Lecture 26: Permeability, Phase transitions, Cholesterol, Biological Membranes:

Goals:

- Predict phase transition temperature
- Predict osmotic effects
- Differentiate between peripheral and integral proteins
- Understand need for detergents in membrane protein purification
- Functional properties of integral membrane proteins
- Structural properties of integral membrane proteins
- Calculate partition coefficient *aqueous* ↔ *membrane* for peptides.

Phospholipids - Permeability properties:

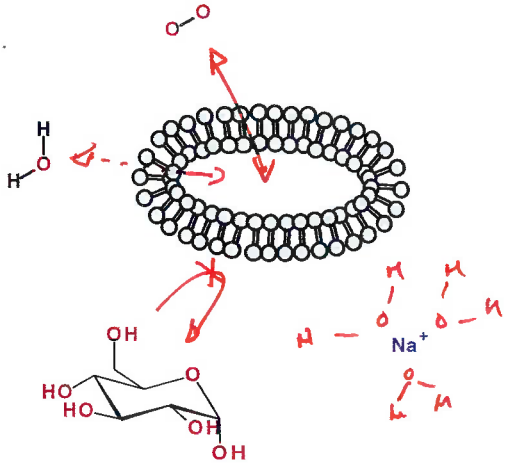
Small non-polar (O_2) fast

Small polar (H_2O)

Large polar (glucose)

Ions (Na^+) slow

rate through membrane



Osmotic Effects;

- A concentration difference across a membrane represents a system that is not at equilibrium.
- Flow is spontaneous from high concentration to low, until equilibrium is reached.
- Ions/large polar will not flow across the membrane (unless a channel is present), so their movement is zero.
- Water will flow across the membrane to establish equal concentration on both sides of the membrane. Will flow from high water concentration to low. High water concentration = low solute concentration.

Hypotonic solution: The concentration of salt is lower outside the cell than inside the cell.

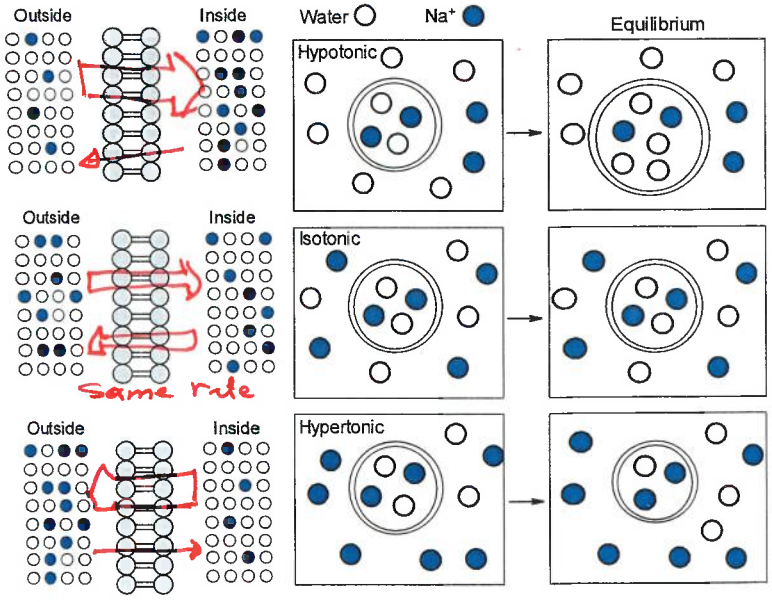
$[H_2O]_{OUT} > [H_2O]_{IN}$

Isotonic solution: The concentration of salt is the same inside and outside the cell.

$[H_2O]_{OUT} = [H_2O]_{IN}$

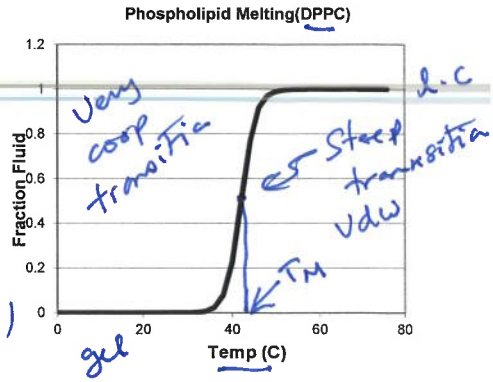
Hypertonic solution: The concentration of salt is higher outside the cell than inside the cell.

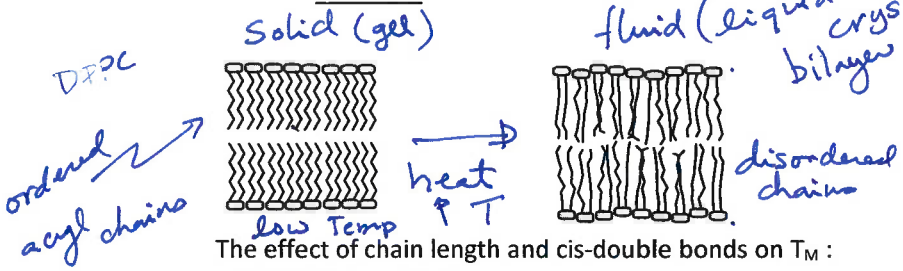
$[H_2O]_{OUT} < [H_2O]_{IN}$



Lipid Phase transition:

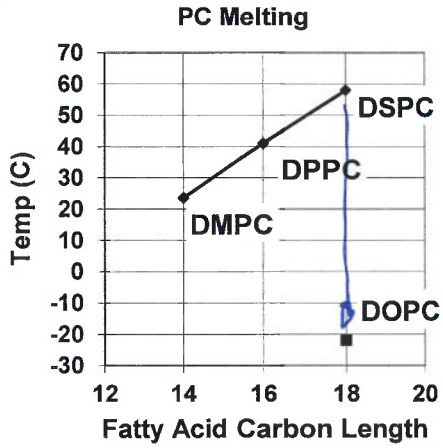
- Lipid bilayers undergo a *highly cooperative* (melts over a narrow temp range) phase transition with a defined T_m.
- Below T_m the lipids exist as a solid-like *gel*; the acyl chains are tightly packed.
- Above T_m the lipids are in a liquid-like *liquid crystal phase*. The acyl chains are disordered. Thus, the phase transition is called the gel to liquid-crystalline transition.

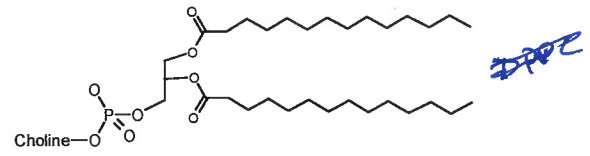


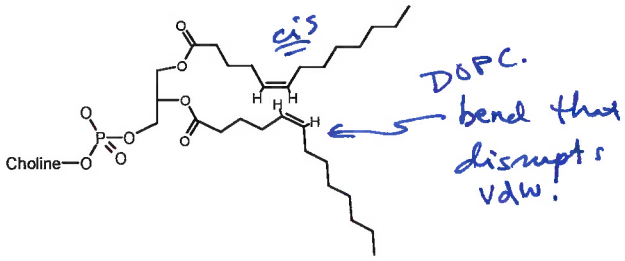


The effect of chain length and cis-double bonds on T_M :

DMPC (C_{14}) = 23 C
DPPC (C_{16}) = 41 C
DSPC (C_{18}) = 58 C
DOPC ($C_{18:1}$) = -22 C

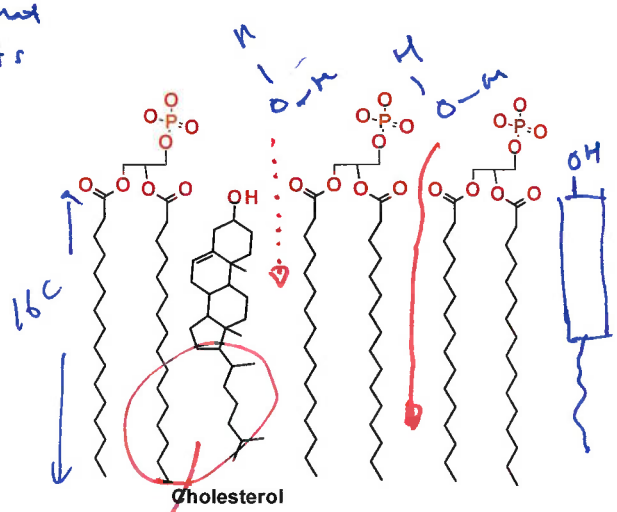


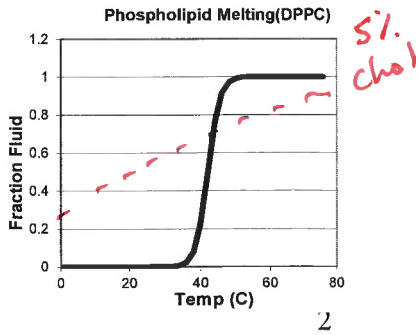




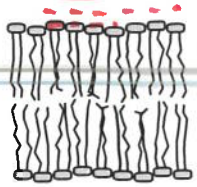
Cholesterol:

1. You produce about 1 g/day!
2. About the same length as C_{16} fatty acid; therefore it reaches across half of the bilayer.
3. *Essential* component of most mammalian membranes.
4. Destroys the phase transition of pure lipid membranes, thereby keeping the membranes fluid below the phase transition and more rigid above the phase transition. Often referred to as a membrane *plasticizer*.
5. Makes membranes less permeable to water.
6. Source of steroid hormones.





Membrane Proteins:

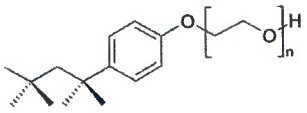


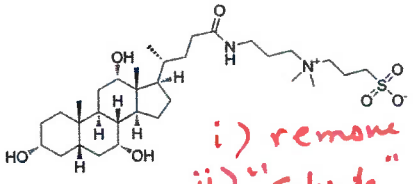
Peripheral Membrane Proteins:

- Loosely attached to membranes via electrostatic interactions – released with high salt.
- Often involved in electron transport (cytochrome C) and act as specific binding proteins, e.g. sugar transport in bacteria.

Integral Membrane Proteins:

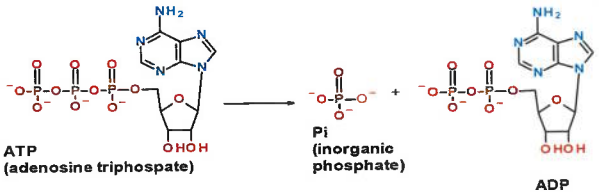
- Considerable hydrophobic surface that interacts with the acyl chains of the lipid.
- Purification usually requires the use of mild detergents to release the protein from the membrane. Triton-X100 (upper) or Chaps (lower) are often used. The detergents replace the non-polar interactions with the lipid to maintain the protein in its native, functional form.





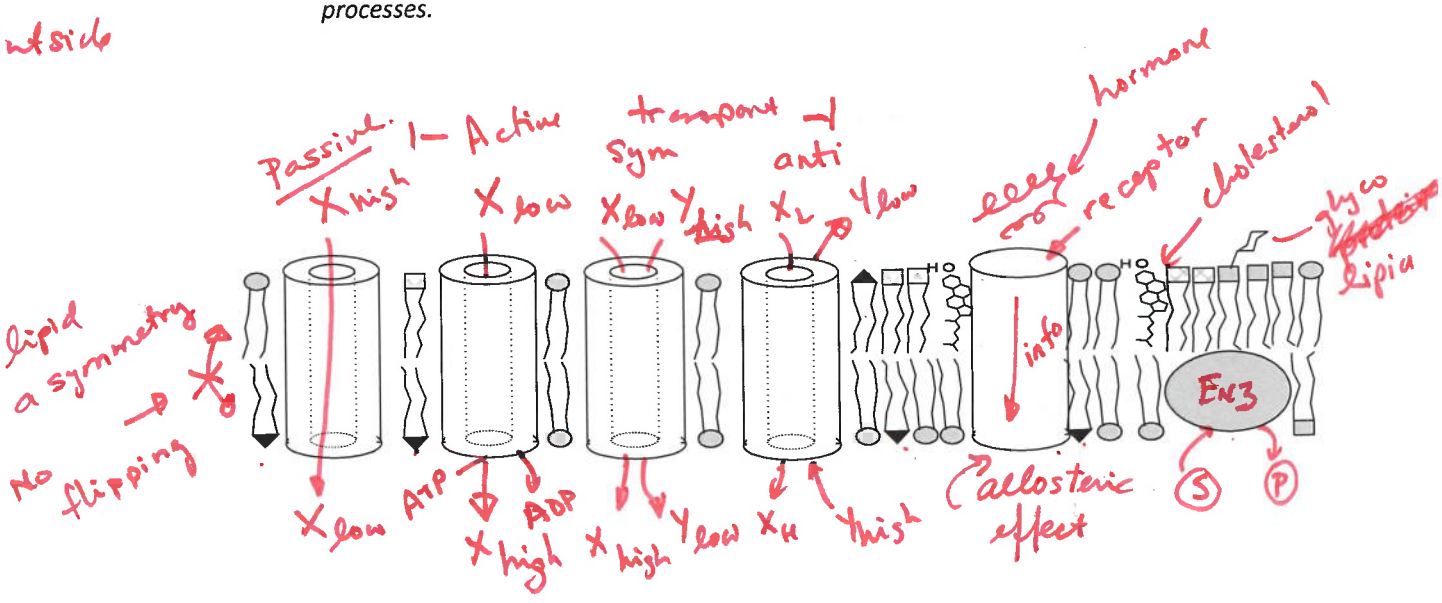
Functional Classes of Membrane Proteins:

1. Transport (*e.g.* of protons, metabolites, electrons)
   a. Passive transport (no energy required, molecules spontaneously go from high concentration to low)
   b. Active transport (energy required, molecules are pushed from low to high concentration.)
      i) symport – transported ligand and "energy source" (e.g. ion gradient) move in the same direction.
      ii) antiport – transported compound and "energy source" move in the opposite direction.
      iii) Transport often coupled to the hydrolysis of ATP.
2. Signal transduction.
3. Enzymes.



Biological Membranes – "*Fluid mosaic model.*"

- Fluid: *Components can move laterally in the plane of the membrane.*
- Mosaic: *Many different components*
- Heterogeneous: *Clustering of molecules – "lipid rafts"*
- Asymmetric: *One side is different than the other, asymmetry is maintained by active processes.*



Structure of Integral Membrane Proteins:

- Largely contained within the membrane.
- Stability energetics are similar to water soluble proteins, except that membrane proteins are "inside-out".

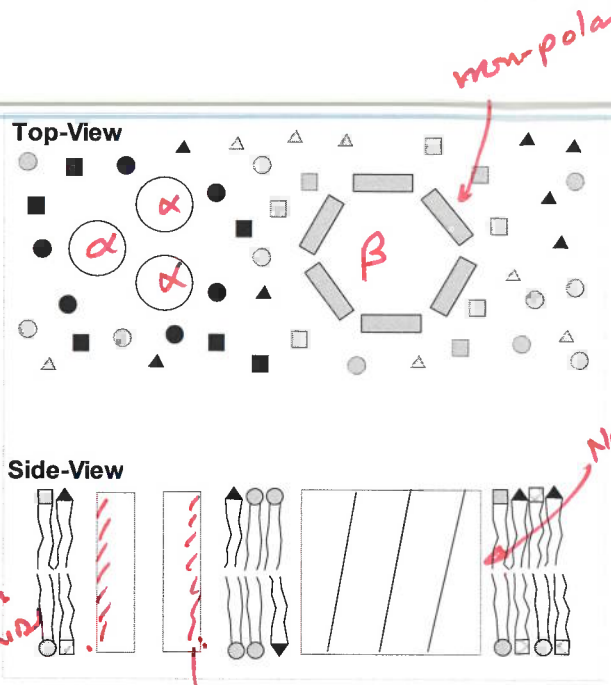
i. Non-polar groups interact with acyl chains in the membrane. The general rule is: hydrophobic outside--hydrophilic or hydrophobic inside, depending on function.

ii. The following secondary structures are observed:

β-barrel

α-helix

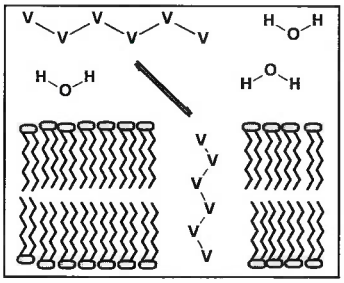
* ***Reflection:*** *β-strands and unstructured regions are not found within the membrane. Why?*



α & β provides a way to reform H-bonds that were to water.

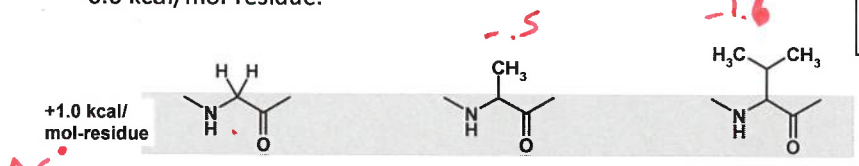
- Usually, the secondary structures are **amphipathic**, displaying a non-polar face to the lipids and a polar face to the interior of the protein. The polar groups in the interior are responsible for function.

Energetics of Membrane Insertion: Non-polar side chain must provide sufficient energy to overcome the unfavorable energy of +1 kcal/mol that is required to bury polar **mainchain** atoms in the non-polar environment.



The *overall* transfer energy for glycine is +1.0, alanine +0.5, and valine

-0.6 kcal/mol-residue.



Example: A peptide is made of 6 Val residues.

i) Calculate the **partition coefficient** for the peptide into a membrane at 300K. $K_P = [X_{MEM}]/[X_{Aq}]$

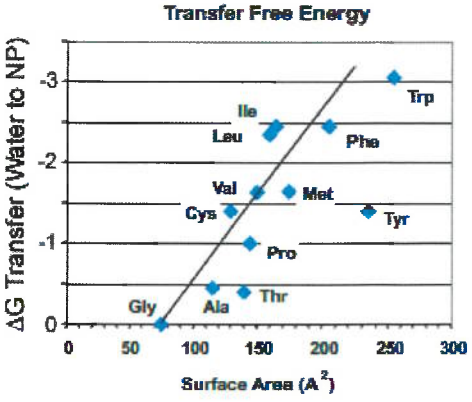
ii) What fraction of the peptide is in the membrane?

i) Calculate $\Delta G^\circ = 6 \times (-0.6 \text{ kcal/mol}) = -3.6$ kcal/mol

Calculate equilibrium constant: $\Delta G^\circ = -RT \ln K_{EQ}$

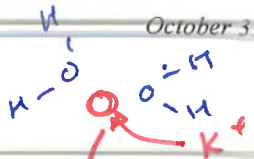
$K_{EQ} = K_P = e^{-\Delta G/RT} = e^{3600/(1.98 \times 300)} = 428.$

ii) $f_{membrane} = K_{EQ}/(1+K_{EQ}) = 428/429 \approx 1.0.$



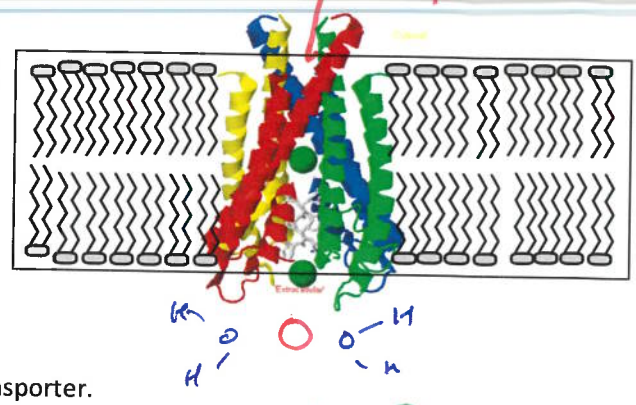
Examples of Integral Membrane Proteins:

A. Potassium Channel – specific K^+ transporter (passive)



i) what provides energy to desolvate

ii) Why is the chan. specific for K^+
- Why doesn't Na^+ go through?



B. Porins – non-selective (< 600 Da) passive transporter.