Reading for lecture 6

1. **Lipids and Lipid Bilayers**
2. **Membrane Proteins**

- Voet and Voet, Chapter 11
- Alberts et al Chapter 6

- Jones, R.A.L, "Soft Condensed Matter"
  195pp Oxford University Press,
  ISBN 0-19-850590-6

[Reading for lecture 6] (1)

Biological Membranes are predominantly **phospo-lipid bilayers**

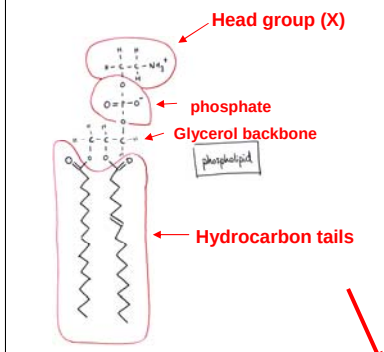
Bilayers are self-organized structures

Purposes of the membrane…

Physical barrier – separates inside cell from outside, compartments

Electrical barrier – separation of charges across membrane is primary form of free-energy storage for living cells. (Lecture 7)

Specialized, for example for transmission of nerve impulses.



Name of X-OH	Charges on X	Total charge of head (including phosphate)
Ethanolamine	+	0
Choline	+	0
Serine	+, -	-
Inositol	0	-
Glycerol	0	-

- 12 to 24 carbon atoms in tail chains
- 0 to 5 double bonds
- Double bonds introduce 30-degree bend

Lipids and Bilayers

Self-organization of amphiphilic molecules

[Biological phospholipids] (2)

Heads are negative or neutral ("Zwitterionic", one each of positive and negative charges) therefore biological membranes are negatively charged.

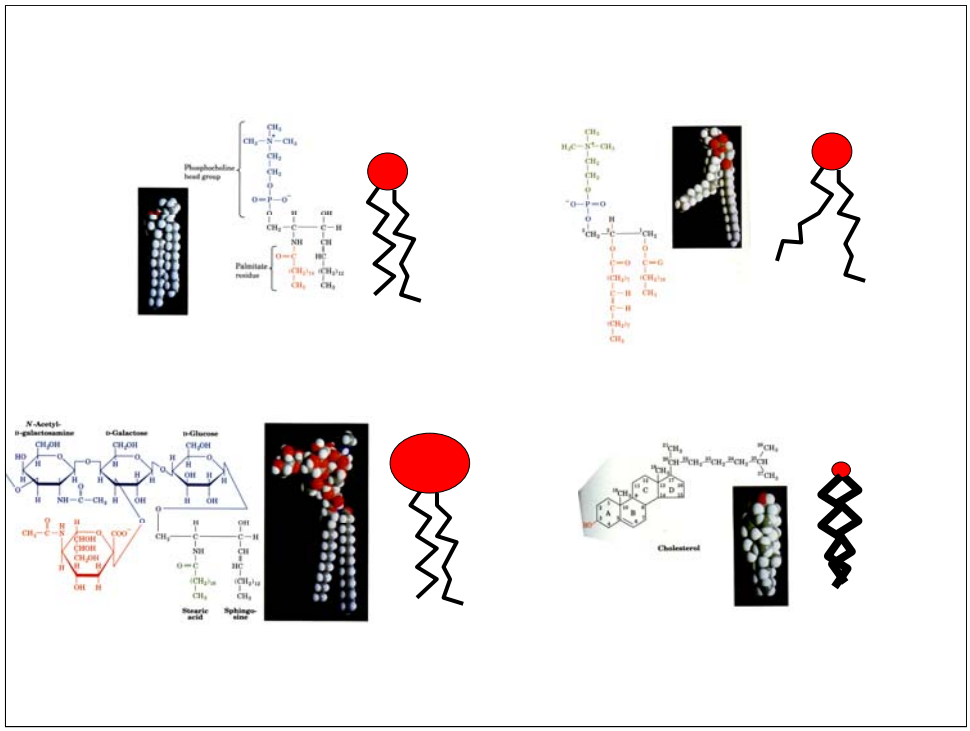
Tails have even number of Carbons due to synthesis from C2 units. 16-18 are most common

About half of tails contain double bonds. Double bonds introduce a 30-degree bend in the tail.

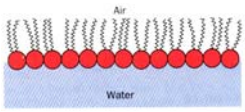
Shape and size of head and tail determine how lipids will self-organize when mixed with water.

Charge of head also matters.

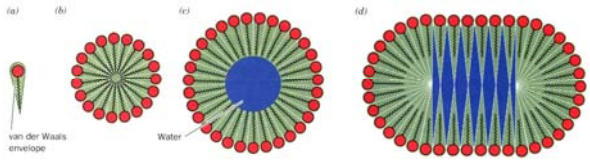
> Charge-charge repulsion increases effective head size
>
> Negative surface charge means that membranes have a negative surface potential.



[Examples of phospholipids] (3)

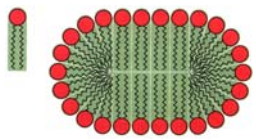


Surface monolayer



Micelles:

- Form when tails are smaller than heads
- Larger micelles (c,d) will break up to optimum size (b)



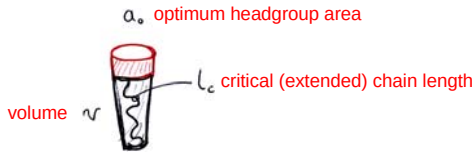
Bilayers:

- Form when tails and heads are similar size
- Curved ends have higher free energy than flat middle

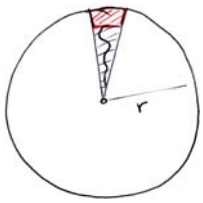
[Some common phases of amphiphilic molecules in water] (4)

Increased free energy at the edge means small bilayers (as shown here) will aggregate to form extended sheets, minimizing free energy.

A simple 3-parameter model



Spherical micelle with N molecules:



Volume: $\frac{4}{3}\pi r^3 = Nv$

Surface area: $4\pi r^2 = Na_o$

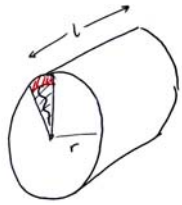
No hole in middle: $\Rightarrow r = \frac{3v}{a_o} \leq l_c$

Condition for Spheres: $\frac{v}{l_c a_o} \leq \frac{1}{3}$

[Simple model of amphiphile phases] (5,6)

“optimal head-group area” depends on electrostatic repulsion as well as actual size.

Cylindrical micelle:



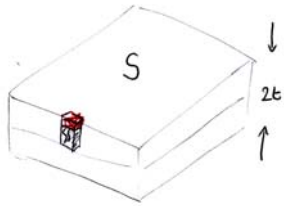
Volume: $\pi r^2 l = N v$

Surface area: $2\pi r l = N a_0$

No hole in middle: $\Rightarrow r = \frac{2v}{a_0} \leq l_c$

Condition for Cylinders: $\frac{v}{l_c a_0} \leq \frac{1}{2}$

Planar bilayer:



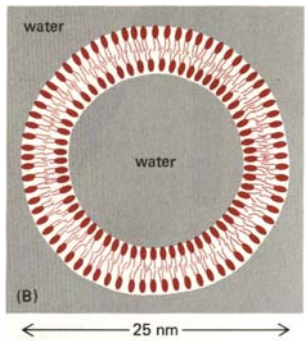
Volume: $2St = Nv$

Surface area: $2S = Na_0$

$\Rightarrow \quad t = \frac{v}{a_0} \leq l_c$

Condition for bilayers: $\frac{v}{l_c a_0} \leq 1$

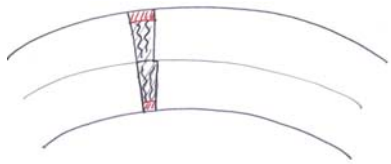
"Phase diagram"



A (very small) “vesicle” or “liposome”

Bilayer curvature

Head groups have more space on the outside…



… and less space on the inside

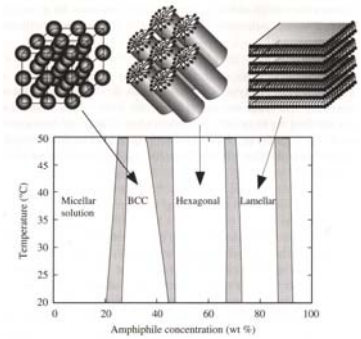
[Vesicles / Liposomes] (7)

Biological membranes are basically planar bilayers.

The edges of a bilayer can seal to form a closed “vesicle” or “liposome”.

- A bit like a cell membrane
- Free energy is reduced by eliminating edges, but may increased by adding membrane curvature
- For large vesicles, curvature is negligible. For small vesicles, it limits their size.
- Vesicles have many (potential) uses, eg delivery of drugs.

Lipid heads do not cross the bilayer, and biological membranes are asymmetrical w.r.t. lipid composition (control of curvature to form desired structures may be one of the reasons for this)



(For more on self-organized amphiphile phases see Jones, Chapter 9.)

"Cubic Phase"

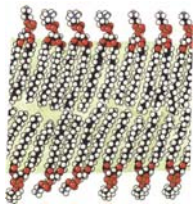
[More complicated phases] (8)

As the fraction of amphiphile increases, concentration becomes important and new phases are seen

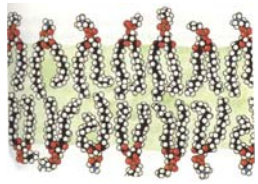
Eg. The lamellar phase is found in bars of soap.

Cubic phases have zero mean curvature everywhere (all saddle)

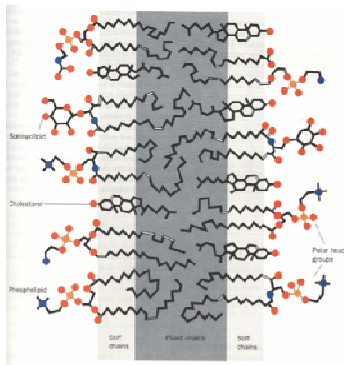
Cubic phases have been designed to help with determination of membrane protein structures.



Below transition temperature – “frozen” bilayer



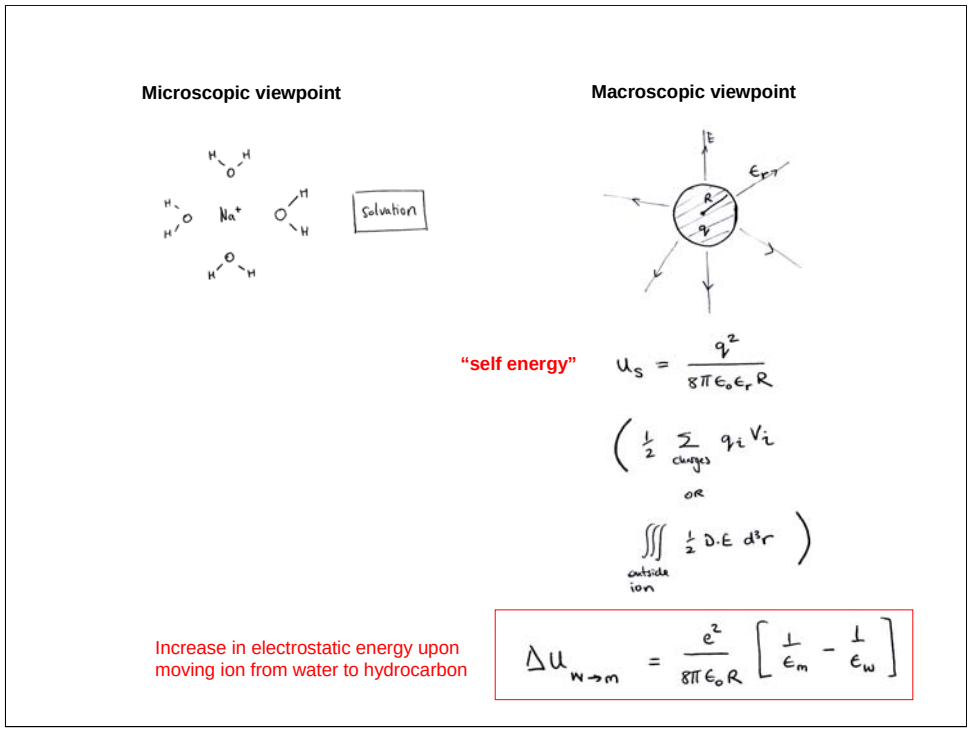
In living organisms – “2-D liquid”



[Biological phospholipid bilayers] (9)

At “physiological” temperatures (ie. when things are alive and molecules are functional), bilayers form a 2-D fluid.

- Membrane fluidity is increased by double-bonds in hydrocarbon tails, also by cholesterol.
- Lipid mobility within each half of the bilayer is high.
- Proteins etc embedded in the bilayer are also mobile
- Very recently, structure in bio-membranes is being discovered – “lipid rafts”, “corrals” and “fences”.



The simplest possible view of the bilayer appeals to the physicist…

Electrical Properties of lipid bilayers

Lipid bilayers are EXTREMELY effective barriers to charged molecules

In particular, small ions

[de-hydration energy] (10)

"Self energy" can be thought of as the work required to assemble the charge distribution by gradual charging (integrate V dq with fixed geometry) – OR as stored throughout space in the electric field due to the charge (integrate ½ D.E).

note: we are interested in the effect of changing the ion's surroundings from water to membrane, so we can assume all charge is at the surface of the ion if we want.

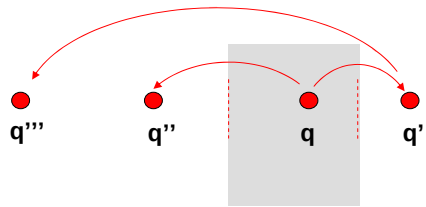
For K+, *R* ~= 0.2 nm. Dielectric constant of hydrocarbon lipid tails *em* ~= 2. Therefore hydration energy is ~260 x 10-21 J = 65 kT

exp(-65) ~ 10-28, so there is essentially no chance of spontaneous partition of the ion into the membrane.

In both microscopic and macroscopic views, it is re-orientation of water molecules that is responsible for the hydration energy of ions and therefore for the high energy for transfer to the membrane

In fact, the first "hydration shell" of water molecules is the most important, and thus the microscopic view is necessary for accuracy.

Method of image charges to calculate the electric field in the membrane due to an ion



How big is the effect of finite membrane thickness?

$\varepsilon_w \sim 80 >> \varepsilon_m$:

1. Approximate water as a conductor
   => $q' = q'' = -q = -e$
2. Ignore $1/\varepsilon_w$ term in de-hydration energy

With **q** in the centre of membrane, thickness **t,**
Interaction energy with the first two charges:

$$(\Delta\Delta u) \approx \frac{-e^2}{4\pi\epsilon_0\epsilon_m t} \times 2$$

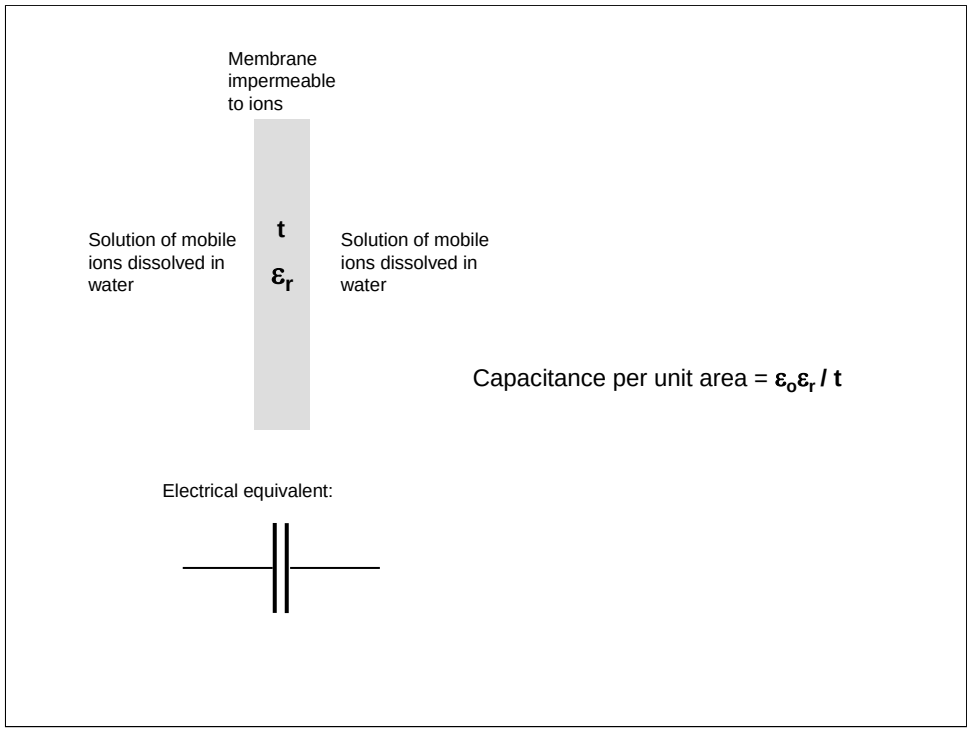
$$\Rightarrow \frac{(\Delta\Delta u)}{\Delta u_{w\to m}} \approx \frac{-8R}{2t} \approx \frac{4R}{t}$$

$R \approx 0.2\text{nm}$, $t \approx 5\text{nm}$ $\Rightarrow \frac{(\Delta\Delta u)}{\Delta u_{w\to m}} \approx 16\%$

[Finite membrane thickness] (11)

An infinite series of ever smaller of image charges can be used to model the effect of the water on the potential inside the membrane and thus on the self-energy.

The reduction of the energy barrier in the centre of a typical biological membrane compared to bulk hydrocarbon is of order 10-20%.



[Membrane Capacitance] (12)

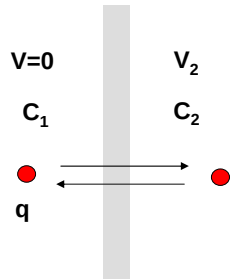
The membrane is an insulating plane between two conducting solutions, ie) a capacitor.

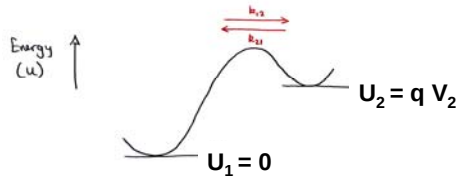
The specific capacitance of the membrane is relatively high because it is only two molecules thick.

(For membrane calculations, it's useful to remember that an infinite plane of charge produces a uniform electric field, from Gauss Law).

"Nernst" or "Diffusion" Potential

If ions are allowed to cross the membrane:





At equilibrium: $C_1 k_{12} = C_2 k_{21}$

Nernst Equation

$$\frac{C_2}{C_1} = \frac{k_{12}}{k_{21}} = e^{-\frac{qV_2}{kT}}$$

(see lecture 5)

By mass action: $rate_{1\to} = AC_1 \equiv Ae^{\frac{\mu_1}{kT}}$

This defines μ , the "Chemical Potential"

$\therefore \mu_1 = kT \ln C_1$

$\Delta\mu_{12} = \mu_1 - \mu_2 = kT \ln \frac{C_1}{C_2}$

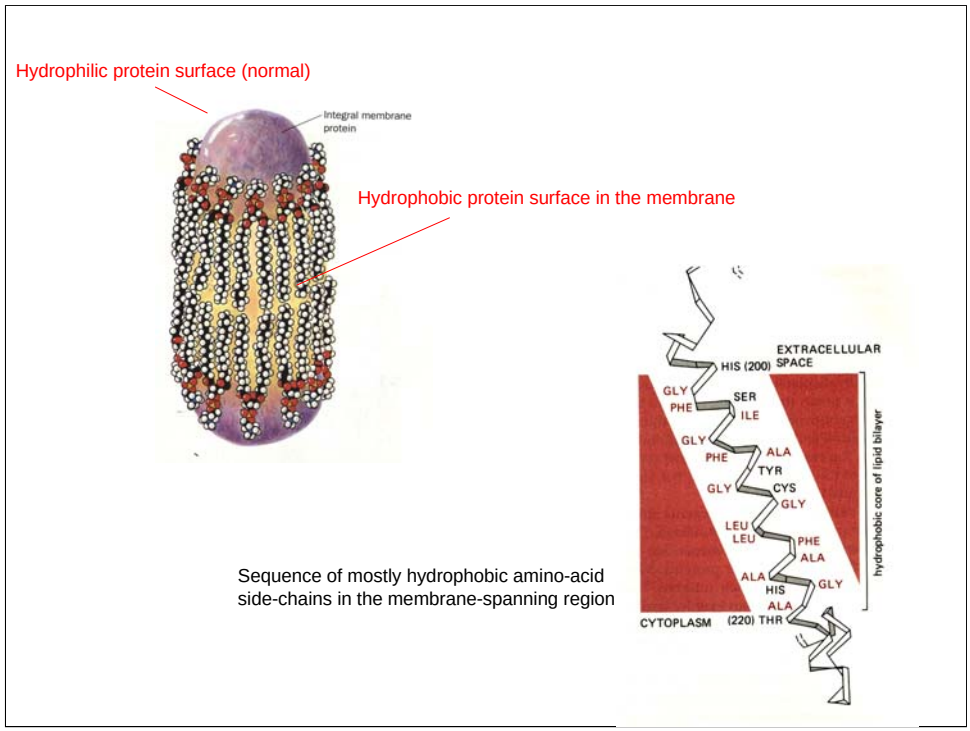
["Nernst" or "diffusion" Potential] (13)

"chemical potential" is essentially a way to formulate mass action as an equivalent free energy, via Boltzmann's law.

Chemical and electrical potentials contribute (add) to the free energy of a "state"

> The sum of electrical and chemical potentials is called the "electrochemical potential".
>
> The Nernst potential is simply the *electrical* potential when the *electrochemical* potential is zero
>
> Electrical and chemical potentials are the two forces that can drive ions across the membrane. The first is electrostatic, the second is entropic.

Despite the relatively high membrane capacitance, the number of charges that need to move to establish a typical membrane potential is VERY SMALL compared to the number present in a typical solution. (Try some numbers – compare the capacitance charge with the total number of ions in a typical cell. Ion concentrations are on the order of 100 mM)



Membrane Proteins

Total isolation of a cell is not an option – the membrane must be made permeable.

Hydrophobic amino acids at the surface of proteins will allow them to insert into the membrane (ie. proteins can be amphiphilic too).

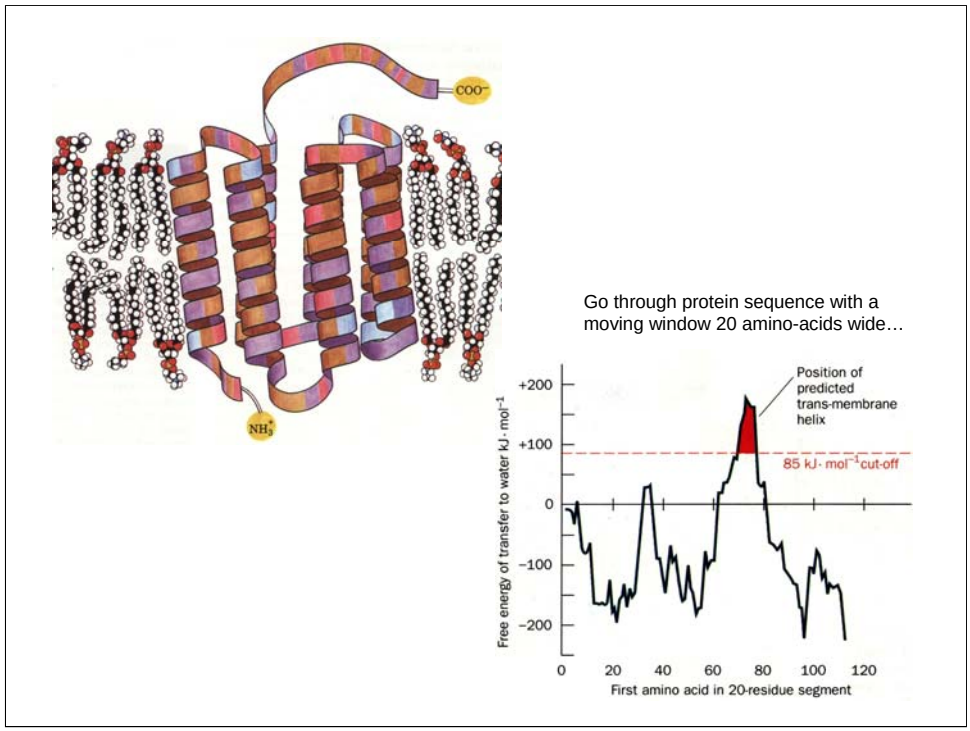
[Integral Membrane Proteins] (14)

How do they get there? (The hydrophilic parts on the outside have to cross the membrane)

- Actually inserted into membranes while being synthesized, before folding
- A “Signal Sequence” of ~20 amino acids at the N-terminus identifies membrane proteins to the machinery that does this, and is later chopped off.

Membrane spanning segments are usually alpha-helices

- Backbone H-bonds are all satisfied within the helix – no loss to put them in the membrane
- Side-chains all stick out – makes a hydrophobic rod.

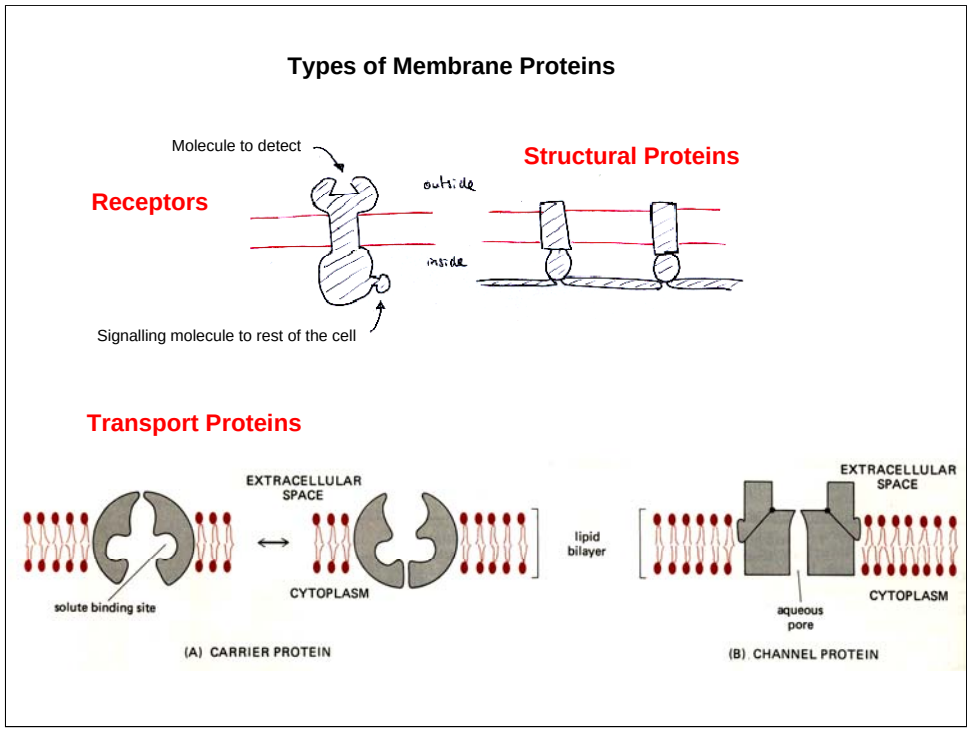


[Alpha-helix bundles] (15)

20 amino-acids will span the membrane as an alpha-helix.

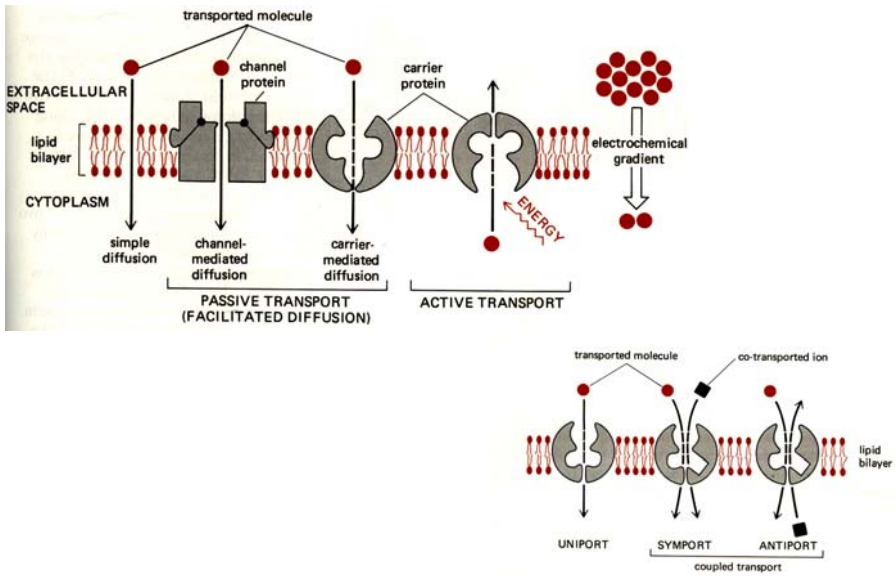
These helices usually form bundles in membrane proteins.

Prediction of membrane-spanning regions from primary sequence is an example of **bioinformatics**.



[Types of membrane proteins] (16)

Active and Passive transport



[Active and passive transport] (17)

Active transporters work against the electrochemical gradient – "pumps"

This can be achieved by coupling transport to another process that is energetically "downhill" (DG < 0). Examples…

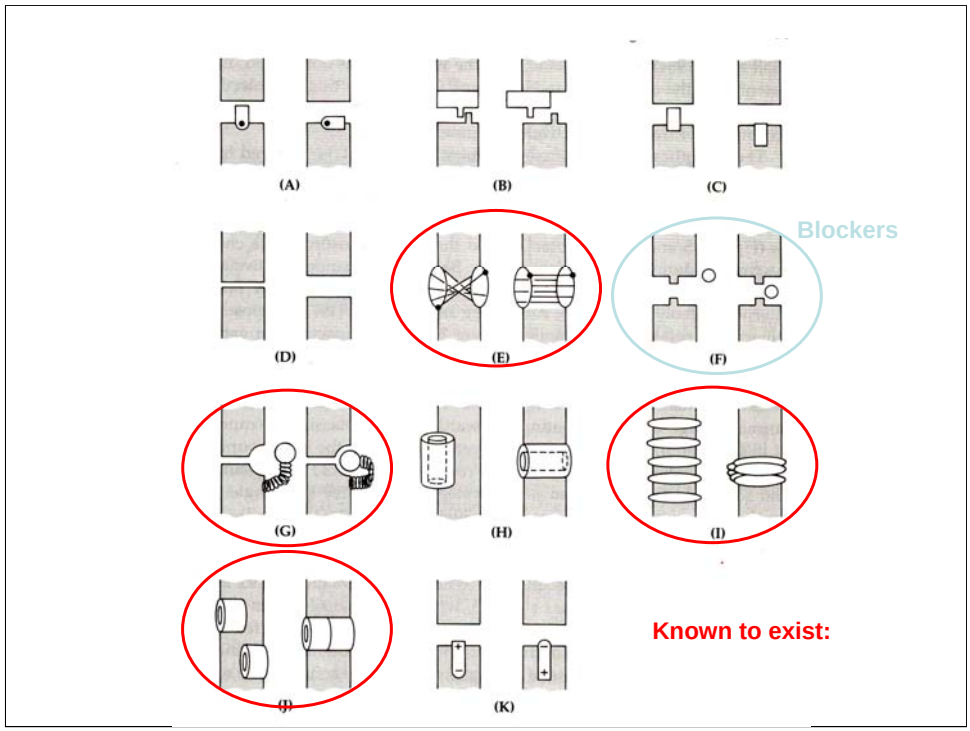
Transport of something else – "co-transporters".

Absorption of photons – photosynthesis, light generates an electrochemical gradient of protons called the "protonmotive force" or "pmf". The pmf is the primary form of free-energy storage for living cells. (Lecture 7)

Breaking down food – "respiration"

Hydrolysis of ATP – pumping "ATPases".

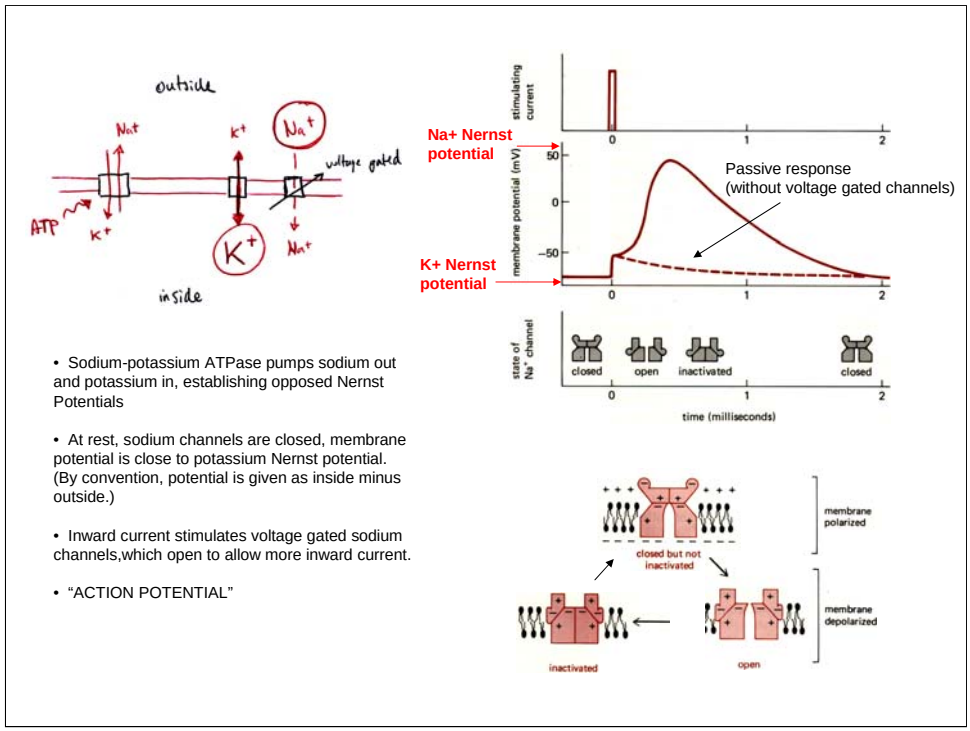
The coupling of downhill processes (burning food, light absorption) to drive uphill processes (movement, growth, the generation of order in spite of the 2nd law of Thermodynamics) is the essence of life. The coupling is done by the molecular machines of life, mostly proteins. How it works will be discussed in lecture 9.



[Possible gating mechanisms in Ion channels] (18)

Gating can be in response to

- Binding a target molecule – eg) smell
- Absorbing light – eg) sight
- Force – eg) hearing, osmotic stress channels
- Voltage – eg) nerve transmission



- Sodium-potassium ATPase pumps sodium out and potassium in, establishing opposed Nernst Potentials
- At rest, sodium channels are closed, membrane potential is close to potassium Nernst potential. (By convention, potential is given as inside minus outside.)
- Inward current stimulates voltage gated sodium channels,which open to allow more inward current.
- "ACTION POTENTIAL"

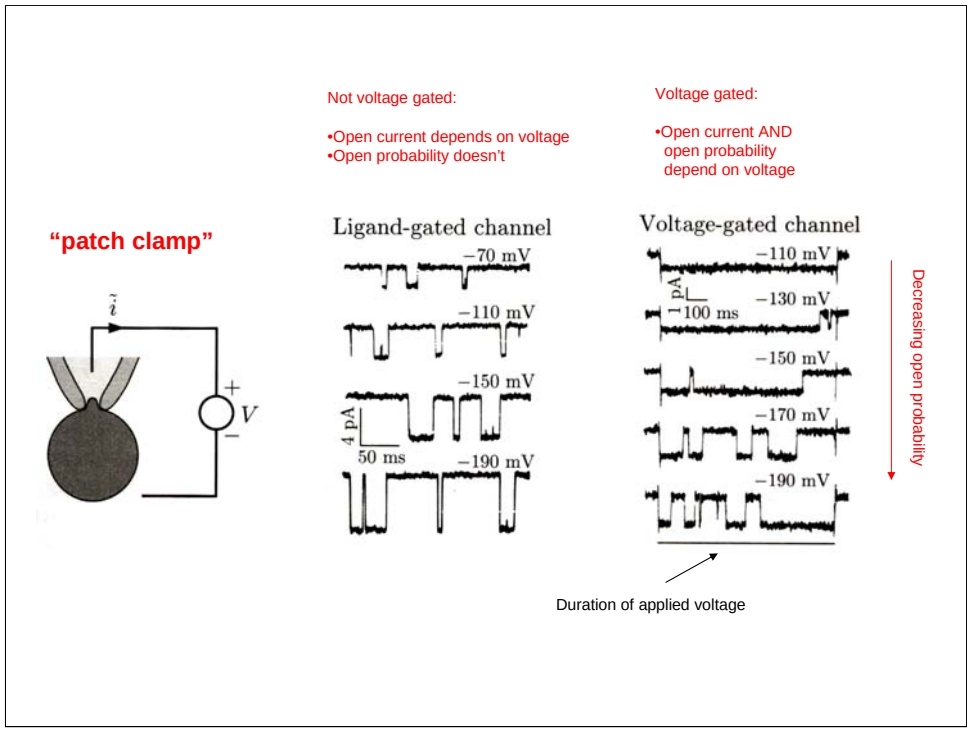
[Nerve Action potentials] (19)

A transient depolarization of the membrane due to Na+ channels opening (fast) and then inactivating (slow) propagates along the nerve.

"forwards" part of the nerve is triggered by diffusion of de-polarizing ions.

"backwards" part of nerve is inactivated. Reactivation is slower still, and determines the maximum rate at which pluses can be sent down the nerve.

Nb: Gating current – capacitive or displacement current due to moving voltage sensor – is measurable. It must be there if the channel is to be sensitive to voltage.



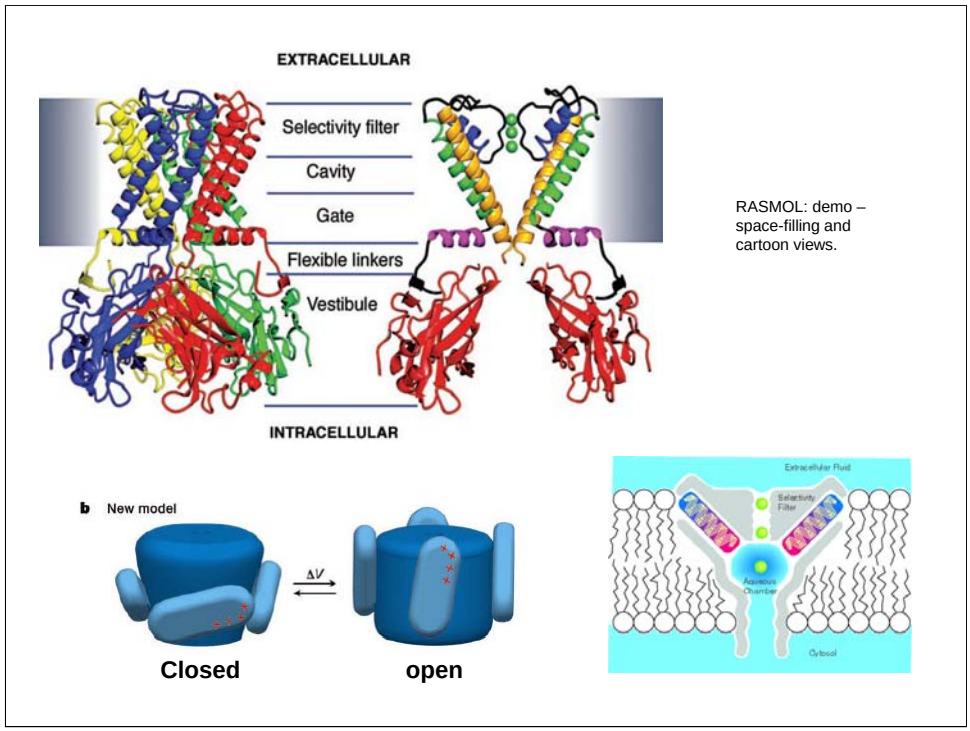
[Single-channel current recording] (20)

"Stochastic" opening and closing is common signature of ion channels.

Gating – changes the probability of being open

First "single-molecule" technique in biology – now there are many more (Lectures 10 & 11).

The process has built-in "gain". One molecular event (channel opening) results in the flux of millions of ions, which can be detected relatively easily.



RASMOL: demo – space-filling and cartoon views.

[Potassium channel crystal structure] (21)

KcsA – a small, simple bacterial K+ channel. Typical of K+ and Na+ channels.

water-filled cavity with short "selectivity filter" – to minimize energy barrier of membrane.

Also, alpha-helix dipoles and

H-bonds from water and protein backbone in selectivity filter.

Nb: This is probably the closed structure – opening by twisting the helices.

Multiple ion occupancy in single-file with water molecules

NB: There are very few crystal structures of membrane proteins (order 10 compared to 1000s of soluble proteins). This is because they depend upon the membrane for their correct structure.

"Molecular Dynamics" –Giant computer simulations to try and work out the details of the conduction mechanism (rates ~107 ions per second).