MCB 110: Biological Membranes

Eva Nogales
708C Stanley Hall
Enogales@lbl.gov

Office Hours:
3-4 PM, Thursdays and Fridays

MCB 110

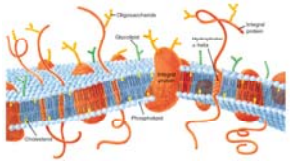
BIOLOGICAL MEMBRANES

1 MEMBRANE LIPIDS AND LIPID BILAYER

- I Intro to Biological Membranes
- II Membrane Lipids
- III Lipid Self-Assembly
- IV Membrane Fluidity

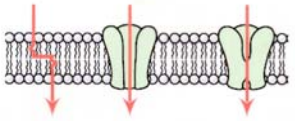
2 MEMBRANE PROTEINS

- I Introduction to Membrane Proteins
- II Integral Membrane Proteins
- III Peripheral Membrane Proteins
- IV Lipid-Anchored Membrane Proteins
- V Diffusion of Membrane Proteins



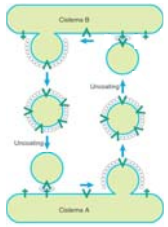
3 TRANSPORT ACROSS MEMBRANES

- I Intro: Permeability of the Cell Membranes
- II Diffusion
- III Facilitated Diffusion
- IV Active Transport
- V Membrane Potential and N



4 MEMBRANE TRAFFICKING

- I Introduction: Secretory Pathway
- II Endoplasmic Reticulum
- III Golgi
- IV Vesicle Transport
- V Lisosomes
- VI Endocytosis



5 CELL SIGNALING

- I Introduction
- II G Protein-coupled Receptors
- III Receptor Tyrosine Kinases
- IV Integrin Signaling
- V Cell Signaling and Apoptosis
- VI Relationships between

MEMBRANE LIPIDS AND LIPID BILAYER

I Intro to Biological Membranes
- A. Membrane Functions
- B. Lipid bilayer

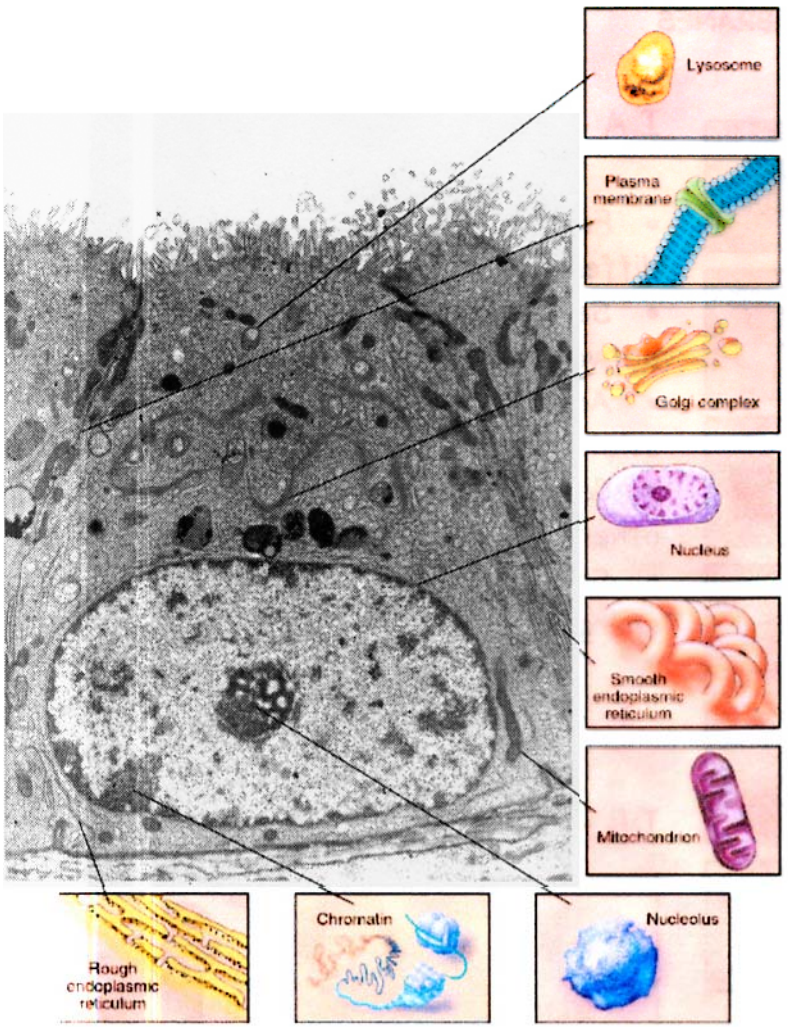
II Membrane Lipids
- A. Phosphoglycerides
- B. Sphingolopids
- C. Cholesterol
- D. Lipid variability; membrane asymmetry

III Lipid Self-Assembly

IV Membrane Fluidity
- A. Definition and function
- B. Effect of lipid composition
- C. Cell control of membrane fluidity
- D. Lateral mobility and flip-flop

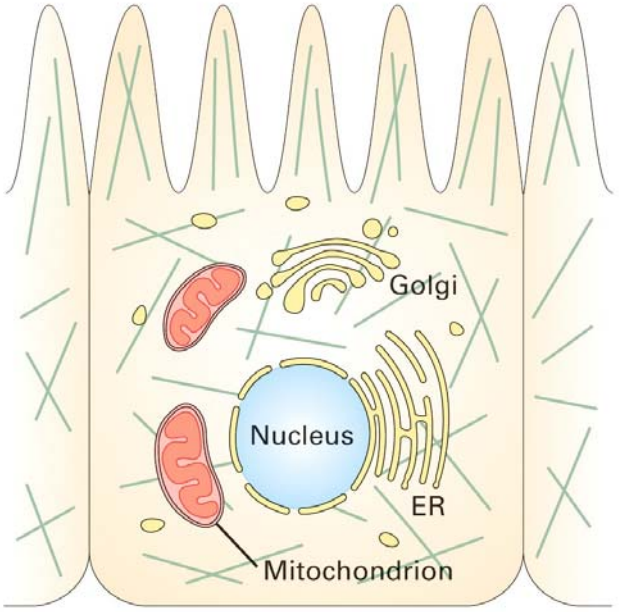
Suggested Reading: Lodish, Chapter 5 - 5.1 **(Chapter 10.1 in new edition!);** Alberts, Chapter 10

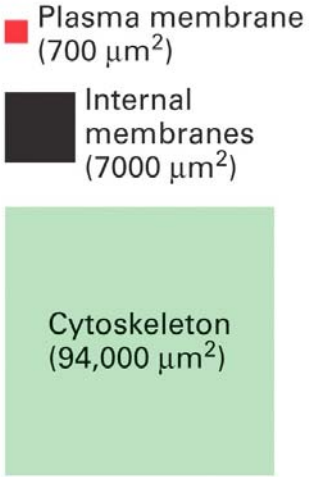
I INTRO TO BIOLOGICAL MEMBRANES



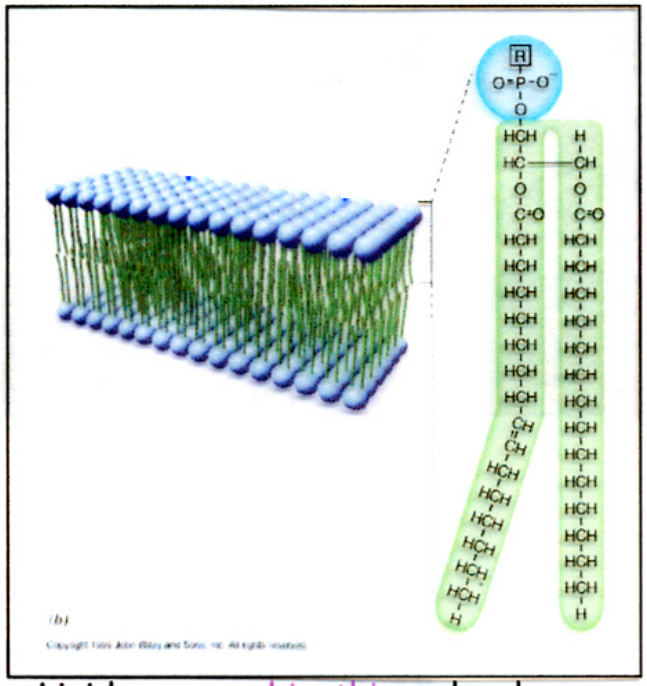
I A - Membrane Functions

- Enclose cells or organelles separating different activities
- Selective exchange of molecules between compartments
- Active transport and accumulation of solutes
- Communication with environment and other cells
- Scaffold for biochemical activities; energy transduction

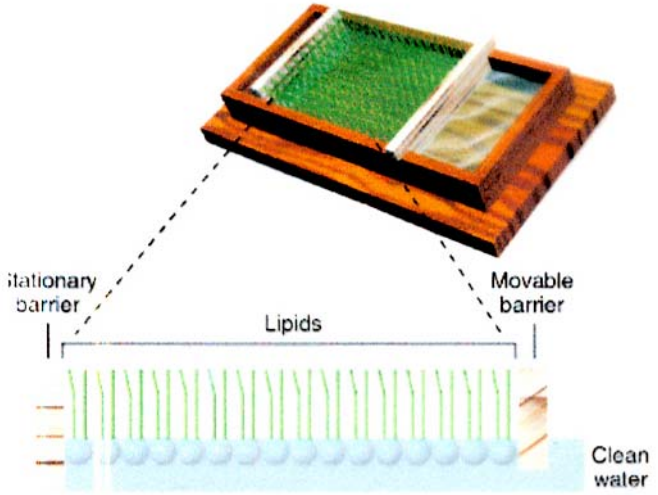




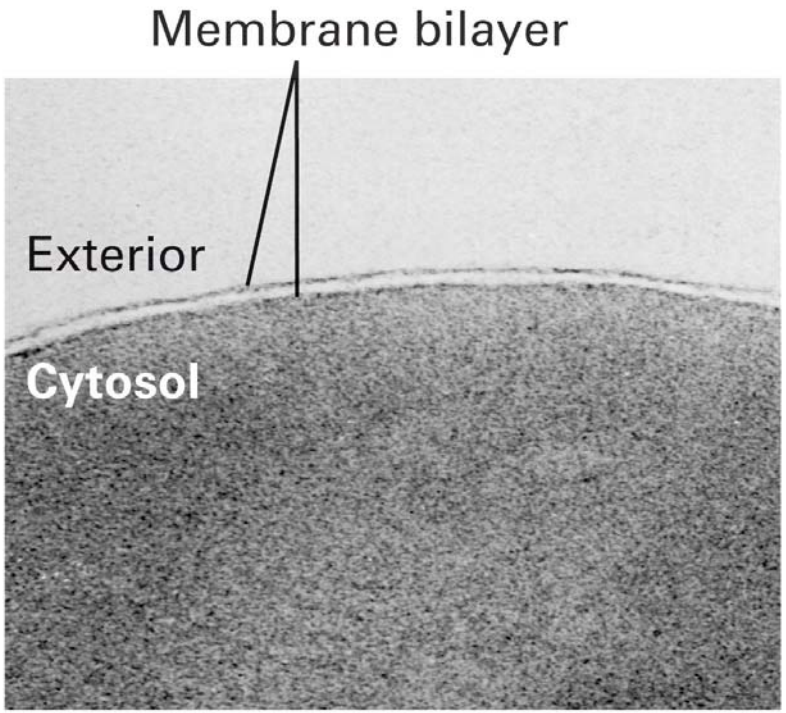
I B - Lipid Bilayer



Lipids are amphipathic molecules

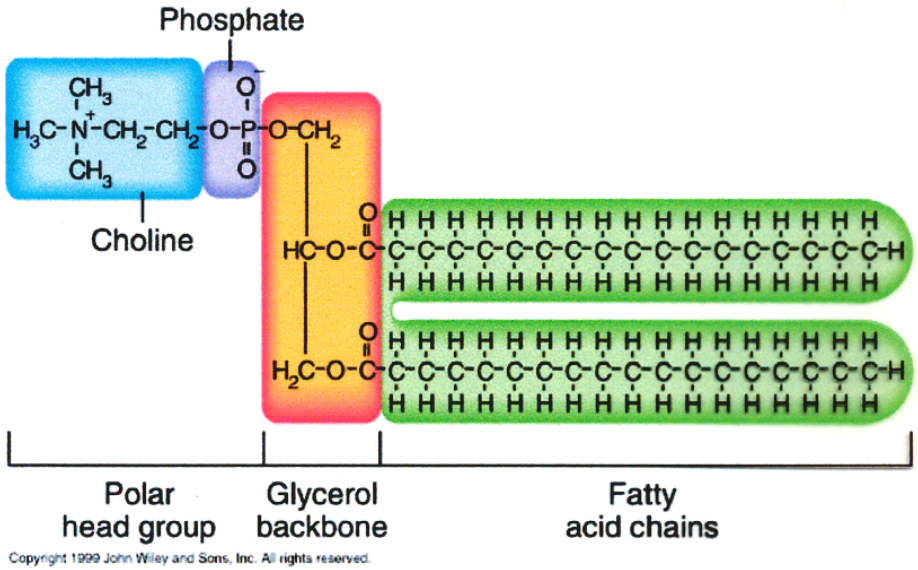


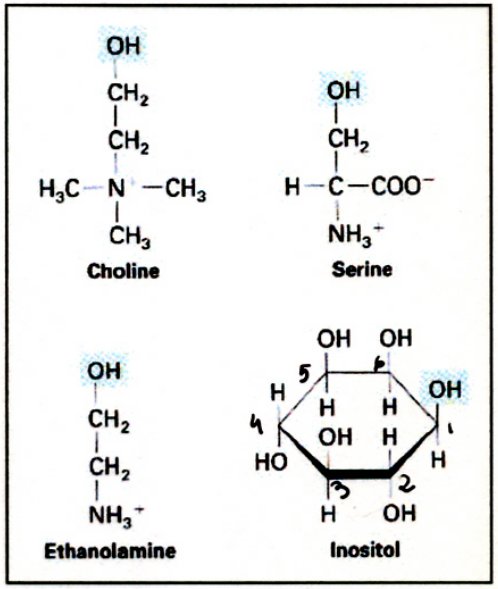
Gorter and Grendel experiment



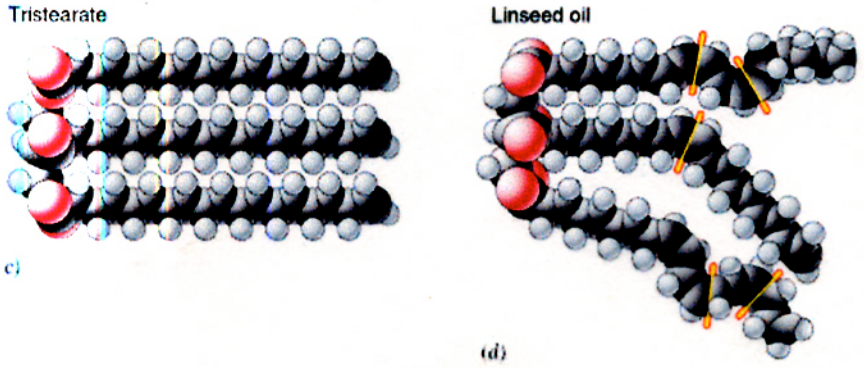
II MEMBRANE LIPIDS

II A – Phosphoglycerides





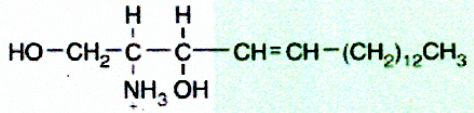
- Common phosphoglycerides groups: choline, ethanolamine, serine, inositol



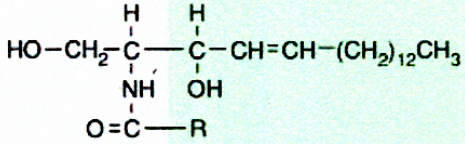
- Saturated and unsaturated fatty acyl chains

II B – Sphingolipids

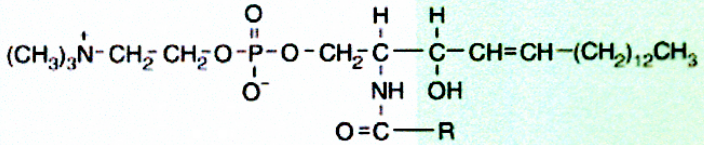
Sphingosine

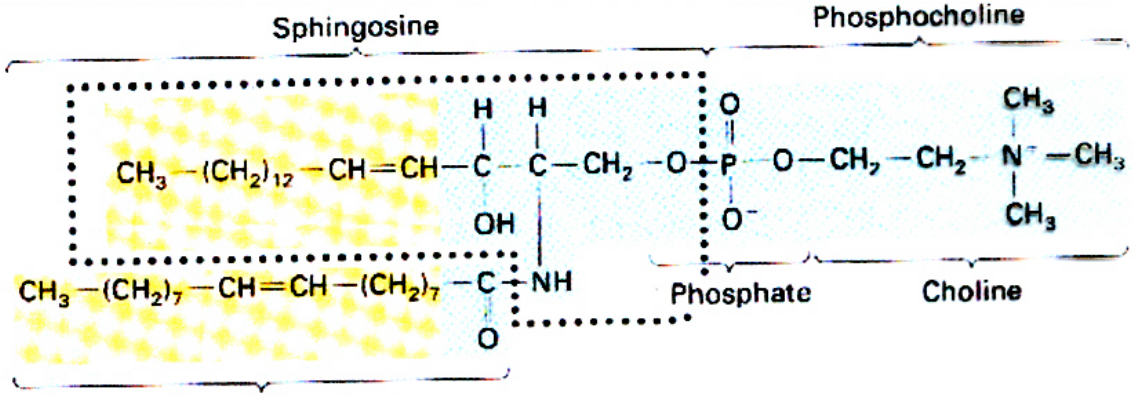


Ceramide



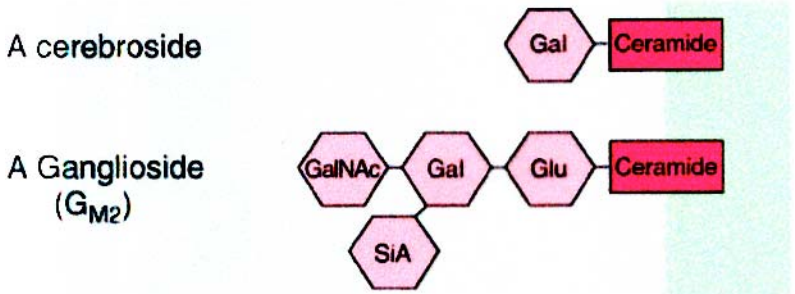
Sphingomyelin





Sphingomyelin

Glycolipids



- **Cerebrosides**
- **Gangliosides – Blood group antigens**

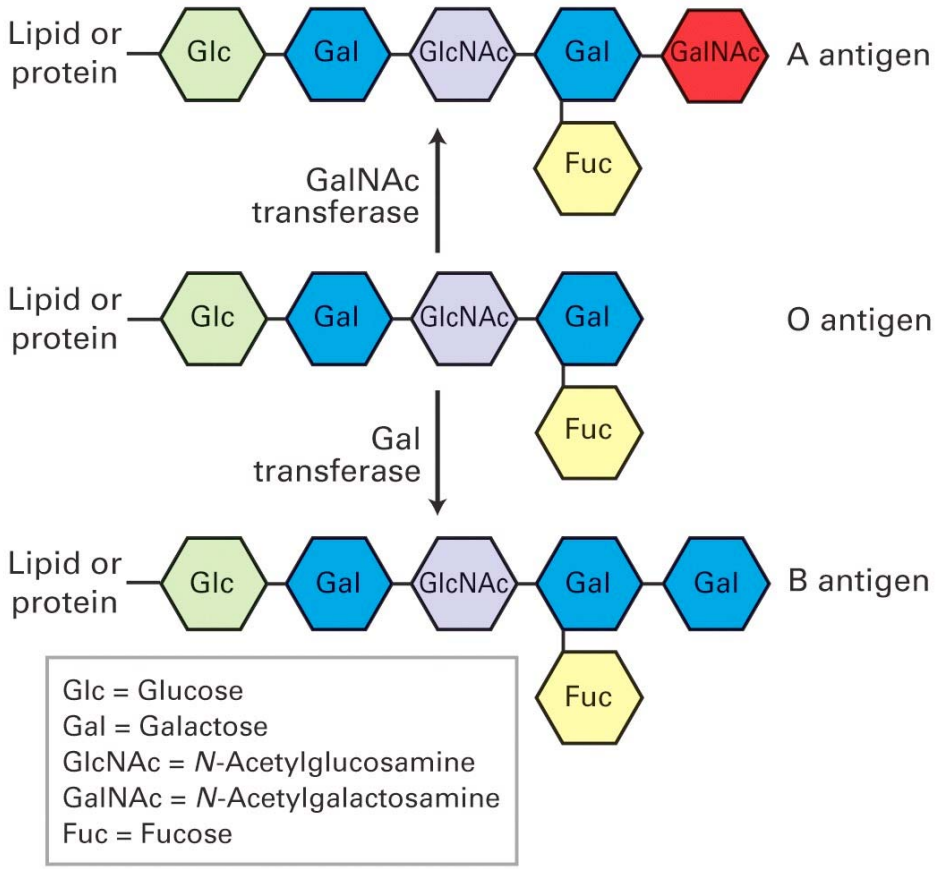


TABLE 5-2 ABO Blood Groups

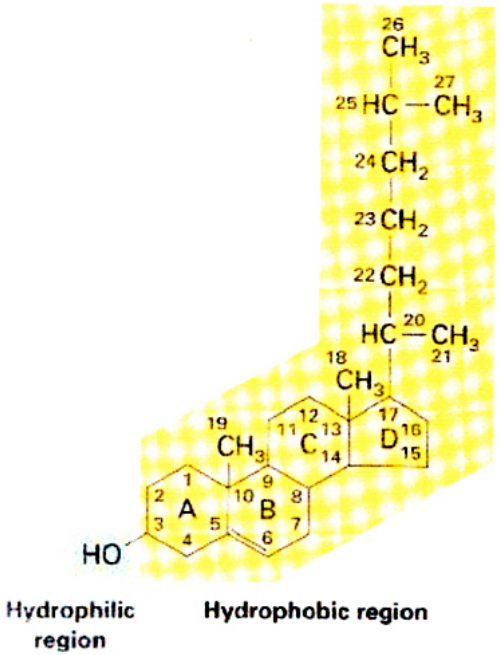
Blood-Group Type	Antigens on RBCs*	Serum Antibodies	Can Receive Blood Types
A	A	Anti-B	A and O
B	B	Anti-A	B and O
AB	A and B	None	All
O	O	Anti-A and anti-B	O

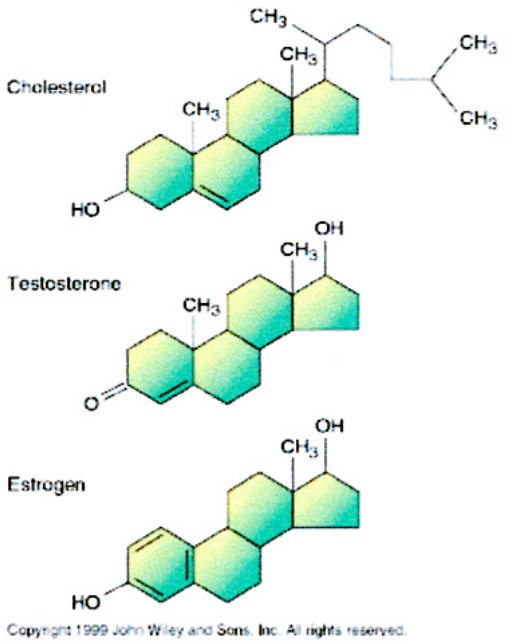
*See Figure 5-16 for antigen structures.

Error in original table!!

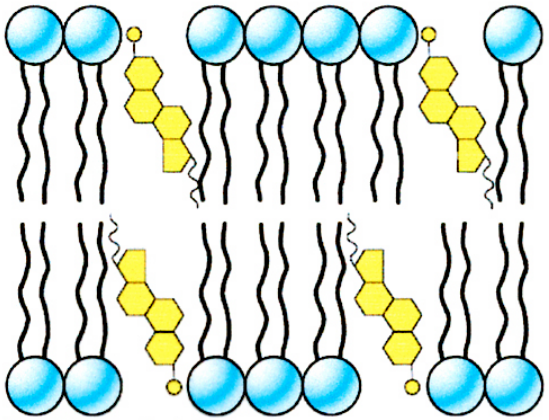
II C – Cholesterol

1- Structure





2- Alignment in bilayer



II D – Lipid variability

1- Head group x fatty acyl chain - Up to 100 distinct lipids in a membrane
2- Determine physical state of the membrane, activity of proteins, etc
3- Lipid distribution is different in the two leaflets – different properties for the cytosolic and exoplasmic face: Glycolipids on the outer surface where they serve as receptors; red blood cells treated with phospholipase: 80% PC, 20% PE, less than 10% PS.

III LIPID SELF-ASSEMBLY

Bilayers

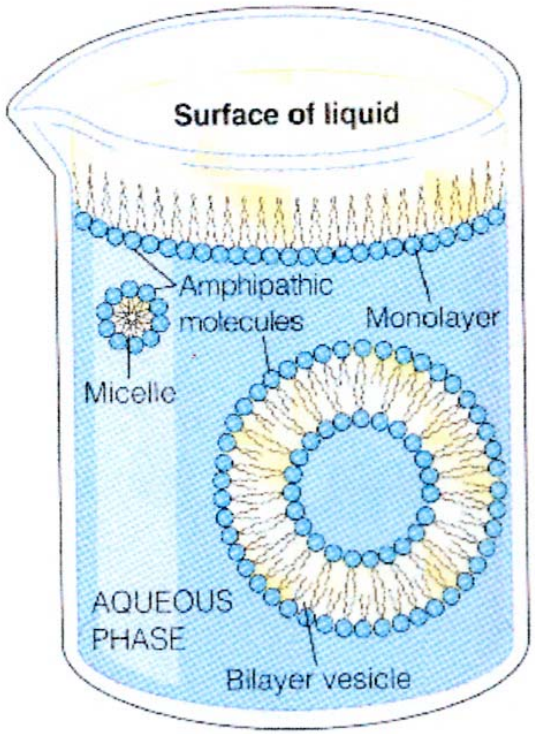
Monolayers

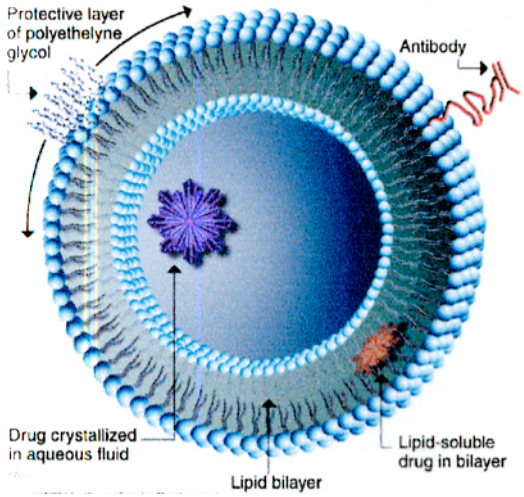
Micelles

Liposomes

- Biochemical characterization of membrane proteins

- Medical application as carriers



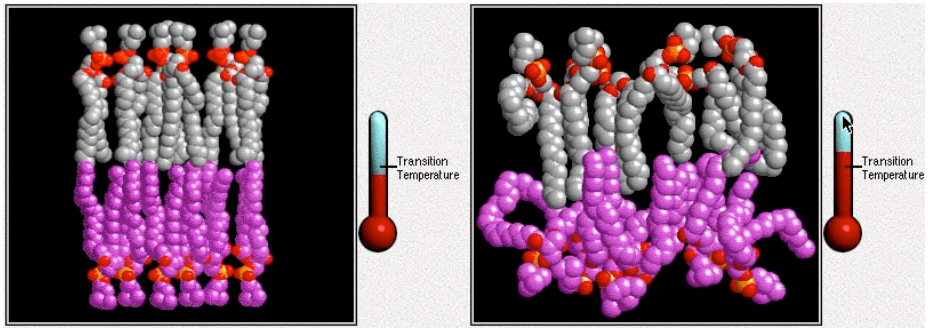


IV Membrane Fluidity

IV A – Definition and Function

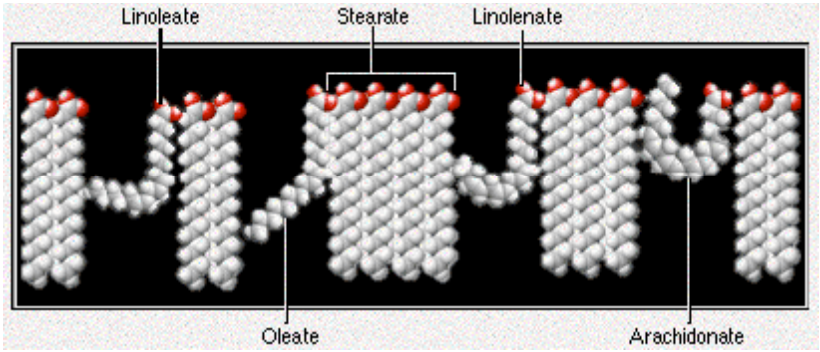
- *Fluidity* is defined as "easy of flow" and is opposed to *viscosity* (resistance to flow).

Lipids bilayer are fluid in the liquid crystal state, but under the melting temperature Tm become rigidified: liquid crystal to gel transition.

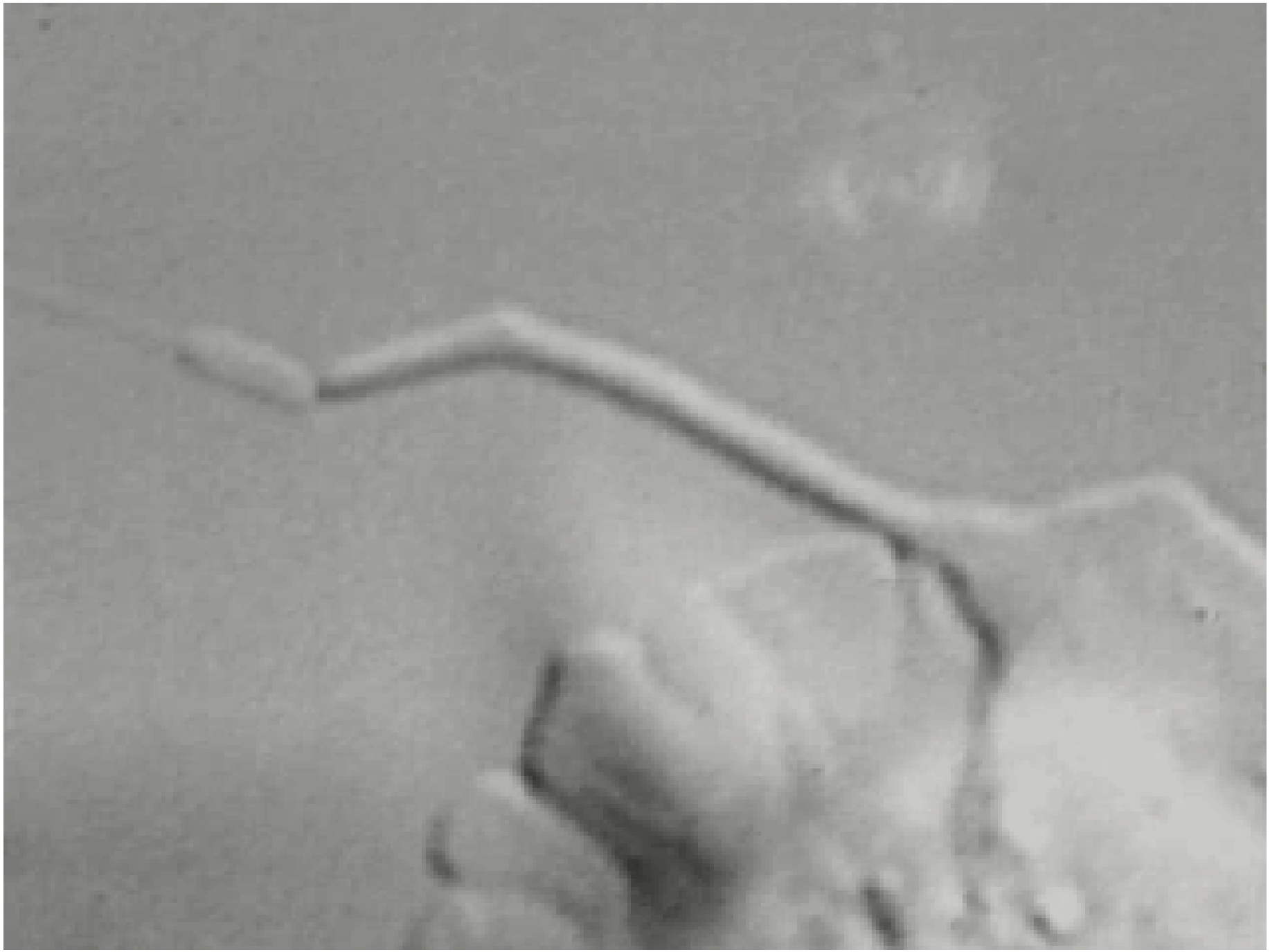


Fluidity of a biological membrane is required for movement of membrane proteins, and for processes requiring membrane fusion such as cell division or exocytosis

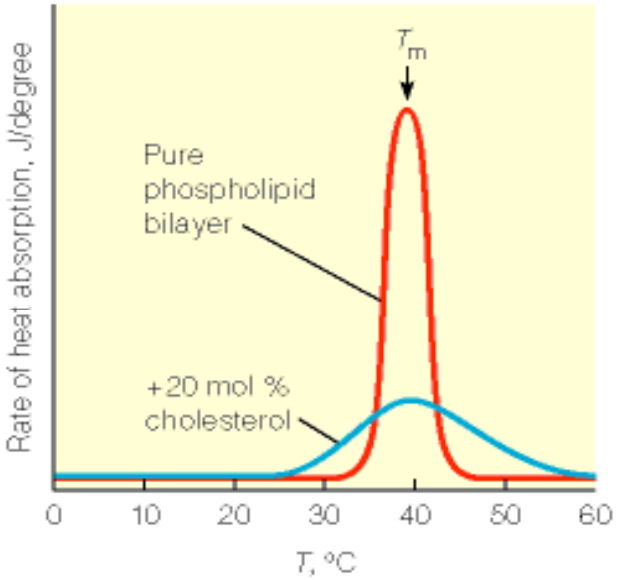
IV B – Effect of Lipid Composition



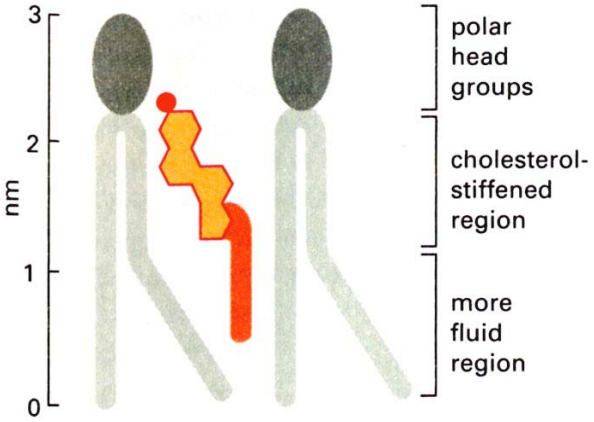
- Very sensitive to the presence of unsaturated fatty chains (one or more double bonds) that make packing difficult and thus decrease the Tm. In contrast saturated lipids have very high melting temperatures.
- The length of the fatty acid chain also influences Tm. The longer the chain the larger the energy of packing. Thus, shorter chains result in lower melting temperatures.
- Bacteria control the fluidity of their membrane in changing environments by regulating the synthesis of lipids (with shorter of longer chains) and by desaturation.
- Exp. – Doubling time larger at the restricted temperature for bacteria with mutant desaturases.



- Cholesterol h as an important effect in membrane fluidity. Its ring structure rigidifies the membrane, but it also makes transition to gel phase more difficult by hindering packing of phospholipids. The net effect is a broadening of the transition.

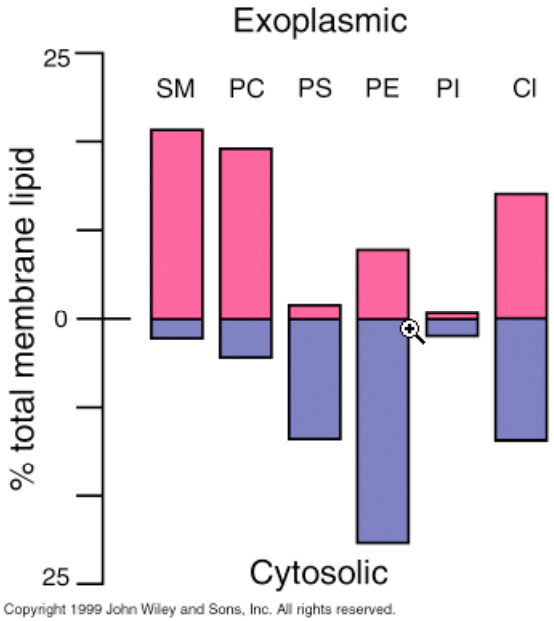


(b) Transition with and without cholesterol

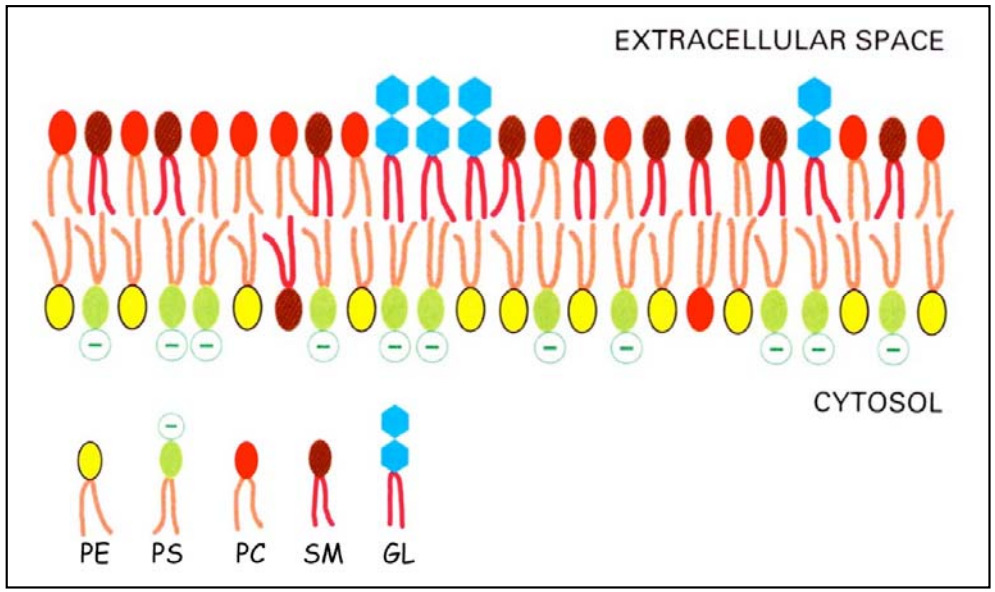


- Eukaryotes control fluidity by changes the amount of cholesterol present in the membrane.

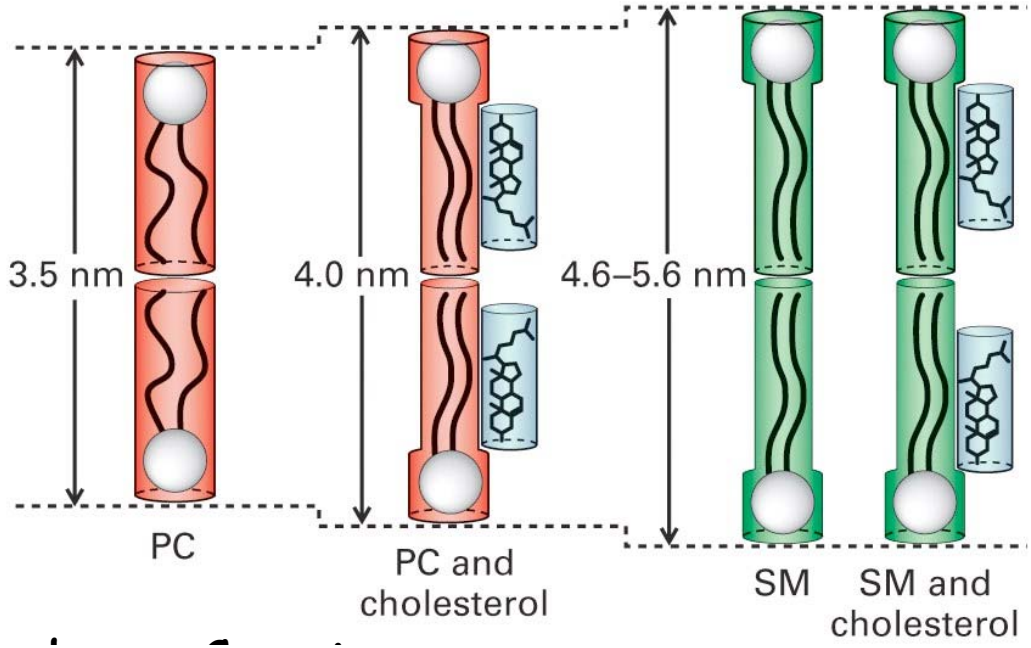
IV D – Lipid Distribution in the leaflets

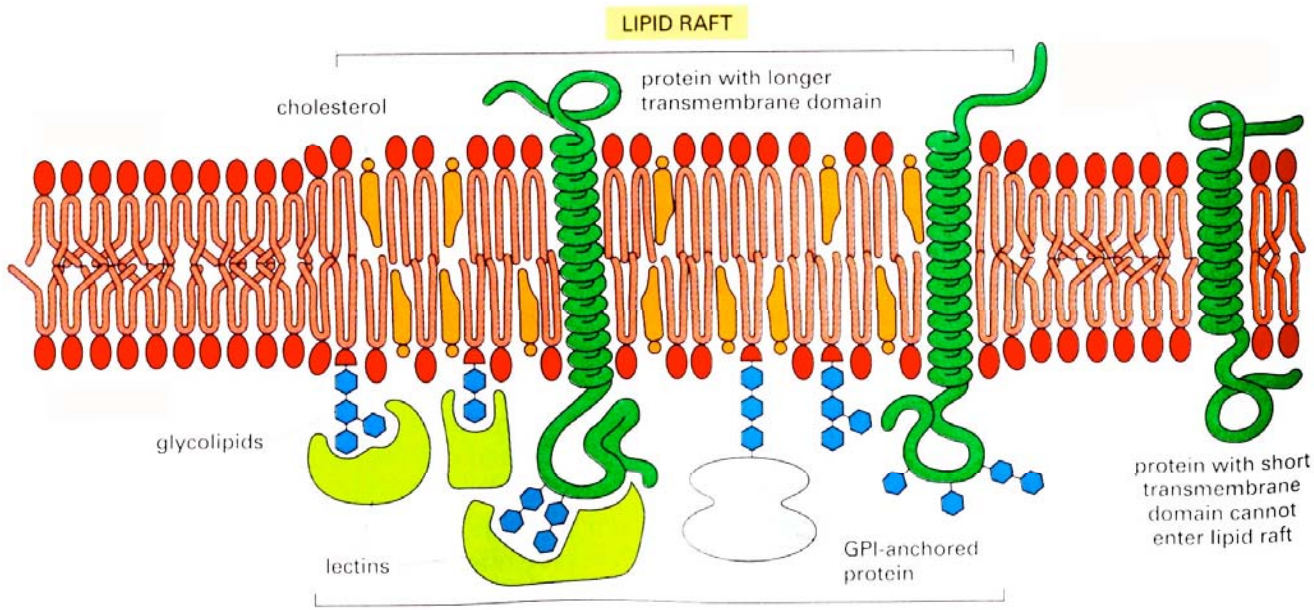


- The lipid composition of the bilayer in the two leaflets is unequal at the cell membrane, giving the two sides very specific properties.



Membrane Thickness

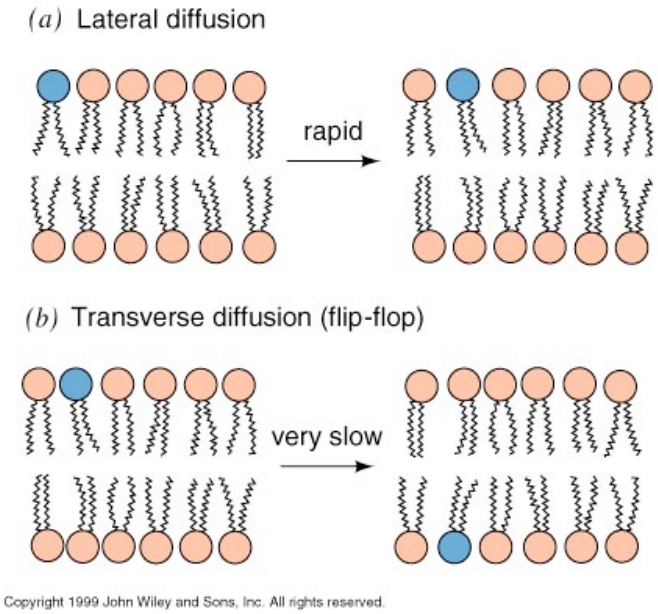


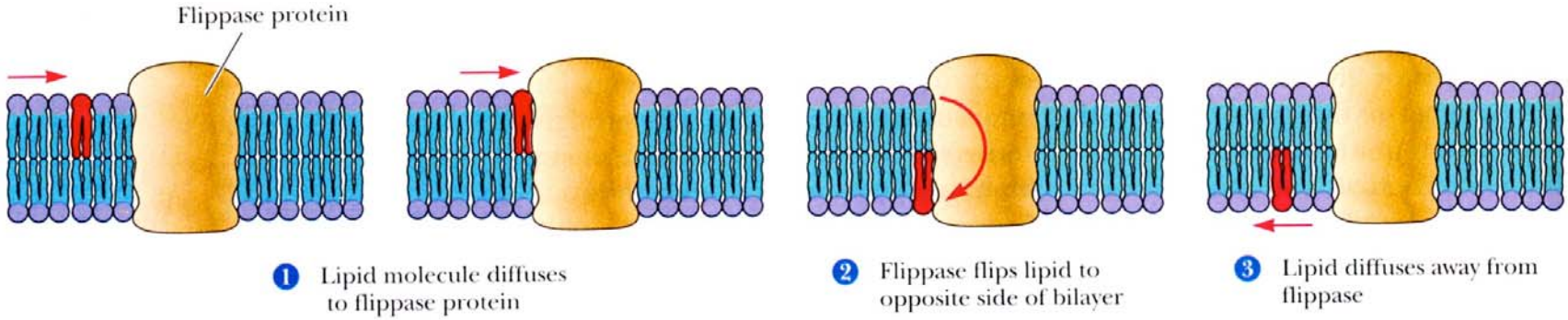


Cholesterol and sphingolipids form rigid microdomains or rafts (~ 70 nm in diameter). These rafts concentrate certain membrane proteins and are important in cell signaling.

IV C - Lipid Mobility: lateral movement and flip-flop

- Lipids are free to move laterally in a lipid bilayer
- Flip-flop movement is very energetically unfavorable and in the cell is catalyzed by flippases.

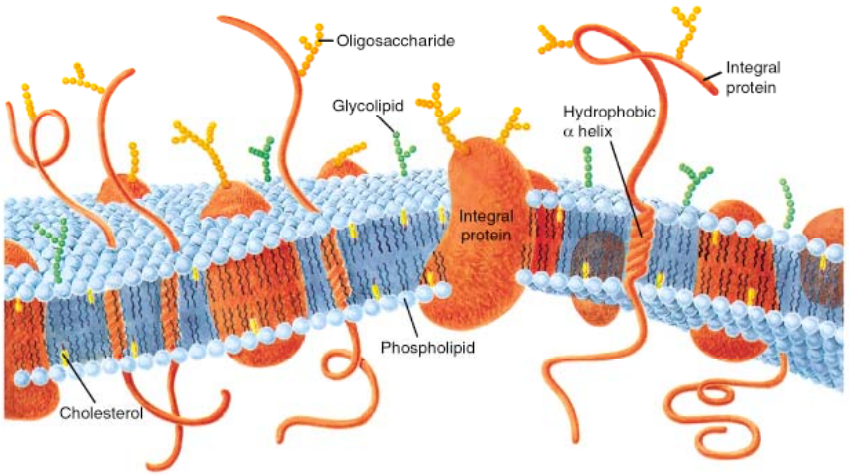




MEMBRANE PROTEINS

I Introduction

- Models of biological membranes
- Protein/lipids ratios
- Asymmetric orientation in membranes
- Types of membrane proteins



II Integral Membrane Proteins

- Amphipatic character
- Visualization by freeze-fracture
- Purification
- Structural characterization

III Peripheral Membrane Proteins

A. Non-covalent association. Extraction methods

B. Functions

IV Lipid-anchored Proteins

A. Outside leaflet. GPI

B. Inside leaflet

V Diffusion of Membrane Proteins

A. Cell fusion experiments

B. FRAP

C. SPT

Suggested Reading: Lodish, Chapter 5 - 5.2 **(Chapter 10.2 in new edition!)**

Alberts, Chapter 10

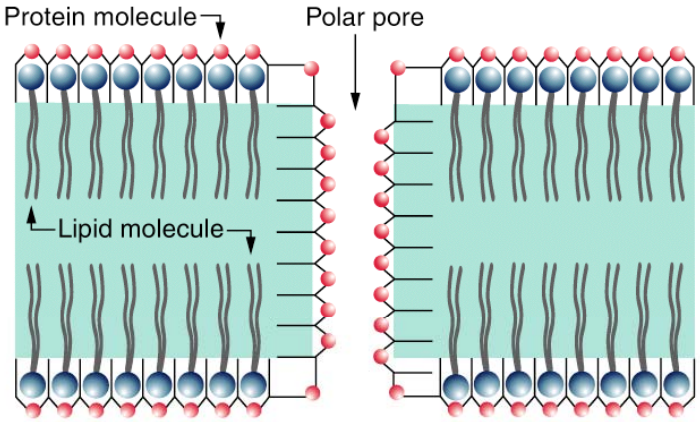
I INTRODUCTION

II A - MODELS OF BIOLOGICAL MEMBRANES

Initial models of biological membranes included only lipids.

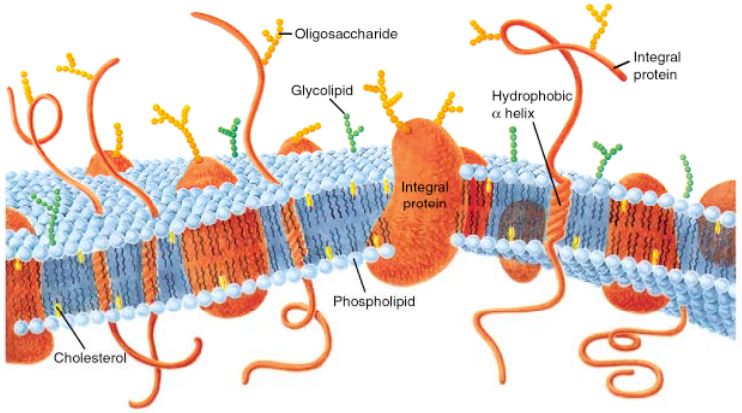
In the 20's and 30's hints from solubility and surface tension experiments indicated that there were other components in addition to lipids.

An initial model of a biological membrane that included proteins had the proteins lining the lipid bilayer with proteins.



In 1972 Singer and Nicholson proposed the fluid mosaic model of the membrane that we used today. In this model proteins both link to lipids or across the bilayer move in a sea of fluid lipid.

While this model is overall correct is has its limitations. For example, we now know that certain components of the cell membrane can be fixed in space by attachment to the underlying cytoskeleton.



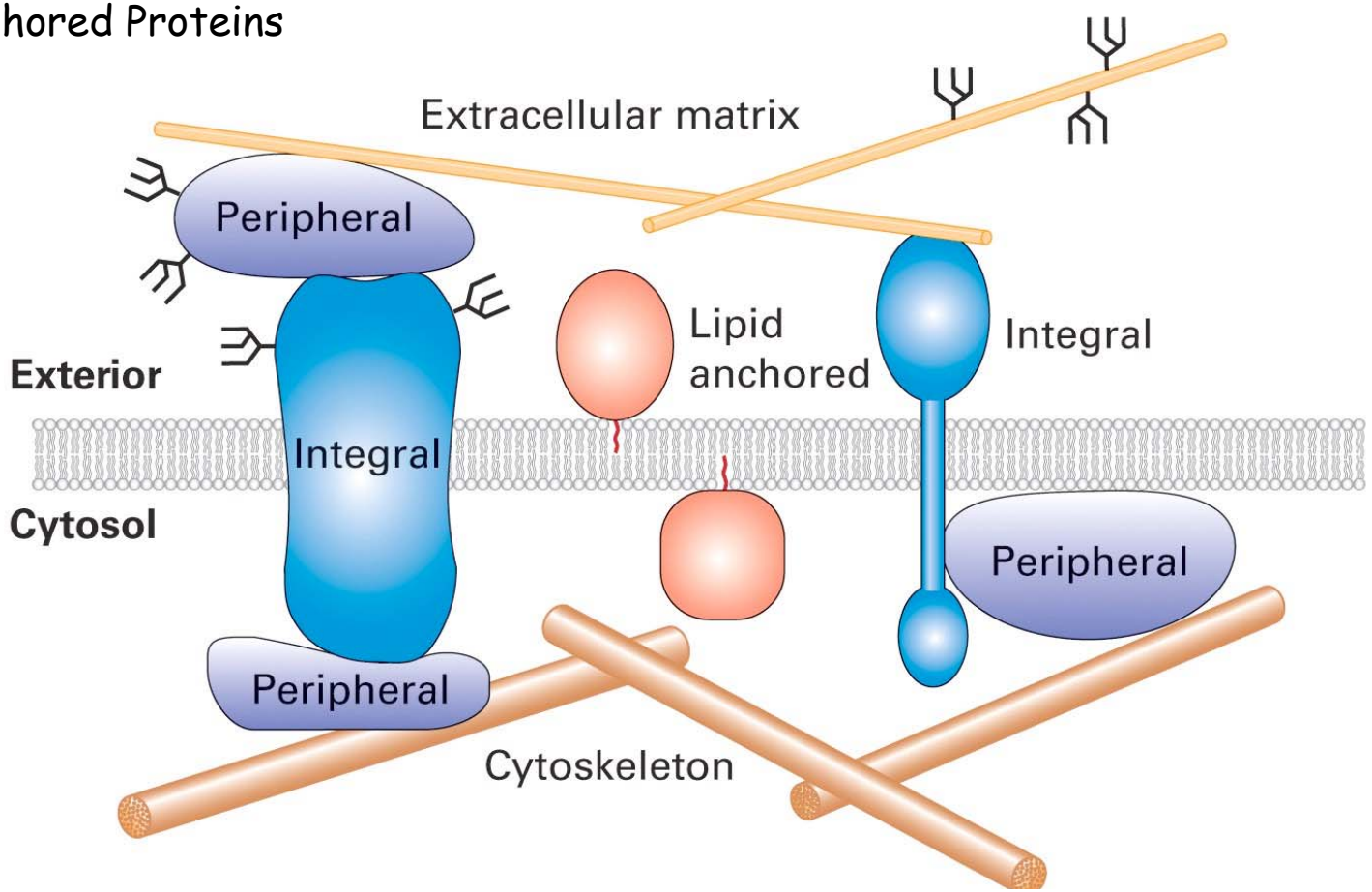
IC – Types of Membrane Proteins

Proteins associated with membranes can be classified into three different types depending on the type of interaction they have with the bilayer.

1 – Integral Membrane Proteins pass through the lipid bilayer

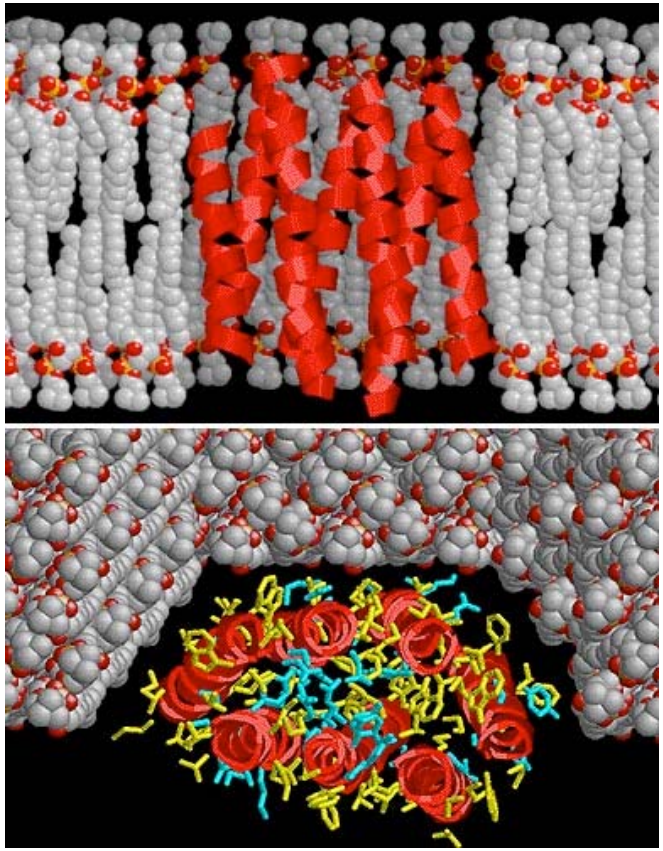
2 – Peripheral Proteins associate with the bilayer by non-covalent interactions

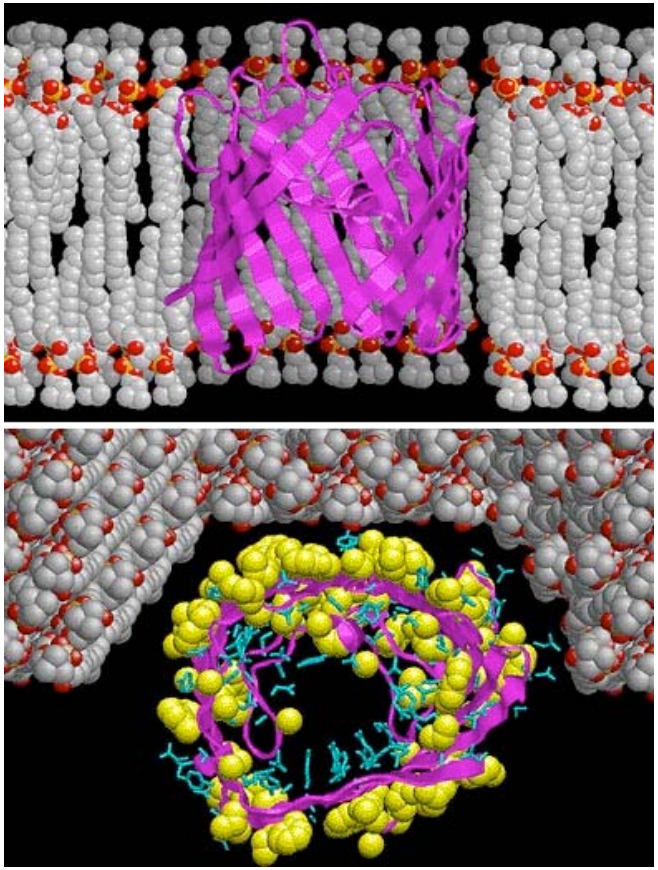
3 – Lipid-anchored Proteins



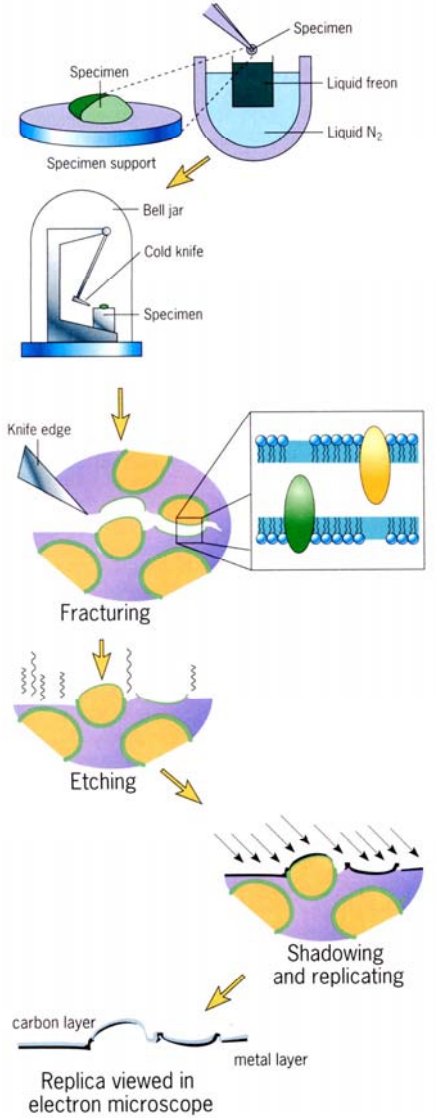
II Integral Membrane Proteins

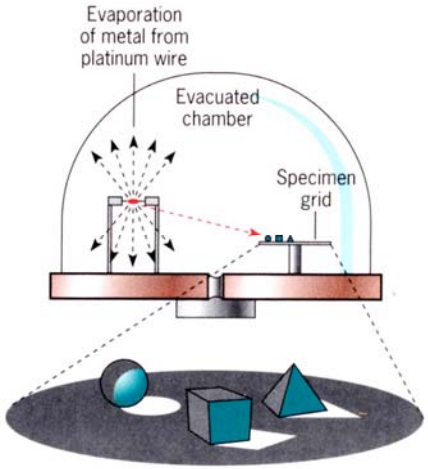
II A – Proteins that traverse the lipid bilayer have a surface with amphipathic character, in contrast with the hydrophilic nature of the surface of soluble proteins.
Hydrophilic residues are present also on the inside of the protein, making hydrogen bonds with ligands or with one another, or forming aqueous channels.

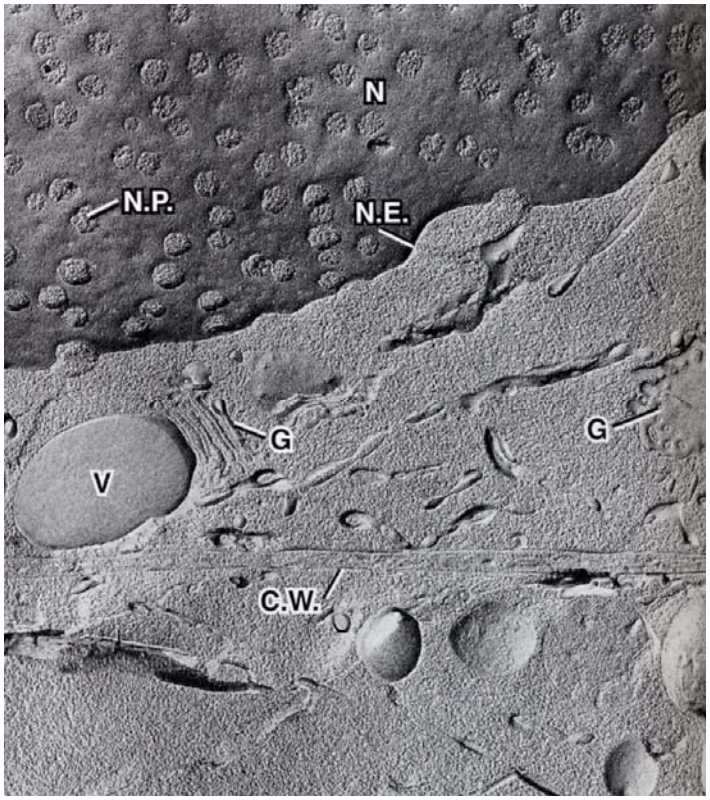




II B – Integral membrane proteins can be directly visualized by freeze-fracture and electron microscopy.







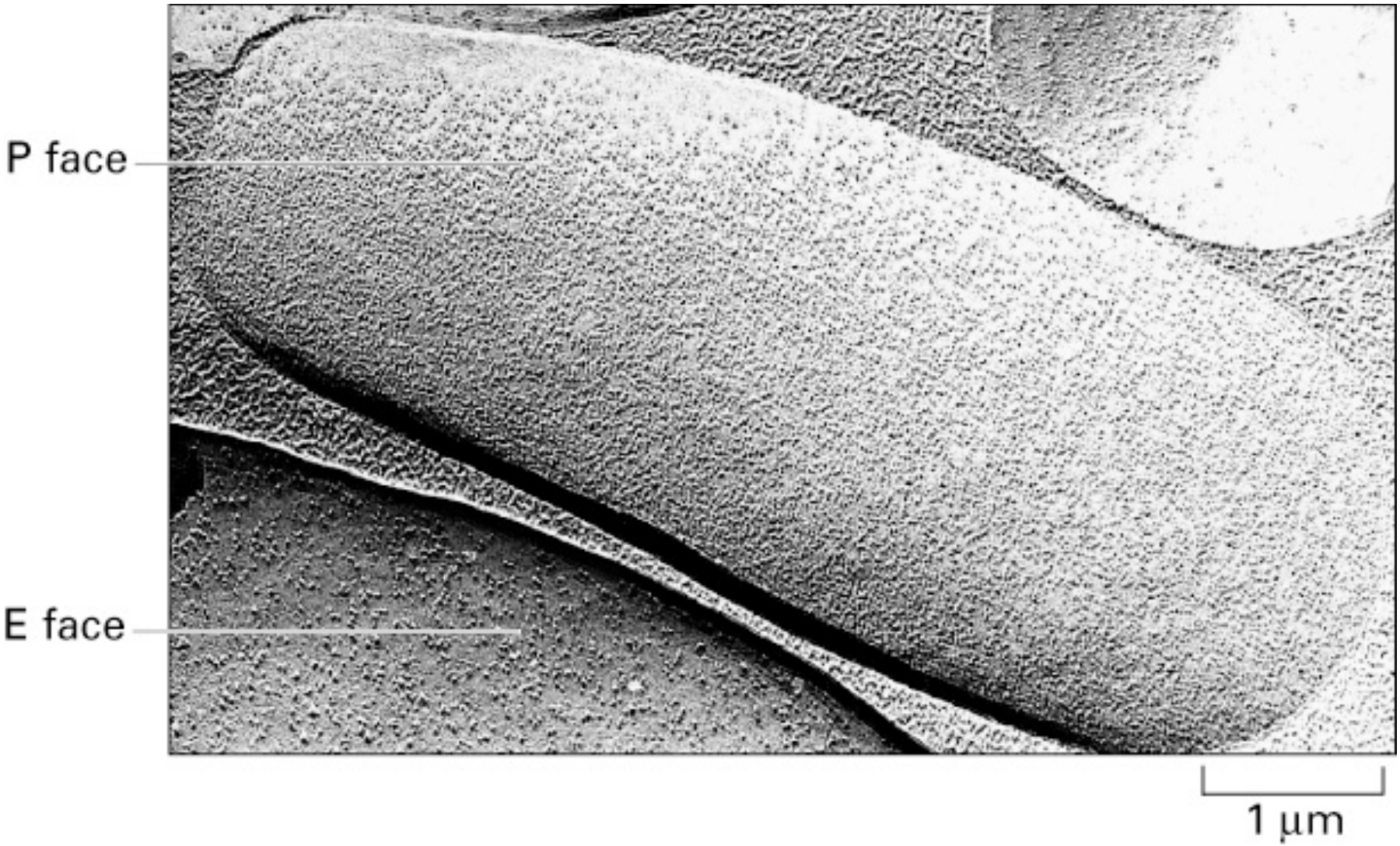


Figure 10–34. Molecular Biology of the Cell, 4th Edition.

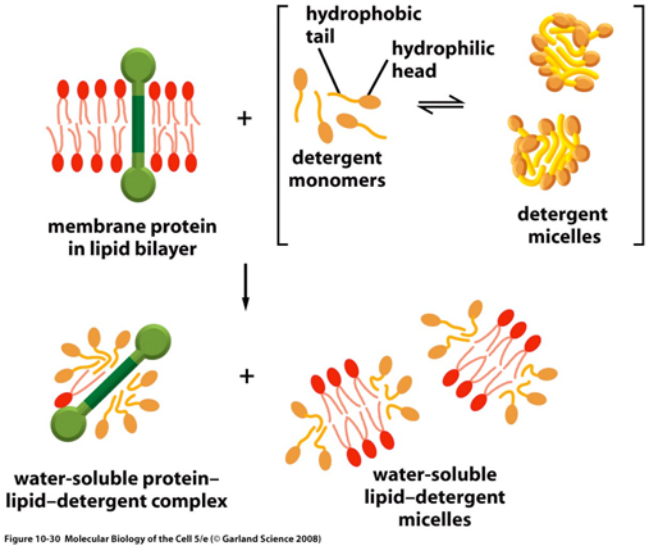


Figure 10-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

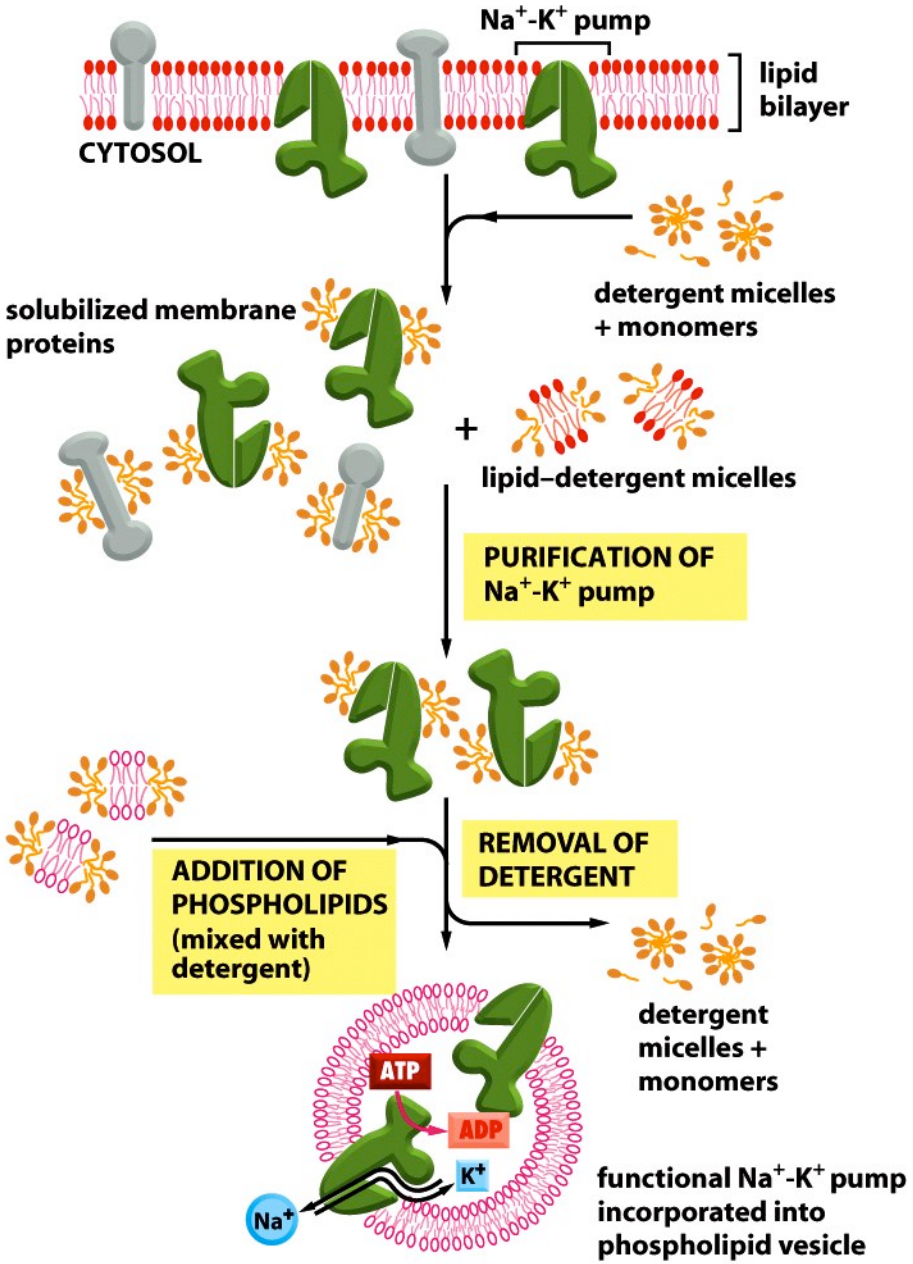
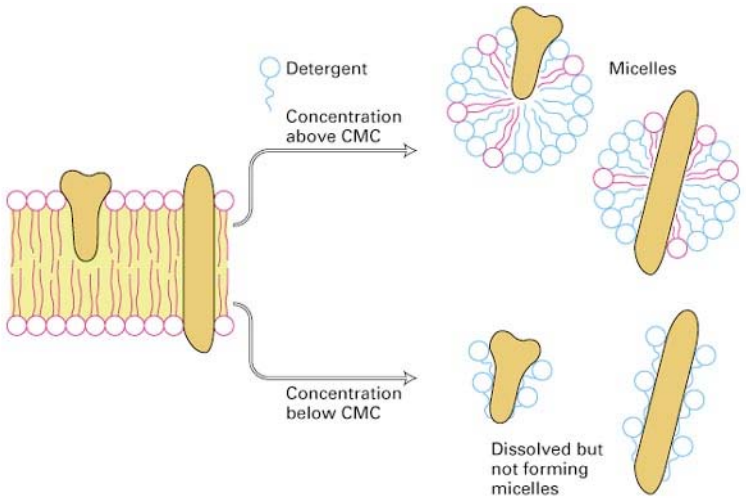
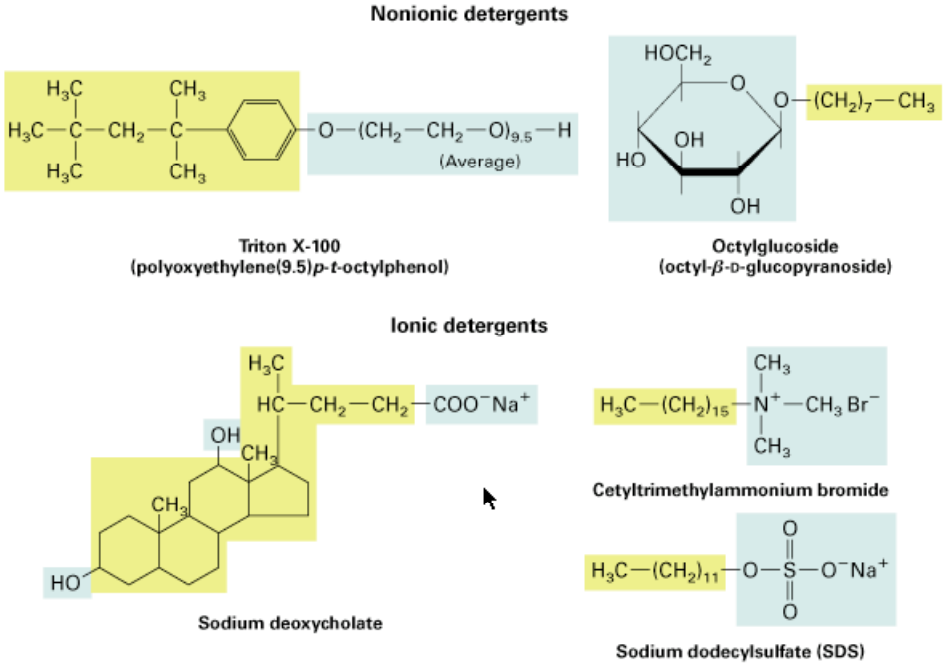


Figure 10-31 Molecular Biology of the Cell 5/e (© Garland Science 2008)

II C – Purification of membrane proteins involves solubilization by detergents that bind to the hydrophobic parts of the protein surface. Below the Critical Micelle Concentration (CMC) the detergent molecules can solubilize the protein without forming detergent micelles



Ionic detergents like SDS (sodium dodecyl sulphate) result in the denaturation of the protein. Milder, non-ionic detergents like Triton X-100 are commonly used for solubilization while preserving the structure of the protein.



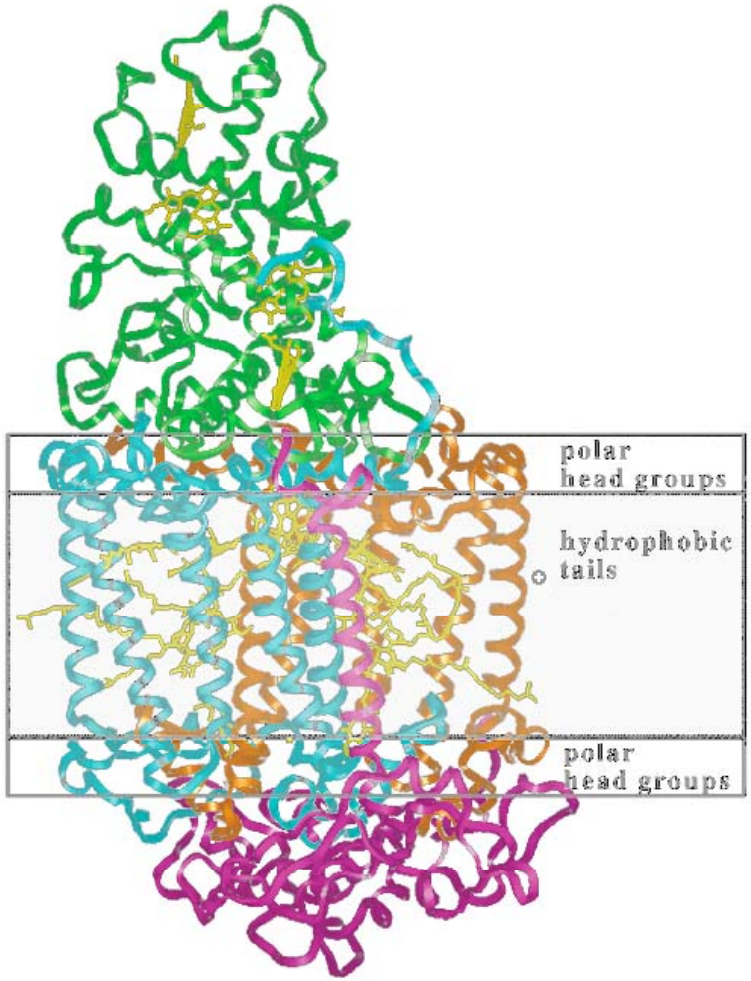
II D – Structural Characterization

- Special problems of Integral Membrane Proteins
  - Difficult overexpression – Formation of inclusion bodies in host cell
  - Need to solubilized
  - Possible loss of function in detergent

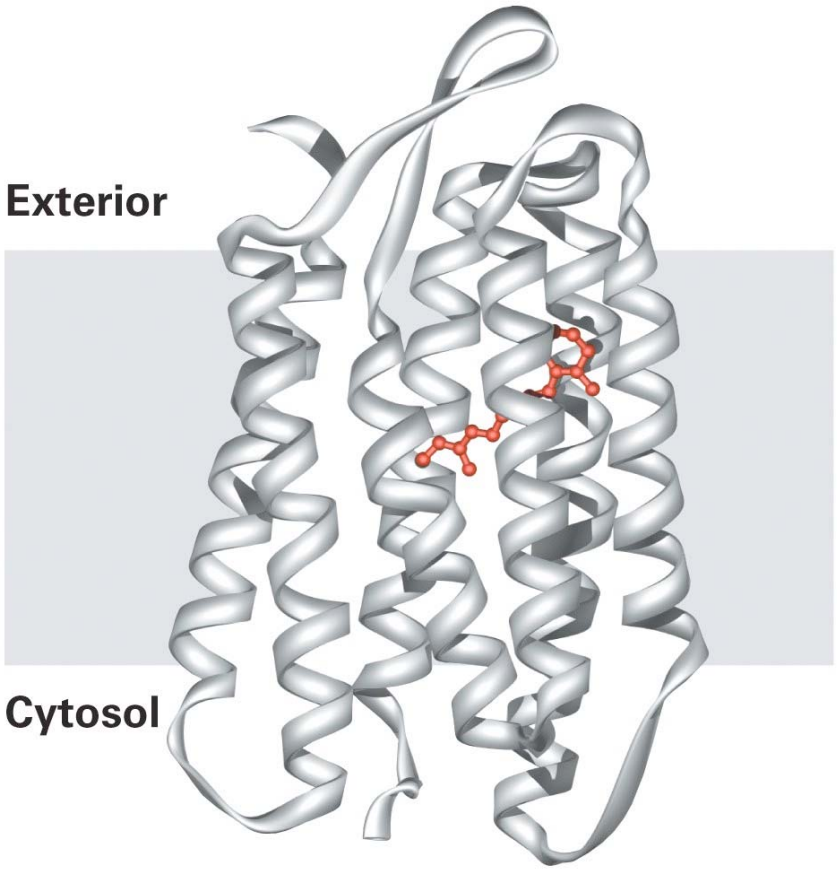
- X-ray and Electron Crystallographies

Because of the difficulty of producing and solubilizing integral membrane proteins, only the structures of a few have been obtained to date by X-ray crystallography. The first structure was that of the bacterial photosynthetic reaction center, which resulted in the Nobel price for J. Deisenhofer. This complex of 4 proteins carries out the primary photochemical process of photosynthesis in purple bacteria. The transmembrane section is formed by the H, M and L subunits (purple, blue and orange), with 11 transmembrane helices. The chromophores are shown in yellow. The green part is on the external side of the membrane.

Recently a number of ion channel structures have also been obtained by this method.

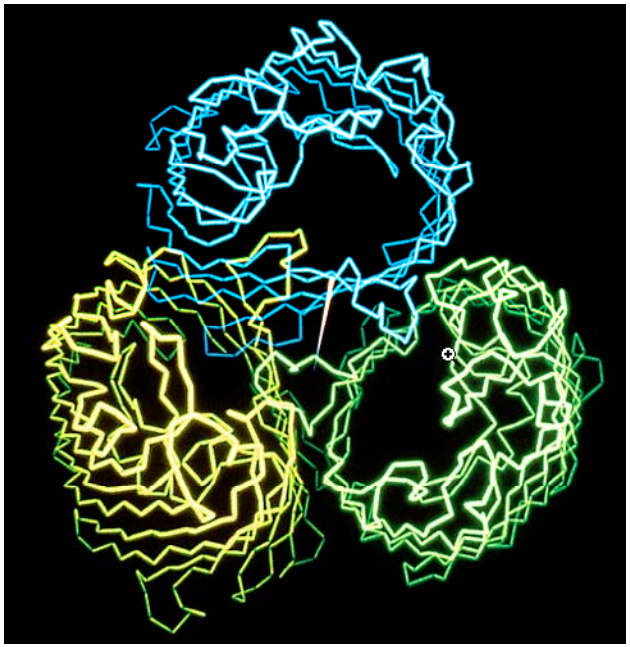


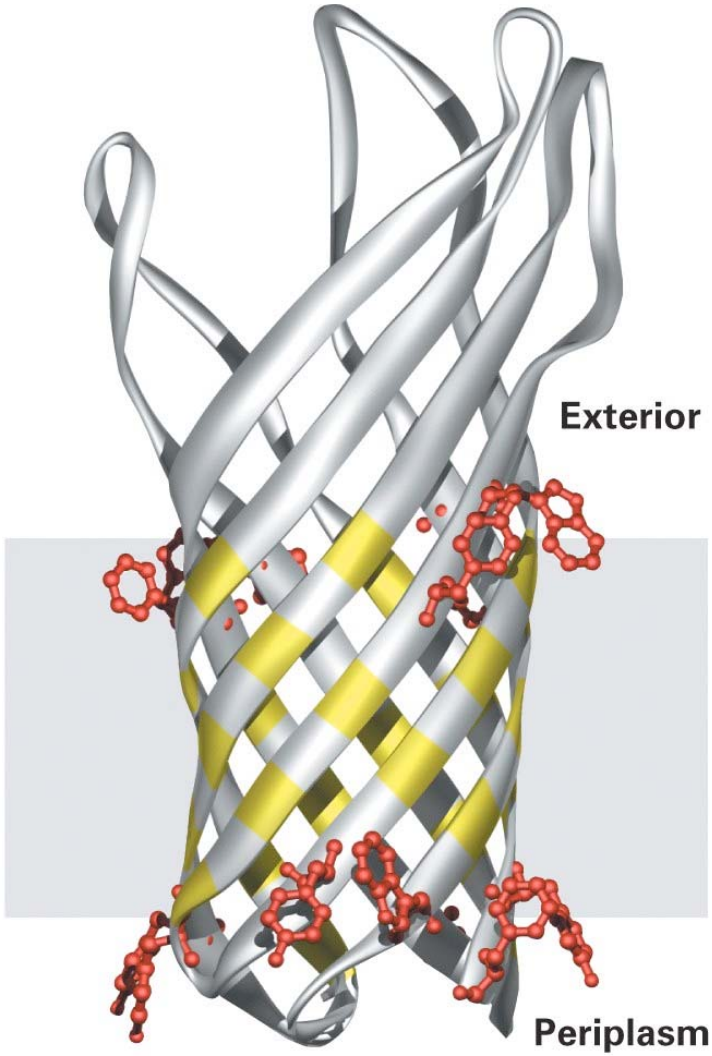
An alternative is **electron crystallography**, where crystals are 2-dimensional and are studied in an electron microscope. The advantage is the possibility of studying naturally occurring 2-D crystalline patches of membrane proteins as they occur in the cell (e.g. bacteriorhodopsin, gap junctions) or to form 2-D crystals in vitro by reconstitution of solubilized proteins into lipid bilayers. The first structure solved by this method was that of bacteriorhodopsin (bR) by R. Henderson. This proton pump is formed by 7 transmembrane helices and a covalently bound retinal chromophore. Light changes the structure of the retinal, which in term changes that of the protein resulting in the movement of one proton from the inside to the outside of the cell.



• **Transmembrane Domains**

The most common motive for transmembrane segments in an integral membrane protein is an alpha helix or a bundle of helices. An alternative is the beta-barrel structure observed in bacterial porins. These proteins exist in the outer membrane of gram-negative bacteria and in mitochondria. They are involved in the passive transport of small molecules across the membrane. They exist as trimers, each of the monomers with a central hydrophilic channel.

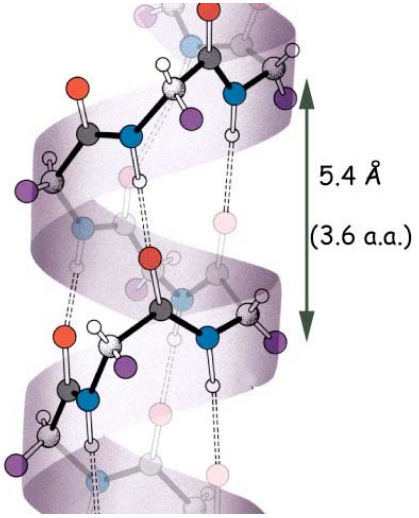


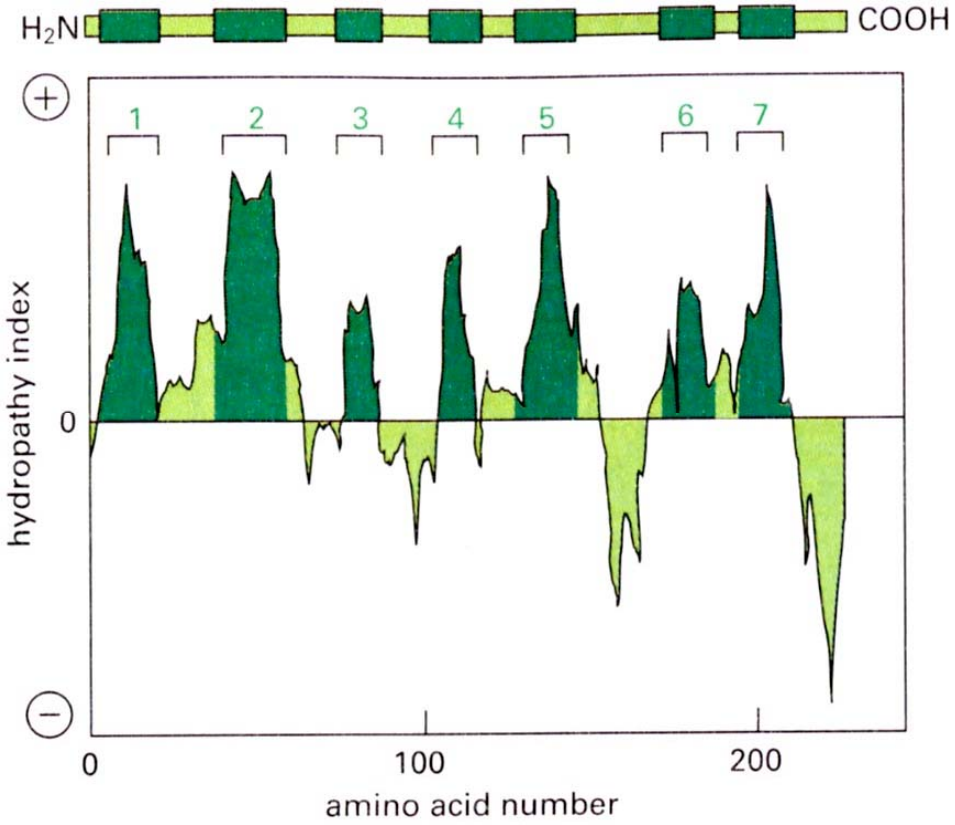


- Hydropathy Plots

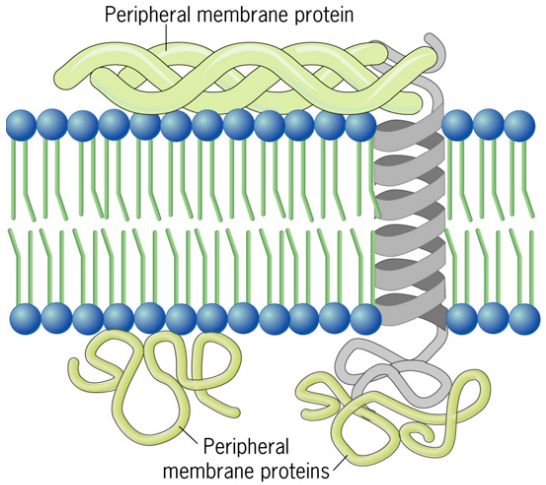
  In many cases the structure of membrane proteins cannot be obtained, but their integral character and the number of helical transmembrane segments can be predicted by analysis the amino acid sequence using hydropathy plots. These plots give an estimation of the local hydrophobicity for each residue in the structure. Hydrophobic stretches of 20-30 amino acids are a good indication of a transmembrane region.

The pitch of an alpha helix is 5.4 Å and involves 3.6 residues. Thus, the rise per residue is 1.5 Å. Given that the hydrophobic section of a lipid bilayer is about 30 Å, it takes about 20 residues to go across it, more if the helix is not perpendicular to the bilayer.





III Peripheral Membrane Proteins

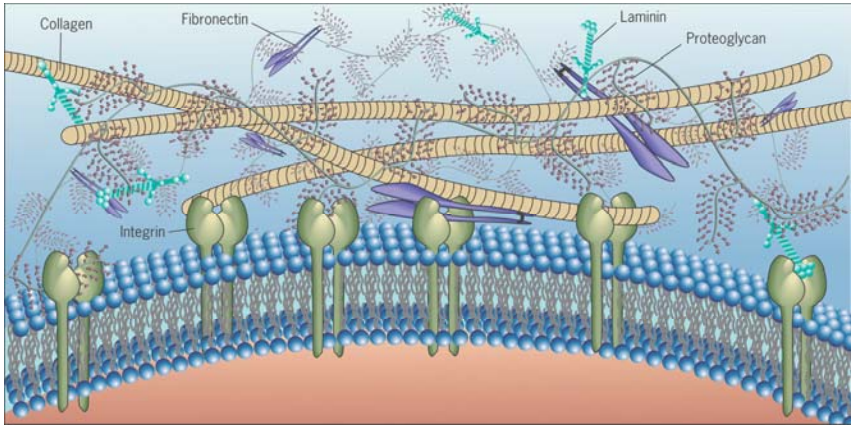


1 - Peripheral membrane proteins are located outside of the membrane bilayer, either on the citosolic or extracellular side, and are associated to the membrane surface by non-covalent bonds.

2 – They can be extracted by treatment of the membrane with high salt or alkaline pH.

3 – Inside the cell peripheral membrane protein function include: linking the membrane to th e underlying cellular cytoskeleton (permanent attachment to the membrane); serving as signal transduction elements (the protein comes on and off the membrane).

4 – Peripheral proteins associated to the external side of the plasma membrane are typically associated with the extracellular matrix (ECM). They are mostly fibrous proteins that confer mechanical properties to tissues.

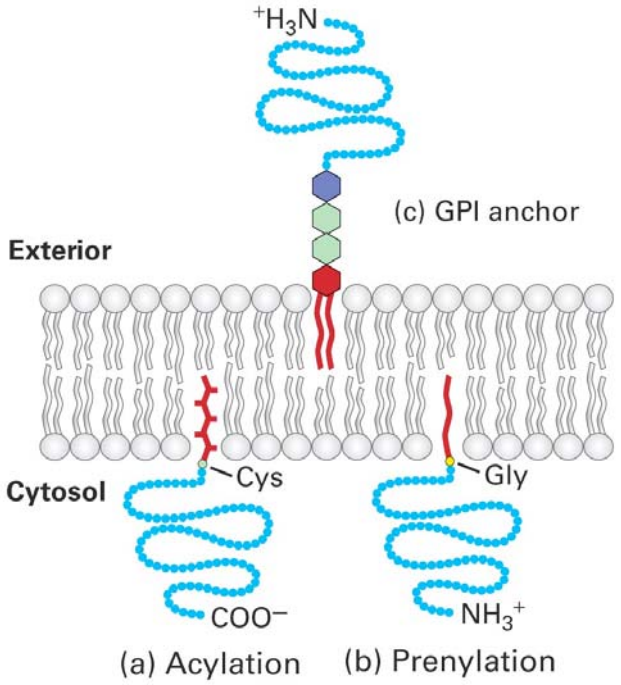


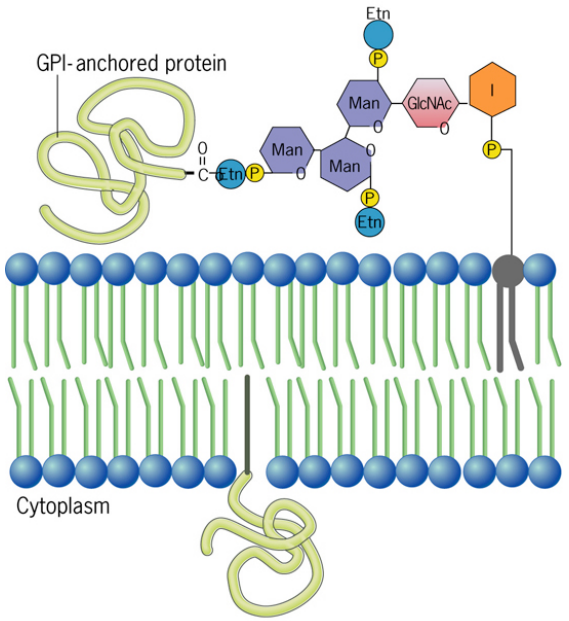
IV Lipid-anchored proteins

1 – Proteins on the outside surface are covalently linked to the membrane by a short oligosaccharide linked to a molecule of glycophosphatidylinositol (GPI) that is embedded in the outer leaflet. They are released by a phospholipase specific for inositol-containing phospholipids.

One of such proteins is responsible for sleeping sickness. The tsetse flies carry an extracellular protozoan parasite that survives in the blood by virtue of a dense cell surface coat made of a GPI anchored glycoprotein. The parasites have several hundred variants of these glycoproteins so that they evade the host immune system by antigenic variation.

2 – In the inside of the plasma membrane some proteins are linked to the membrane by a long hydrocarbon chain embedded in the inner leaflet. Examples include oncogenes such as Src and Ras involved in cell proliferation signaling.





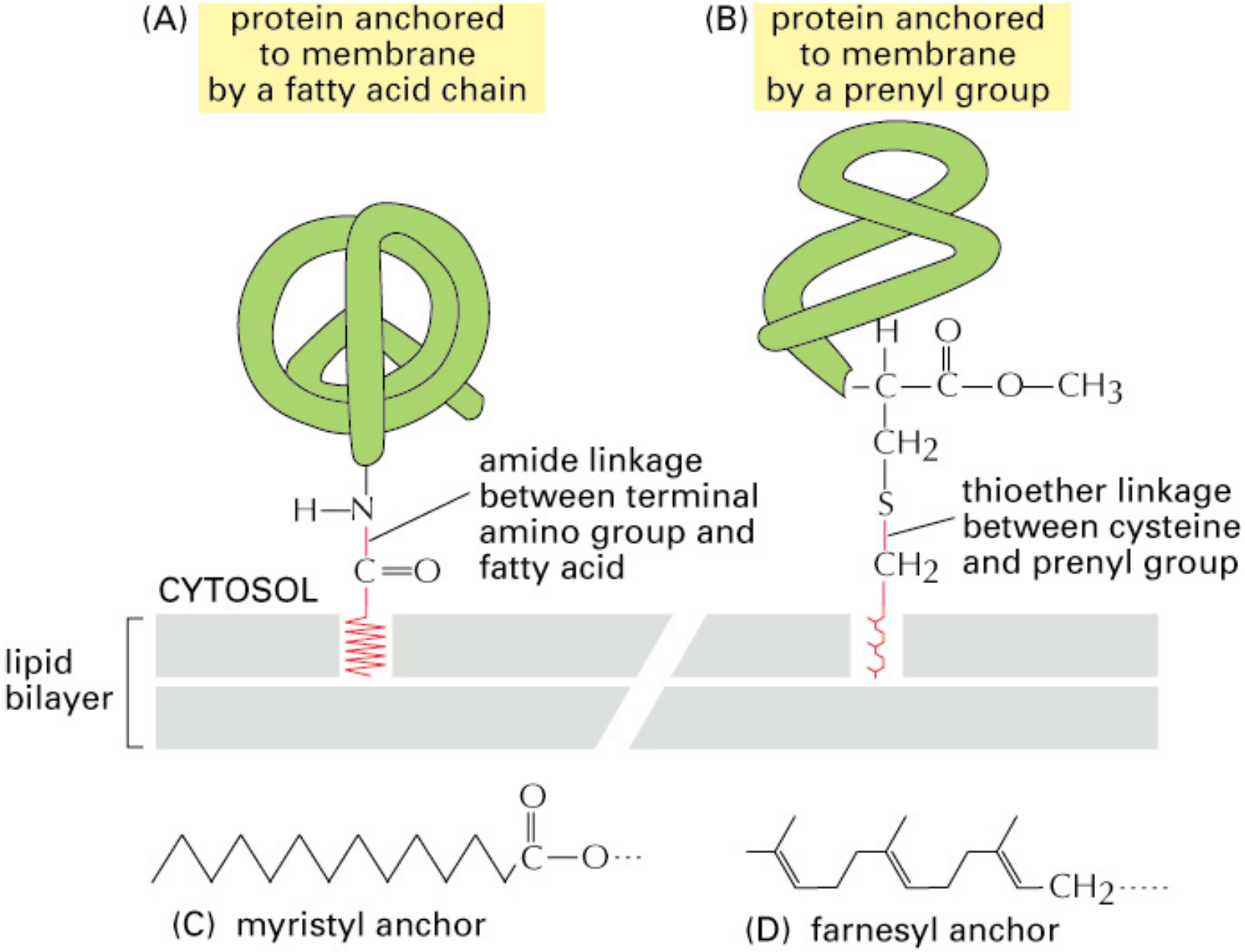
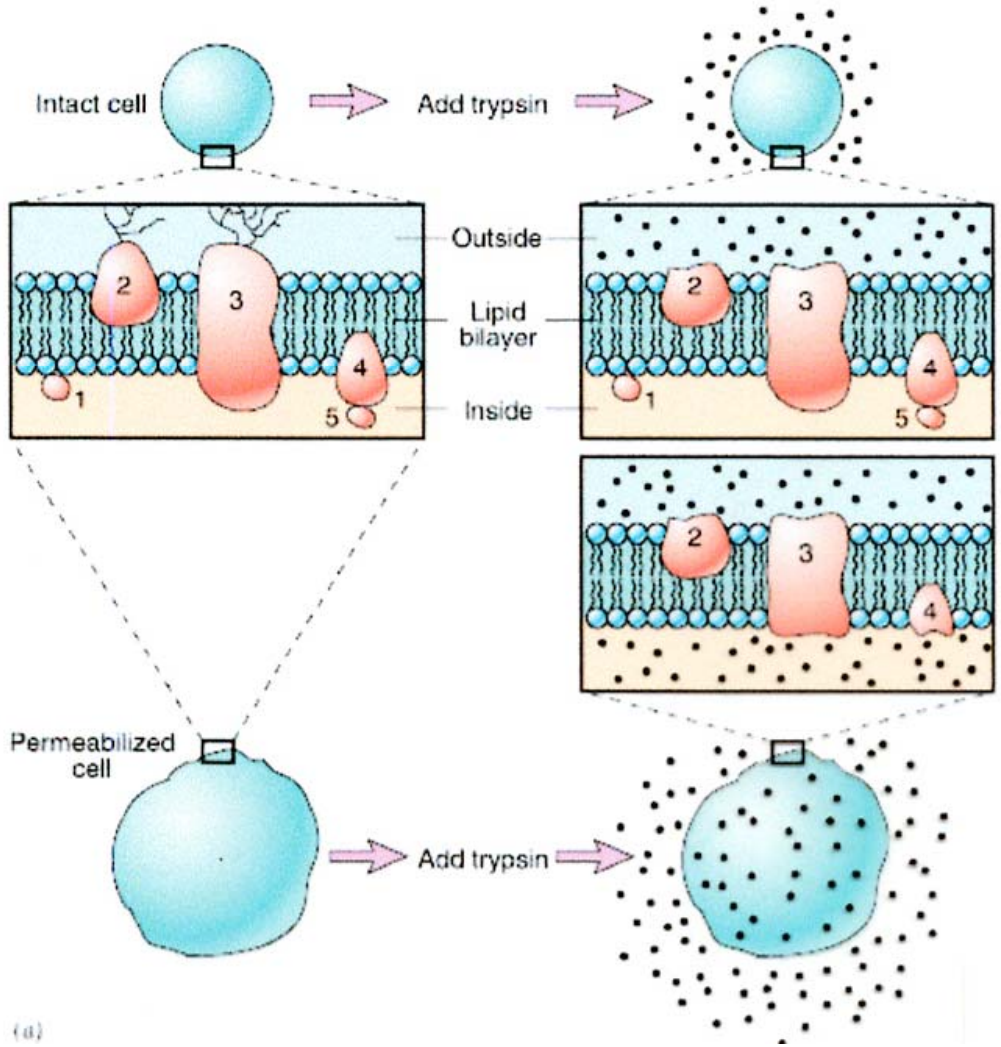
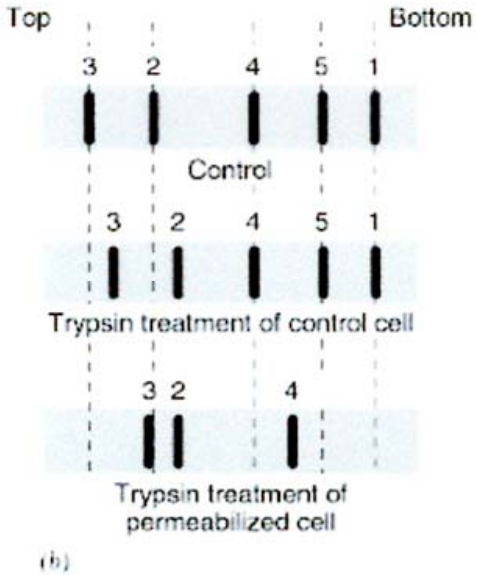


Figure 10–18. Molecular Biology of the Cell, 4th Edition.

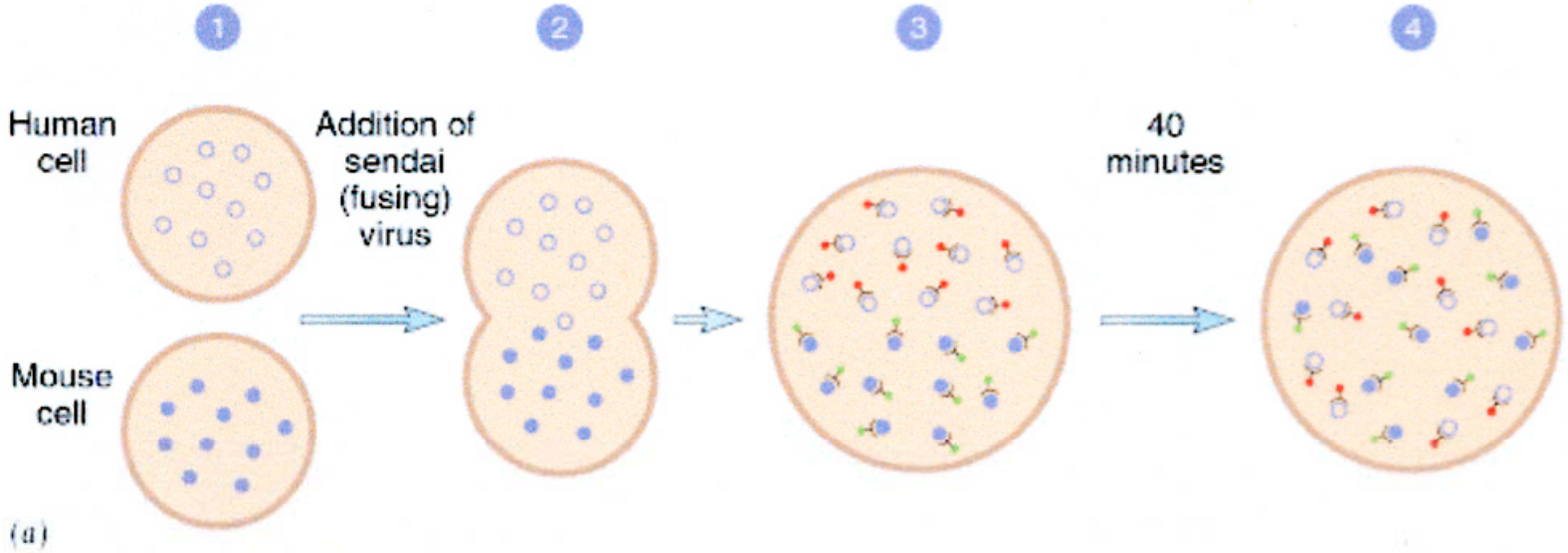
Proteins are assymetrically oriented in membranes, with one side of the protein oriented towards the cytosolic and the other towards the exoplasmic sides. In the plasma membrane the parts of the proteins that protrude one way or the other can be experimentally determined by proteolysis of intact and permeabilized cells.





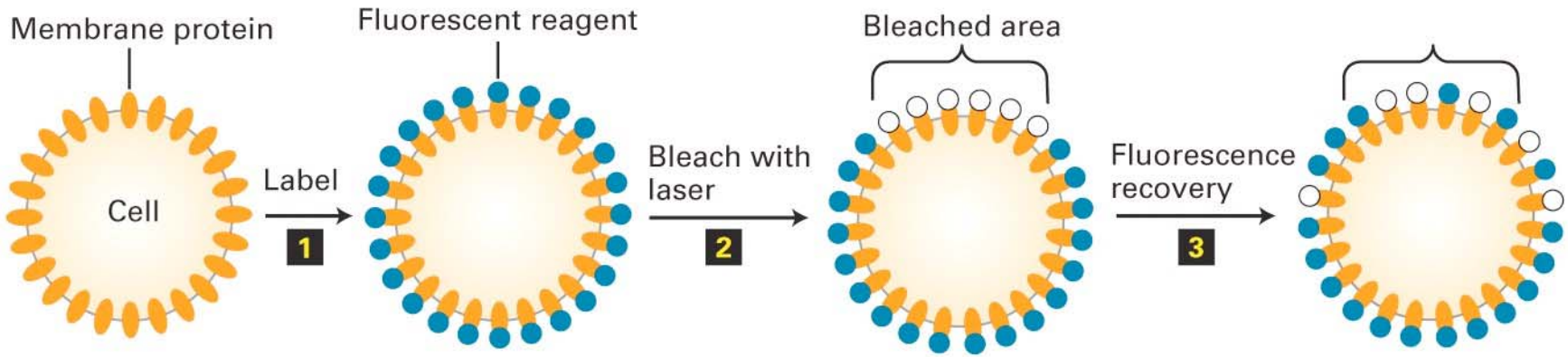
V DIFFUSION OF MEMBRANE PROTEINS

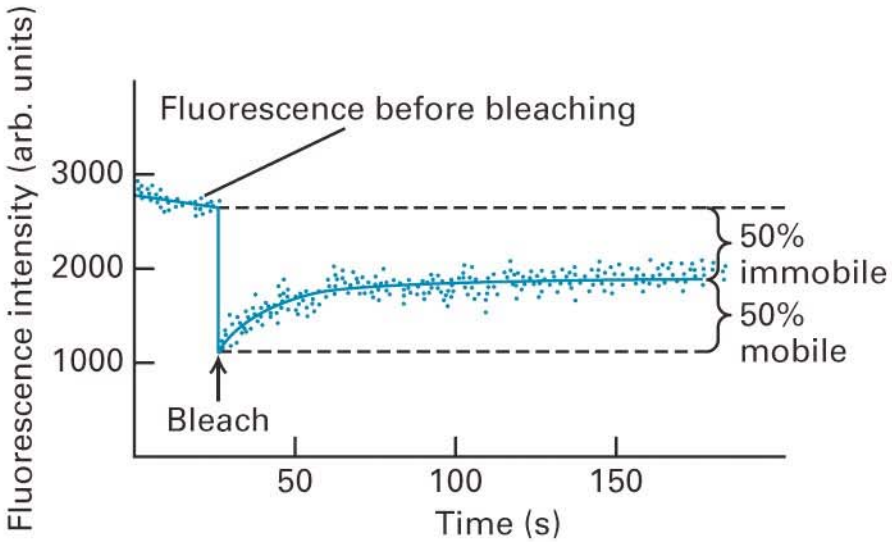
V A - Cell fusion experiments

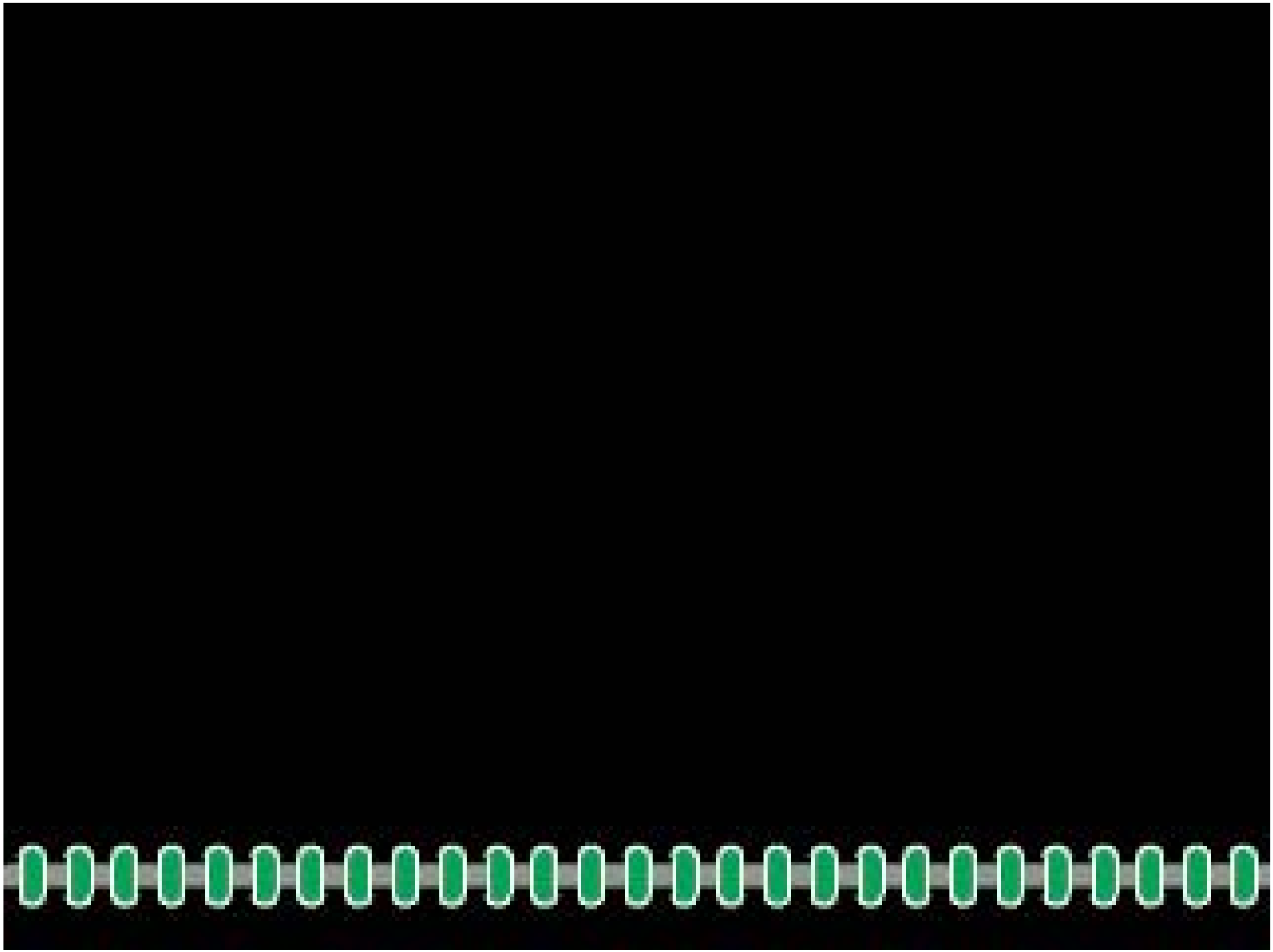


(a)

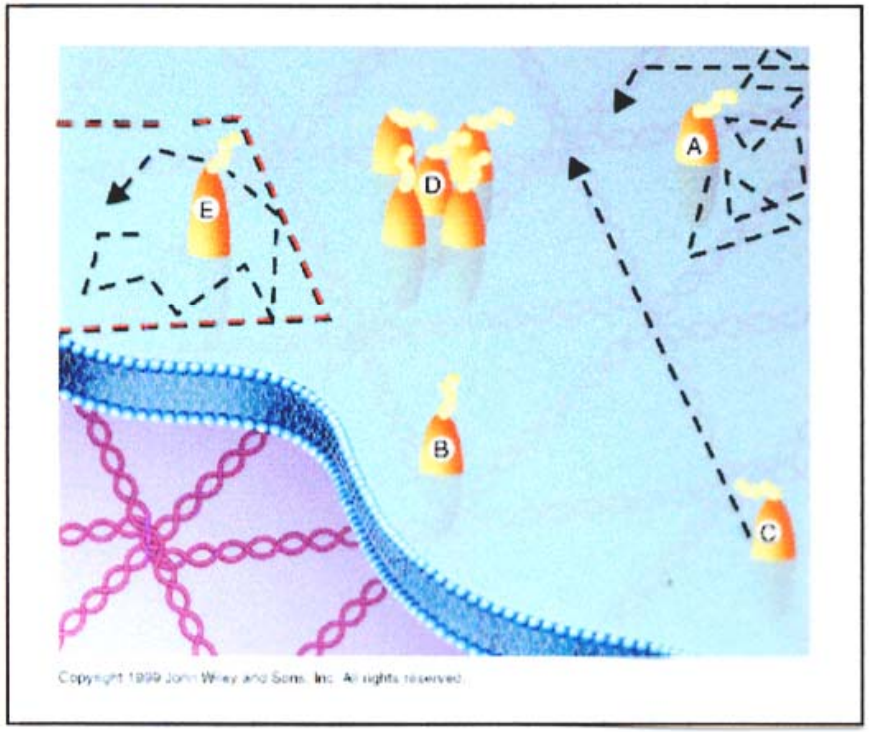
V B - FRAP


Membrane protein
Fluorescent reagent
Bleached area
Cell
Label
1
Bleach with laser
2
Fluorescence recovery
3


Fluorescence intensity (arb. units)
Fluorescence before bleaching
3000
2000
1000
50% immobile
50% mobile
Bleach
50
100
150
Time (s)



V C - SPT (Single-Particle Tracking)



Gold-labeled antibodies and enhanced video microscopy

Different behaviors