Introduction to Biological Membranes

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Biological membranes serve 5 distinct functions:

1) Define boundaries and serve as permeability barriers plasma membrane (surrounding cell), and intracellular membranes

2) Sites of specific functions (transport proteins, vesicle sorting, ER)

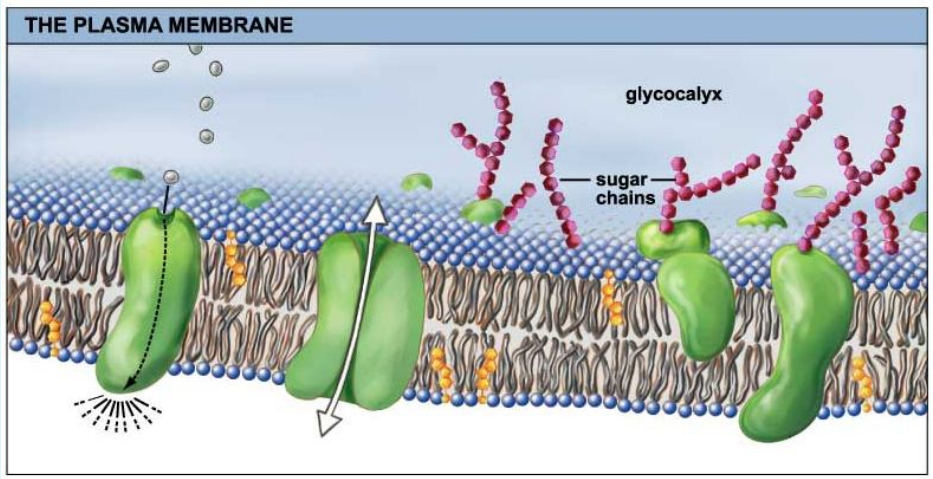
3) Regulate transport of solutes
Facilitated diffusion: transport across membrane by specific proteins
Active transport: energy requiring transport against a gradeint (pump)

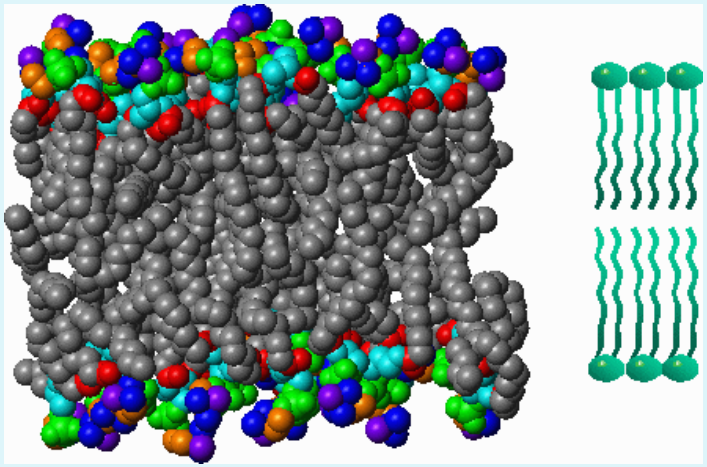
4) Membranes Detect and Transmit Electrical and Chemical Signals
signal transduction: detection and transmission of signals from the membrane to the cell interior (eventually the nucleus)

5) Mediate Cell-Cell communication
adhesion proteins, gap junction- cytoplasmic connection in animal cells

Main Concepts

- Membrane models: historical perspectives
- The Singer-Nicolson "fluid mosaic" model
- Dynamics of lipids and proteins in membranes
- Physical state of lipids in membranes; influence of cholesterol
- Membrane asymmetry: proteins, lipids, carbohydrates





Historical Perspective: Evolving Concepts of Membrane Structure

- Overton (1895) - Found that the ability of a substance to pass through membrane was related to its chemical nature.
- *Non*polar substances pass *more* quickly through membranes into cells than polar molecules. [Contrary to prevailing view at the time; the exception being water.]

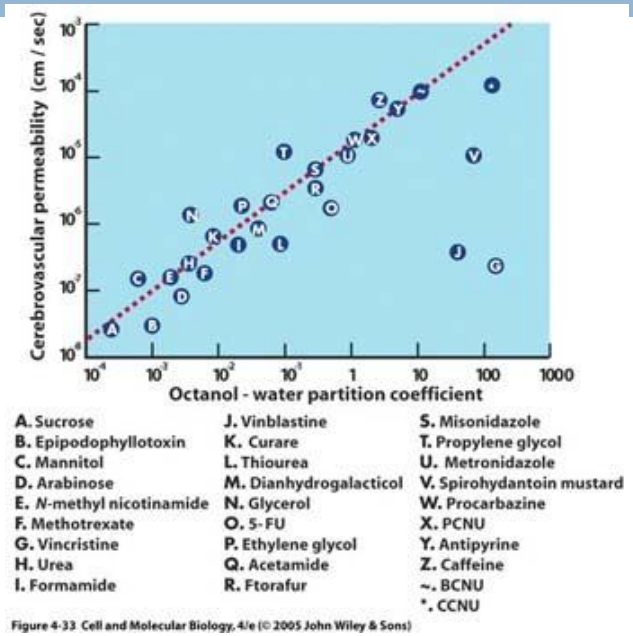
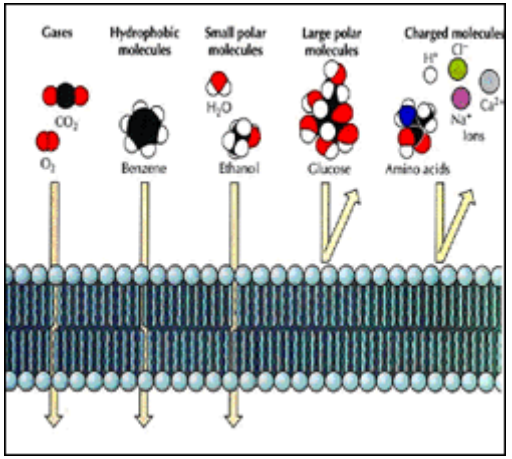
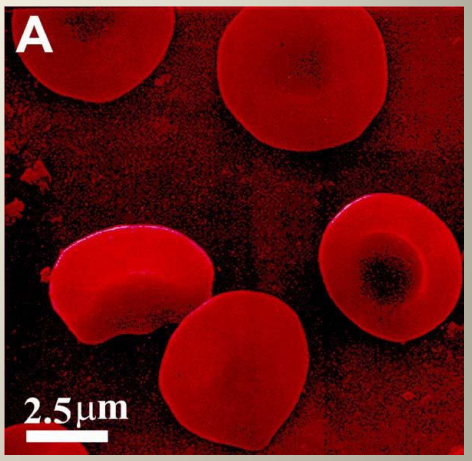


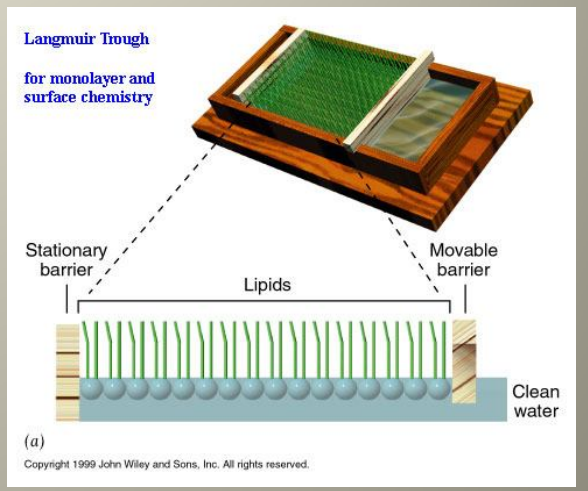
Figure 4-33 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

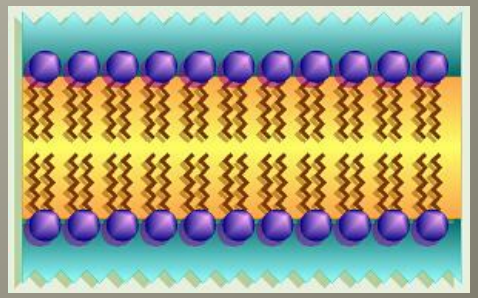


Gorter & Grendel (1925)



- a) Does the red blood cell (RBC) plasma membrane contain lipid? b) If so, how much?
- Prepared RBC membranes, extracted them with organic solvent (acetone)
- Spread lipid extract onto water surface in Langmuir trough (acetone evaporated)
- Applied lateral pressure with glass bar to compress surface film; measured Force (dynes/cm) necessary to compress film
- Measured surface area of film (A_{film}) at point where resistance to compression detected
- Measured RBC dimensions and computed cell surface area (A_{cell})
- Calculated area ratio (A_{film}/A_{cell}) ~ 2 $\Rightarrow$
- **LIPIDS MUST BE ARRANGED AS BILAYER**





(E. Gorter, F. Grendel (1925) *J. Exp. Med*. 41: 439)

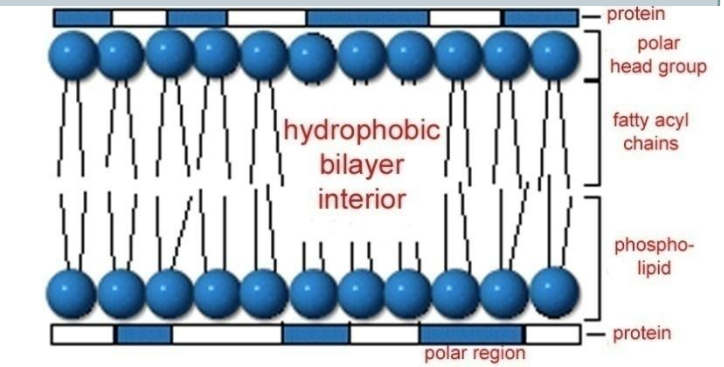
Thoughts about the Gorter-Grendel Experiment: Good idea / Dumb luck

- Acetone does not quantitatively extract all the lipids-- they under-estimated the lipid content of the RBC membrane
- Their calculation of membrane surface area also less than actual figure
- These two errors fortuitously cancelled one another, providing the correct answer after all!

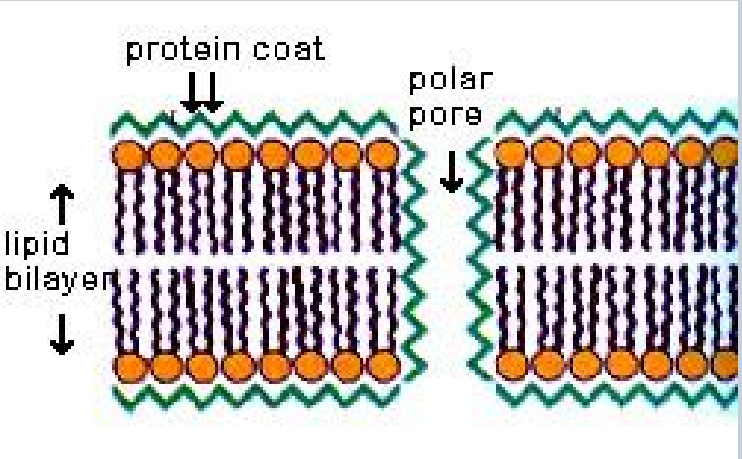
NOTE: Although the Langmuir trough method is “old”, it is still used today to gain useful information about membrane structure and packing of lipids (*e.g.*, see A.B. Serfis, S. Brancato, and S.J. Fliesler (2001) Comparative behavior of sterols in phosphatidylcholine-sterol monolayer films. *Biochim. Biophys. Acta* 1511: 341-348)

Danielli-Davson (1930's-40s)

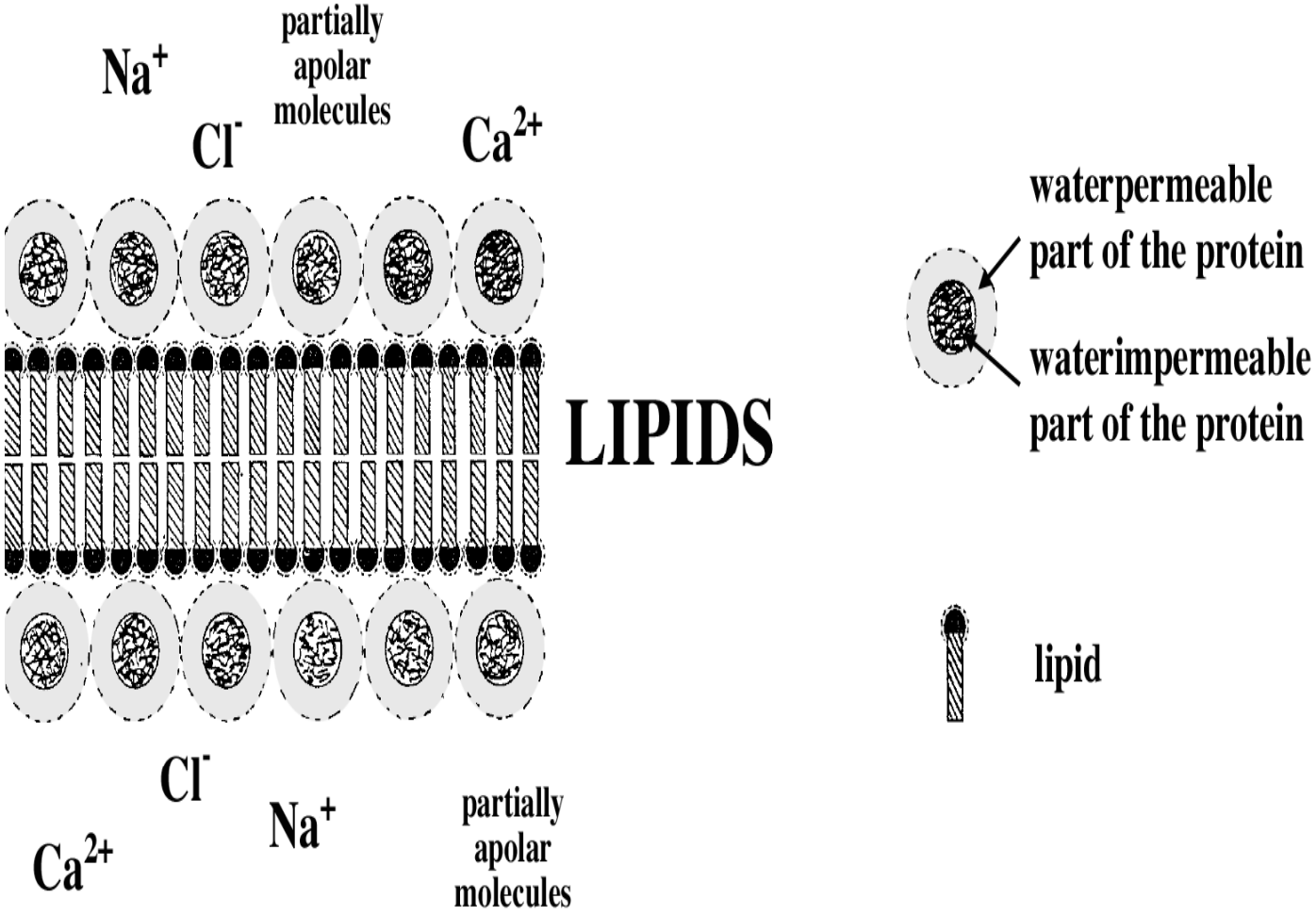
- "Sandwich" Model
- Lipid bilayer with PL polar headgroups facing outwards and fatty acyl "tails" inside.
- Globular proteins coat bilayer.



Subsequently refined model to include protein channels ("pores") interrupting bilayer to be consistent with water and ion permeability .

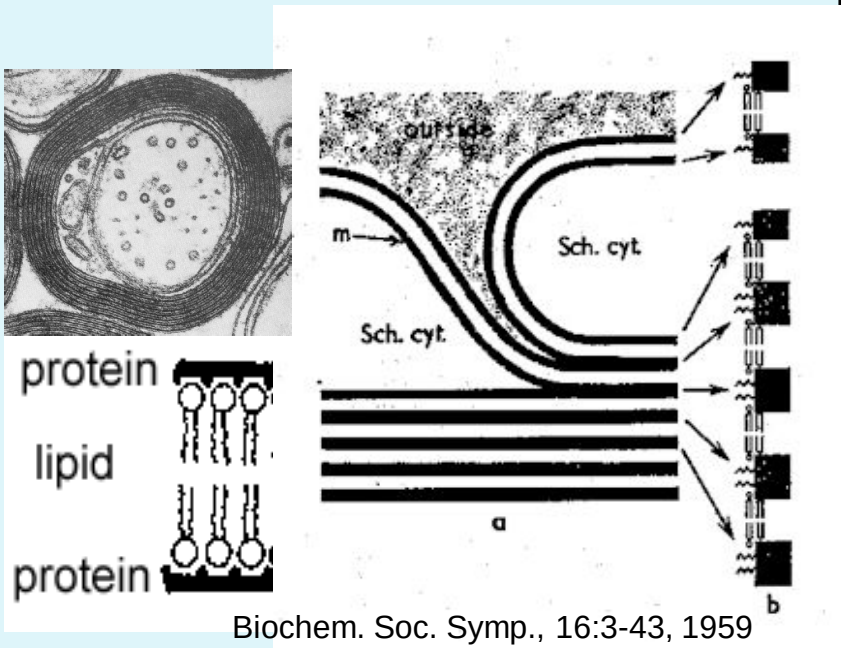


J.F. Danielli, H. Davson (1935) *J. Cell Comp. Physiol.* 5: 495.


Na+
partially apolar molecules
Cl-
Ca2+
LIPIDS
waterpermeable part of the protein
waterimpermeable part of the protein
lipid
Cl-
Ca2+
Na+
partially apolar molecules

J.D. Robertson (1957): "Unit Membrane" Hypothesis

- Based upon $KMnO_4$-stained electron microscopic (EM) images of myelin, and various tissues and cells
- Characteristic **"trilaminar" unit-** two outer dark lines (interpreted as monolayer of protein) separated by a lighter "inner core" line (interpreted as lipid bilayer)
- Proposed ALL cellular membranes are like this!

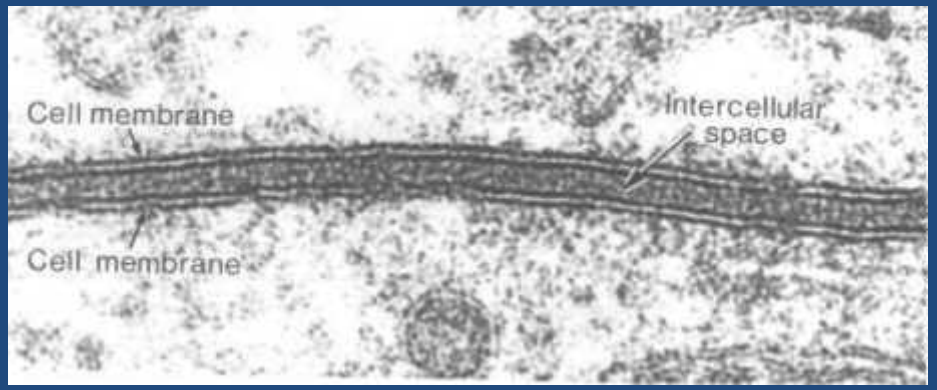


Biochem. Soc. Symp., 16:3-43, 1959

Problems with D-D Model

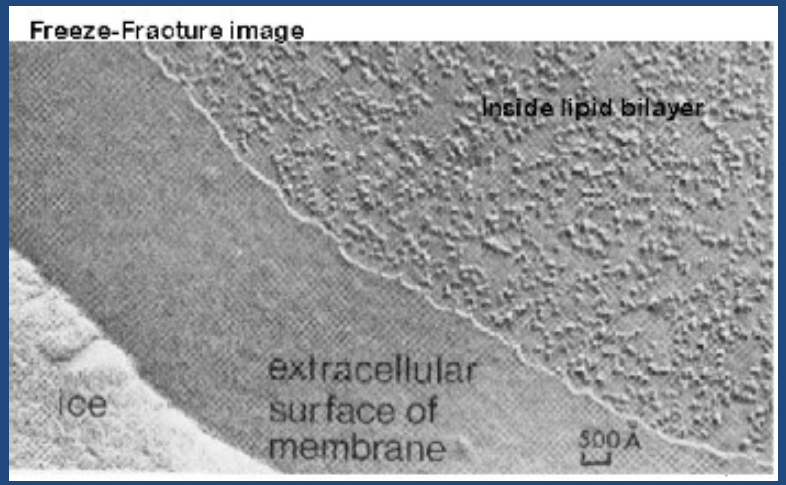
- Proteins are *amphipathic*- protein layer as interface between PL polar head groups and water exposes *hydrophobic* residues of protein to water/charge (energetically unfavorable)
- Largely assumed predominant β-sheet conformation of proteins (later found not to be true)

Electron Microscopy Images

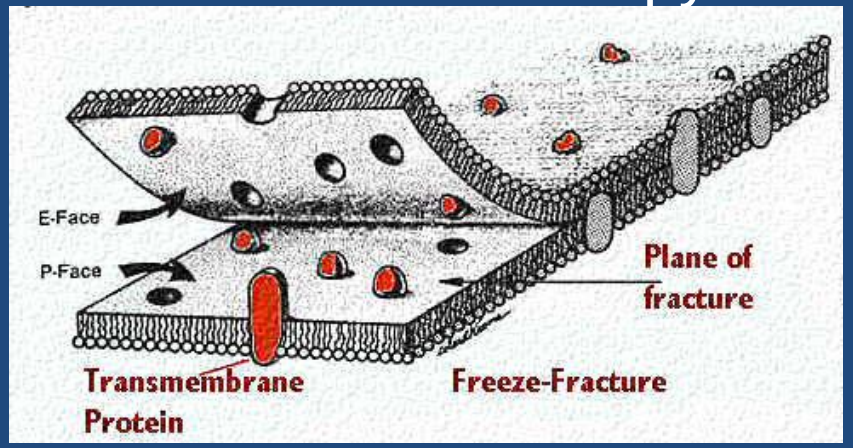


(1950's-1960's)

Transmission electron microscopy (TEM)



Freeze-fracture electron microscopy



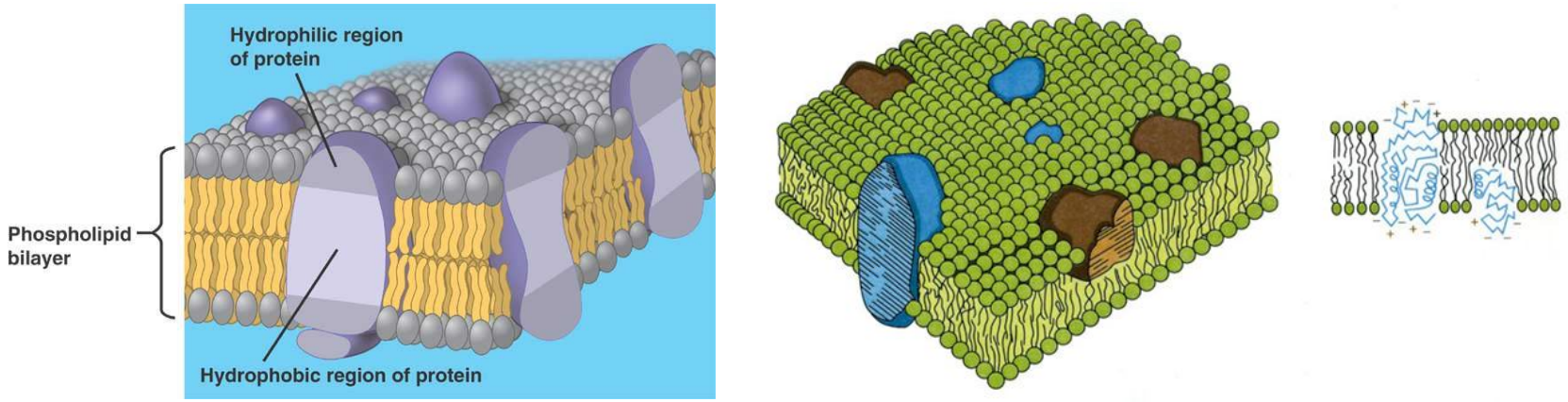
- "cobblestone" appearance
- proteins embedded in and traverse membrane bilayer

D. Branton (1969) *Annu. Rev. Plant Physiol*. 20: 209-238

Problems with Historical Models

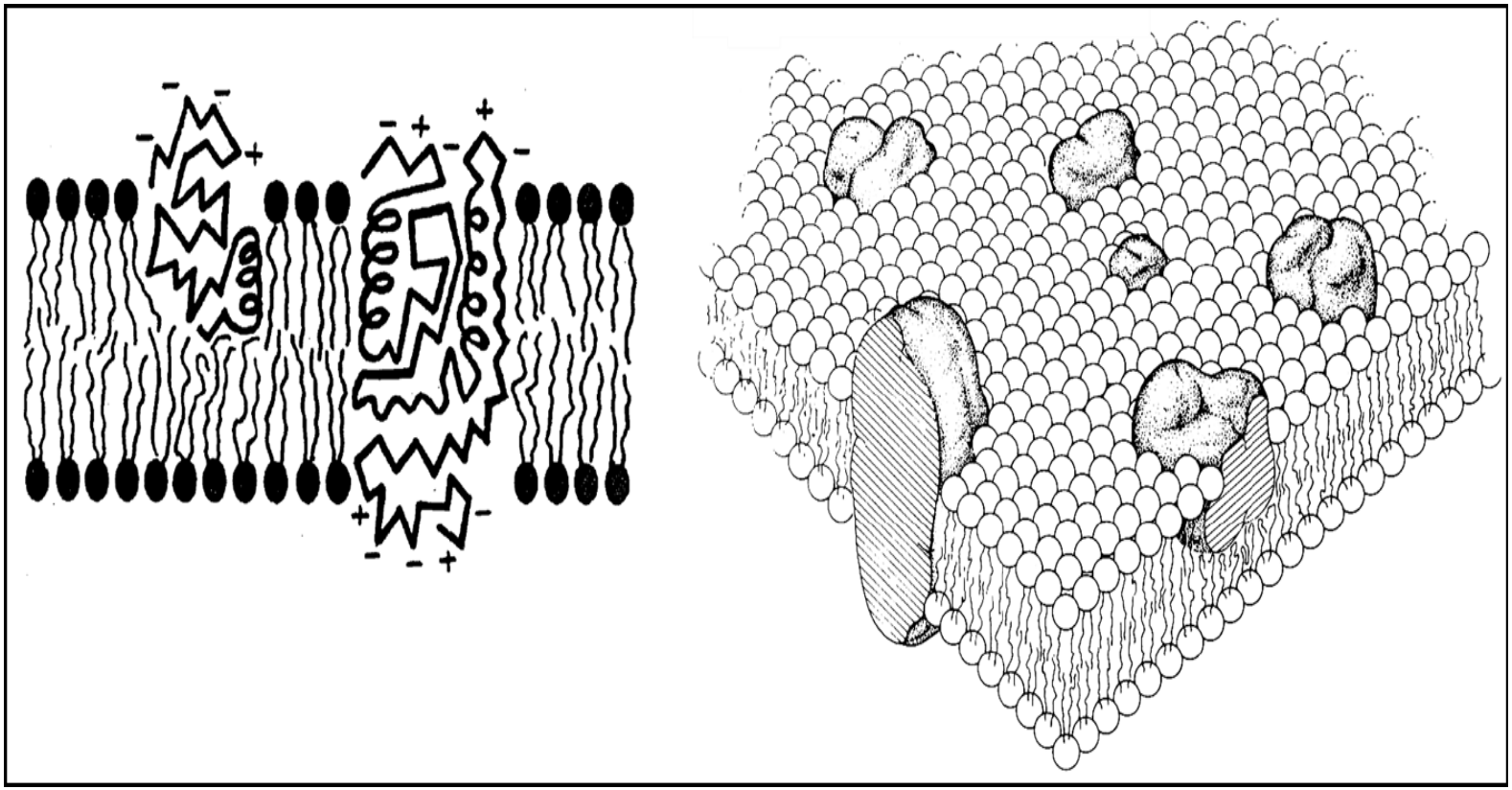
- Assume membrane constituents are *static* (not moving/movable)
- Most do not account for differential permeability of ions, water, small molecules of varying polarity (pores, channels, transporters)
- Assume *all* membranes alike, disregarding known differences in morphology, thickness, and biological function
- Do not take into account α-helical and random coil motifs of proteins (assume dominant *beta* sheet)

Singer-Nicolson "Fluid Mosaic" Model



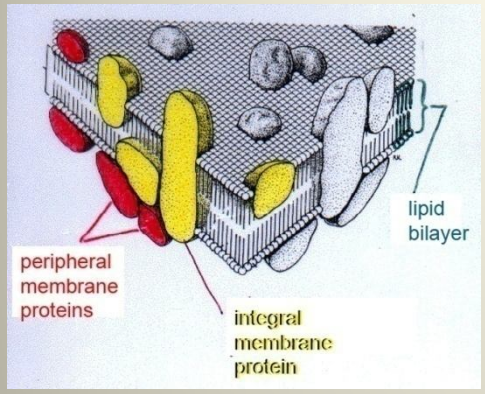
- **The proteins interact with the lipid bilayer by electrostatic interactions (extrinsic proteins) or penetrate partially or completely span the hydrophobic domain of the lipid bilayer (intrinsic proteins).**
- **The lipids of the bilayer matrix are in a liquid-crystal (fluid) state and can diffuse laterally in the plane of the membrane.**
- **The matrix of the membrane consists of a lipid bilayer.**
- **Proteins are able to freely diffuse within the bilayer plane and about their axes perpendicular to the plane of the membrane.**
- **There is no long-range order in the arrangement of components other than that which results from summation of short-range intermolecular interactions.**

S.J. Singer & G. Nicolson (1972) *Science* 175: 720-731.



Essential Concepts

- **Phospholipid bilayer is the major structural feature (forms the matrix of the membrane); asymmetric distribution of lipids in the bilayer.**
- **"FLUID"-- Lipids and proteins diffuse freely in plane of membrane; Proteins "float" in a "sea" of lipid (no constraints indicated). Allowed because protein-lipid and lipid-lipid interactions *weak*, compared to covalent bonds.**
- **"MOSAIC"-- membrane composed of heterogeneous mixture of lipids and proteins, organized in dynamically changing patterns. Proteins also asymmetrically distributed.**
- **Proteins distributed asymmetrically: attached to either side of bilayer, or partially or fully embedded in the bilayer, even traversing (penetrating) bilayer- NOT just coating the bilayer.**
- **THERMODYNAMICS taken into account: Maximize hydrophobic-hydrophobic and hydrophilic-hydrophilic interactions. *Alpha*-helical portions of proteins maximize hydrophobic residue interactions with hydrophobic lipid bilayer interior, allows for hydrophilic residues to be exposed to water (channels) or polar, charged PL head groups.**



Two Types of Membrane Proteins

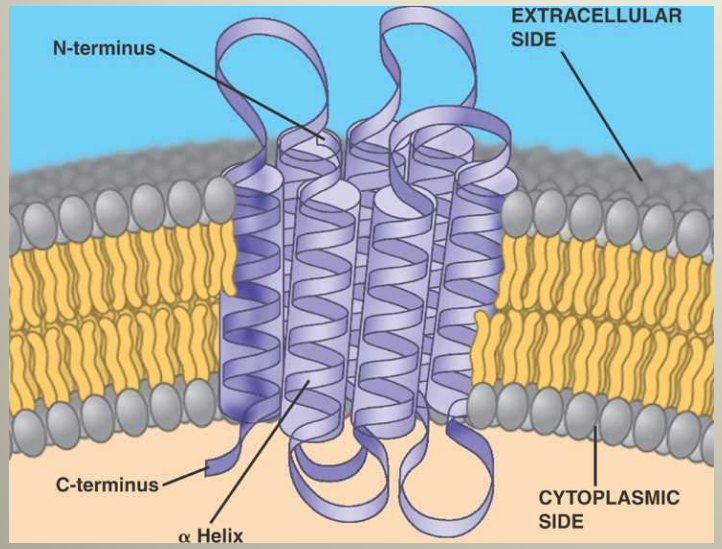
Peripheral ("extrinsic") membrane proteins

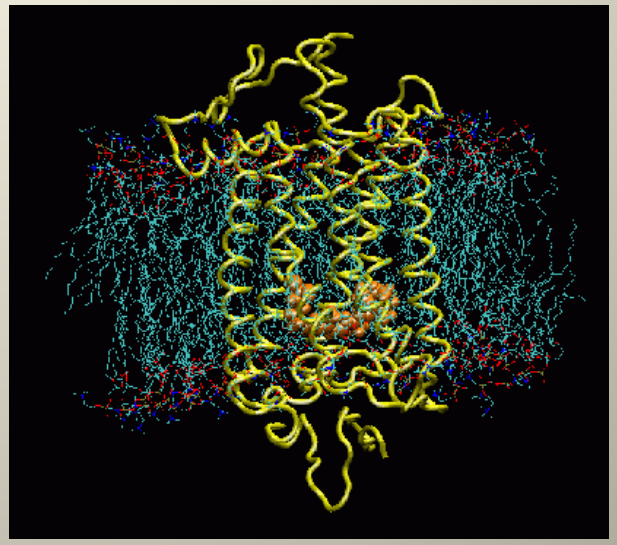
- loosely associated with bilayer
- weak, electrostatic forces (non-covalent)
- removable with mild treatments (ΔpH, Δ ionic strength)
- *examples*: spectrin; ankyrin; actin

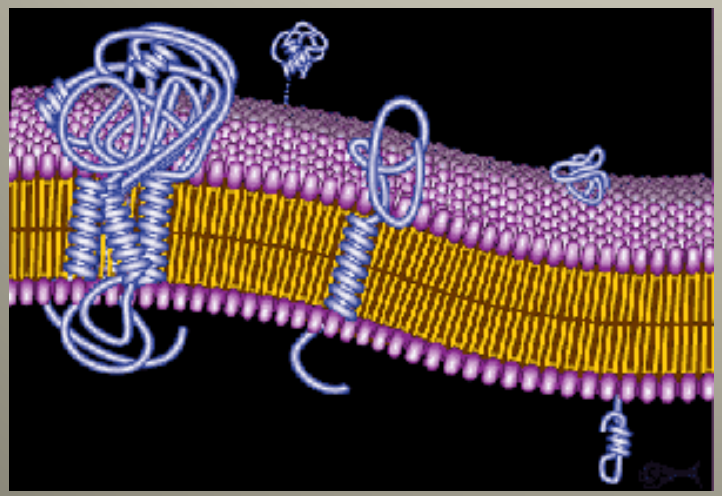
Integral ("intrinsic") membrane proteins

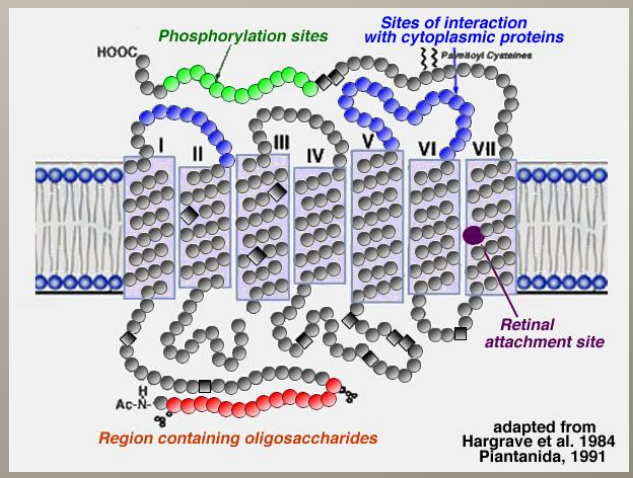
- strongly associated with bilayer
- strong, hydrophobic (van de Waals') forces
- harsh treatments required to remove: detergents (SDS, CHAPS); chaotropic agents (urea; guanidine-HCl)
- *examples*: glycophorin; rhodopsin; β-adrenergic receptor

Multi-Spanning Transmembrane Proteins



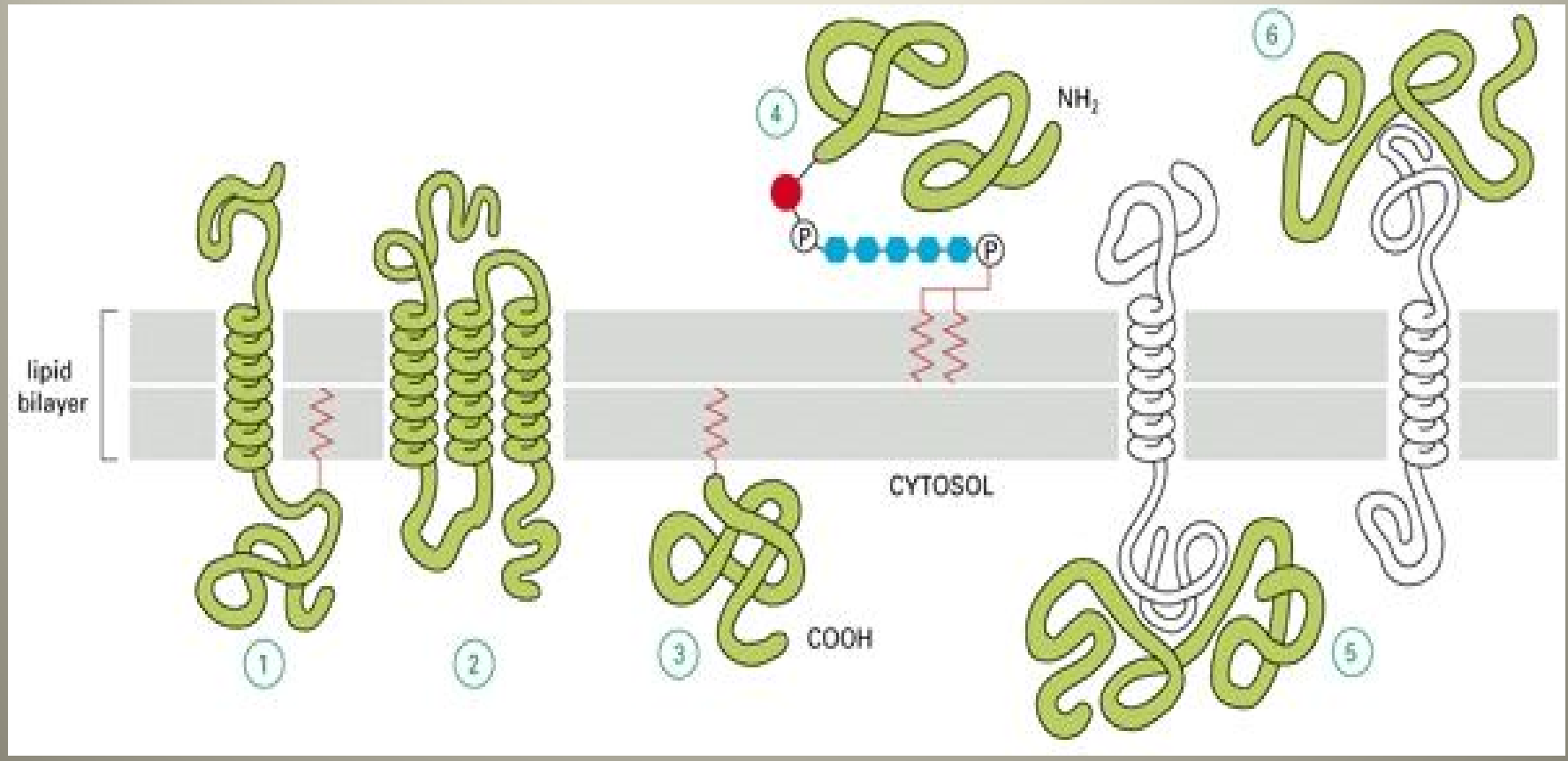




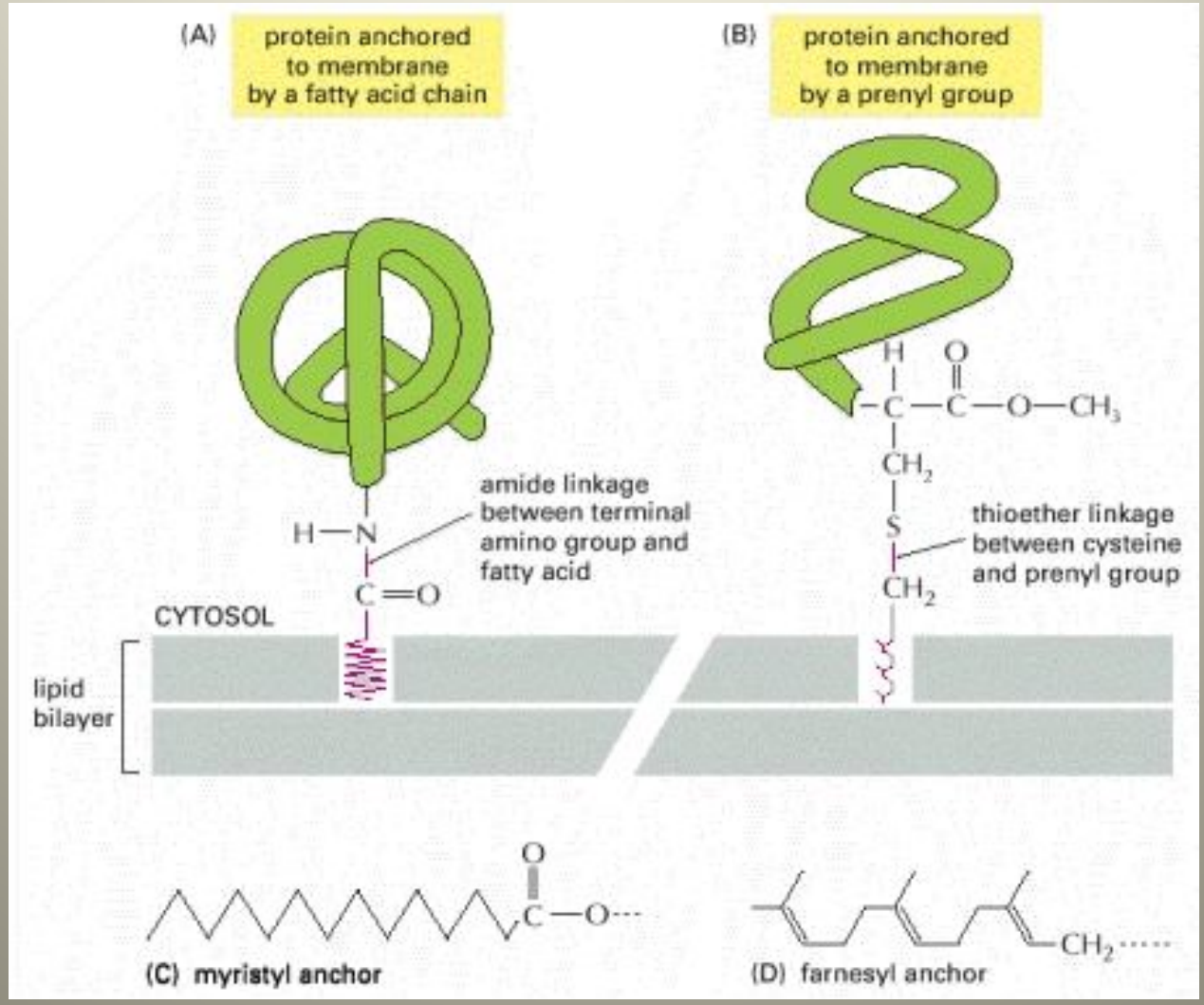


Rhodopsin in disk membrane

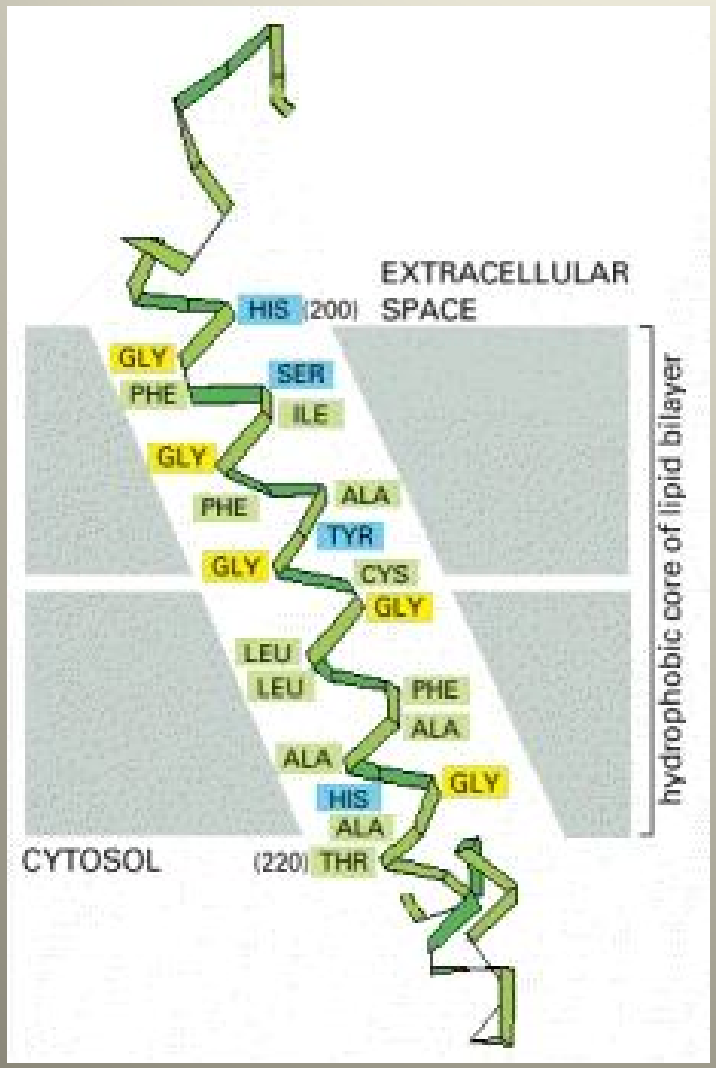
Six ways in which membrane proteins associate with the lipid bilayer



Membrane protein attachment by a fatty acid chain or a prenyl group

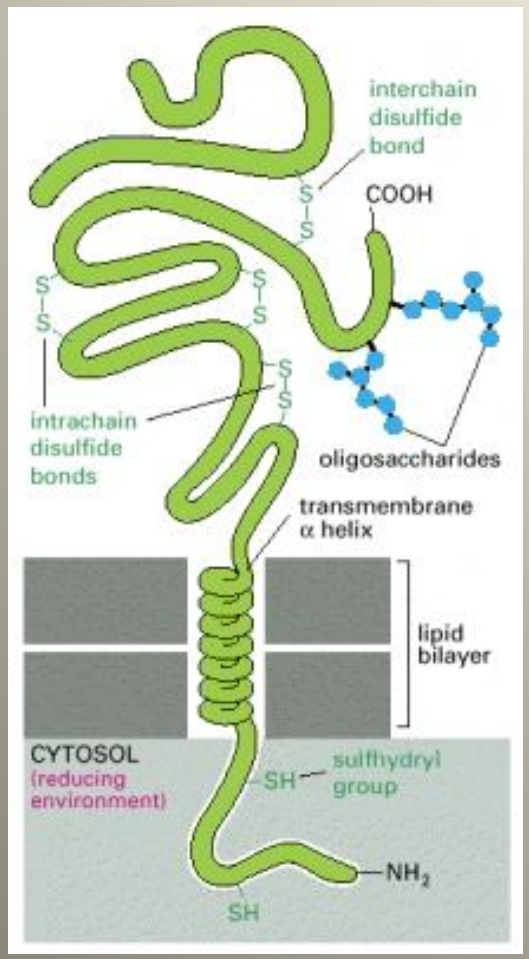


A segment of a transmembrane polypeptide chain crossing the lipid bilayer as an α helix



- Only the α-carbon backbone of the **polypeptide** chain is shown, with the hydrophobic **amino acids** in *green* and *yellow*

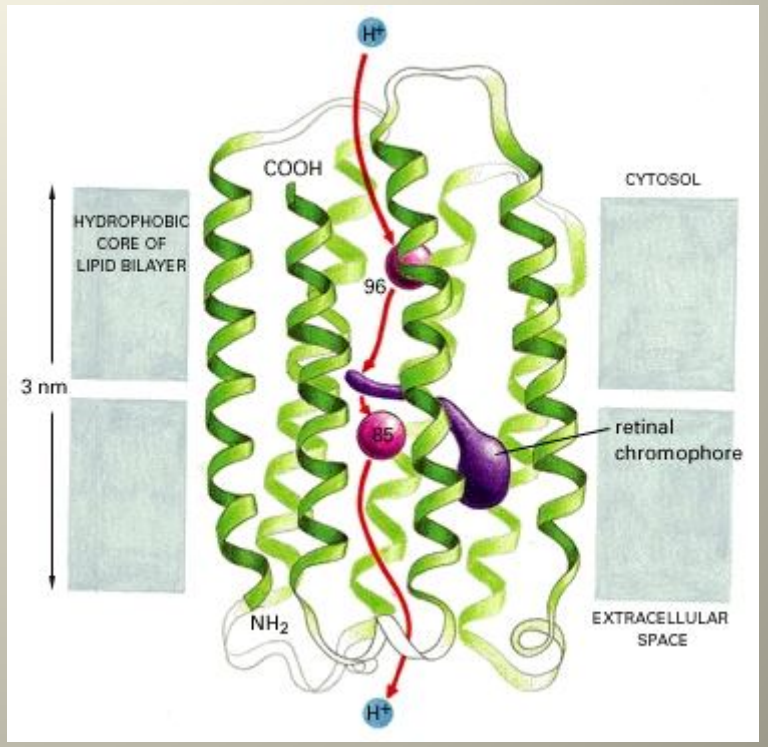
A single-pass transmembrane protein



- Note that the **polypeptide** chain traverses the **lipid bilayer** as a right-handed **α helix** and that the **oligosaccharide** chains and disulfide bonds are all on the noncytosolic surface of the **membrane**. The sulfhydryl groups in the cytosolic **domain** of the **protein** do not normally form disulfide bonds because the reducing environment in the **cytosol** maintains these groups in their reduced (-SH) form

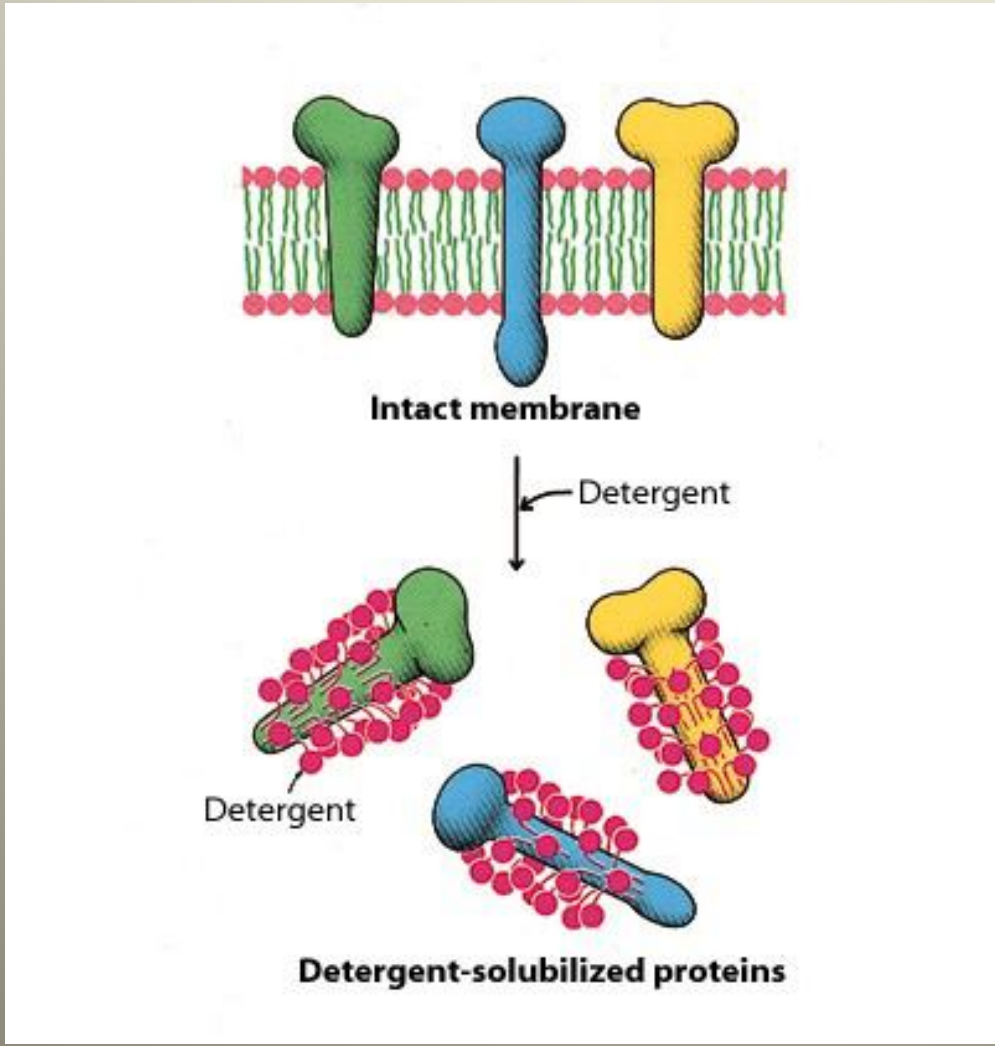
- **Schematic drawing of the bacterium *Halobacterium halobium* showing the patches of purple membrane that contain bacteriorhodopsin molecules**

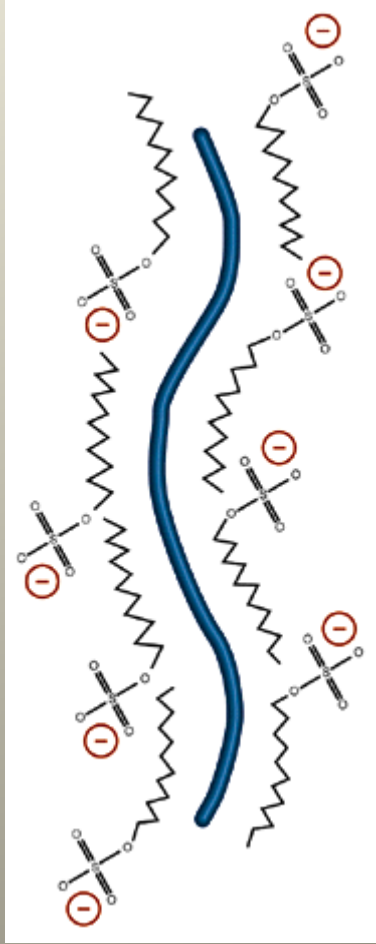
These bacteria, which live in saltwater pools where they are exposed to a large amount of sunlight, have evolved a variety of light-activated proteins, including bacteriorhodopsin, which is a light-activated proton pump in the plasma membrane.



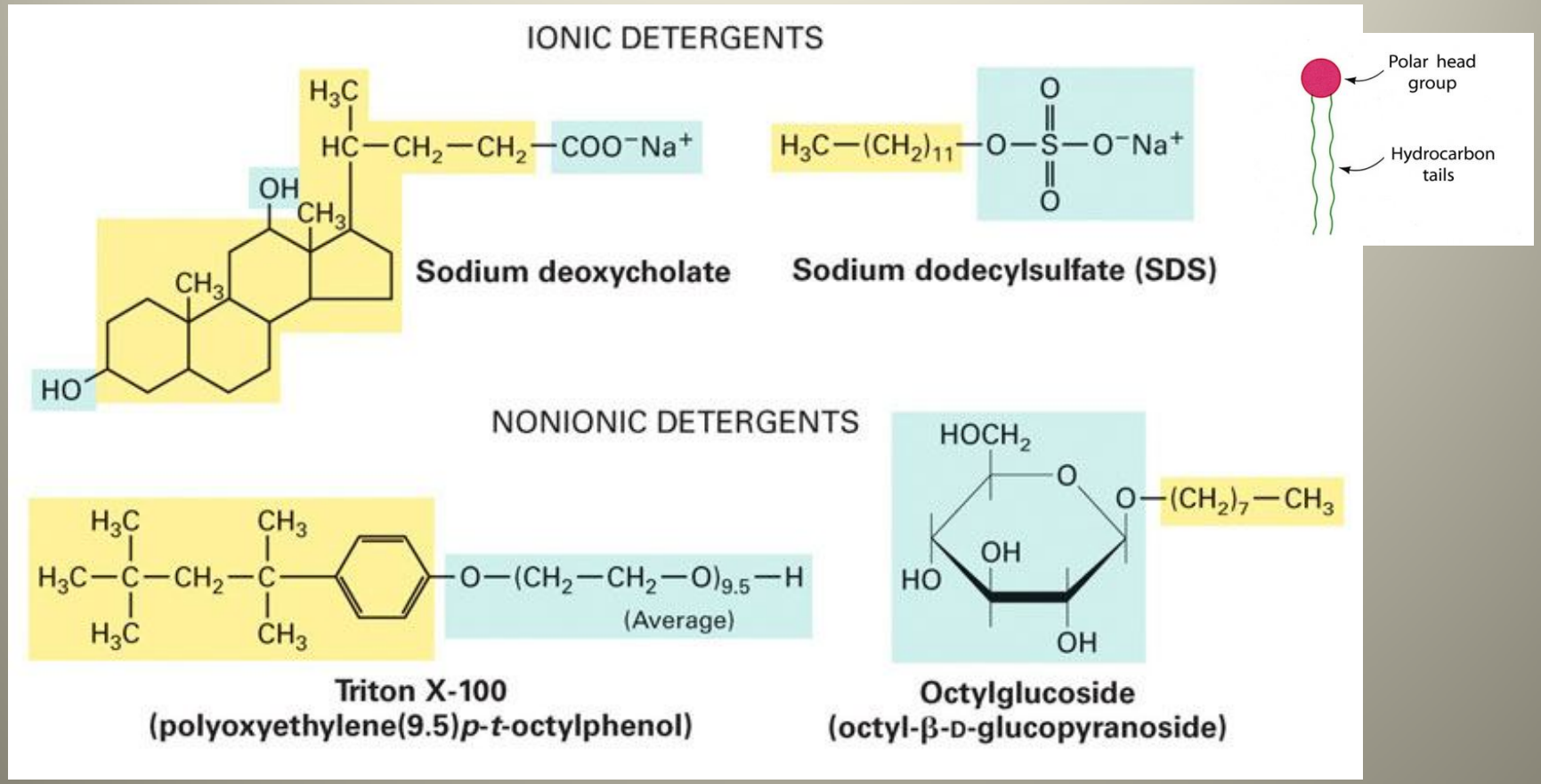
The polypeptide chain crosses the lipid bilayer as seven α helices. The location of the chromophore and the probable pathway taken by protons during the light-activated pumping cycle are shown. When activated by a photon, the chromophore is thought to pass an H+ to the side chain of aspartic acid 85 (*pink sphere marked 85*).

Solubilization of Integral Membrane Protein

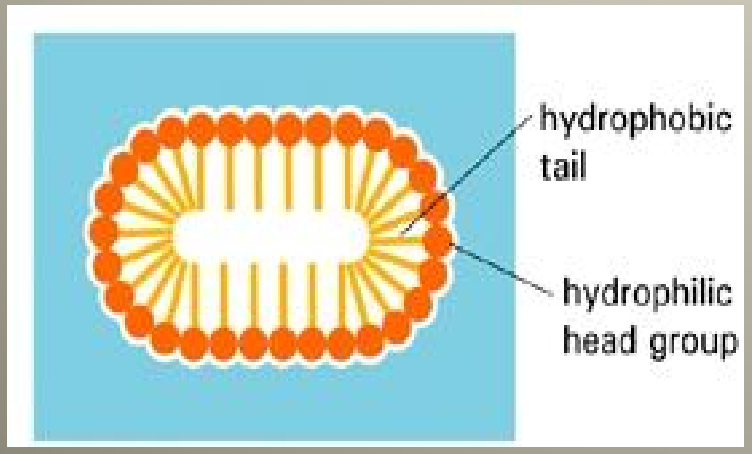


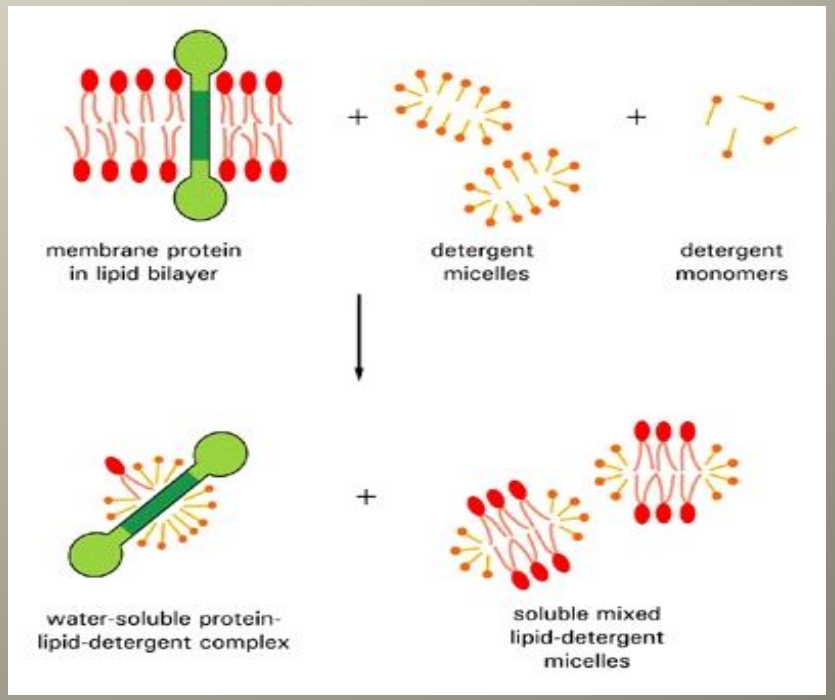


Commonly used detergents for membrane protein solubilization



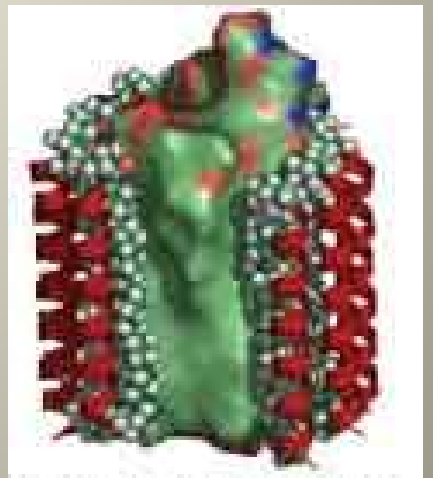
- Transmembrane proteins can be solubilized only by agents that disrupt hydrophobic associations and destroy the lipid bilayer. Detergents, which are small amphipathic molecules that tend to form micelles in water. When mixed with membranes, the hydrophobic ends of detergents bind to the hydrophobic regions of the membrane proteins, thereby displacing the lipid molecules. Since the other end of the detergent molecule is polar, this binding tends to bring the membrane proteins into solution as detergent-protein complexes (although some tightly bound lipidmolecules may also remain. The polar (hydrophilic) ends of detergents can be either charged (ionic), as in the case of *sodium dodecyl sulfate (SDS)*, or uncharged (nonionic), as in the case of the *Triton* detergents.





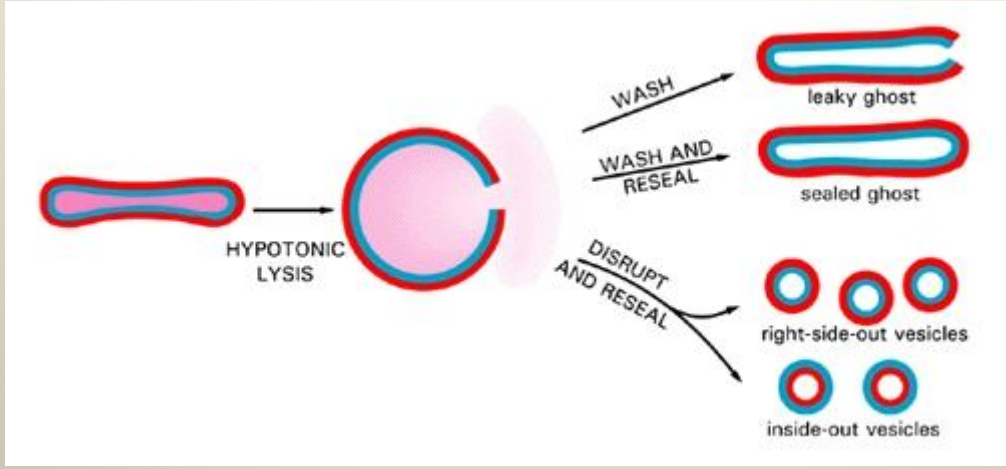
New Approach: Lipopeptide Detergents (LPDs)

- Efficiently solubilizes membrane proteins
- Retains native conformation
- Does not harm protein (retains biological activity)



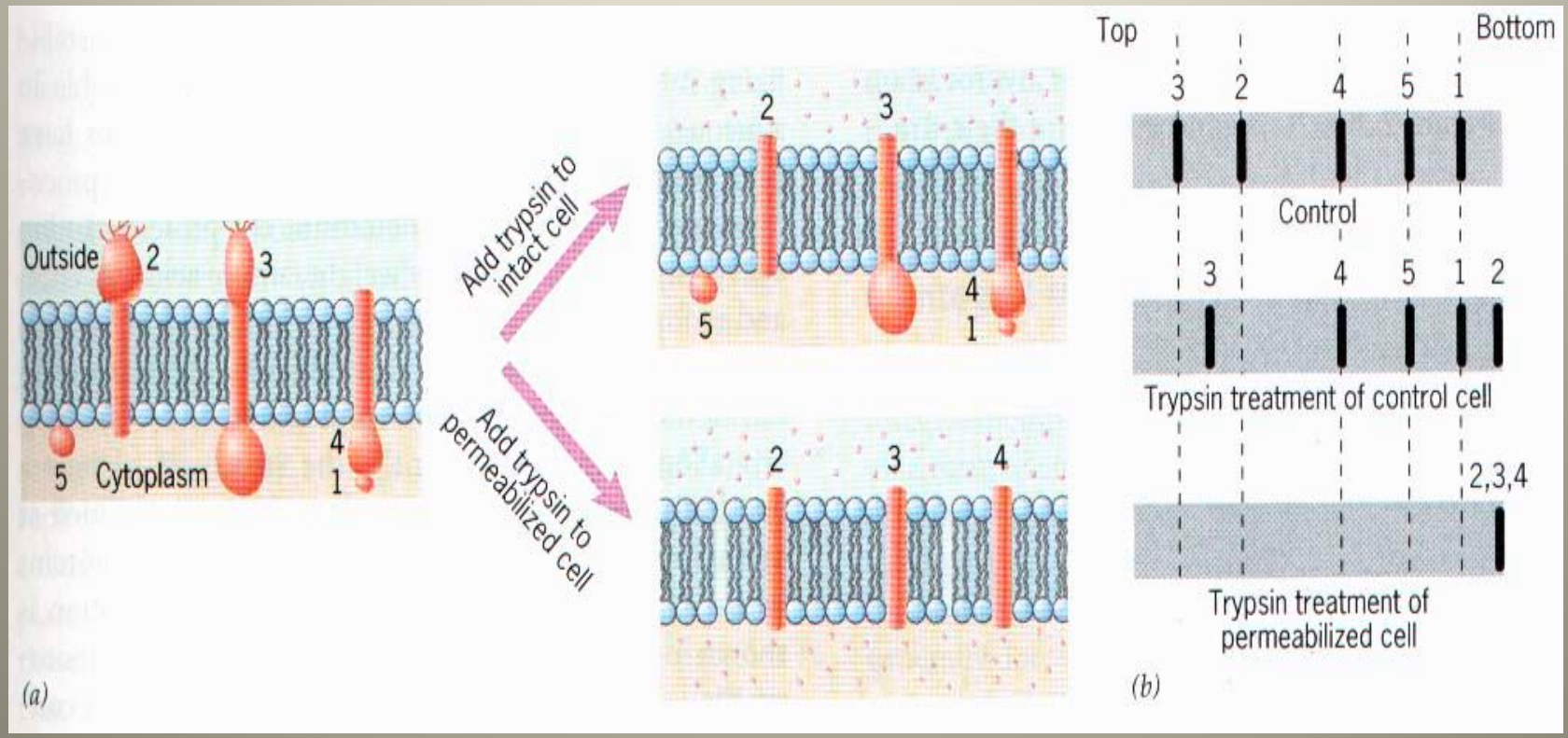
LPDs surrounding a membrane protein (green)

McGregor et al. (2003) *Nat Biotechnol 21(2):171-176*
Lipopeptide detergents designed for the structural study of membrane proteins.

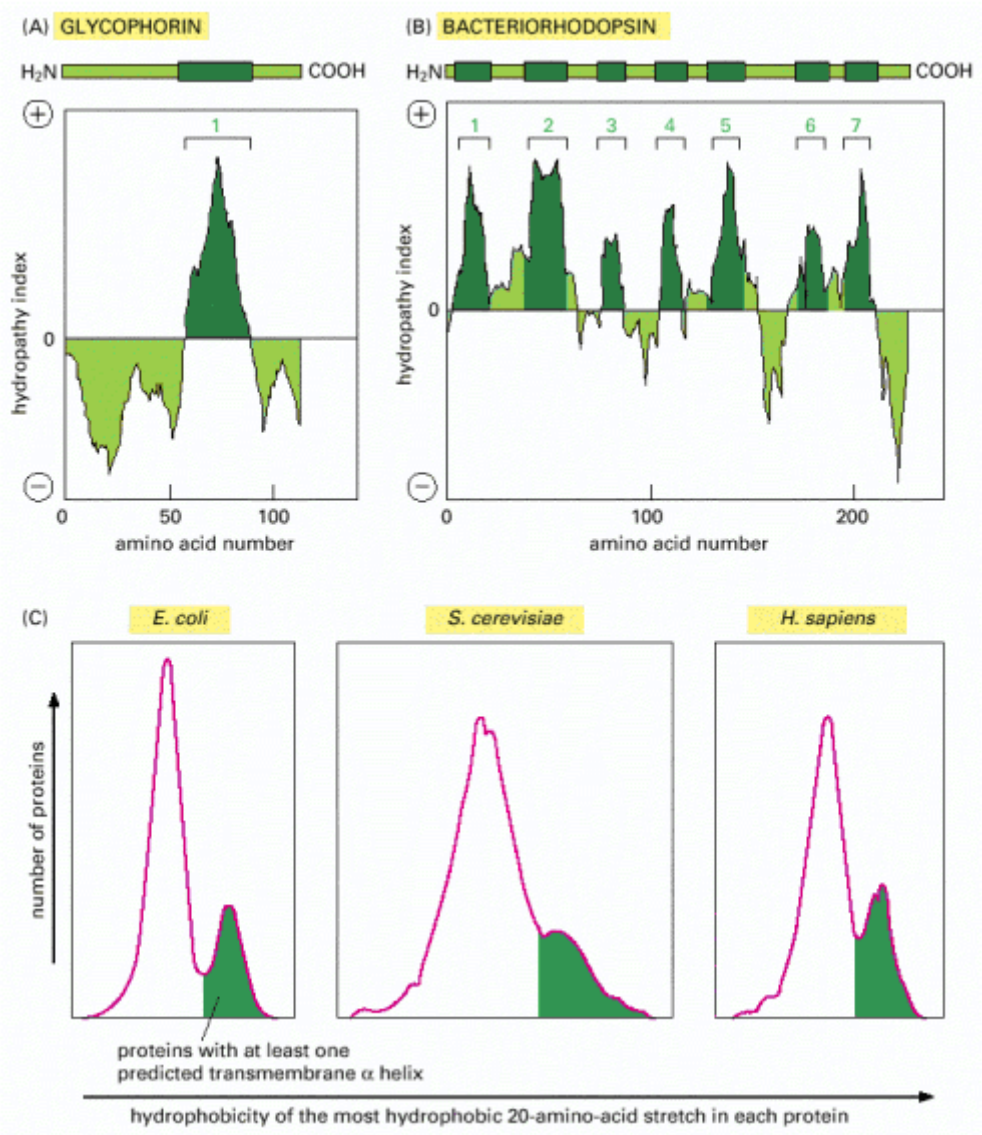


- It is easy to prepare empty red blood cell membranes, or "ghosts," by putting the cells in a medium with a lower salt concentration than the cell interior. Water then flows into the red cells, causing them to swell and burst and release their hemoglobin (the major nonmembrane protein). Membrane ghosts can be studied while they are still leaky (in which case any reagent can interact with molecules on both faces of the membrane), or they can be allowed to reseal so that water-soluble reagents cannot reach the internal face. Moreover, since sealed *inside-out*vesicles can also be prepared from red blood cell ghosts, the external side and internal (cytoplasmic) side of the membrane can be studied separately. The use of sealed and unsealed red cell ghosts led to the first demonstration that some membrane proteins extend across the lipid bilayer and that the lipid compositions of the two halves of the bilayer are different. Like most of the basic principles initially demonstrated in red blood cell membranes, these findings were later extended to the membranes of nucleated cells.

The orientation of integral proteins can be determined using nonpenetrating agents that label the proteins.

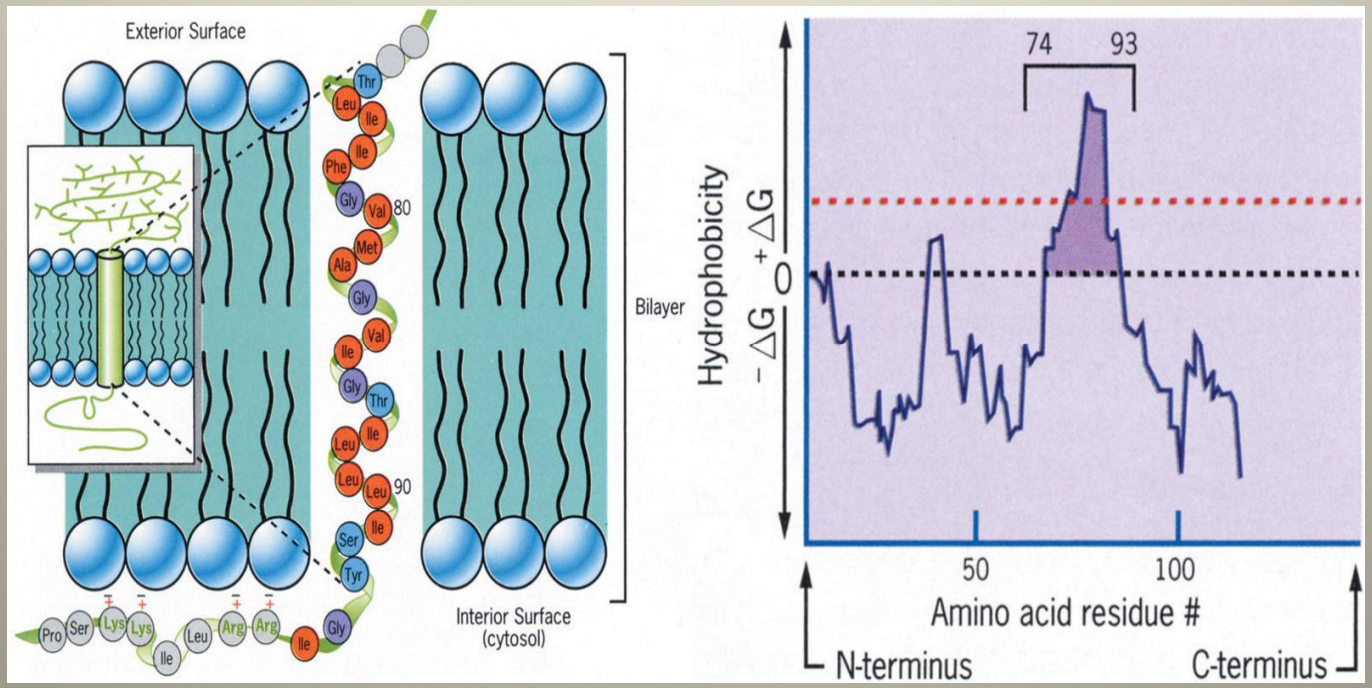


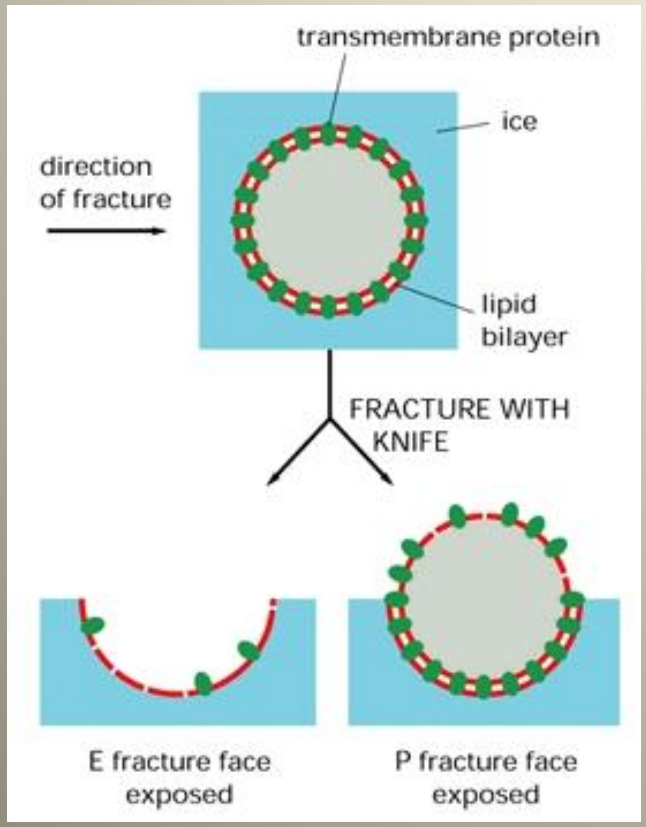
Hydropathy Plots: Predicting Membrane Protein Structure



Using hydropathy plots to localize potential a-helical membrane-spanning segments in a polypeptide chain. The free energy needed to transfer successive segments of a polypeptide chain from a nonpolar solvent to water is calculated from the amino acid composition of each segment using data obtained with model compounds. This calculation is made for segments of a fixed size (usually around 10 20 amino acids), beginning with each successive amino acid in the chain. The "hydropathy index" of the segment is plotted on the y axis as a function of its location in the chain. A positive value indicates that free energy is required for transfer to water (*i.e.*, the segment is hydrophobic), and the value assigned is an index of the amount of energy needed. Peaks in the hydropathy index appear at the positions of hydrophobic segments in the amino acid sequence. (A and B) Two examples of membrane proteins discussed later in this chapter are shown. Glycophorin (A) has a single membrane-spanning a helix and one corresponding peak in the hydropathy plot. Bacteriorhodopsin (B) has seven membrane-spanning a helices and seven corresponding peaks in the hydropathy plot. (C) The proportion of predicted membrane proteins in the genomes of *E. coli, S. cerevisiae,* and human. The area shaded in *green* indicates the fraction of proteins that contain at least one predicted transmembrane helix. The curves for *E. coli* and *S. cerevisiae* represent the whole genome; the curve for human proteins represents an incomplete set; in each case, the area under the curve is proportional to the number of genes analysed. (A, adapted from D. Eisenberg, *Annu. Rev. Biochem.* 53:595 624, 1984; C, adapted from D. Boyd et al., *Protein Sci.* 7:201 205, 1998.)

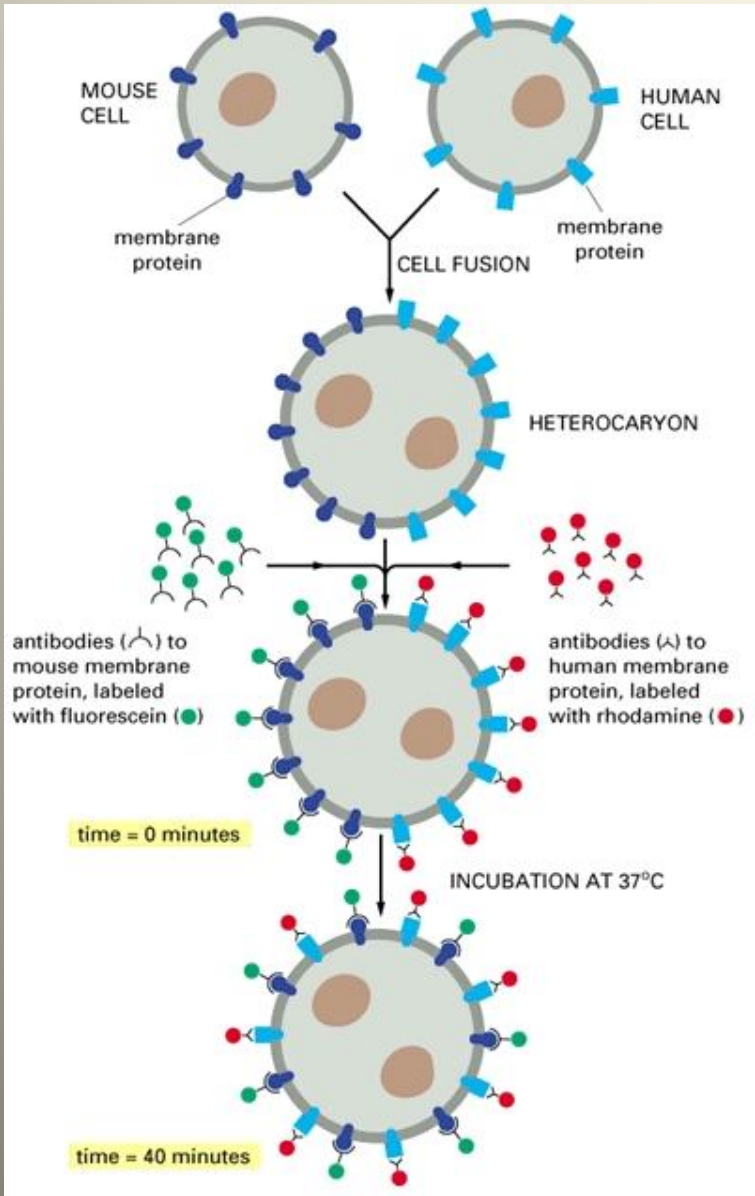
Identification of transmembrane domains can be predicted from the amino acid sequence using a hydropathy plot



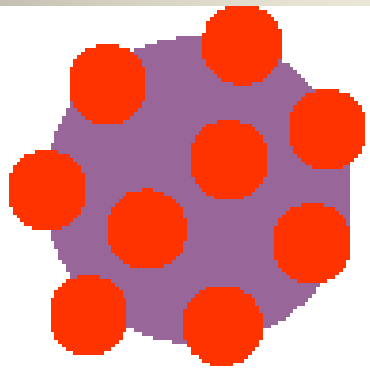


- **Freeze-fracture electron microscopy**
- The drawing shows how the technique provides images of the hydrophobic interior of the cytoplasmic (or protoplasmic) half of the bilayer (called the P face) and the external half of the bilayer (called the E face). After the fracturing process shown here, the exposed fracture faces are shadowed with platinum and carbon, the organic material is digested away, and the resulting platinum replica is examined in the electron microscope

Experiment demonstrating the mixing of plasma membrane proteins on mouse-human hybrid cells

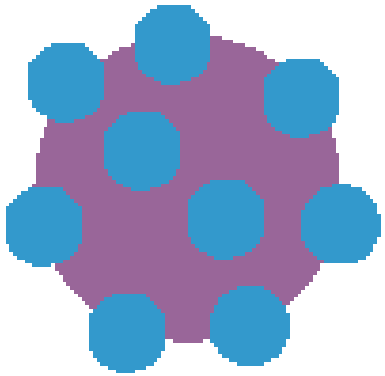


The mouse and human proteins are initially confined to their own halves of the newly formed heterocaryon plasma membrane, but they intermix with time. The two antibodies used to visualize the proteins can be distinguished in a fluorescence microscope because fluorescein is green whereas rhodamine is red. (Based on observations of L.D. Frye and M. Edidin, *J. Cell Sci.* 7:319-335, 1970, by permission of The Company of Biologists.)

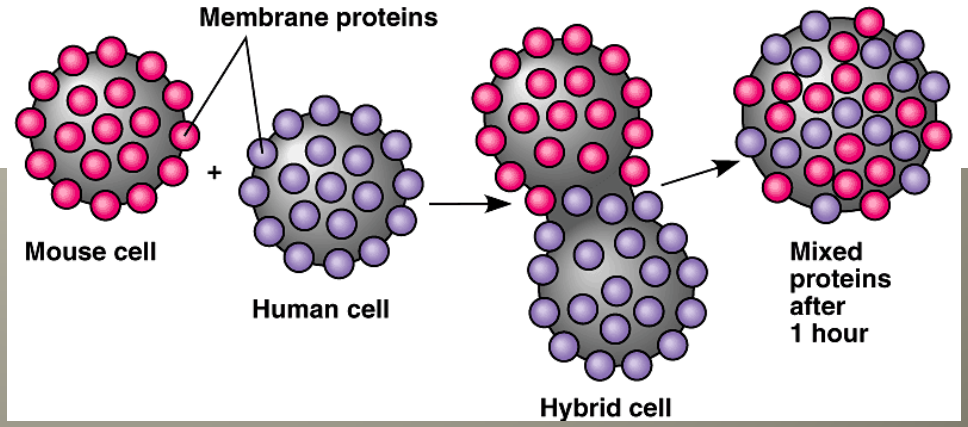


Mouse Cell

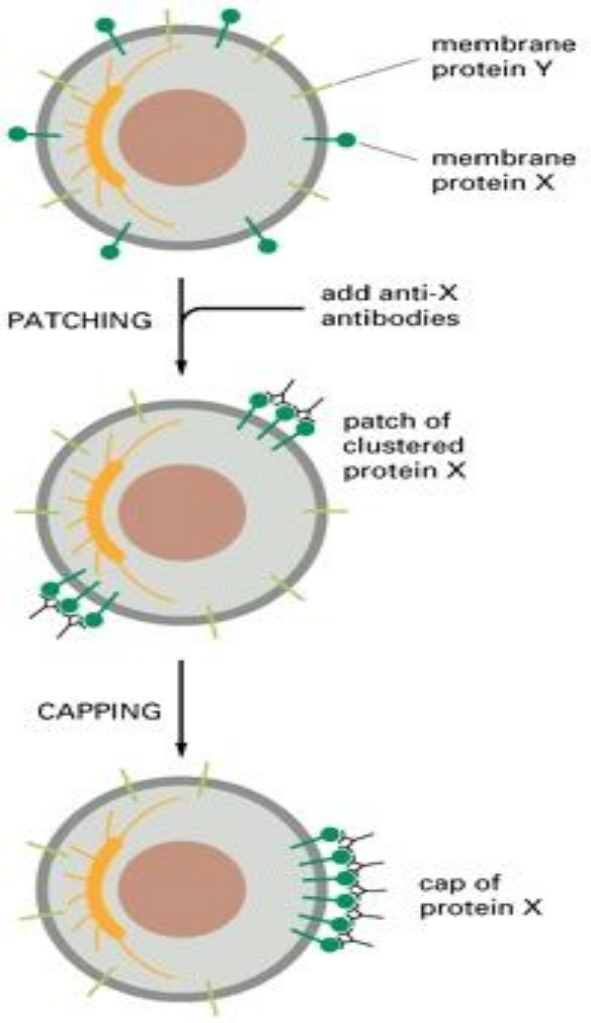
Time after fusion 0 : 0 0



Human Cell

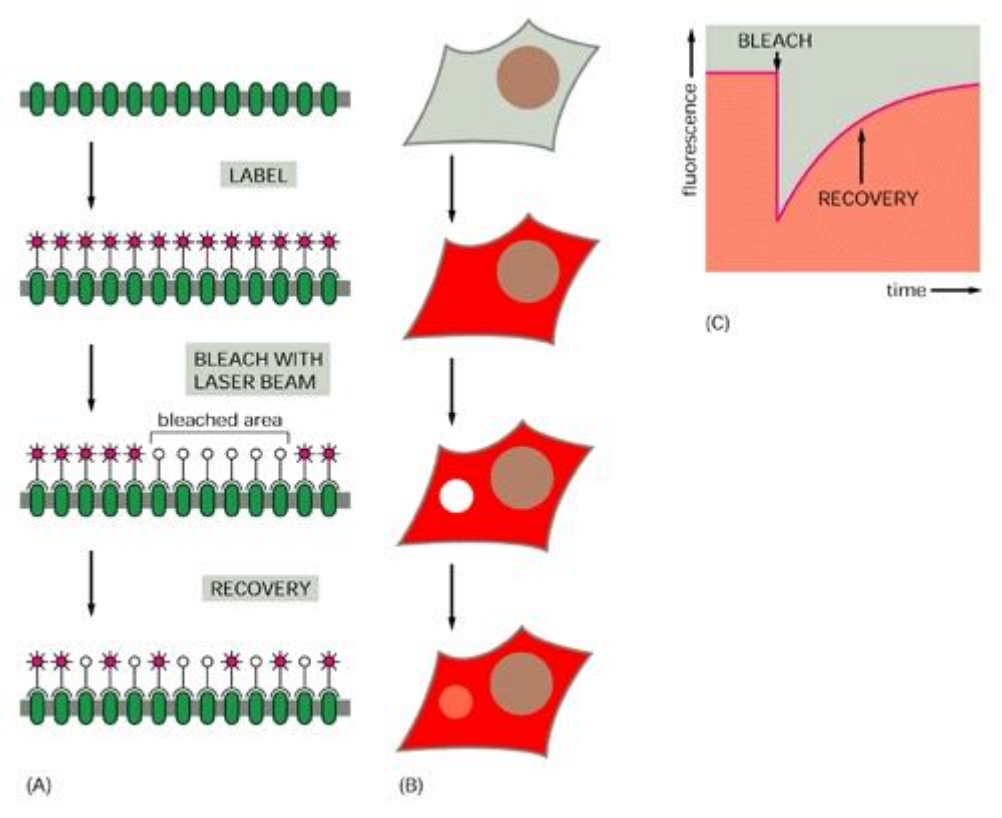


Antibody-induced patching and capping of a cell-surface protein on a white blood cell



- The bivalent antibodies cross-link the protein molecules to which they bind. This causes them to cluster into large patches, which are actively swept to the tail end of the cell to form a "cap." The centrosome, which governs the head-tail polarity of the cell, is shown in *orange*.

Measuring the rate of lateral diffusion of a plasma membrane protein by the FRAP technique

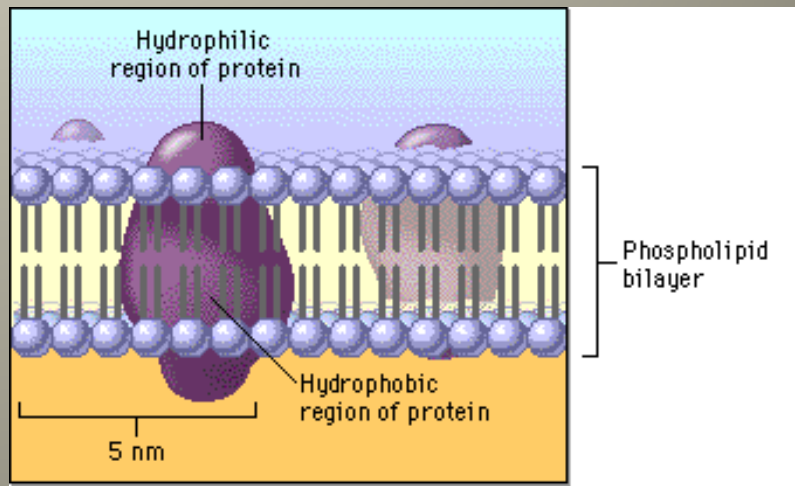


A specific protein is labeled on the cell surface with a fluorescent monovalent antibody that binds only to that protein (for simplicity, no other proteins are shown). After the antibodies are bleached in a small area using a laser beam, the fluorescence intensity recovers as the bleached molecules diffuse away and unbleached molecules diffuse into the irradiated area (shown in side view in A and top view in B). (C) A graph showing the rate of recovery. The greater the diffusion coefficient of the membrane protein, the faster the recovery.

Fluorescence Recovery After Photobleaching

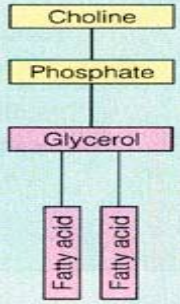
Motion of Proteins

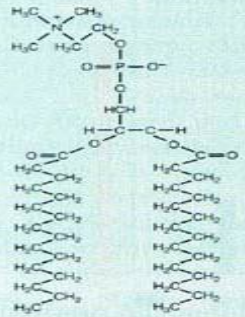
- Consider relative mass of protein, *vs*. lipid
- Lateral diffusion ~10-10^4X slower than for lipids (D ~ 10^{-9} – 10^{-12} cm^2 sec^{-1})
- Rotational diffusion (generally relatively rapid)
- Transverse (flip-flop) diffusion NOT OBSERVED (thermodynamically not allowed)- would require moving highly polar/charged mass through a low dielectric (nonpolar) medium

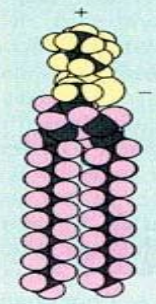


(a) PHOSPHOLIPIDS

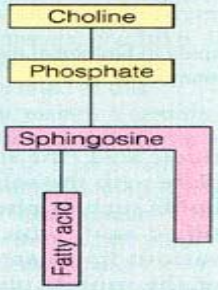
Phosphatidylcholine (shown)
Phosphatidylethanolamine
Phosphatidylserine
Phosphatidylthreonine
Phosphatidylinositol
Phosphatidylglycerol
Diphosphatidylglycerol (cardiolipin)

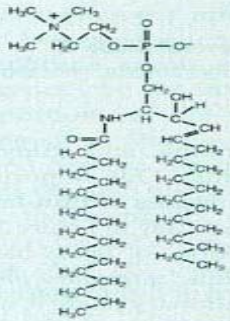


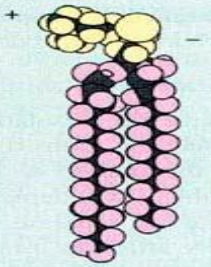




Sphingomyelin (a sphingolipid)

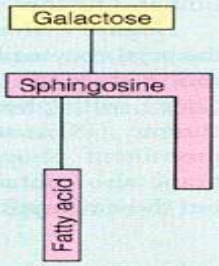


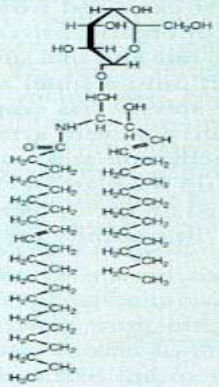


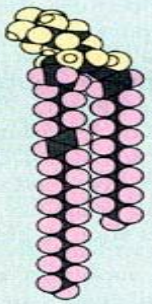


(b) GLYCOLIPIDS

Cerebrosides
(galactocerebroside shown)
Gangliosides

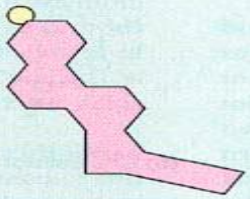


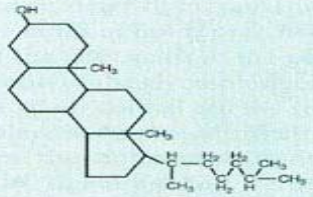


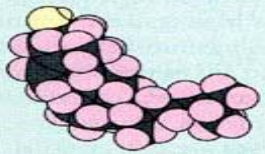


(c) STEROLS

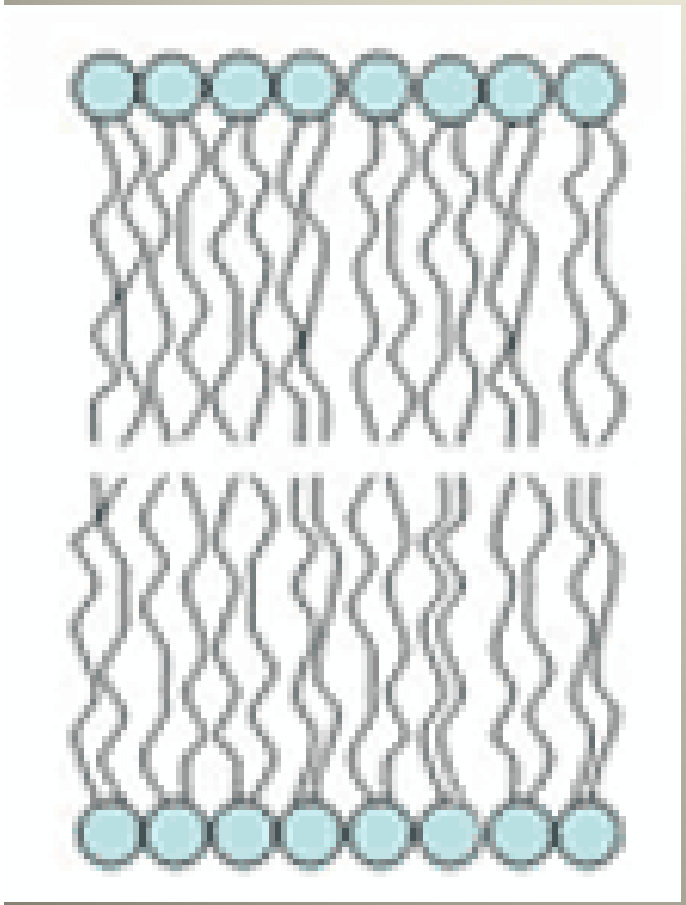
Cholesterol (shown)
Campesterol
Sitosterol
Stigmasterol







Motion of Membrane Lipids



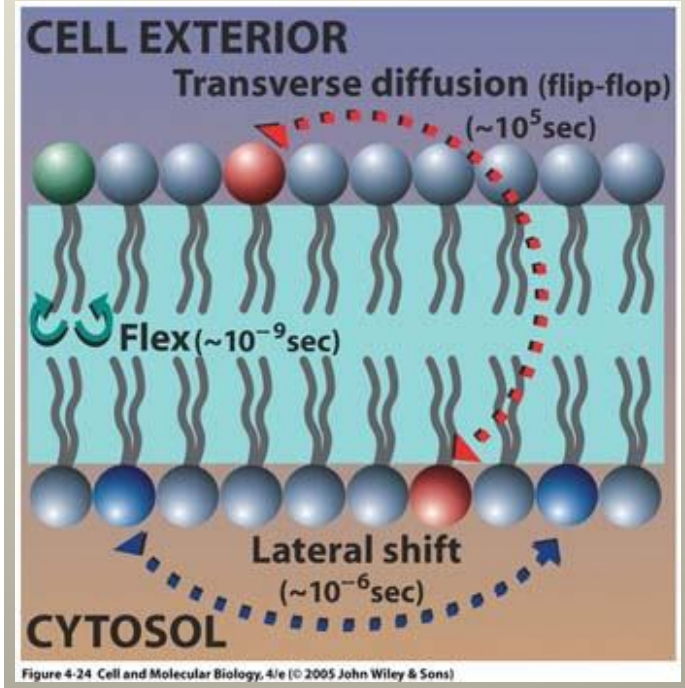
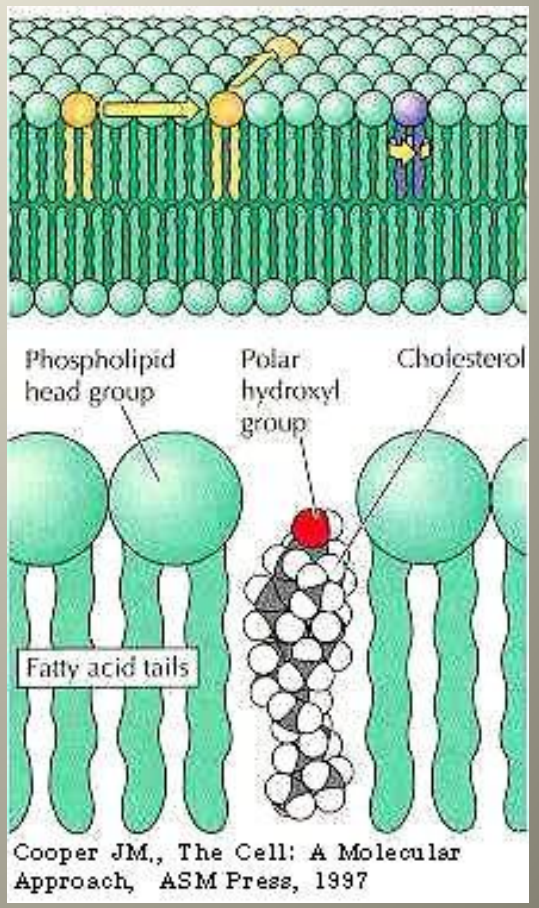


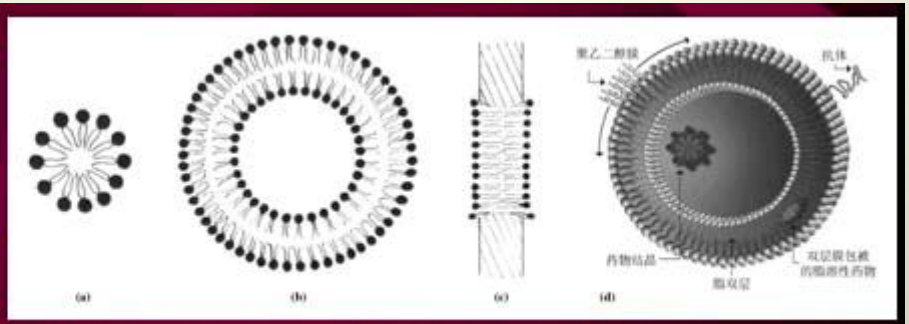
Figure 4-24 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)



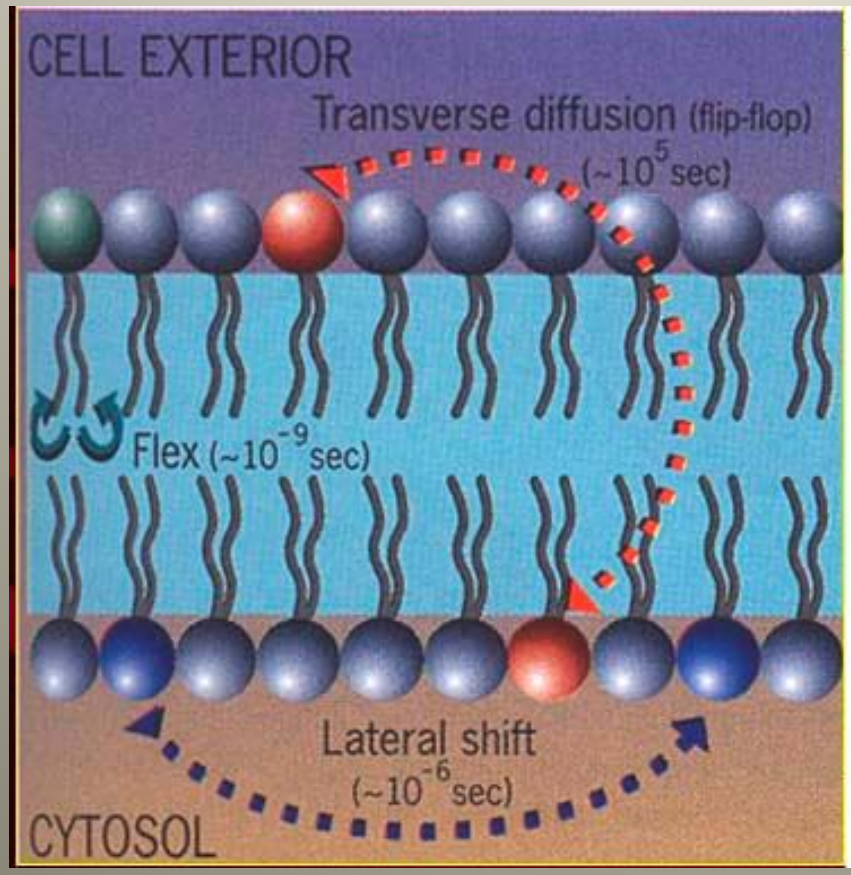
Cooper JM., The Cell: A Molecular Approach, ASM Press, 1997

http://www.aber.ac.uk/gwydd-cym/cellbiol/cellmembrane/index.htm

❖ **Liposome and application**



Study on nature; gene transfer; as a carrier.



Three kinds of movement:

Rotation about its long axis;

Lateral diffusion by exchanging places;

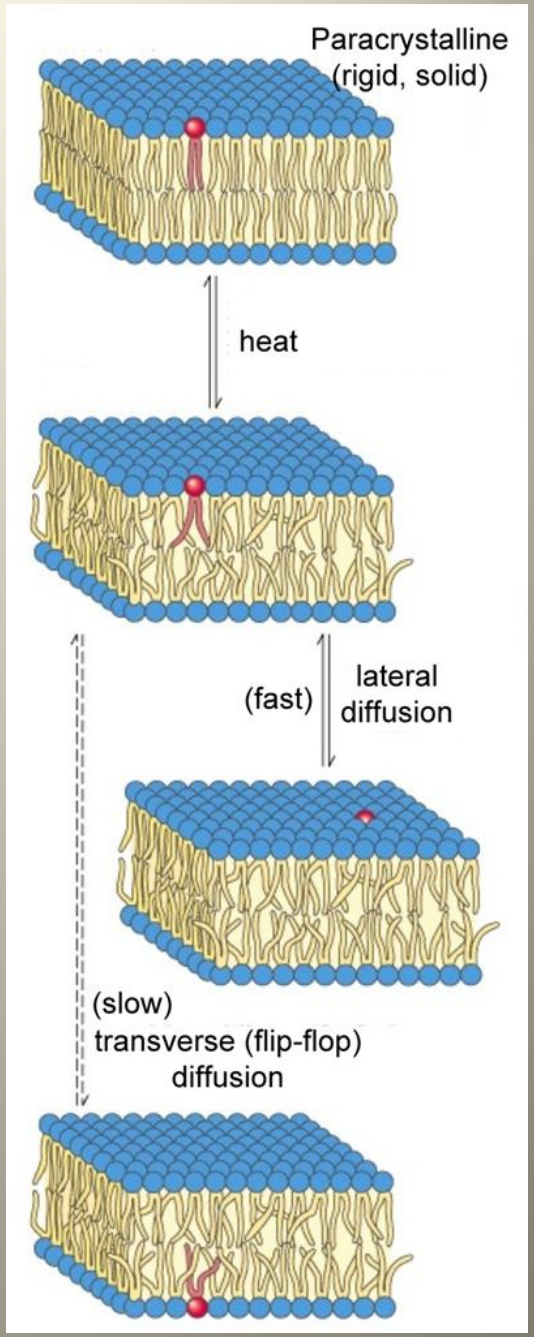
Transverse diffusion, or "flip-flop" from one monolayer to the other. Flippases catalyze the flip-flop.

Lipid Motion

- Lateral (in-plane) diffusion (relatively rapid: $r \sim 10^6 sec^{-1}$ $D \sim 10^{-8}\ cm^2 sec^{-1}$)
- Rotational diffusion (rapid)
- Flexing of acyl chains (rapid: $r \sim 10^9\ sec^{-1}$)
- Transverse (flip-flop) diffusion

  -spontaneous: very slow (hours, days: $r \geq 10^5$ sec)

  -catalyzed by flippase or scramblase: rapid (seconds)



Membrane Dynamics

Lateral (In-Plane) Diffusion

http://www.d.umn.edu/~sdowning/Membranes/phospholipidlateralmovanim.html

Rotational Diffusion

http://www.d.umn.edu/~sdowning/Membranes/phospholipidrotationalanim.html

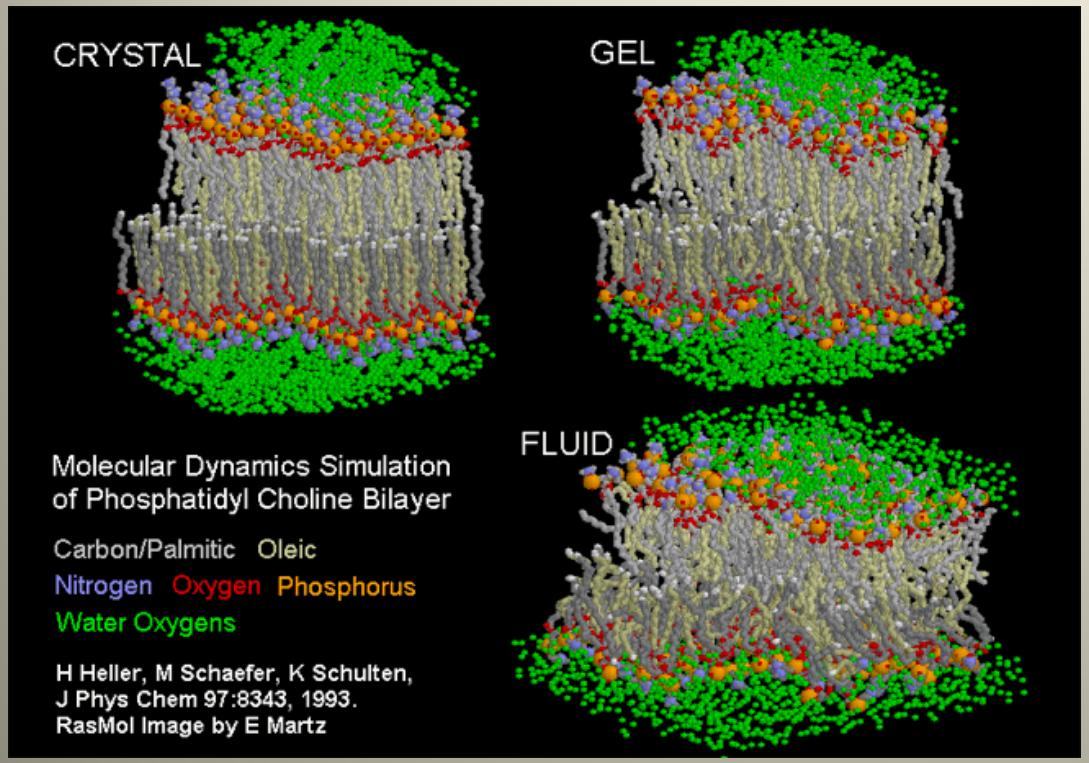
Flippase (ER)

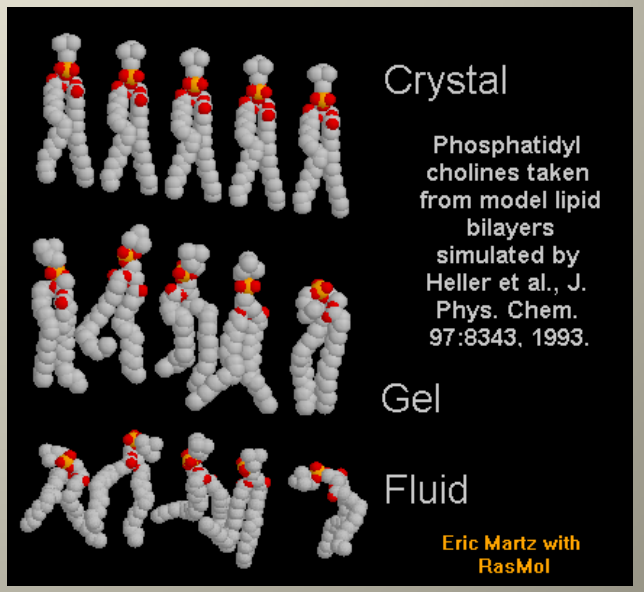
http://www.d.umn.edu/~sdowning/Membranes/flippaseanim.html

Protein Mobility

http://www.d.umn.edu/~sdowning/Membranes/proteinmobilityanim.html

Physical States of Lipids in Bilayer





Determined by: a) Lipid composition
b) Temperature

http://courses.cm.utexas.edu/archive/Fall2001/CH339K/Hackert/Membranes/membranes.htm

- Membrane fluidity is influenced by **temperature** and by **composition**.
- As temperatures cool, membranes switch from a fluid state to a solid state as the phospholipids are more closely packed.
- Membranes rich in *unsaturated* fatty acids are *more fluid* that those dominated by saturated fatty acids, because the kinks in the unsaturated fatty acid tails prevent tight packing.

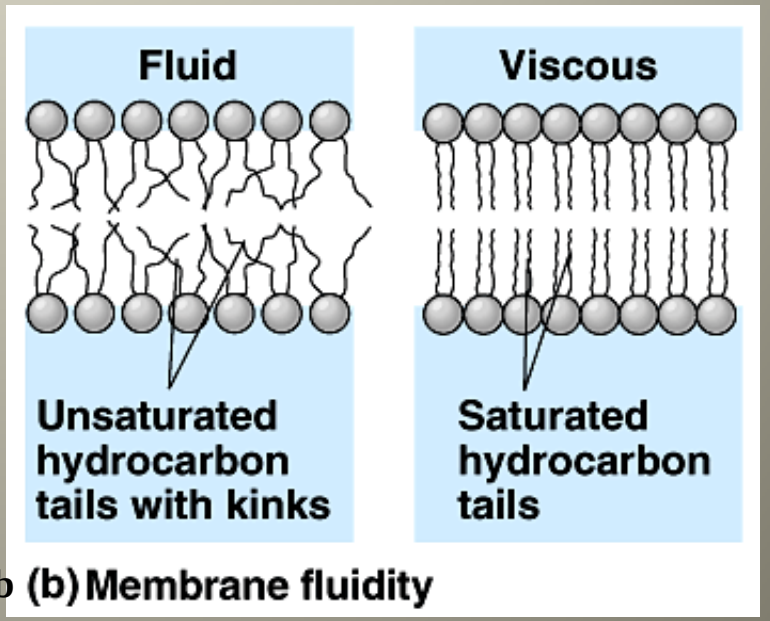
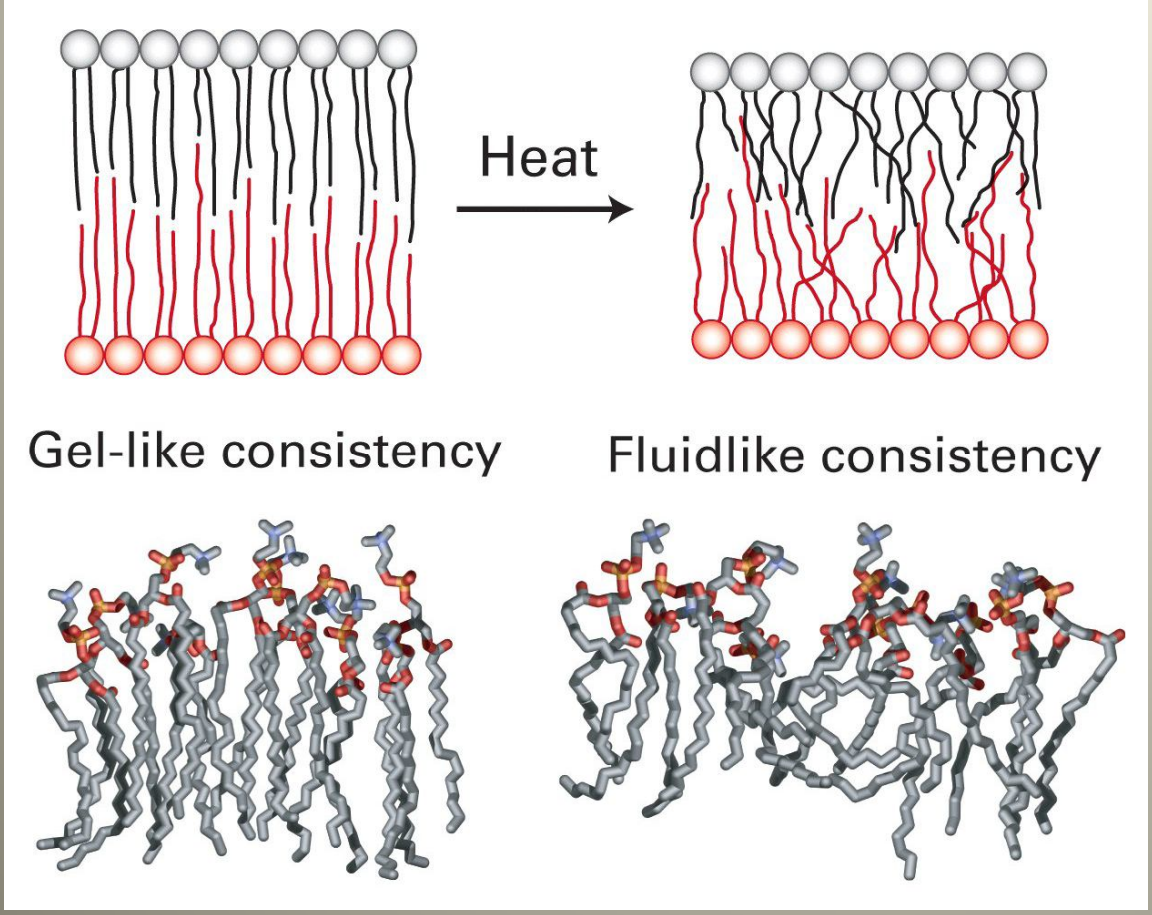


Fig. 8.4b (b) Membrane fluidity

Most lipoid and many protein are laterally mobile in biomembrane



Below the phase transition temperature fatty acyl chains are in a gel-like (crystalline) state
Above the phase transition temperature, fatty acyl chains are in rapid motion

Heat disorders the nonpolar tail and induces a transition form gel to fluid

Membrane fluidity important for membrane function; determined by phospholipid composition

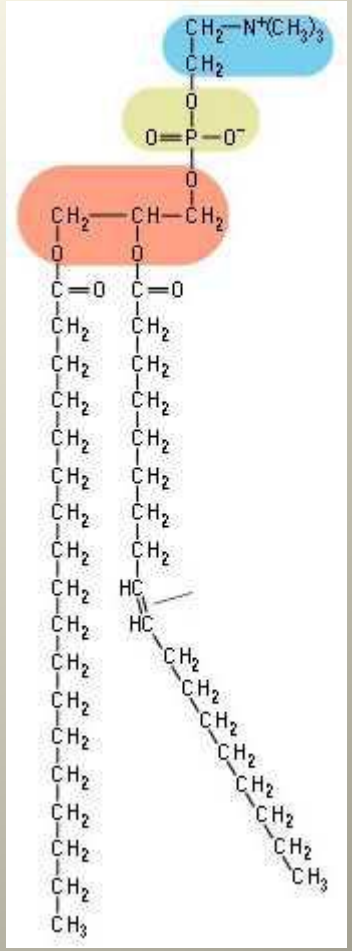
Close packing of hydrocarbon tails ⇒less fluidity (increased viscosity)

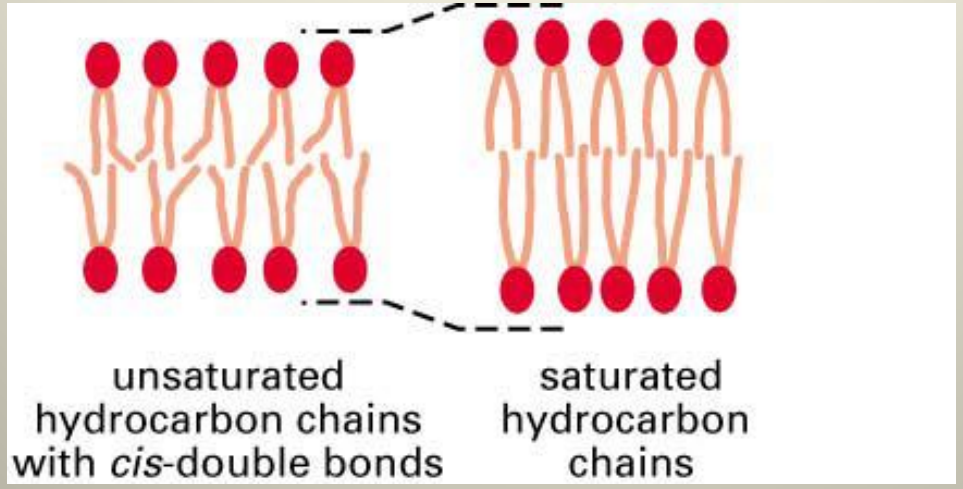
Length and *unsaturation (no of double bonds) determine closeness of packing*

Length varies from 14-24 C atoms; shorter chain length ⇒less interaction ⇒increased fluidity

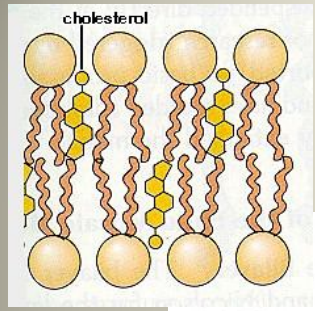
One tail of molecule has one or more double bonds - unsaturated (H atoms); other tail has no double bonds - saturated

Double bonds ⇒kinks (曲) ⇒less packing

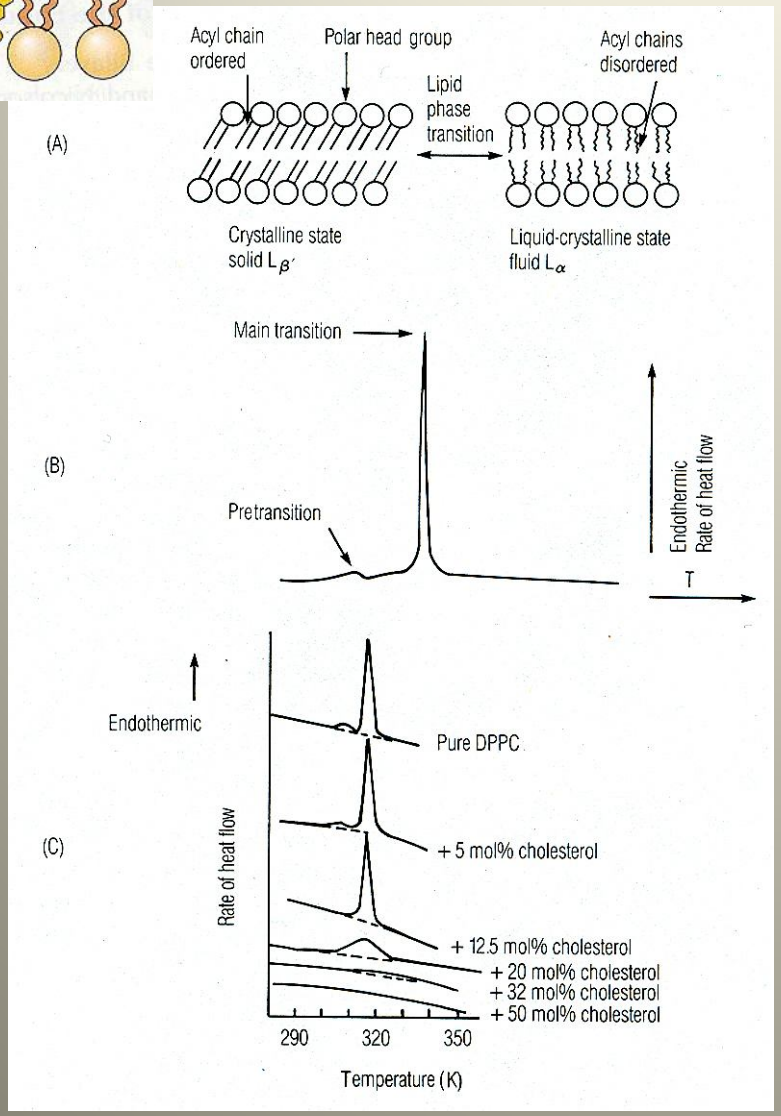




Van der Waals interactions between fatty acyl chains are the main determinants of acyl chain mobility Double bonds reduce the number of potential van der Walls interactions between fatty acyl chains



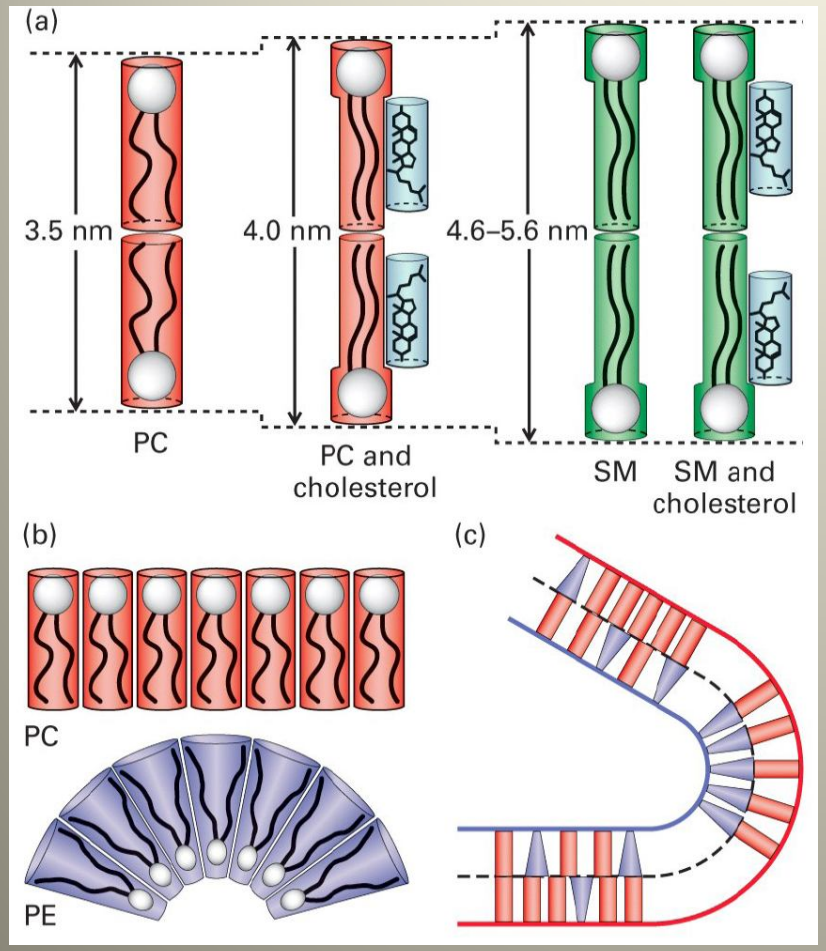
Cholesterol: A "Fluidity Buffer"



- Below T_m - cholesterol disrupts close packing of acyl chains $\Rightarrow$ *increases* fluidity
- Above T_m - cholesterol constrains motion of acyl chains $\Rightarrow$ *decreases* fluidity
- Broadens/abolishes phase transitions

From P.R. Cullis & M.J. Hope, In:
D.E. Vance & J.E. Vance (1985)
Biochemistry of Lipids and Membranes

4. Influence thickness of membrane ; 5. Local curvature



Cholesterol can increase membrane (PC) thickness, but no effect of SM

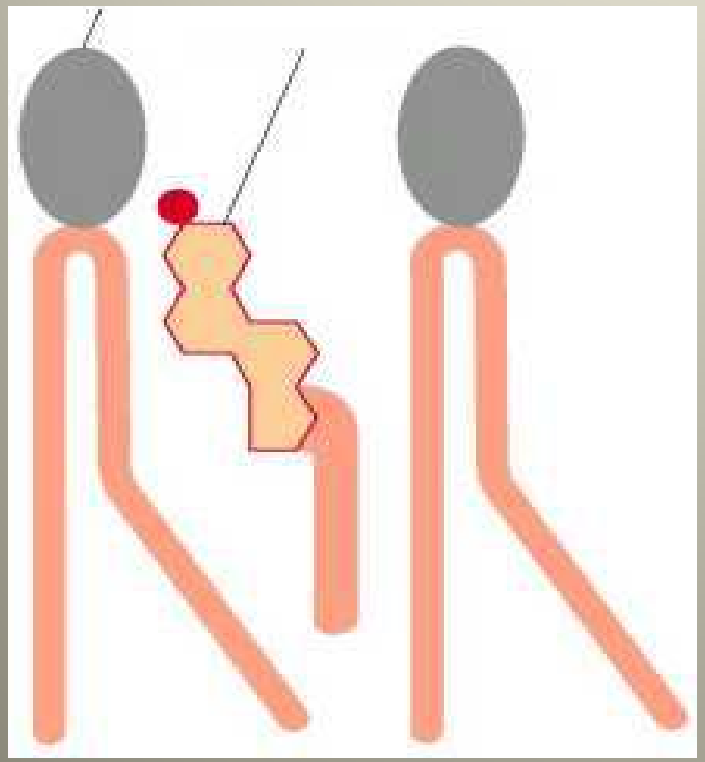
Exoplasmic leaflet has enrich PC
Cytosolic face has enrich PE, so more curvature

bilayer enriched with PC in the exoplasmic leaflet and with PE in the cytoplasmic face would cause the natural curvature

Cholesterol →Membrane fluidity↓

In animal cells, cholesterol used to modulate membrane fluidity - fills gaps between kinks of unsaturated tails

Used particularly in plasma membrane ⇒closer packing ⇒less fluidity/permeability



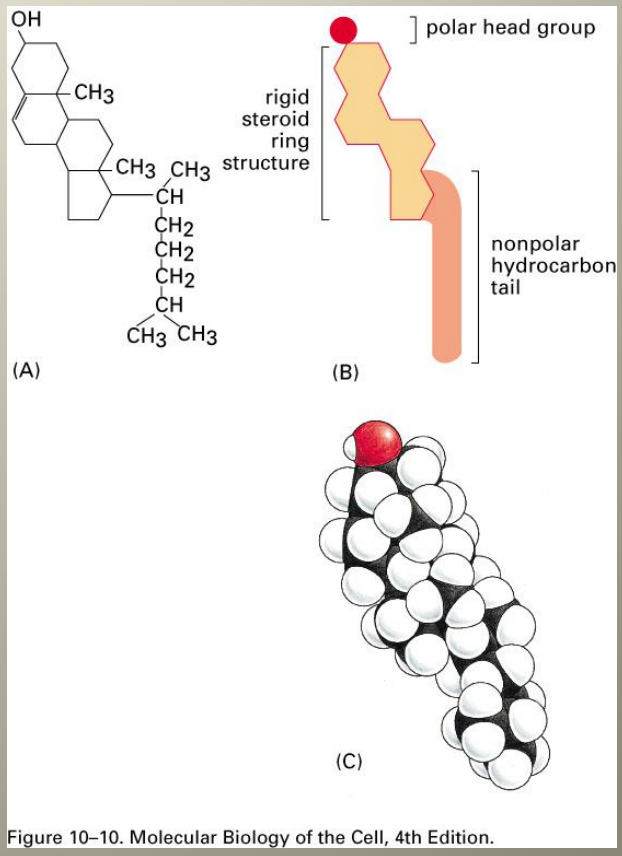
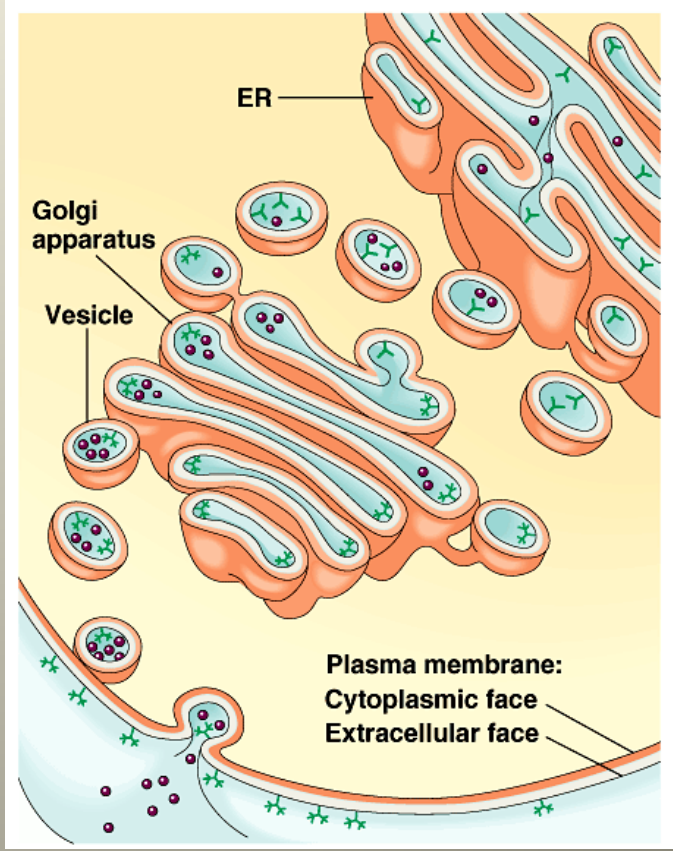


Figure 10–10. Molecular Biology of the Cell, 4th Edition.

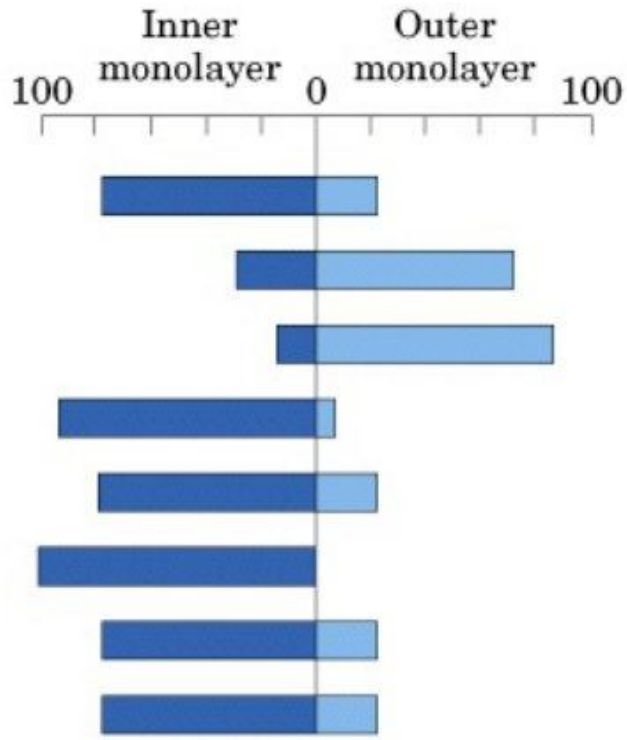
- Membranes are ASYMMETRIC- they have distinctive inside and outside faces.
  - The two layers may differ in lipid composition, and proteins in the membrane have a clear direction.
  - The outer surface also has carbohydrates.
  - This asymmetrical orientation begins during synthesis of new membrane in the endoplasmic reticulum.



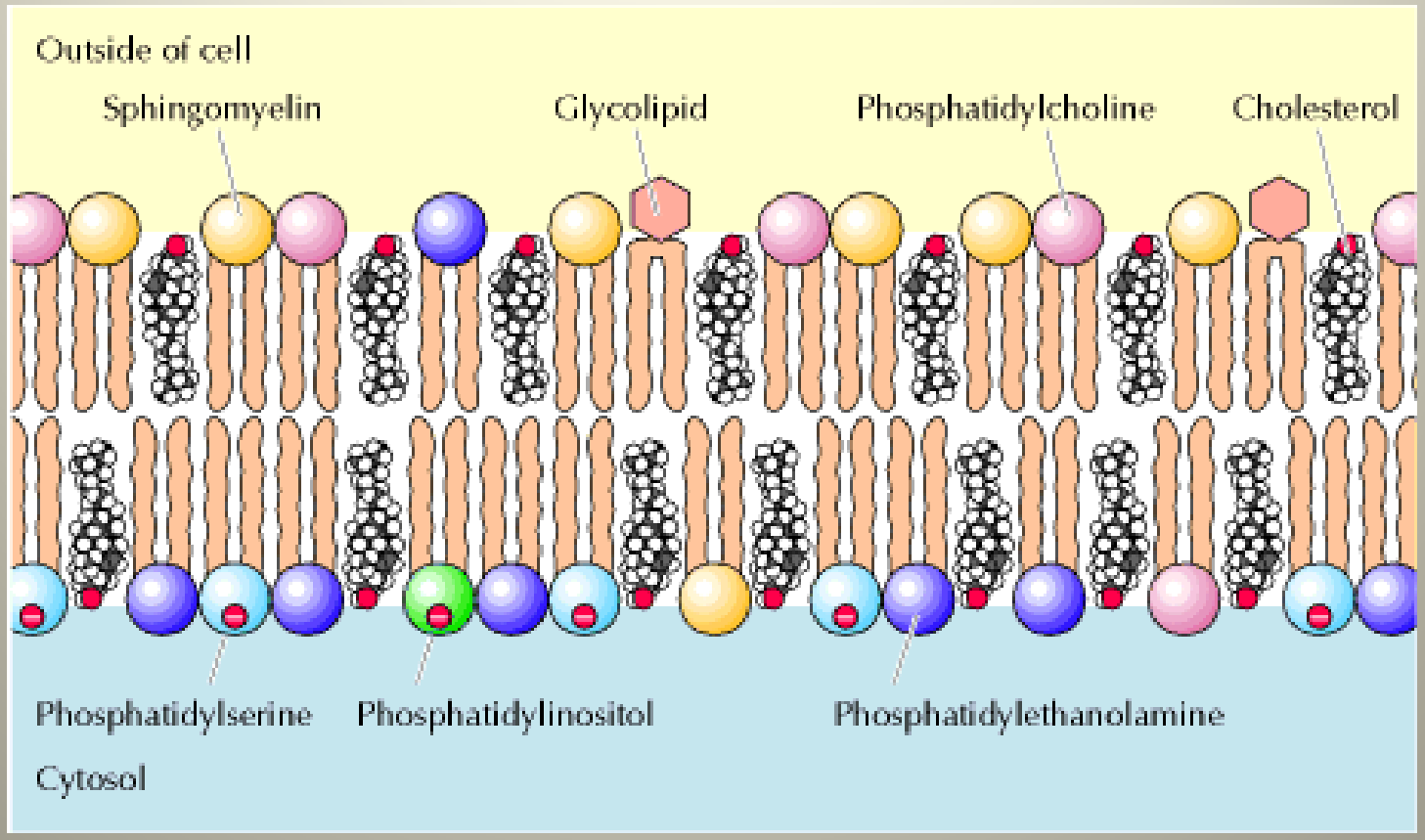
Consequences of Lipid Asymmetry

- Packing of PLs different in the two bilayer leaflets
- Different PL classes have different acyl chain composition (*e.g.*, PC tends to have more saturated FAs, PE and PS tend to have more PUFAs)
- Membrane fluidity and physical state different in the two leaflets of the bilayer
- Can affect enzyme and transport protein activities

Lipid Asymmetry

Membrane phospholipid	Percent of total membrane phospholipid	Distribution in membrane
Phosphatidylethanolamine	30	
Phosphatidylcholine	27	
Sphingomyelin	23	
Phosphatidylserine	15	
Phosphatidylinositol	5	
Phosphatidylinositol 4-phosphate		
Phosphatidylinositol 4,5-bisphosphate		
Phosphatidic acid		

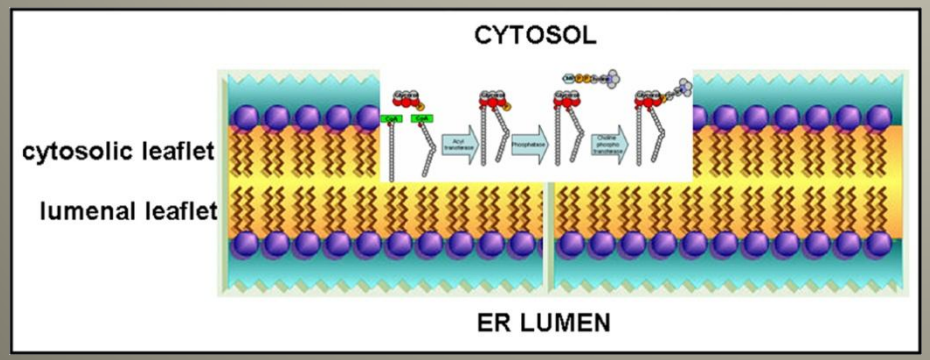
Lipid Asymmetry

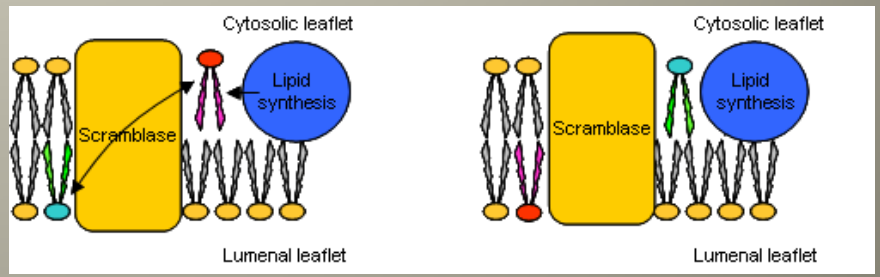


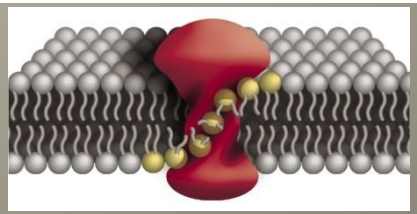
- Amino PLs (PE, PS) tend to face cytoplasm
- Choline PLs (PC, Sph) tend to face outside cell
- Cholesterol in both halves of lipid bilayer
- Glycolipids exclusively on outer leaflet of bilayer

Generation of Membrane Lipid Asymmetry

- Glycerophospholipids synthesized on cytosolic leaflet of SER (topologically equivalent to cytoplasmic face of PM)
- “Flippase” specifically translocates PE and PS (but not PC) to SER lumenal leaflet (topologically equivalent to extraplasmic face of PM)
- “Scramblase” exchanges PC from cytosolic to lumenal leaflet
- Sphingolipids synthesize on lumen leaflet of SER (and Golgi–glycosylation)

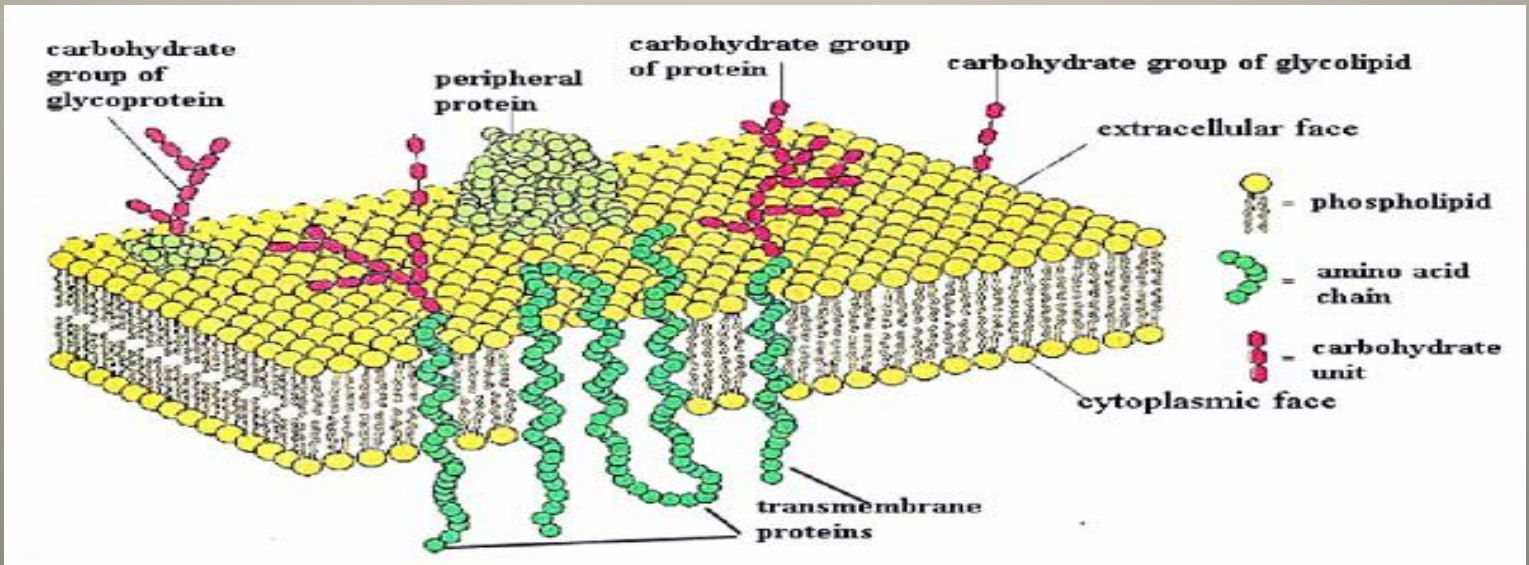


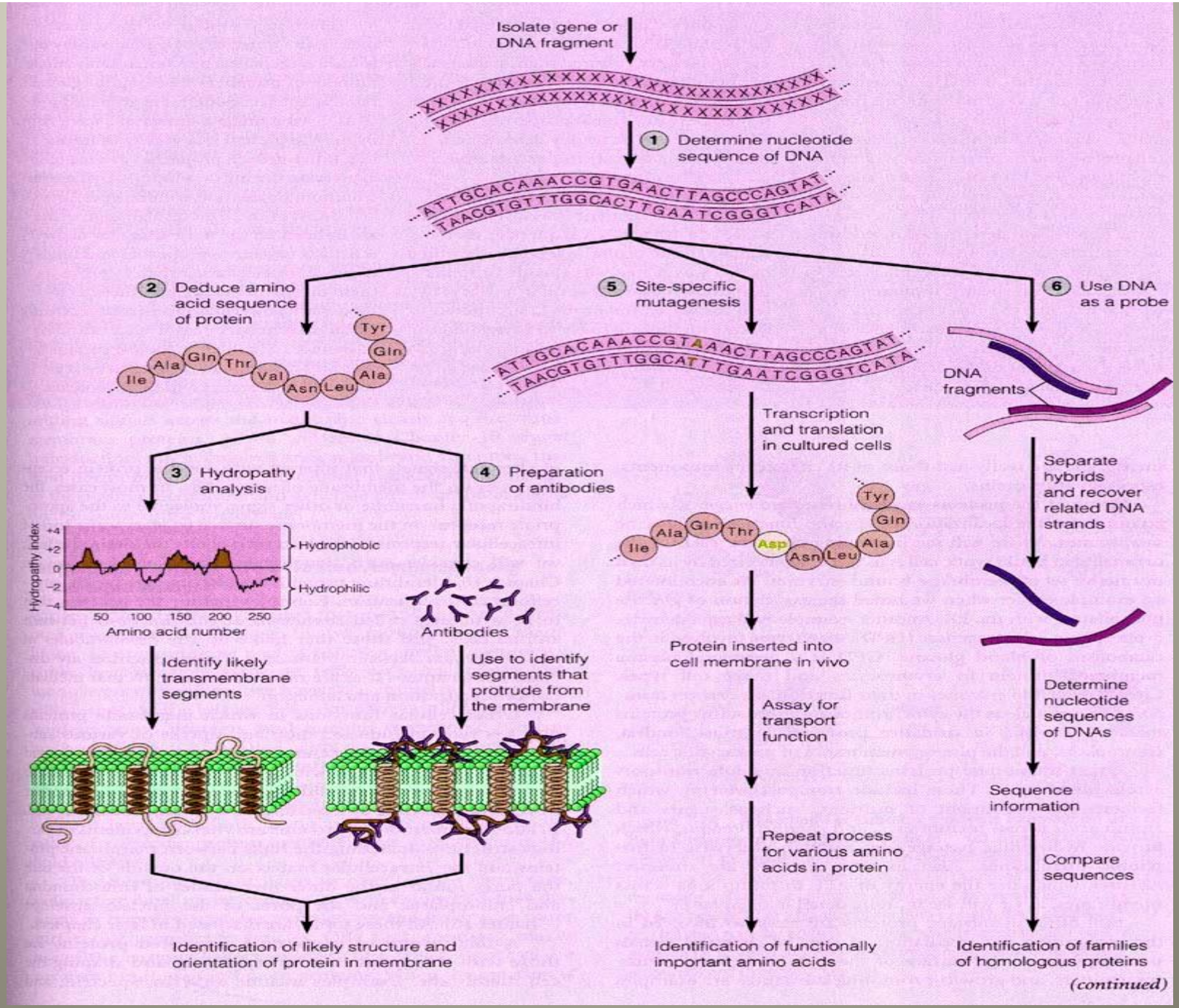




Carbohydrate Asymmetry

- Glycolipids exclusively on external leaflet
- Carbohydrate chains of glycoproteins face outside of cell




Isolate gene or DNA fragment
1 Determine nucleotide sequence of DNA
ATTGCACAAACCGTGAACTTAGCCCAGTAT
TAACGTGTTTGGCACTTGAATCGGGTCATA
2 Deduce amino acid sequence of protein
Tyr
Gln
Ala
Gln
Thr
Val
Ile
Asn
Leu
Ala
3 Hydropathy analysis
Hydropathy index
+4
+2
0
−2
−4
Hydrophobic
Hydrophilic
50
100
150
200
250
Amino acid number
4 Preparation of antibodies
Antibodies
Identify likely transmembrane segments
Use to identify segments that protrude from the membrane
Identification of likely structure and orientation of protein in membrane
5 Site-specific mutagenesis
ATTGCACAAACCGTAAACTTAGCCCAGTAT
TAACGTGTTTGGCATTTGAATCGGGTCATA
Transcription and translation in cultured cells
Tyr
Gln
Ala
Gln
Thr
Asp
Ile
Asn
Leu
Ala
Protein inserted into cell membrane in vivo
Assay for transport function
Repeat process for various amino acids in protein
Identification of functionally important amino acids
6 Use DNA as a probe
DNA fragments
Separate hybrids and recover related DNA strands
Determine nucleotide sequences of DNAs
Sequence information
Compare sequences
Identification of families of homologous proteins
(continued)

Summary

- Concepts about membrane structure have evolved over the past >100 years, based upon principles of physical chemistry and augmented by evidence obtained through biophysical methods (*e.g.*, microscopy, spectroscopy, x-ray diffraction, etc.) and biochemical/cell biological methods (*e.g.*, immunofluorescence, chemical modification, etc.)
- Even methods considered “old” (e.g., Langmuir trough) can provide new and useful insights into current problems concerning membrane structure and function.
- The most common structural motif of ALL biological membranes is the LIPID BILAYER
- The Singer-Nicolson “fluid mosaic” model of membrane structure (1972) replaced prior models; it depicts proteins floating in a “sea” of lipids, with relatively few constraints to diffusion within the bilayer plane

Summary (cont'd)

- Proteins in the fluid mosaic model are depicted as either "peripheral" (extrinsic) or "integral" (intrinsic), depending on the strength and nature of their association with the lipid bilayer
- Integral proteins are strongly associated with the bilayer, requiring harsh means (detergents, chaotropes) to remove them from the membrane; Peripheral proteins are more loosely associated with the membrane, and only require mild treatments (change in pH or ionic strength) to remove them from the membrane.
- The transbilayer distribution of proteins and lipids is ASYMMETRICAL
- Choline-PLs (PC, Sph) favor the extracellular (outer; lumenal) leaflet, while amino-PLs (PE, PS) favor the cytoplasmic (inner) leaflet of the bilayer
- Such asymmetry can generate fluidity differences in the two halves of the bilayer, which can affect biological properties and function

Summary (cont'd)

- Physical state of membrane lipids depends on composition and temperature; Cholesterol is a "fluidity buffer"- can enhance or restrict fluidity, depending on ambient temperature relative to T_m of lipids
- Lateral (in-plane) and rotational diffusion of lipids, and flexing of PL acyl chains, are rapid (in the absence of extrinsic constraints); transverse ("flip-flop") diffusion of lipids is extremely slow in pure lipid bilayers, but is more rapid in biological membranes, facilitated by translocases (scramblases, flippases)
- Proteins diffuse relatively freely within the plane of the membrane, and rotate about an axis perpendicular to the plane of the membrane; however, transverse (flip-flop) diffusion does not occur (energetically highly unfavorable)
- Carbohydrates are also distributed asymmetrically in biological membranes: glycolipids (GSLs) and the oligosaccharide chains of glycoproteins are exclusively found on external leaflet of the plasma membrane bilayer