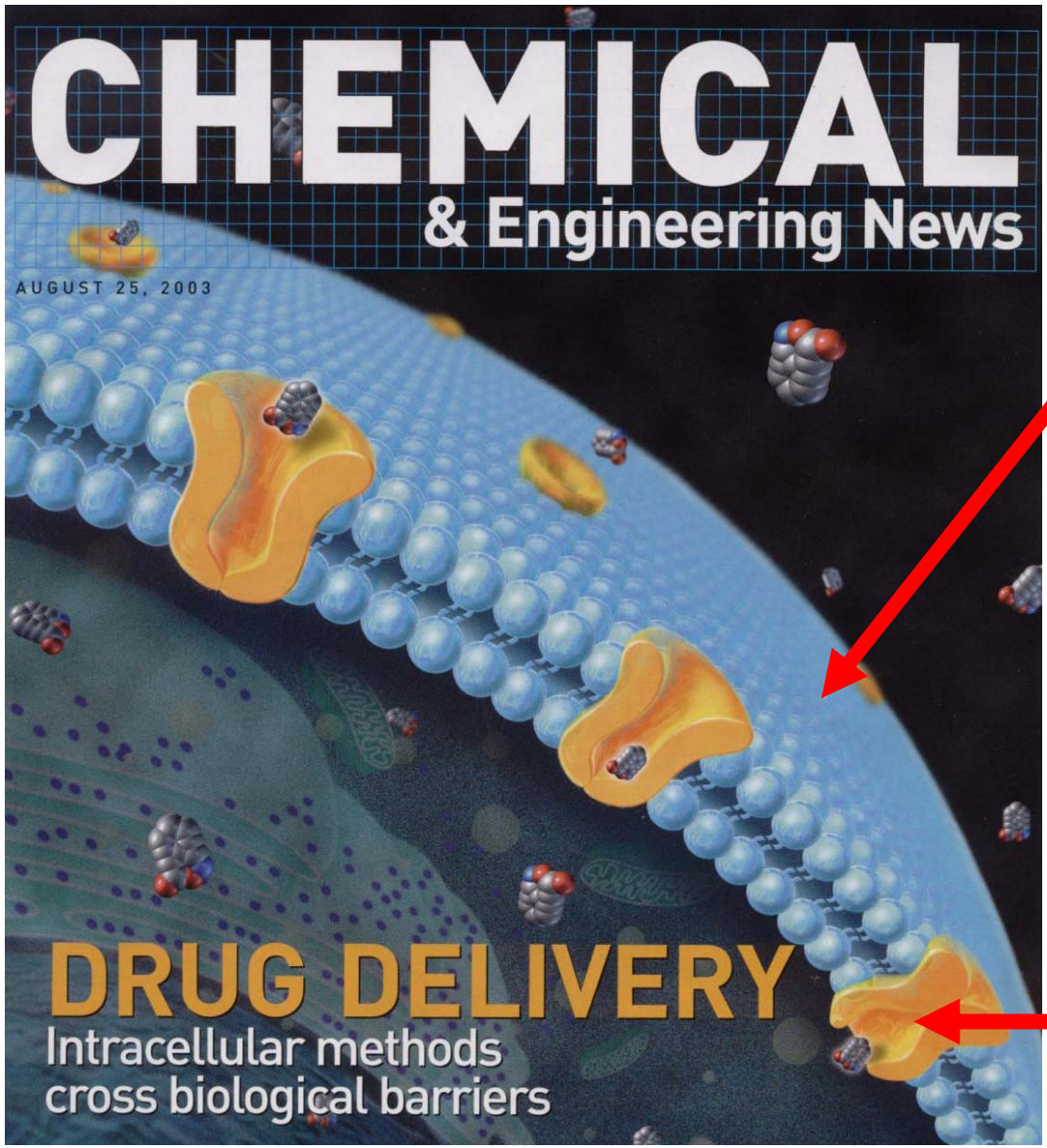
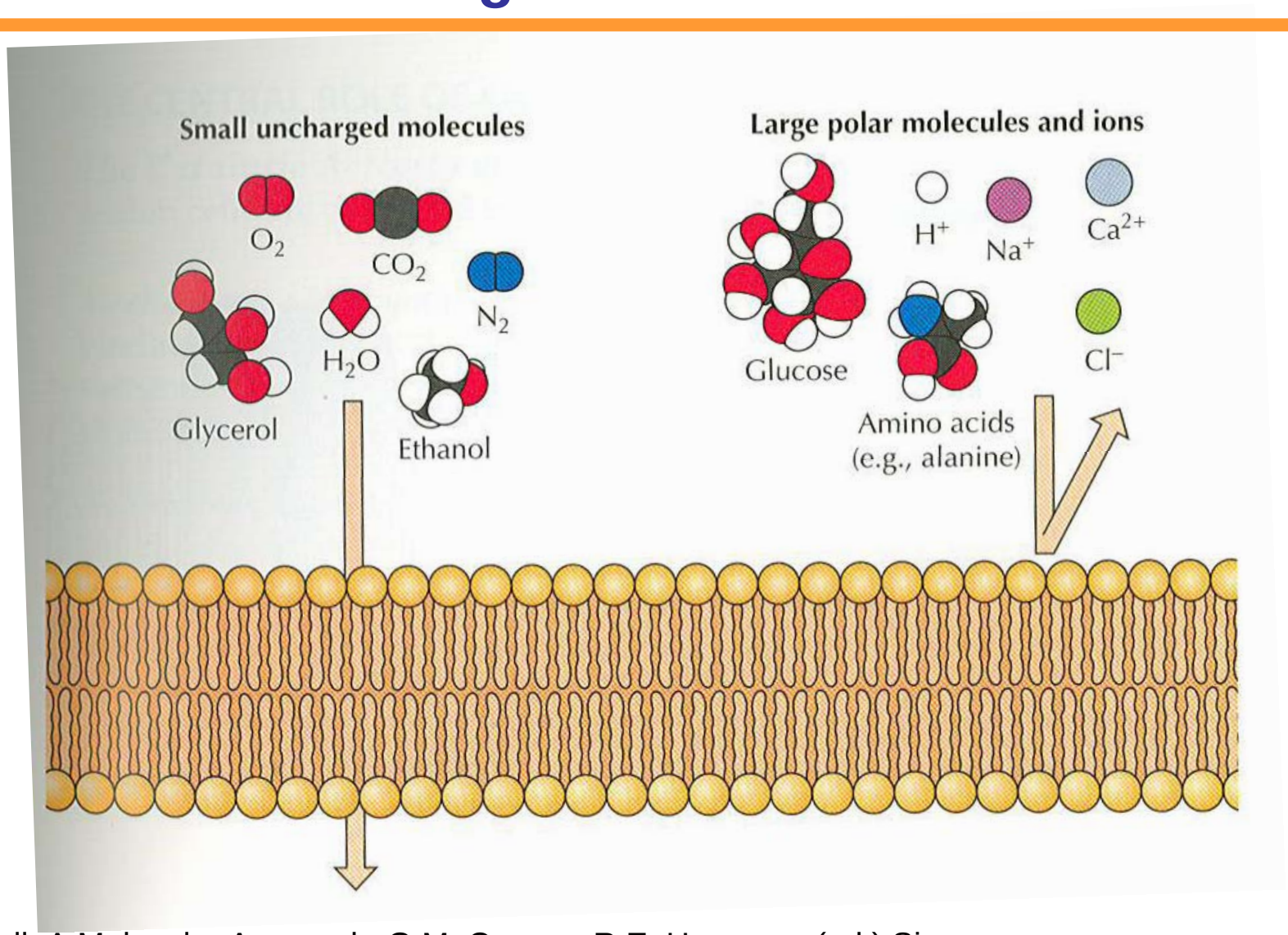
Biological Membranes



Impermeable lipid bilayer membrane

Protein Channels and Pores

Biological Membranes Are Barriers for Ions and Large Polar Molecules

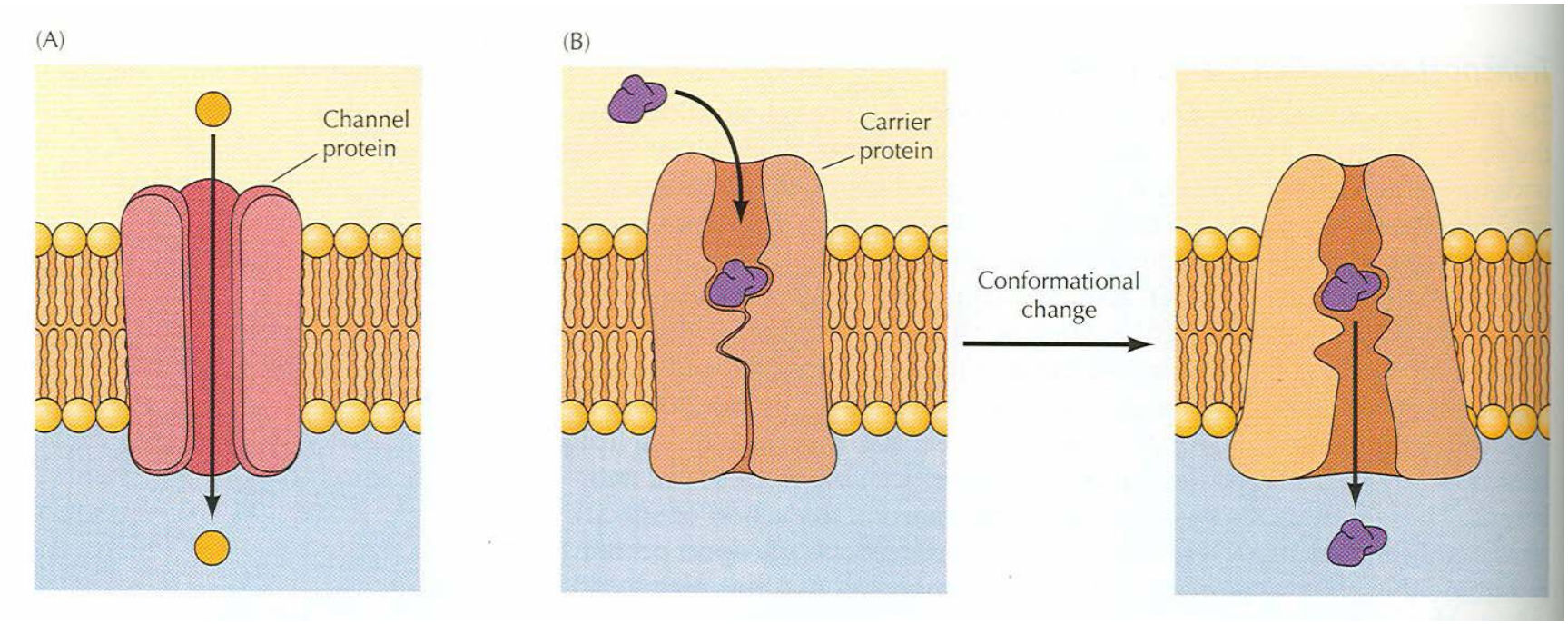


The Cell. A Molecular Approach. G.M. Cooper, R.E. Hausman (ed.) Sinauer Associates, Inc. Washington D.C. (2004)

Passive Diffusion

During passive diffusion the molecules dissolve in the phospholipid membrane, diffuse across it and dissolve in the intracellular medium. The net flow of molecules is always down their concentration gradient. Passive diffusion is therefore a non-selective process. Only small and relatively hydrophobic molecules can dissolve in the lipid membrane and get transported according to the passive diffusion mechanism.

Mechanisms of Transport Through Membranes



Passive Transport **Facilitated Diffusion**

The Cell. A Molecular Approach. G.M. Cooper, R.E. Hausman (ed.) Sinauer Associates, Inc. Washington D.C. (2004)

Active Transport

Transport against electrochemical gradient, coupled with hydrolysis of ATP

Facilitated Diffusion

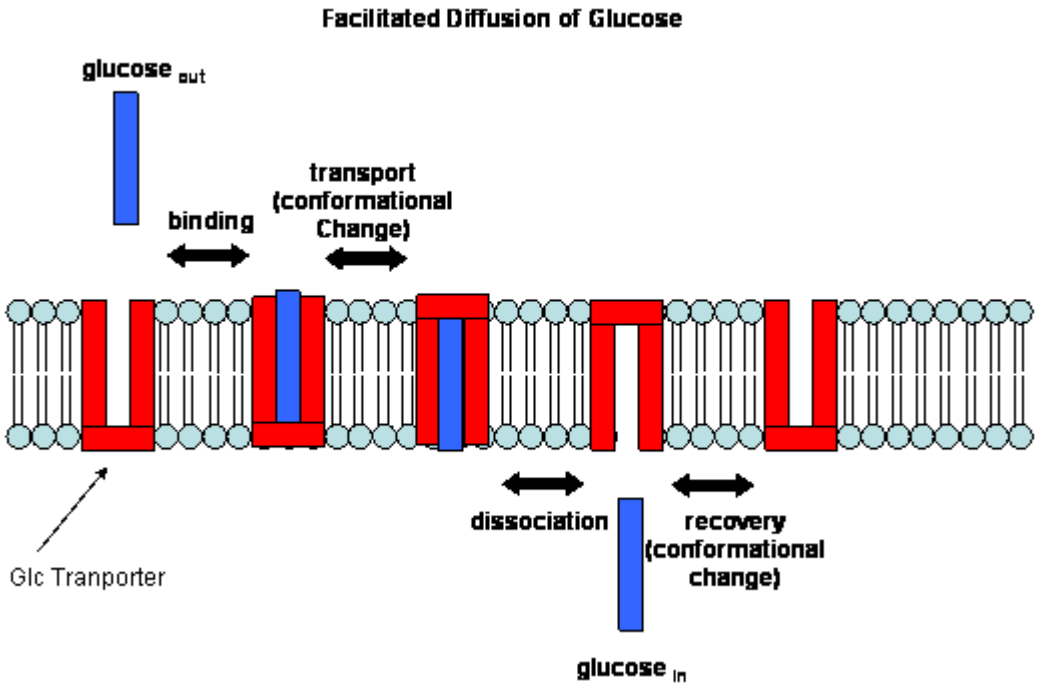
Facilitated diffusion similar to passive diffusion occurs down the electrochemical gradient (no chemical energy input is needed), however facilitated diffusion occurs via protein channels.

Two classes of proteins that mediate diffusion across the membrane are generally distinguished:

- ***carrier proteins*** - responsible for transport of sugars, amino acids, and nucleosides across the plasma membrane of most cells.
- ***channel proteins*** - responsible for transport of ions

Facilitated Diffusion – Carrier Proteins

Carrier proteins bind specific molecules to be transported on one side of the membrane. The carrier proteins undergo subsequently a conformation change that allow the molecule to pass through the membrane and be released on the other side of the membrane.



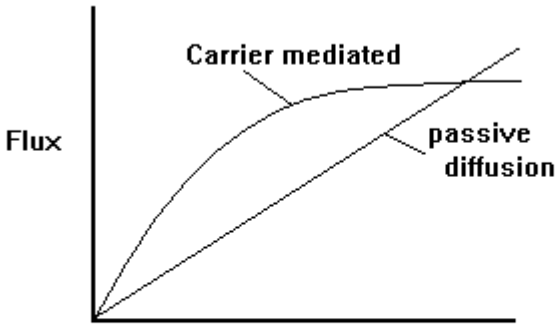
http://employees.csbsju.edu/hjakubowski/classes/ch331/transkinetics/oldiffusioneq.html

Selectivity of Carrier-Mediated Diffusion

A substrate molecule interacts with its carrier at a particular site (the receptor site) on the surface of the carrier protein. That site has a 3-dimensional shape and that shape permits interaction with substrates that have a suitably **matching** 3-dimensional configuration. Molecules with a shape that does not match that of the receptor site cannot bind to the transporter and therefore cannot be transported.

This is the basis of the empirical observation that every category of transport process displays, to one extent or another, **specificity** for a particular structural class of substrate. For example, the glucose carrier of neuronal cells (the GLUT 3 transport protein) shows a high degree of specificity for glucose and other molecules that share certain structural characteristics with glucose: e.g., galactose (with a six-membered pyranose ring) is transported via the glucose carrier, but fructose (with a five-membered furanose ring) is not. Moreover, the process is **stereospecific**; e.g., the D-isomers of hexose sugars are transported while the L-isomers are effectively ignored.

Carrier-Mediated Transport is Characterized by Saturability



Distinction between passive diffusion and carrier mediated solute movement across membranes from kinetic measureme

Carrier-mediated transport is typically described by Michaelis – Menten like kinetics:

$J = J_{max} [S] / (K_m + [S])$

Where [S] is the concentration of the solute to be transported; K_m – solute concentration for which $J=J_{max}/2$

http://www.kcl.ac.uk/kis/schools/life_sciences/life_sci/quinn/teaching/jp0225/MemTransport/facdiff.html

Carrier Proteins Behave Like Membrane Bound Enzyme

$E + S \leftrightarrow ES$, k_a, k_a'

$ES \rightarrow P$ k_b

The rate of product formation is $v = k_b [ES]$

In equilibrium the concentration of enzyme does not change:

$$\frac{d[ES]}{dt} = k_a[E][S] - k_a'[ES] - k_b[ES] = 0$$

$$[ES] = \left(\frac{k_a}{k_a' + k_b} \right)[E][S]$$

$$[E]_0 = [E] + [ES]$$

$$[S] \approx [S]_0$$

$$[ES] = \frac{[E]_0}{1 + \left(\frac{k_a' + k_b}{k_a} \right) \frac{1}{[S]_0}}$$

$$v = \frac{k_b[E]_0}{1 + \left(\frac{k_a' + k_b}{k_a} \right) \frac{1}{[S]_0}}$$

Carrier Proteins Behave Like Membrane Bound Enzyme

$$\frac{k_a^{'} + k_b}{k_a} = K_M$$

K_M is characteristic for a given enzyme

1. When $[S]_0 << K_{M,}$ the rate is proportional to $[S]_0$

$$v = \frac{k_a k_b}{k_a^{'} + k_b}[S]_0[E]_0$$

2. When $[S]_0 >> K_{M,}$ the rate reaches its maximum that is independent of $[S]_0$

$$v = v_{\max} = k_b[E]_0$$

$$v = \frac{v_{\max}}{1 + \frac{K_M}{[S]_0}}$$

Ion Channels

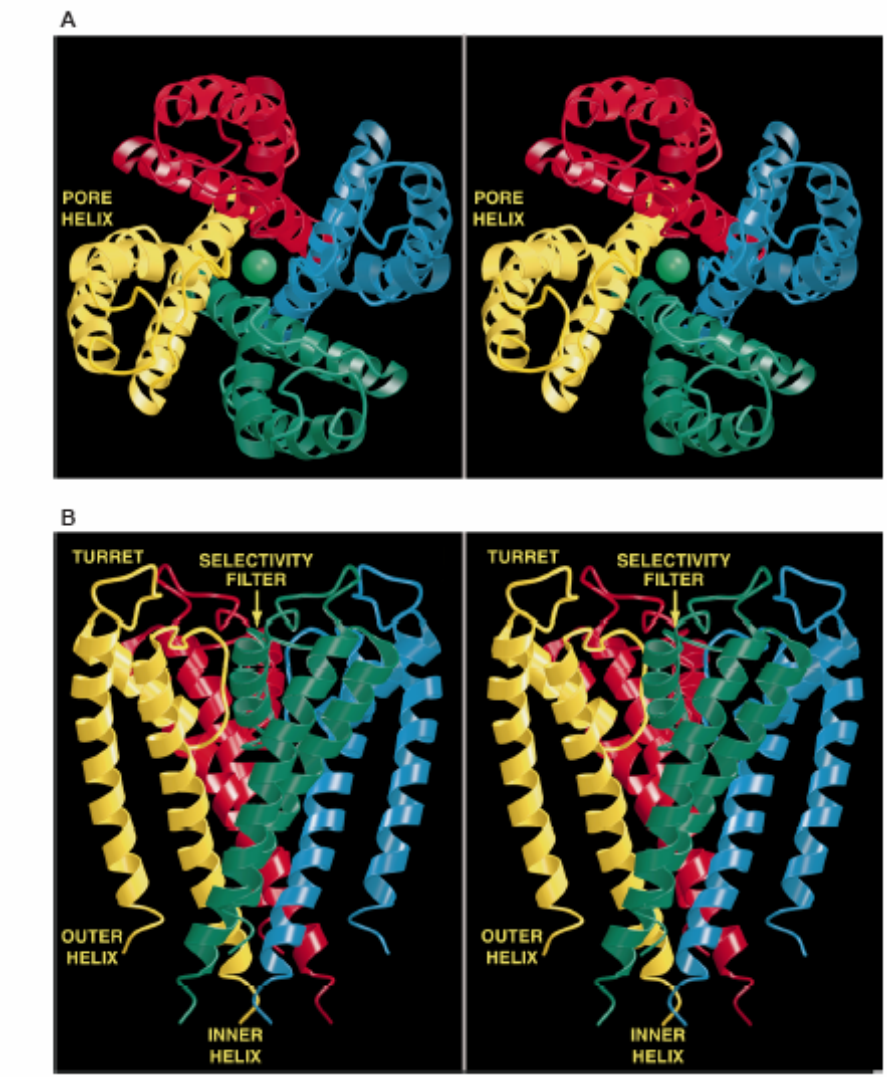
Three types of ion channels are distinguished:

- Voltage-gated channels
- Ligand-gated channels
- Mechano-sensitive channels

Potassium voltage-gated channel is the best studied voltage-gated channel

Nobel Prize 2003

Potassium Voltage-Gated Channel



Voltage-gated channel. It consists of four units (A) View from the extracellular side (B) Stereoview of potassium channel perpendicular to the view shown in (A)

D.A. Doyle et al. Science **280** (1998) 69.

Potassium Voltage-Gated Channel

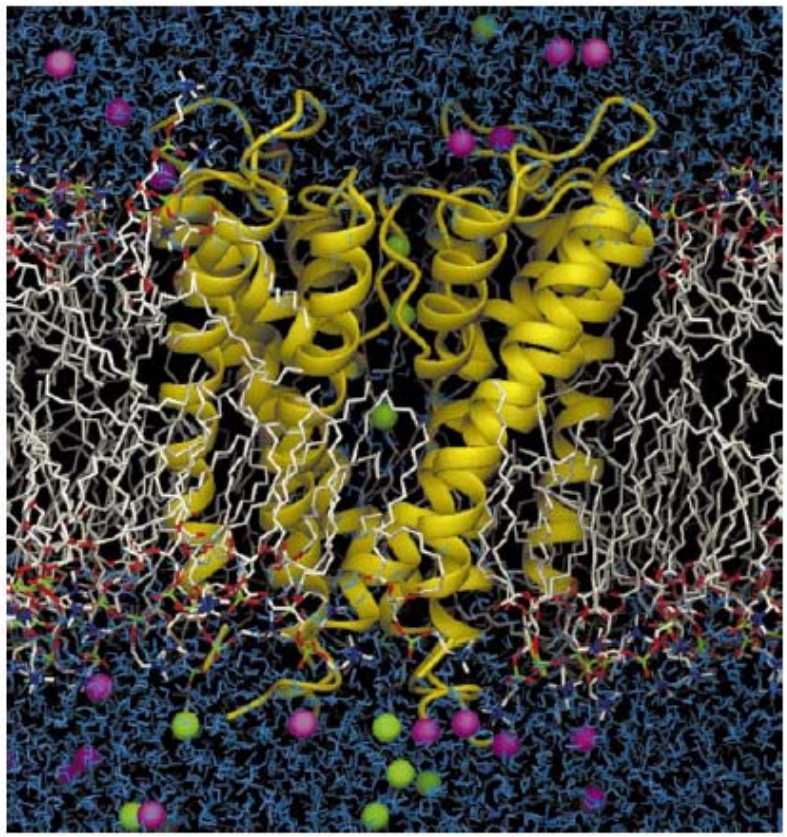


Figure 1 Molecular representation of the atomic model of the KcsA K^+ channel embedded in an explicit DPPC phospholipid membrane bathed by a 150 mM KCl aqueous salt solution[11].

S. Berneche, B. Roux, Energetics of ion conduction through the K^+ channel, Nature **414** (2001) 73-77.

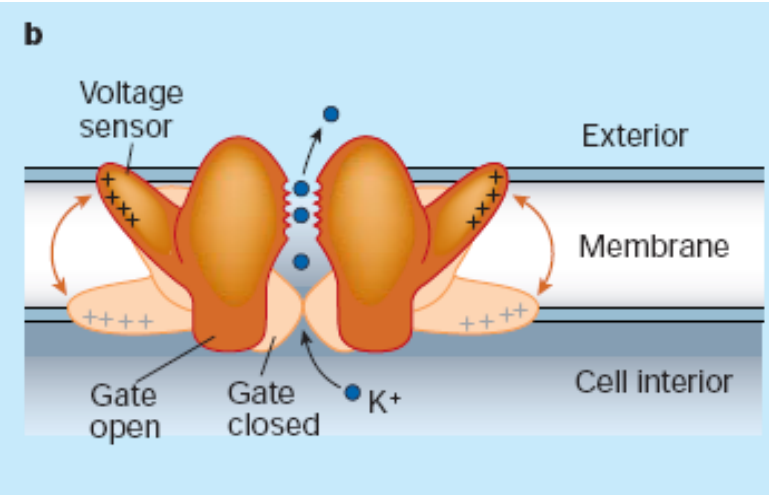
Why Can Potassium Voltage-Gated Channel Transport so Fast

1. There are negative charges inside the potassium voltage-gated channel which stabilize the cations inside the channel.

2. There are 16 carbonyl oxygens identified in the selectivity filter. They replace the water shell of ions in the solution, making entering the pore more energetically favorable.

3. In the middle of the channel there is a wide “central cavity” called also a “lake”, in which the ions can again be hydrated by water molecules. In the central cavity there are four dipoles which further stabilize the cation inside the channel.

Function of Voltage-Gate



F.J. Sigworth, Life's transistors, Nature 423 (2003) 21.

Fingerprints of Voltage-Gated Channels

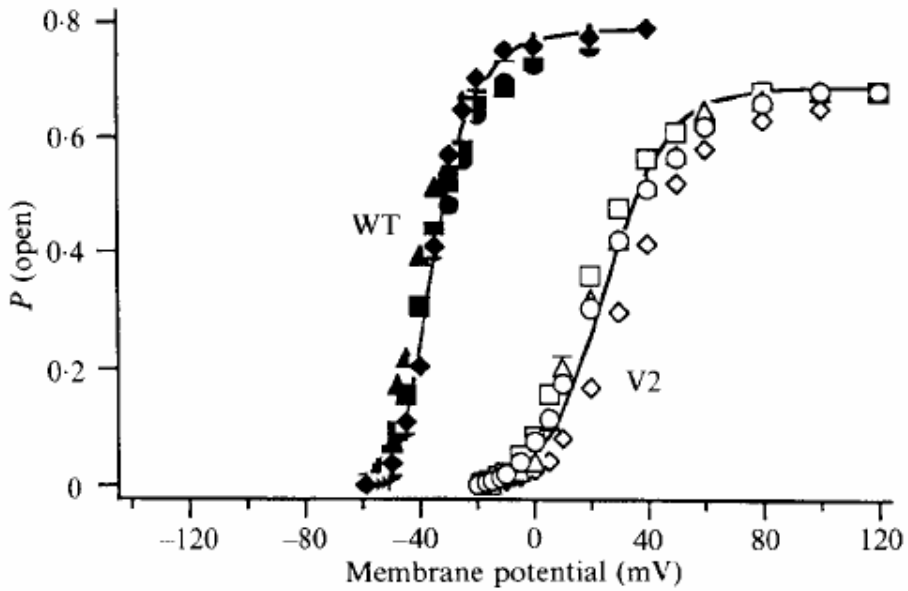


Fig. 7. Gating charge movement and channel open probability as a function of membrane potential for non-inactivating wild-type *Shaker* channels (solid symbols) and V2 mutant channels expressed in *Xenopus* oocytes. Data are from Schoppa *et al.* (1992). (A) Equilibrium charge movement, obtained from integrated gating current records and scaled according to the estimated maximal charge movement of 12·3 e_0 per channel, from oocytes expressing the given channel type. (B) Equilibrium open probability estimated from the steady-state macroscopic current and normalized to open probability values obtained from fluctuation analysis. The various symbols represent data from different membrane patches in parts A and B.

F. Sigworth, Voltage gating of ion channels, Quaterly Reviews of Biophysics, **27** (1994) 1-40.

Description of Gating Kinetics

If the channel fluctuates between two conductance levels we can describe the system as an equilibrium between two structural states of channel protein:

$$C \Leftrightarrow O$$

C and O will be treated as an ensemble of various conformation states with different means.

To determine the probability of finding the channel in the open and closed state (p_o and p_c) one can apply Boltzmann's law where the energies describing given states are given as free energies *G*:

$$\frac{p_o}{p_c} = \exp\left[\frac{-\Delta G}{kT}\right] = K_{eq}$$

Bolztmann's law allows us to calculate how a force influences the equilibrium between two (or more) structural states.

Description of Gating Kinetics

At presence of external force, in our case electric force:

$\Delta G = \Delta G^o - V \Delta q$, because application of electric force causes conformation change of a protein coupled with the movement of charge.

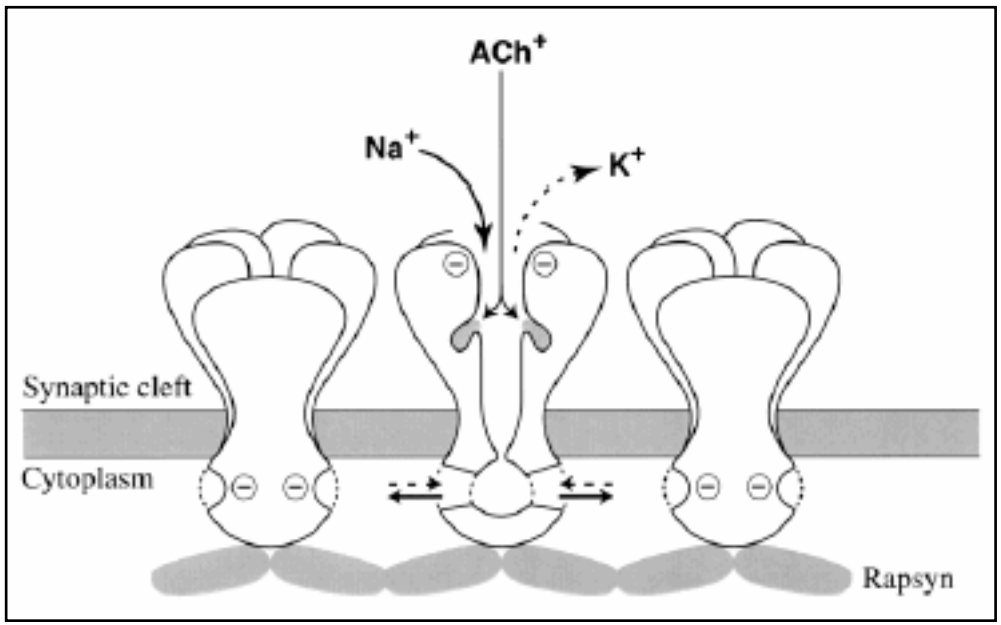
Δq gives information about the so-called gating charge: how many charges have to move through the pore to open the pore. The energy difference between the open and closed states includes the term $V \Delta q$, and this makes the opening sensitive to voltage.

$$\frac{p_o}{p_c} = \exp\left[-\frac{\Delta G}{kT}\right] \cong \exp\left[-\frac{\Delta G^0 - V\Delta q}{kT}\right]$$

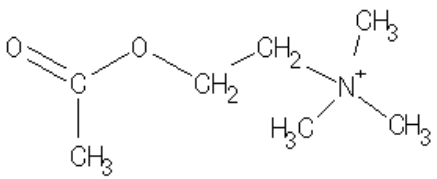
Ligand-Gated Channel

The channel opens as a response to binding of a chemical at a receptor side close to the channel opening. Nicotinic acetylcholine receptor is essential in the passage of electrical signal from a motor neuron to a muscle fiber at the neuromuscular junction. Acetylcholine released by the motor neuron diffuses a few micrometers to the plasma membrane of myocyte (single fiber of a muscle). Acetylcholine binds to the receptor which causes the channel to open. Sodium ions can pass through the channels, it depolarizes the membrane, which subsequently causes contraction of the muscle. There are 2 acetylcholine molecules needed to open the pore.

Miyazawa et al, (1999)



Acetylcholine

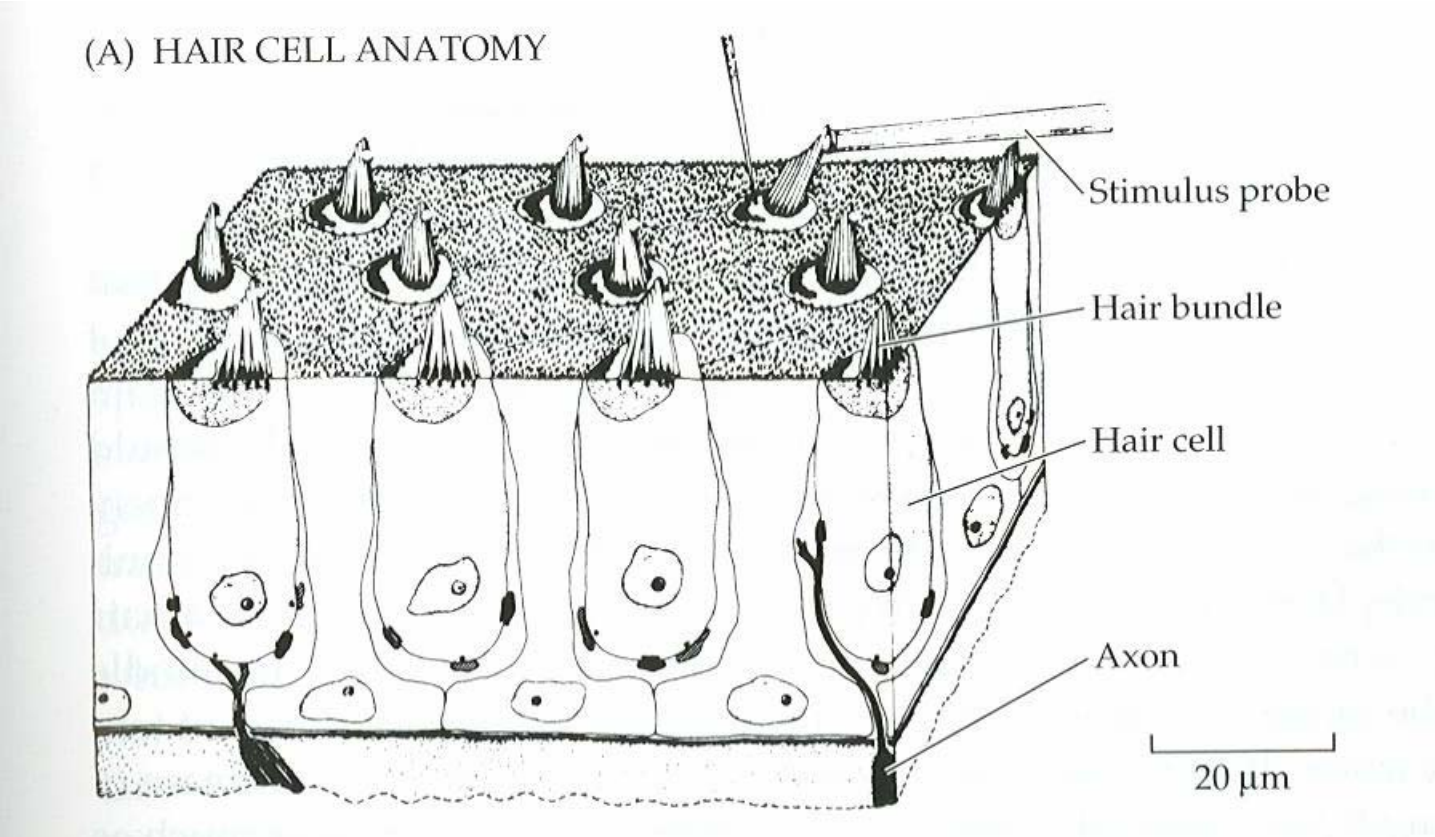


Mechano-sensitive channels

The vertebrate hair cell is a sensitive mechanoreceptor used to detect e.g. sound vibration in the ear. It is a compact receptor whose mechanosensitive channels on hair-like cilia respond to movements as small as a nanometer. In an intact animal current flowing through adjacent hair cells of the inner ear sum up to produce microphonic potential, a signal that follows the vibrations of sound waves up to nearly 20 kHz in humans and as high as 100 kHz in some whales and bats.

There was a mechanism suggested for opening of mechanosensitive channels: when the sensory cilia are moved, a gating spring attached to the channels is stretched which causes opening of the channel.

Mechano-sensitive channels



Ion Channels are Very Selective

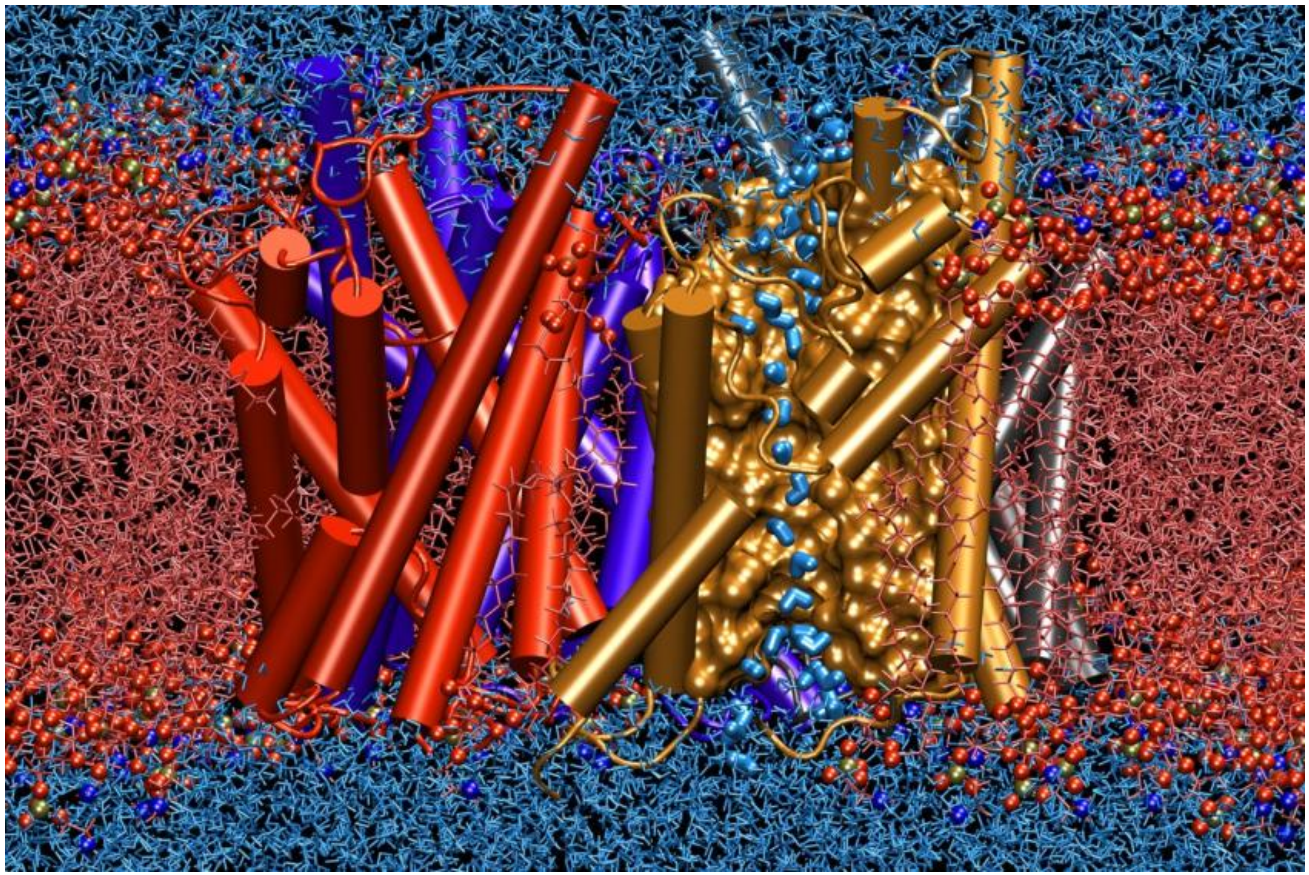
If a channel is highly ion-selective, the pore must be narrow enough to force permeating ions into contact with the wall so they can be sensed. Selection requires interaction.

There are two major mechanisms postulated to explain selectivity of biological channels:

1. Fit of cation inside the selectivity filter of the channel.
2. Specific binding of ions inside the pores.

Voltage-gated channels are selective according to the first mechanism. Potassium voltage-gated channel distinguishes between potassium over sodium more than 1000 fold.

Aquaporins – Water Channels

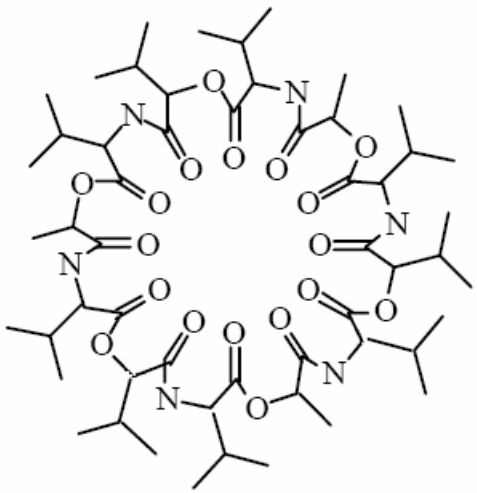


Aquaporins are tetramers transporting water but not allowing to pass through protons

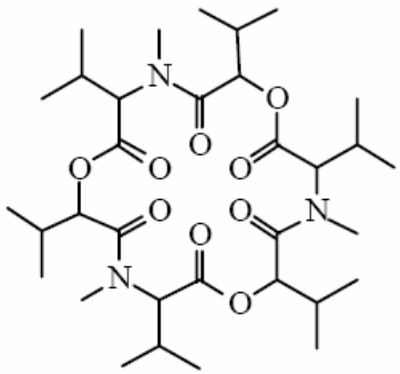
http://www.ks.uiuc.edu/Research/aquaporins/

Transport via Ionophores

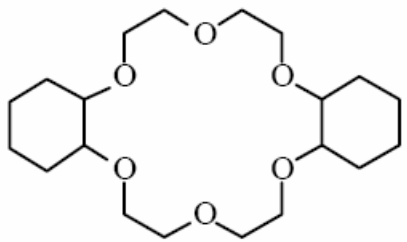
Compounds that can complex ion and transport it on the other side of the membrane.



Valinomycin



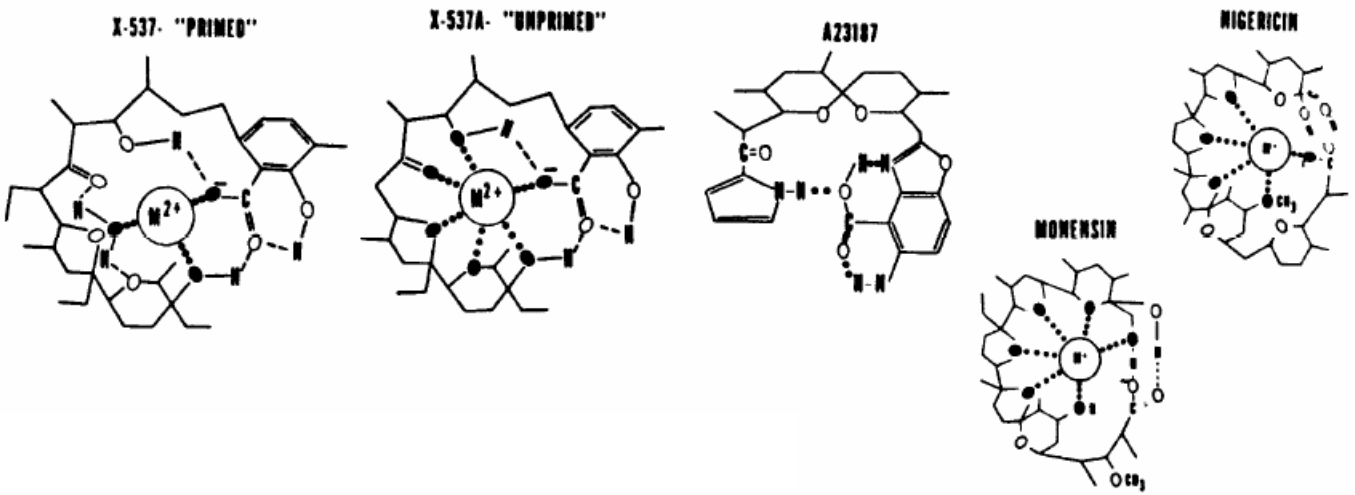
Enniatin B



Dicyclohexyl-18-crown-6

Examples of neutral ionophores

Transport via Ionophores



Examples of carboxylic ionophores