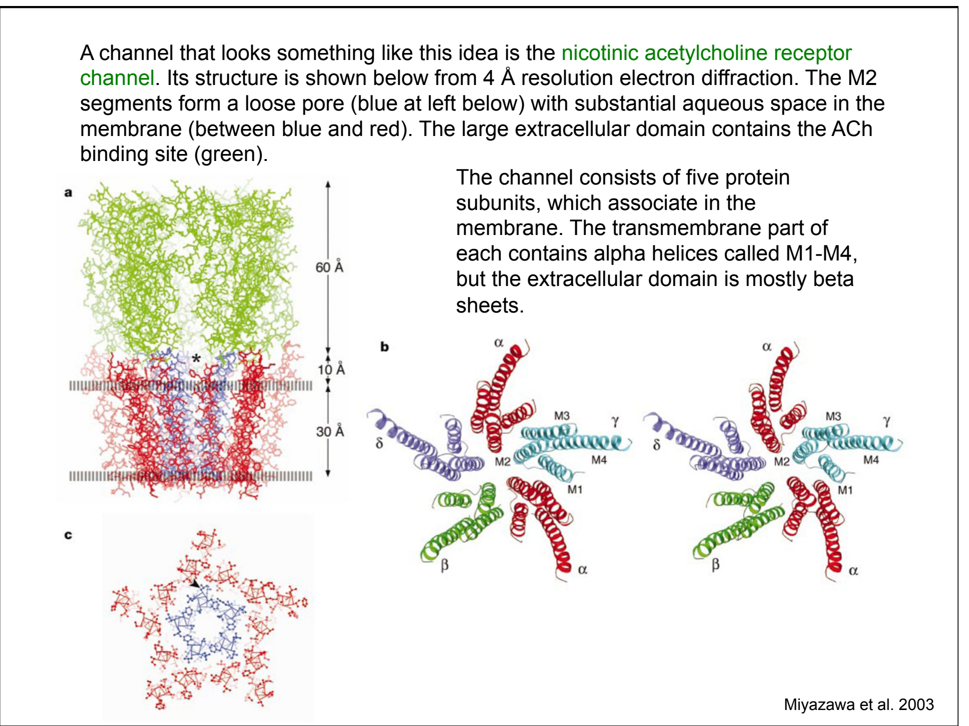
Ion channels are protein molecules with extracellular and intracellular domains and transmembrane domains. The **nicotinic acetylcholine receptor channel** subunit is diagrammed below. Note the **transmembrane segments**, denoted M1 - M4.

Early evidence for channel structure:

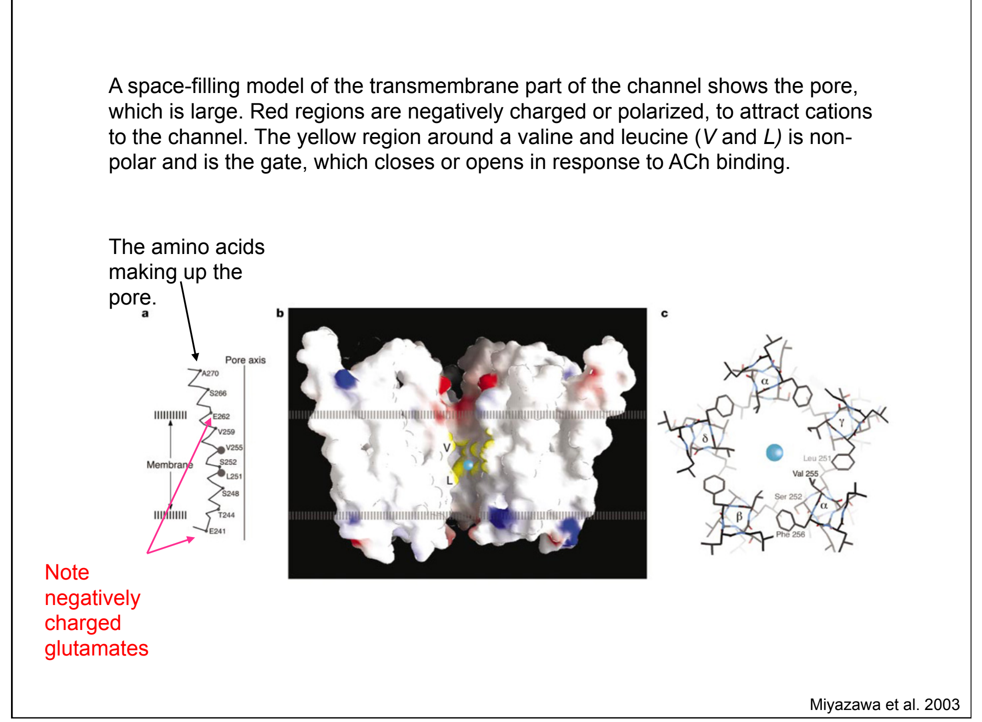
1. Hydropathy plots
2. Binding sites for various ligands

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

Hille, 2001



A channel that looks something like this idea is the nicotinic acetylcholine receptor channel. Its structure is shown below from 4 Å resolution electron diffraction. The M2 segments form a loose pore (blue at left below) 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.
a
60 Å
10 Å
30 Å
b
α
M3
γ
δ
M2
M4
M1
β
α
c
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 cations to the channel. The yellow region around a valine and leucine (V and L) is non-polar and is the gate, which closes or opens in response to ACh binding.
The amino acids making up the pore.
a
Pore axis
A270
S266
E262
V259
V255
S252
L251
S248
T244
E241
Membrane
Note negatively charged glutamates
b
V
L
c
α
γ
δ
β
α
Leu 251
Val 255
Ser 252
Phe 256
Miyazawa et al. 2003

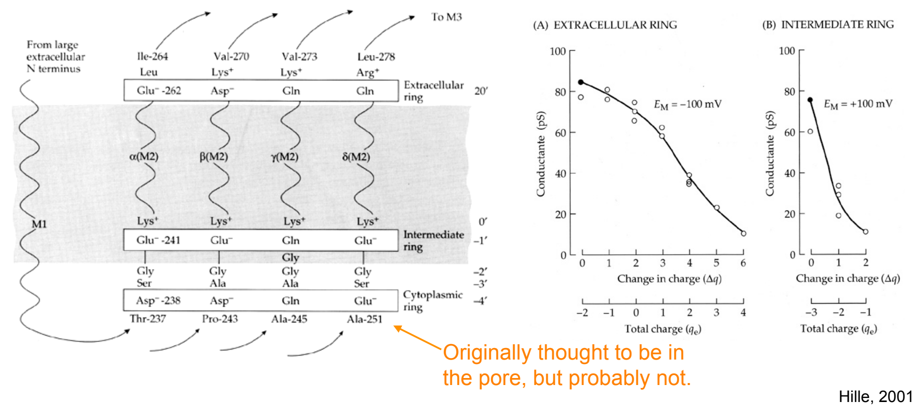
The channel of the NAChR is formed by M2 transmembrane segments.

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.

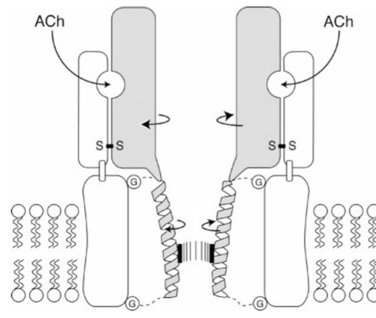
2. An intermediate ring near the narrow spot in the pore contains 3 charges. It is more important in determining channel conductance than the external rings.

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



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

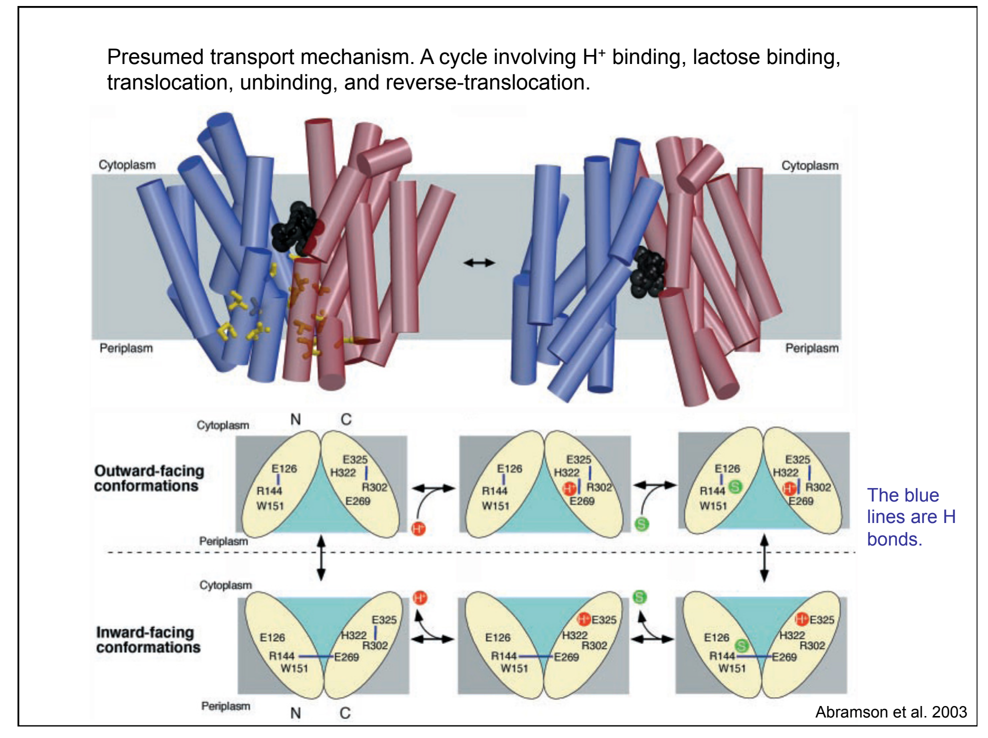
Structure of a bacterial transporter, LacY, which transports lactose into the cell using energy stored in the gradient of H^+ ions.

The molecule has 12 transmembrane ⍰ 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

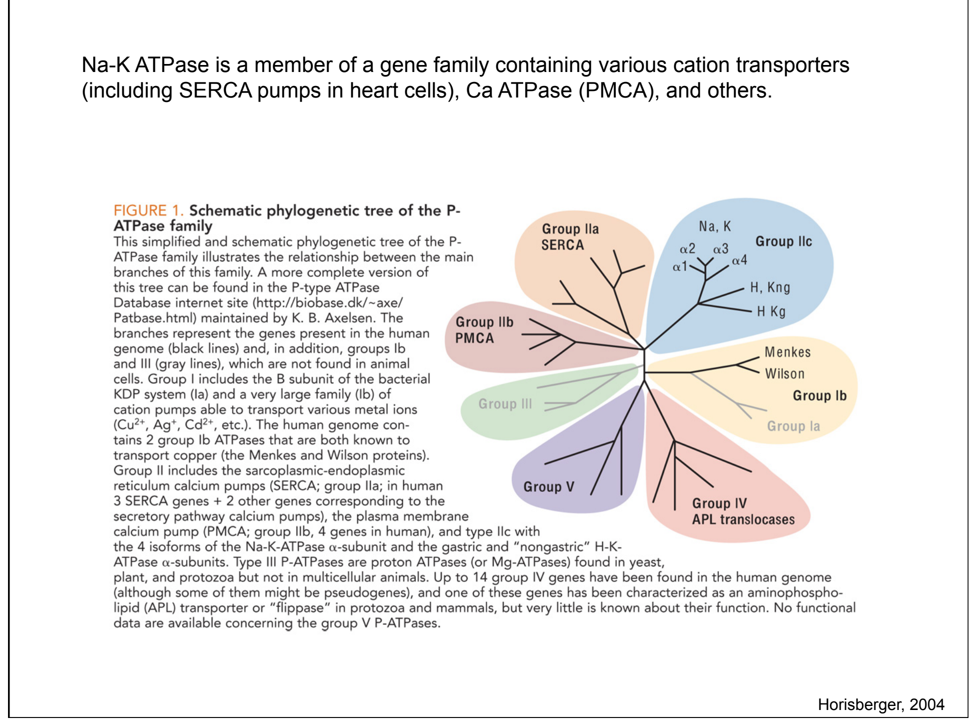
Abramson et al. 2003

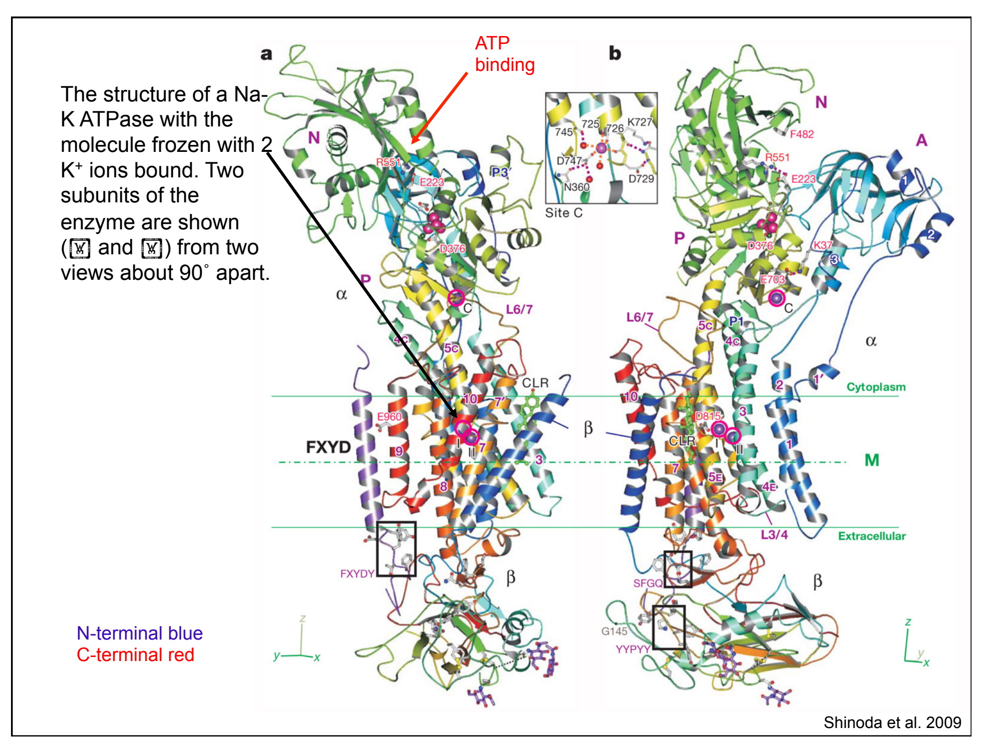
Presumed transport mechanism. A cycle involving H^+ binding, lactose binding, translocation, unbinding, and reverse-translocation.



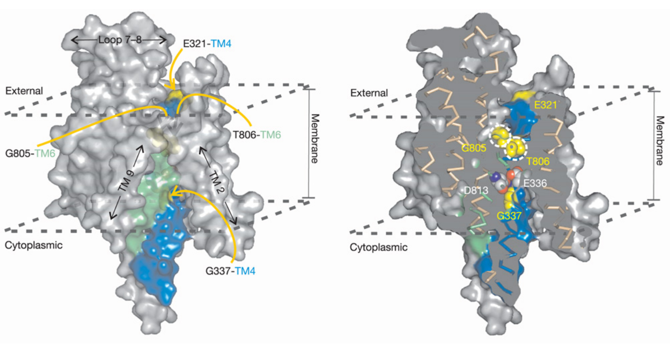
The blue lines are H bonds.

Abramson et al. 2003

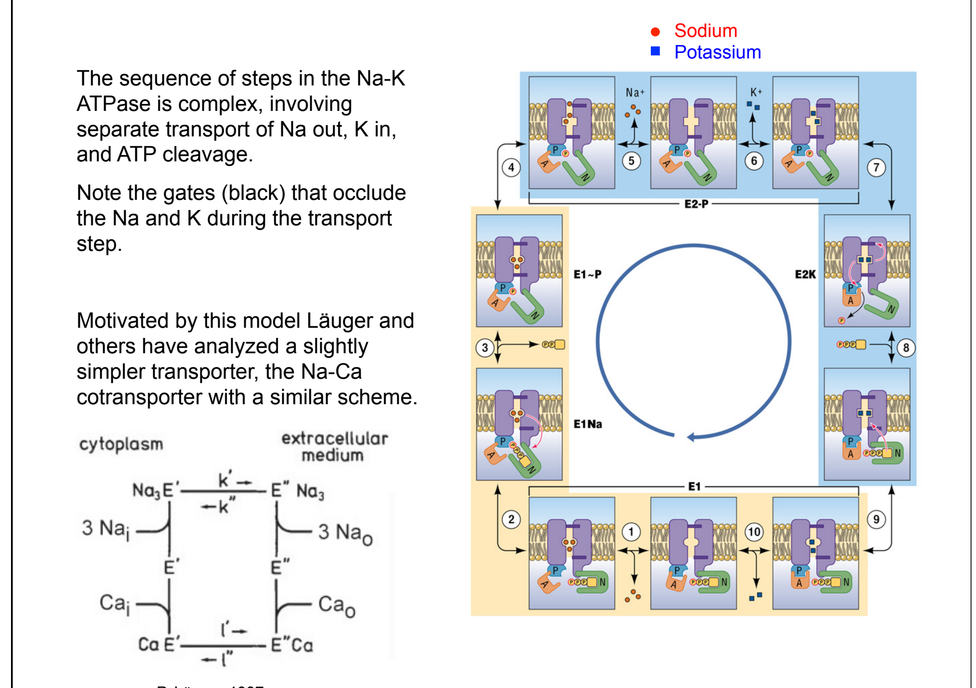

Na-K ATPase is a member of a gene family containing various cation transporters (including SERCA pumps in heart cells), Ca ATPase (PMCA), and others.
FIGURE 1. Schematic phylogenetic tree of the P-ATPase family
This simplified and schematic phylogenetic tree of the P-ATPase family illustrates the relationship 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 (Cu2+, Ag+, Cd2+, 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.
Group IIa
SERCA
Na, K
α2 α3
α1 α4
Group IIc
H, Kng
H Kg
Group IIb
PMCA
Menkes
Wilson
Group Ib
Group III
Group Ia
Group V
Group IV
APL translocases
Horisberger, 2004


The structure of a Na-K ATPase with the molecule frozen with 2 K+ ions bound. Two subunits of the enzyme are shown (and) from two views about 90˚ apart.
ATP binding
a
b
N
P
α
β
FXYD
FXYDY
P3
L6/7
CLR
A
P1
Cytoplasm
M
Extracellular
L3/4
SFGQ
YYPYY
G145
Site C
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.

Motivated by this model Läuger and others have analyzed a slightly simpler transporter, the Na-Ca cotransporter with a similar scheme.

P. Läuger 1987

Horisberger, 2004