

Lecture 4 Membrane structure

Outline

- I. Overview of membrane
- II. The lipid bilayer
- III. Lipid assembly and Detergents
- IV. Membrane proteins

I. Overview of cell membrane

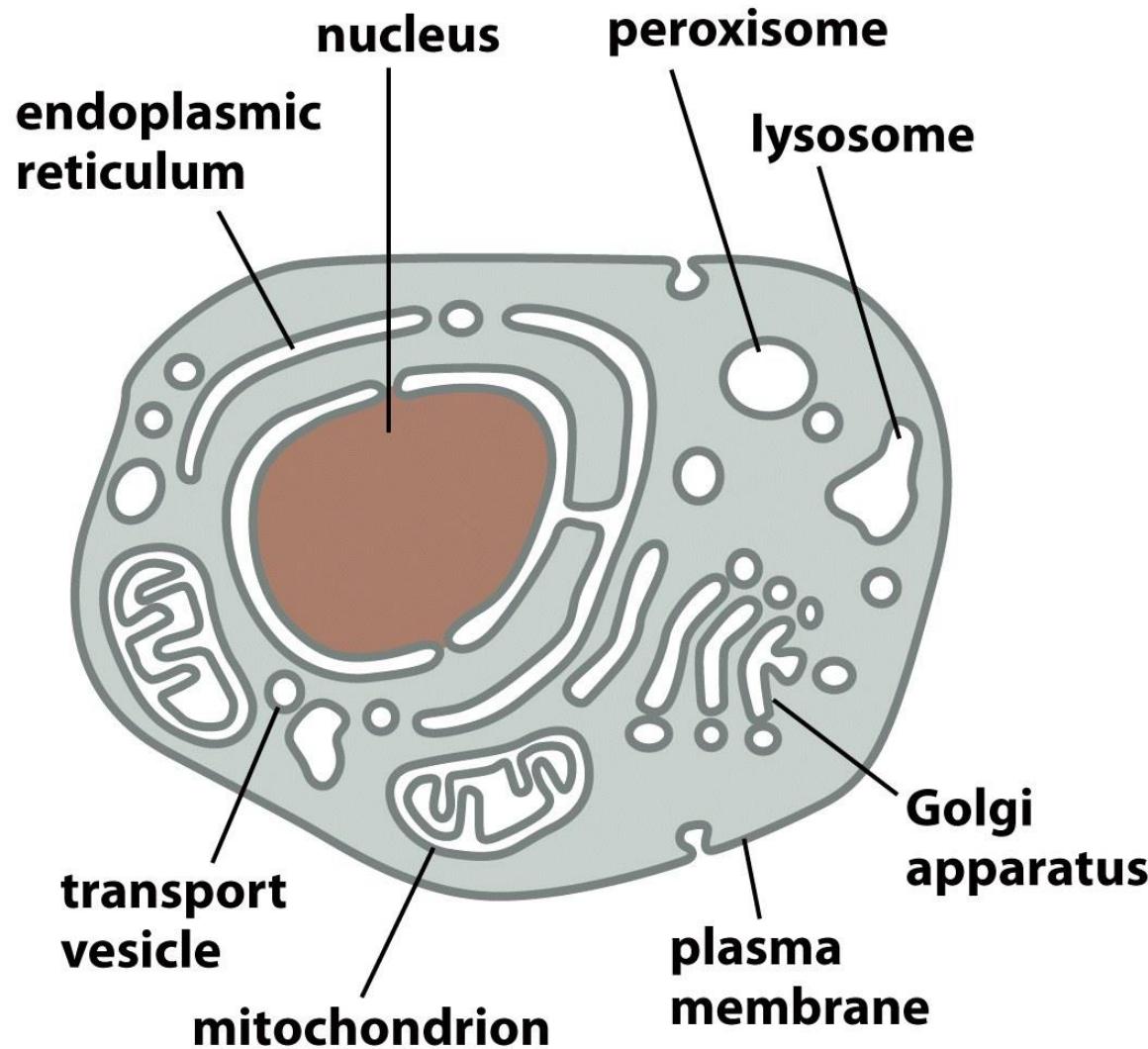


Figure 11-3 Essential Cell Biology 3/e (© Garland Science 2010)

Lipid bilayer is about 5nm thick

membrane that surrounds **cells** and
membranes that surround **organelles** within the cell

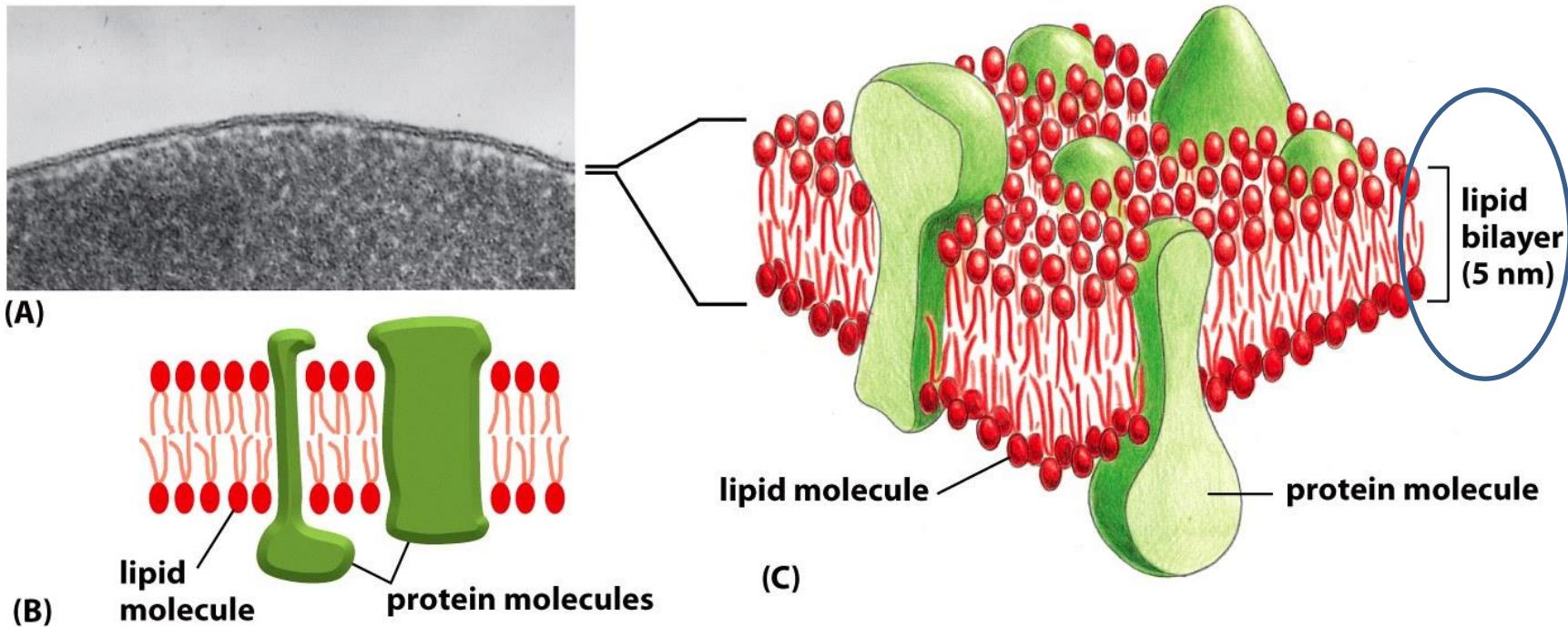


Figure 11-4 Essential Cell Biology 3/e (© Garland Science 2010)

- A: electron microscopic image of a red blood cell membrane
- B: 2-D image of membrane
- C: 3-D image of membrane

Subcellular organelles with membrane

- Nucleus (double membrane)
- Mitochondria (double membrane)
- Chloroplast (double membrane)
- Endoplasmic reticulum (single membrane)
- Golgi apparatus (single membrane)
- Peroxisome, lysosomes, endosomes, vesicles, (single membrane)

Cell Membranes act as selective barriers

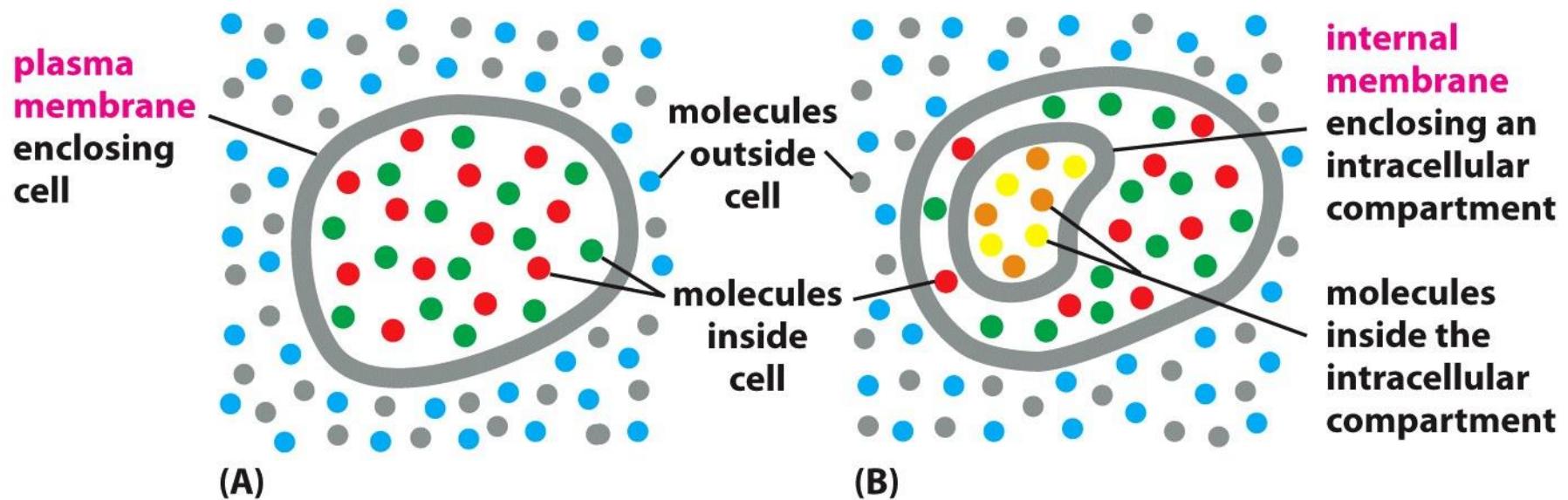
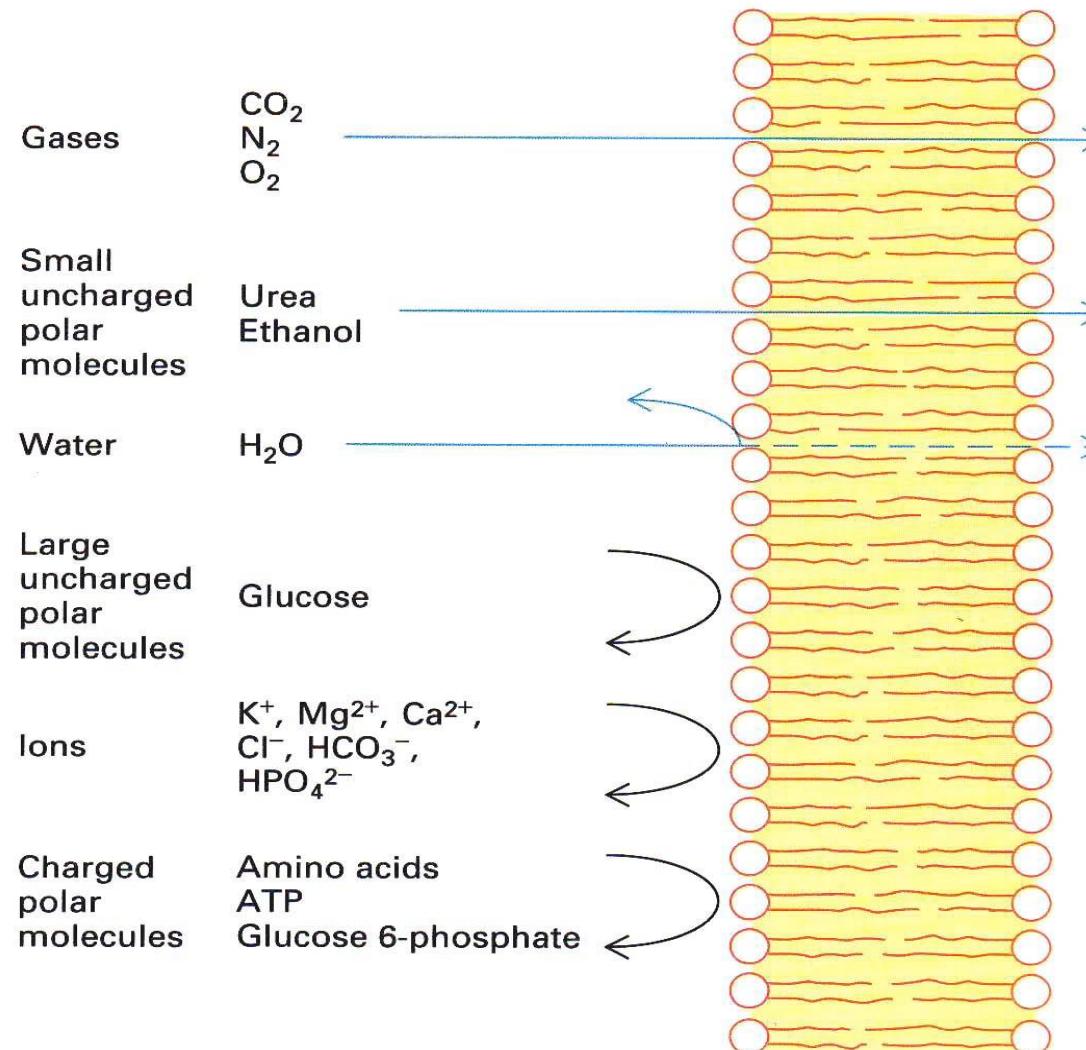


Figure 11-1 Essential Cell Biology 3/e (© Garland Science 2010)

Permeability of membrane



General functions for plasma membrane

- Relatively impermeable barrier
- Fluid, dynamic
- Composed of protein and lipid

Functions of plasma membrane include:

1. Compartmentalization (**relatively impermeable barrier**)
2. Scaffolding (**transmembrane protein connect extracellular matrix or adjacent cells to cytoskeleton**)
3. Gatekeeper (**selectively let some materials in and secret others out**)
4. Sensors of outside signals (**receptors on the membrane signal to other proteins inside the cell**)
5. Energy transduction (**establish ion gradients to drive ATP synthesis , or produce and transmit electric signals**)

Surface of membrane is not smooth

- Various lipids, detergents, metals, and proteins can induce membrane curvature

II. Lipid bilayer

1. Lipid composition overview
2. Phospholipids
3. Sterol
4. Glycolipids
5. Asymmetry of lipids
6. Lipid raft (domains)
7. Motions of lipid molecules
8. Phase transition
9. Lipid storage in cells

1. Lipid bilayer

- Phospholipids (**main ones**), sterols and glycolipids are major constituents for plasma membrane

Table 10–1 Approximate Lipid Compositions of Different Cell Membranes

| LIPID | PERCENTAGE OF TOTAL LIPID BY WEIGHT | | | | | | E. COLI BACTERIUM |
|--------------------------|-------------------------------------|--------------------------------------|--------|---|--------------------------|-------|----------------------|
| | LIVER CELL PLASMA MEMBRANE | RED BLOOD CELL PLASMA MEMBRANE | MYELIN | MITOCHONDRION (INNER AND OUTER MEMBRANES) | ENDOPLASMIC RETICULUM | | |
| Cholesterol | 17 | 23 | 22 | 3 | 6 | 0 | Phospholipids |
| Phosphatidylethanolamine | 7 | 18 | 15 | 28 | 17 | 70 | |
| Phosphatidylserine | 4 | 7 | 9 | 2 | 5 | trace | |
| Phosphatidylcholine | 24 | 17 | 10 | 44 | 40 | 0 | |
| Sphingomyelin | 19 | 18 | 8 | 0 | 5 | 0 | |
| Glycolipids | 7 | 3 | 28 | trace | trace | 0 | |
| Others | 22 | 13 | 8 | 23 | 27 | 30 | |

Eukaryotic cells contain various combination of lipids, ~500-1000 different lipid species, but prokaryotic cells contain much less.

Amphipathic molecules

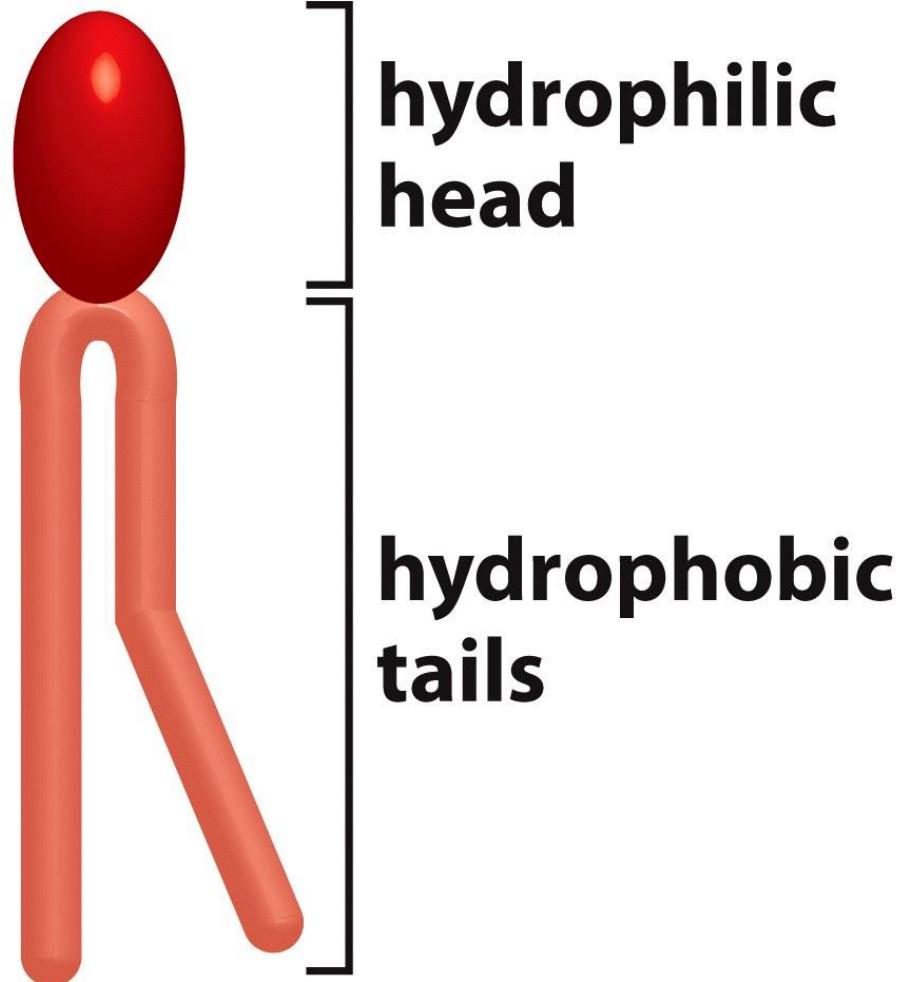
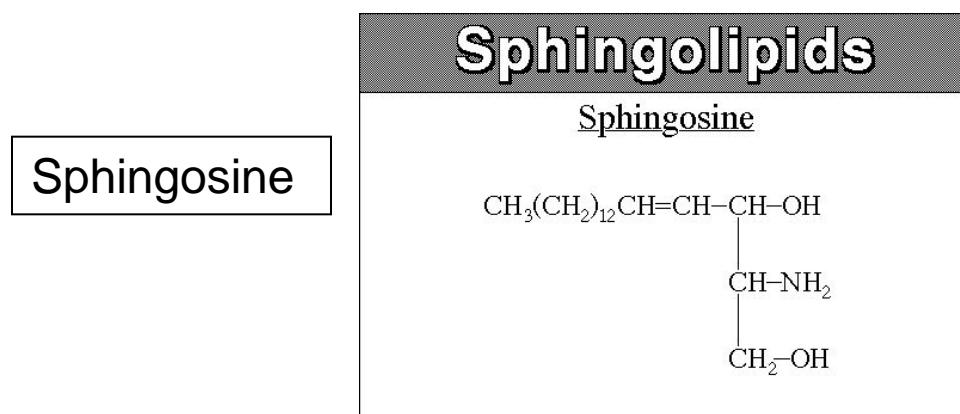
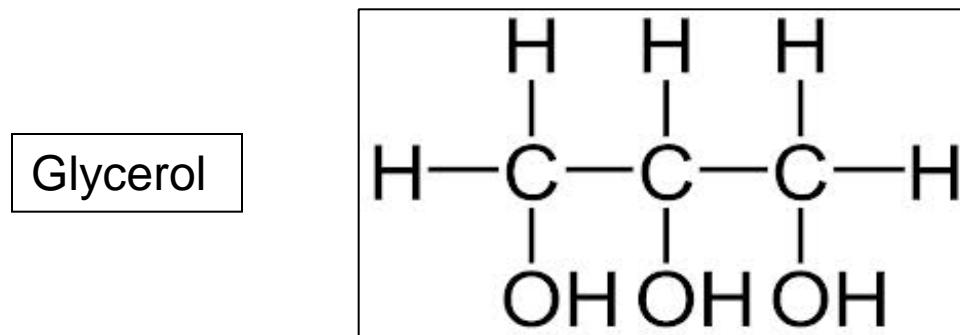


Figure 11-5 Essential Cell Biology 3/e (© Garland Science 2010)

2. Phospholipids

Amphipathic: a hydrophilic head and a hydrophobic tail

- 1. Phosphoglycerides (glycerol as backbone) **main ones**
- 2. Sphingomyelin (sphingosine as backbone)



Phosphoglyceride molecule

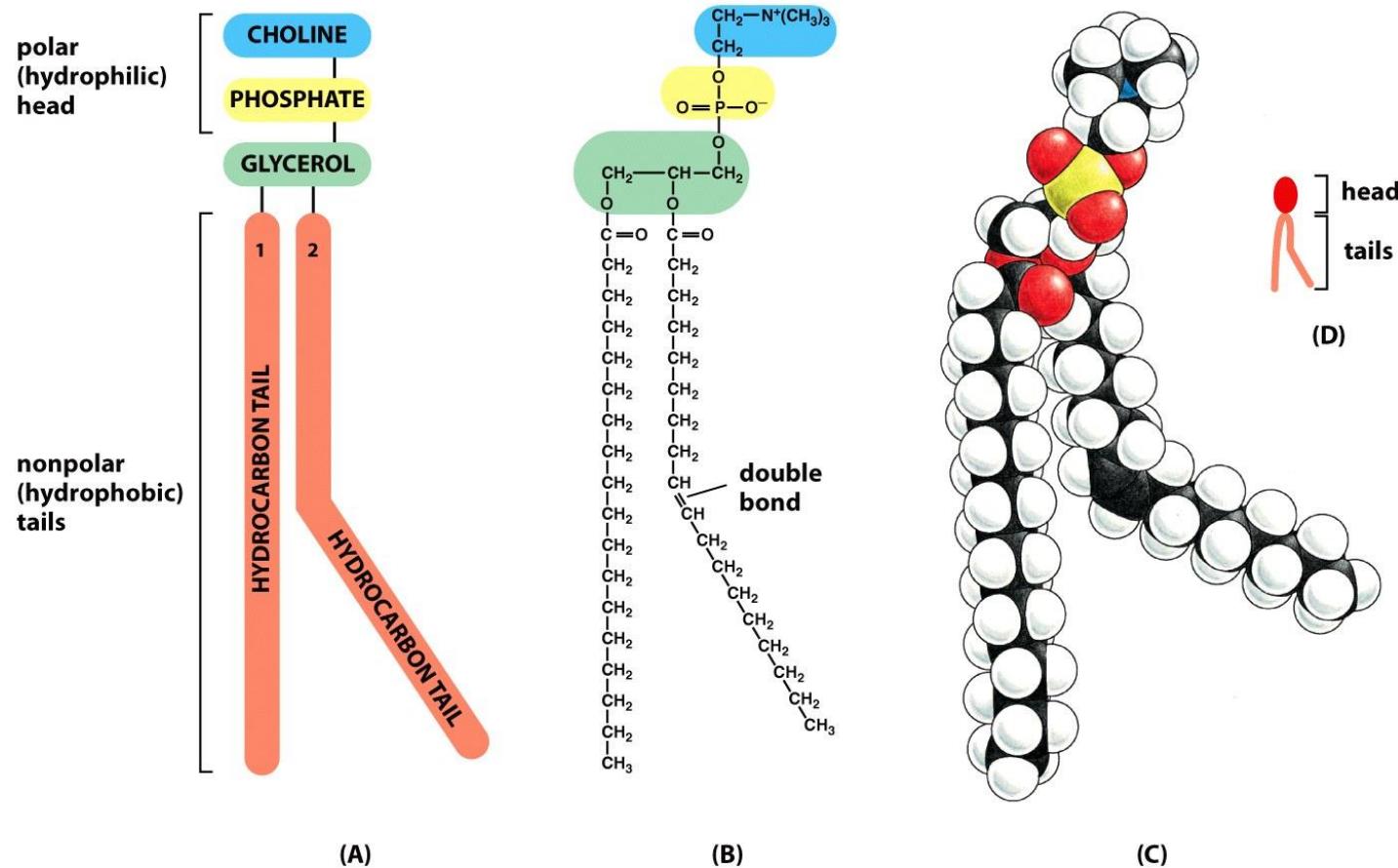
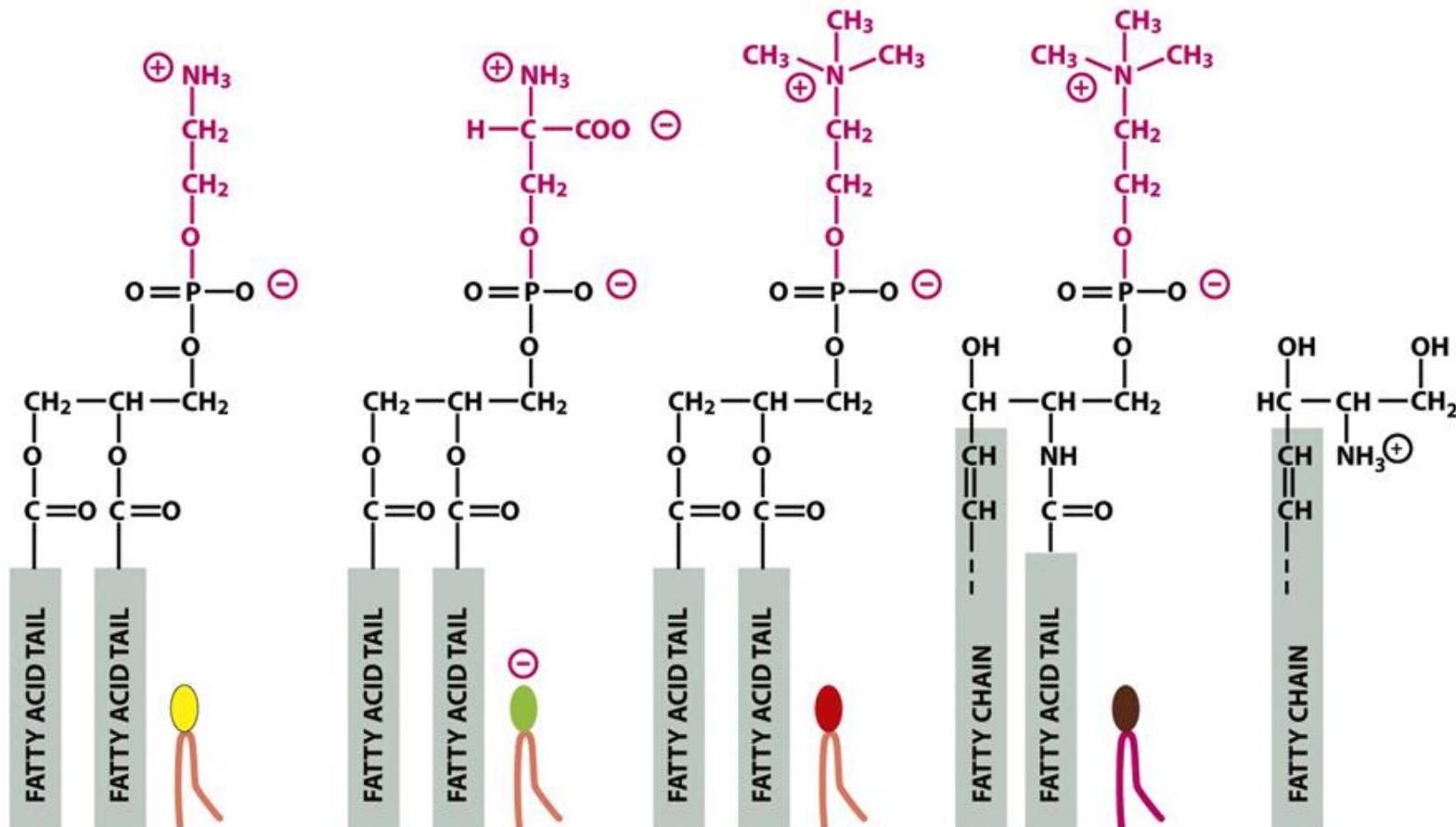


Figure 11-6 Essential Cell Biology 3/e (© Garland Science 2010)

- (1). Two fatty acids linked by ester bonds with glycerol, differ in length 14-24 carbon atoms
- (2). Usually one fatty acid tail contains one or more cis-double bonds (unsaturated), while the other tail is saturated.
- (3). Cis-double bonds creates kinks in the tail, and make the lipid more fluid.

Four major types of phospholipids



phosphatidyl-
ethanolamine
(A)

phosphatidyl-
serine
(B)

phosphatidyl-
choline
(C)

sphingomyelin
(D)

sphingosine
(E)

Charge
state :

neutral

negative

neutral

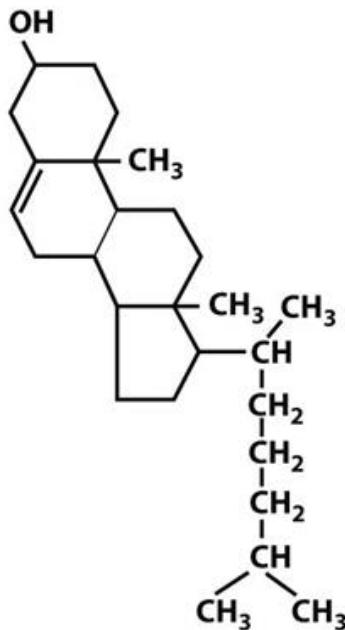
neutral

3. Sterol

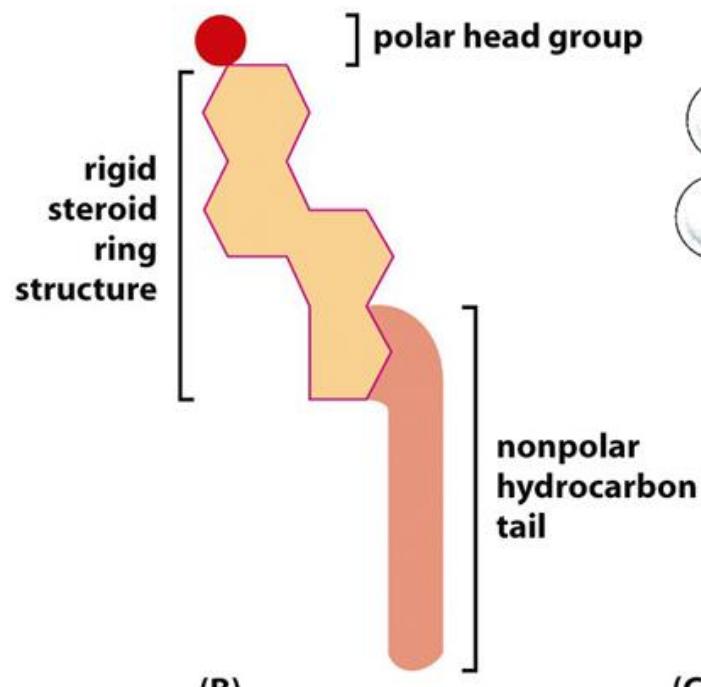
In animals: cholesterol
In plants: phytosterol
In fungi: ergosterol



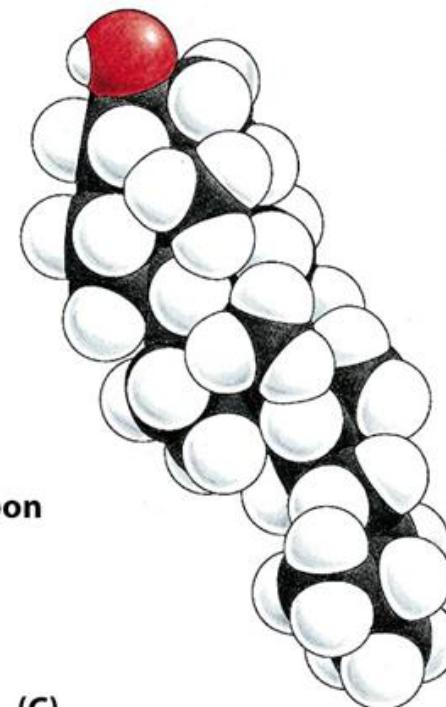
All have the similar 4-ring isoprenoid structure



(A)



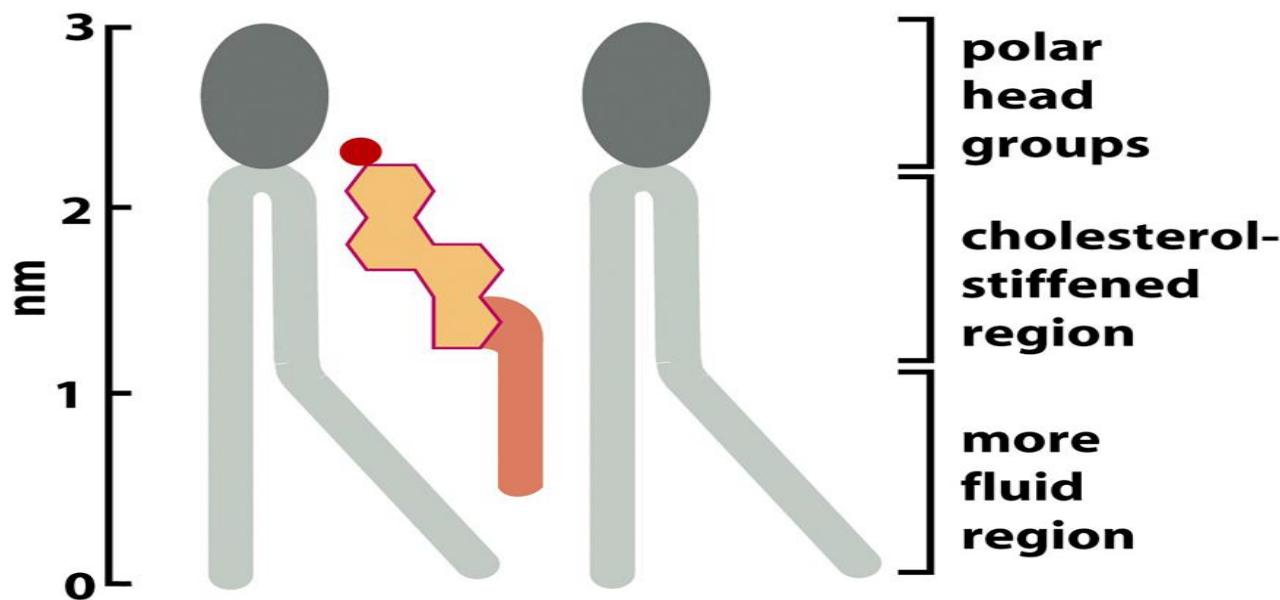
(B)



(C)

Cholesterol

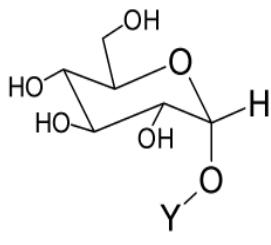
- (1) Mainly **animal** plasma membrane contains large amounts of cholesterol.
- (2) It is a rigid ring structure.
- (3) It has certain orientation: the hydroxyl group close to polar head of adjacent phospholipid molecules.
- (4) At high concentrations, its rigid ring make membrane less flexible and its long hydrocarbon tail make lipids pack more tightly, but at low concentration, it makes lipids more fluid. At higher temperature it reduces membrane fluidity, but not in lower temperature, it prevents the tightening of the membrane.



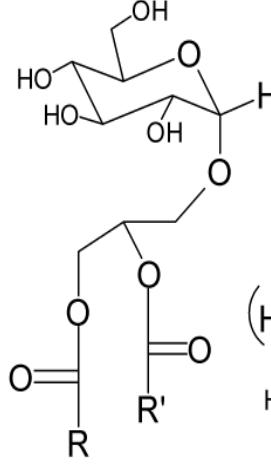
4. Glycolipids

- Relatively small amount, mostly occurs in nerve cells.
- They are on the surface of all plasma membrane
- Sugar part projects outside of membrane

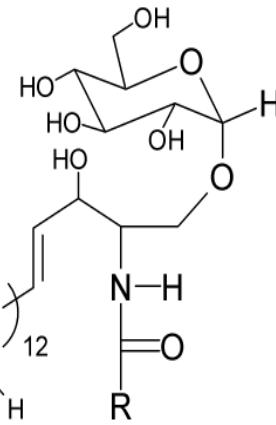
Glycolipids



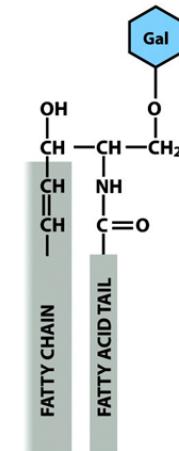
Glycero-Glycolipids



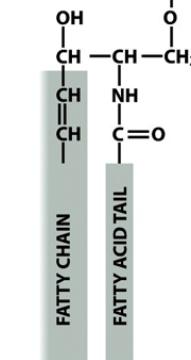
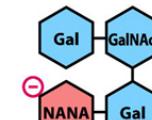
Sphingo-Glycolipids



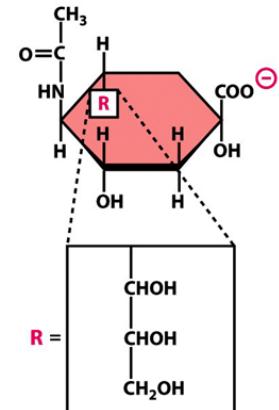
Y = Lipid



(A) galactocerebroside



(B) G_{M1} ganglioside

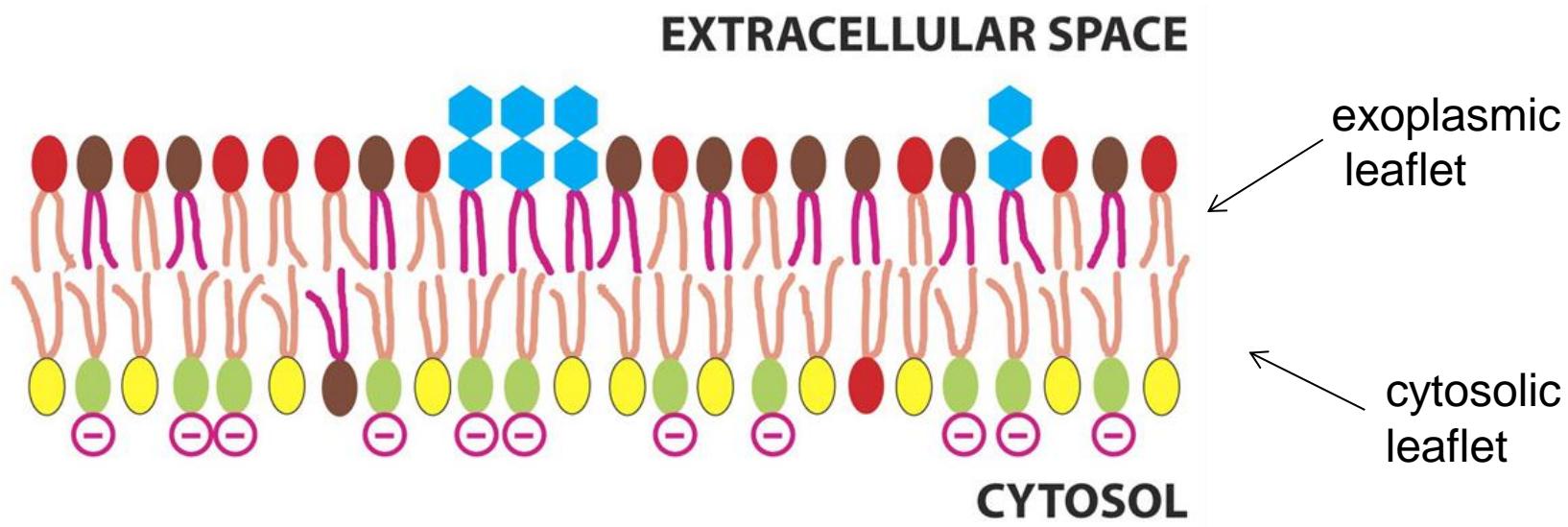


(C) sialic acid (NANA)

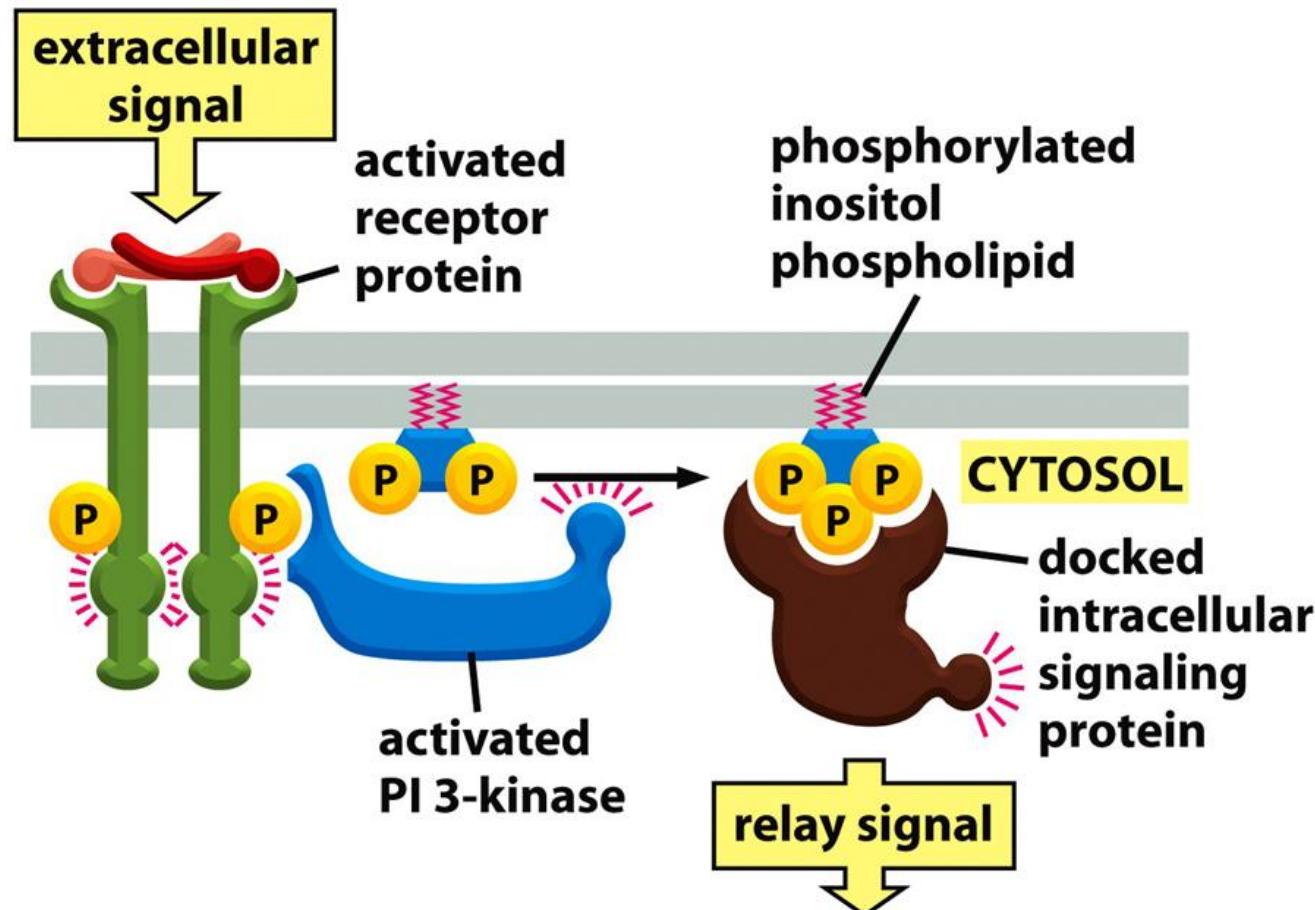
Negative charge

5. Composition for the lipids in two layers of membrane is different---Asymmetry

1. Phosphatidylserine (PS) (-): almost all cytoplasmic
2. Phosphatidylcholine (PC) (neutral): found on both sides, especially exoplasmic
3. Phosphatidylethanolamine (PE) (neutral): found on both sides
4. Phosphatidylinositol (PI) (-): almost all cytoplasmic
5. Sphingomyelin (neutral): mostly exoplasmic
6. Cholesterol equal on both leaflets
7. Glycolipids: mostly on exoplasmic

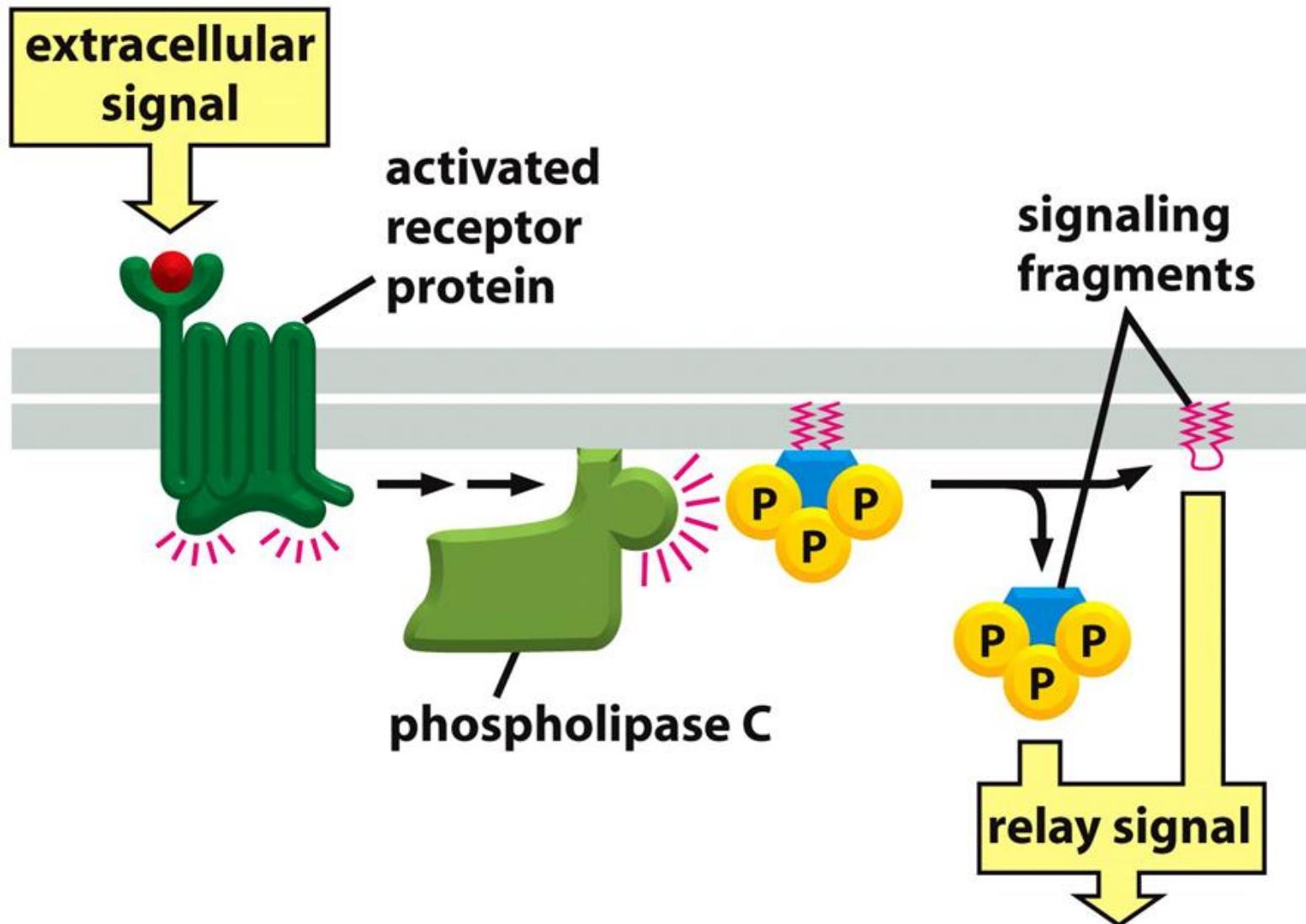


Example 1 for asymmetry: phosphatidylinositol



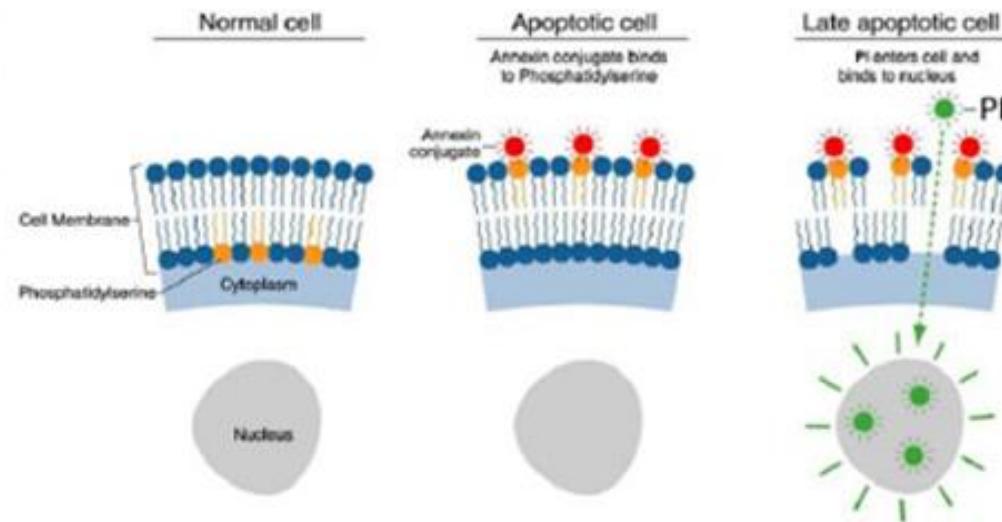
Phosphatidylinositol only locates on the cytosolic side, it acts as substrate for PI3K in signal transduction

Example 2 for asymmetry: phospholipase C



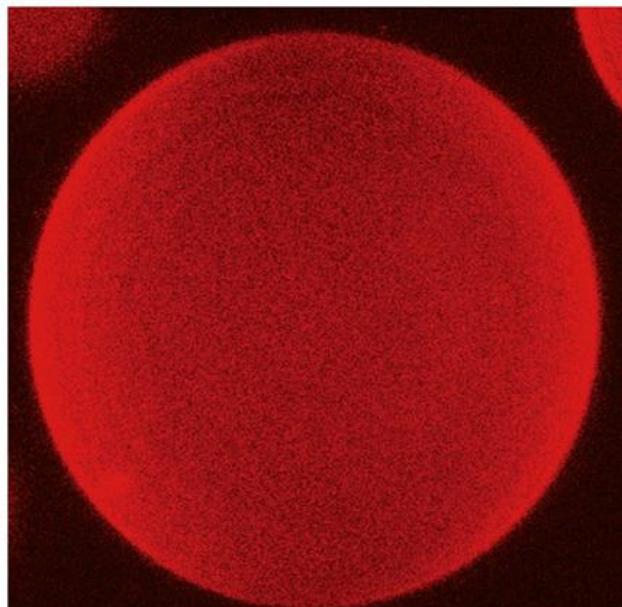
Example 3: phosphatidylserine

Phosphatidylserine locates only on the cytosolic leaflet, when cells undergo programmed cell death, it is translocated to the exoplasmic side, which can be detected by Annexin V for labeling



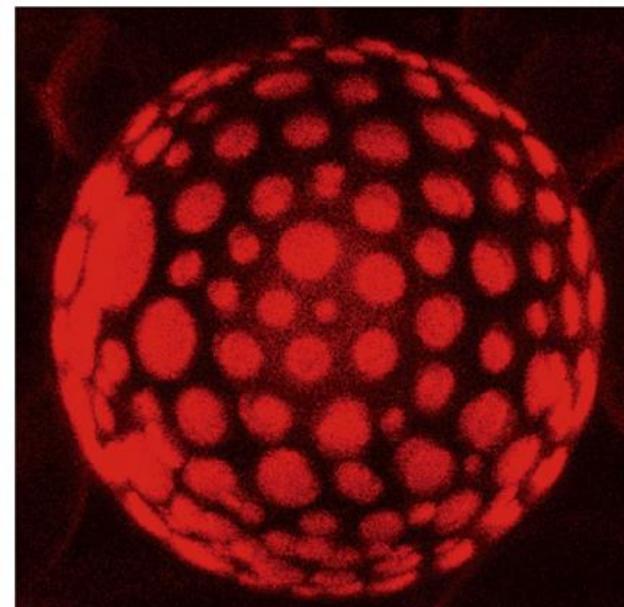
6. Domains in membrane: lipid rafts

Lipid membrane is a dynamic patchwork of different domains, called lipid rafts
Mainly constituted by mixture of sphingomyelin and cholesterol , is thought to concentrate specific membrane proteins



(A)

10 μm



(B)

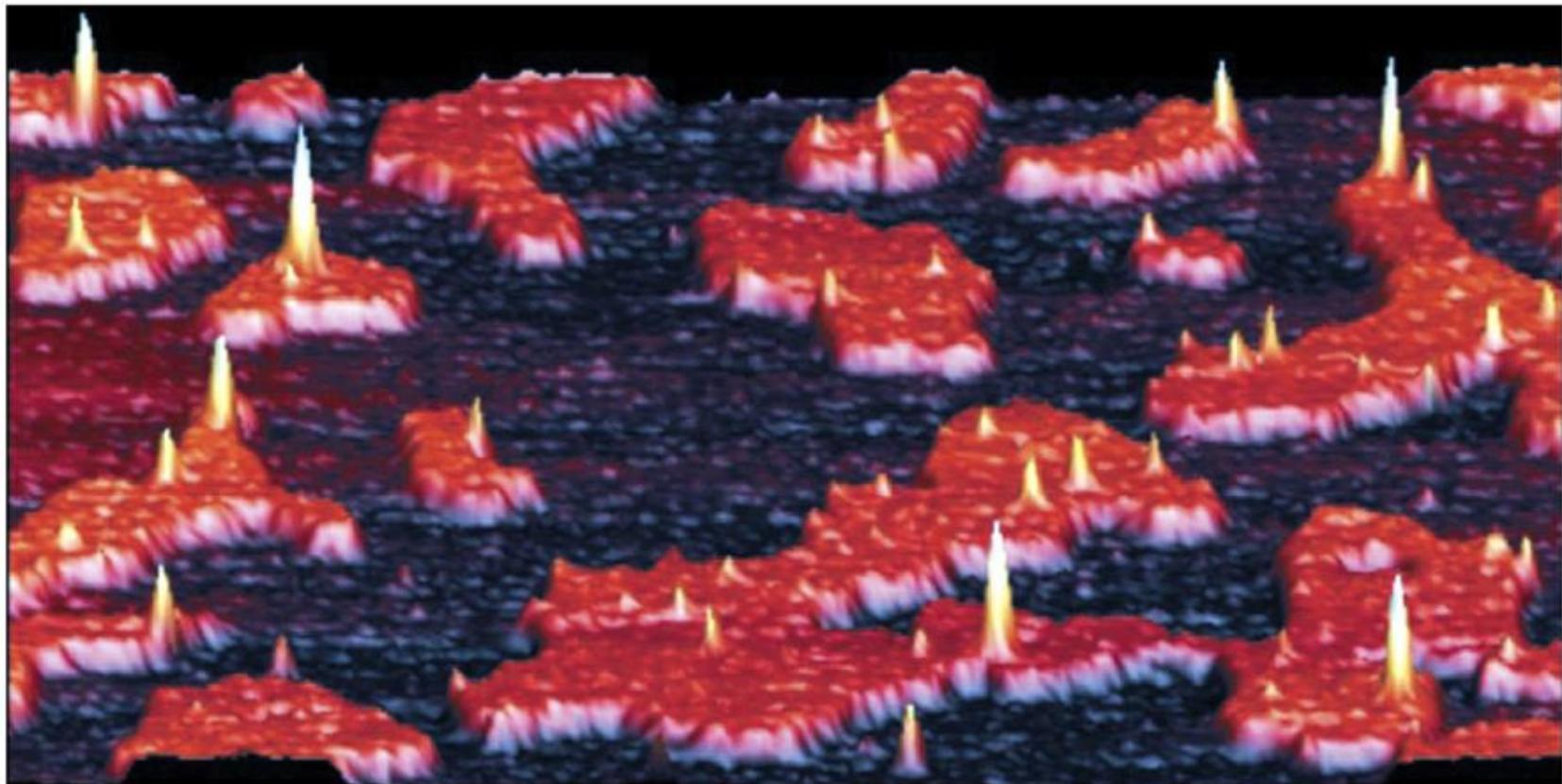
5 μm

Liposome with mixture of:

sphingomyelin: phosphatidylcholine
1: 1

cholesterol:sphingomyelin:phosphatidylcholine
1: 1 :1

Membrane protein locates in the lipid rafts



Atomic force microscopy for artificial membrane

500 nm

Yellow ---membrane proteins
Red--- lipid raft

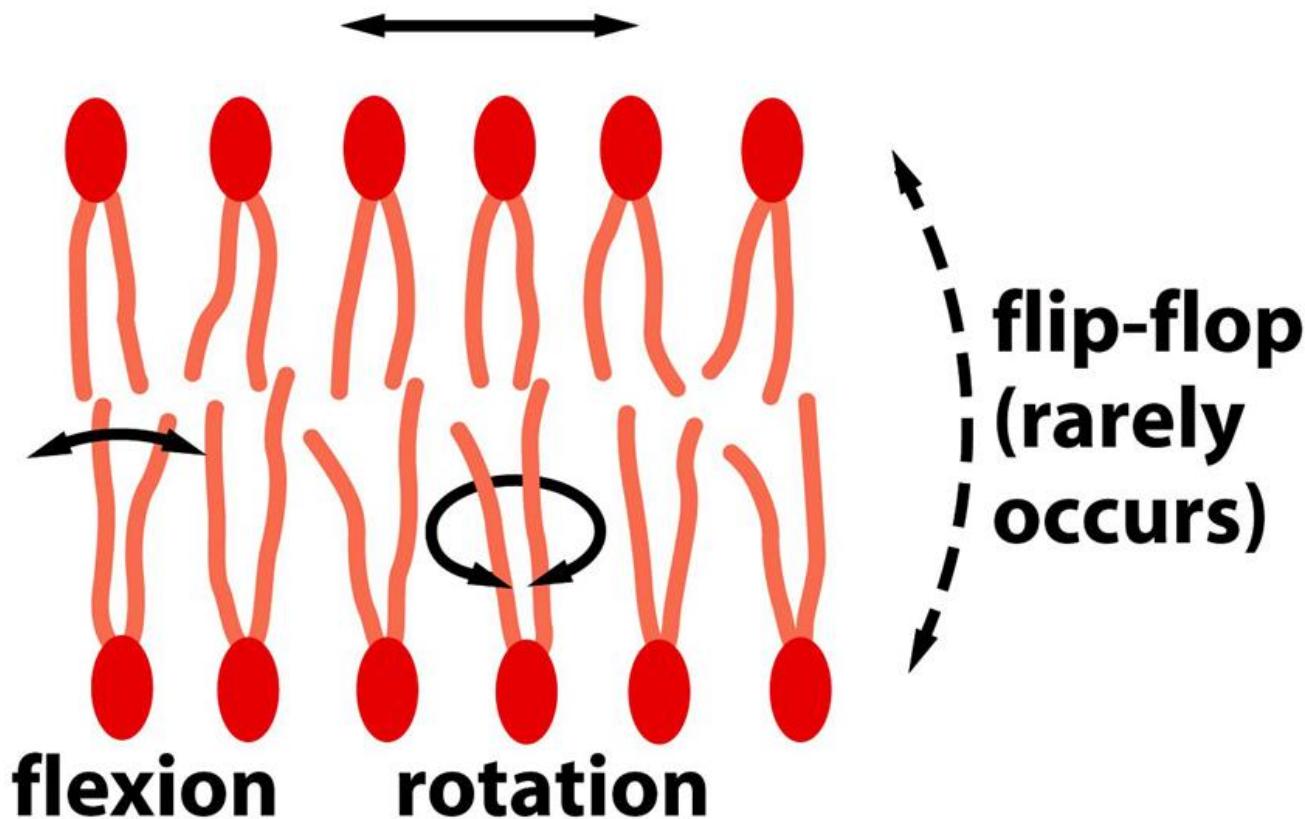
7. Motion of the lipid molecules

- Rapid change places ($\sim 10^7/\text{sec}$) laterally-by FRAP
- Rotate very rapidly along their long axis
- Hydrophobic tail is highly flexible
- Flip-flop in very rare cases (once a month)--- **flip-flop: move from one leaflet to the other.**

A special enzyme on membrane: phospholipid translocators can catalyze the rapid flip-flop

Modes of motion for lipid molecules

lateral diffusion



The two-dimensional lipid bilayer is a fluid

(A)

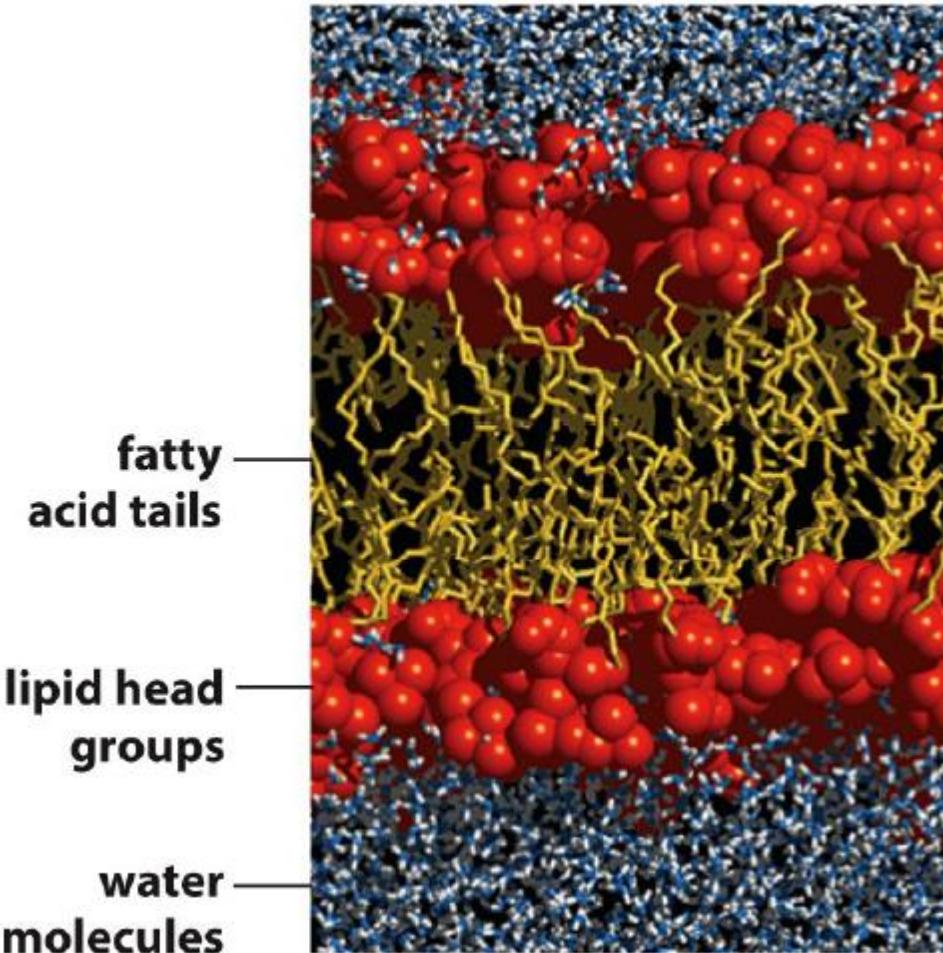


Figure 10-10 Molecular Biology of the Cell 6e (© Garland Science 2015)

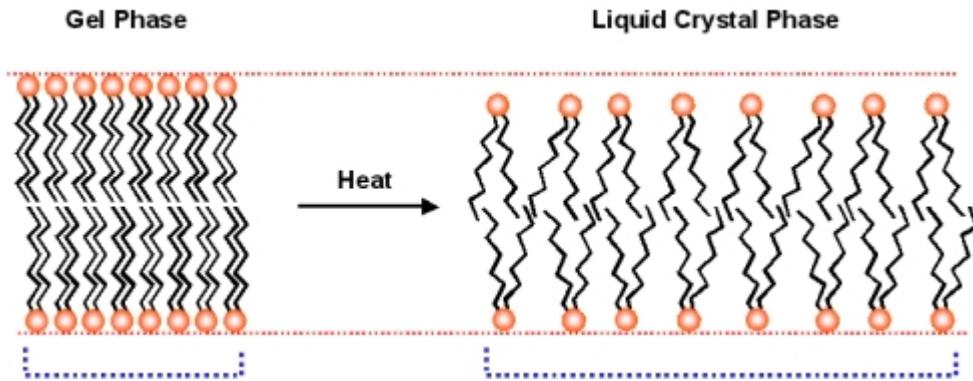
The fluidity of a lipid bilayer depends on its composition and temperature

- Short hydrocarbon chains and double bonds lower Tm.
- Lower organisms can adjust lipid composition to keep membrane fluid at different environmental temperatures.
- Cholesterol modulates the property of membrane in two ways:

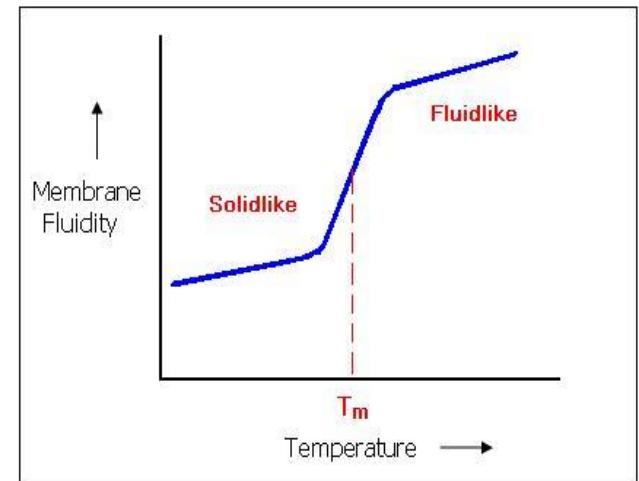
At lower temperature/lower concentration: it inserts into lipid molecules and prevents the tightening.

At higher temperature/higher concentration: it tightens the packing of the lipids

8. Phase transition of lipid membrane

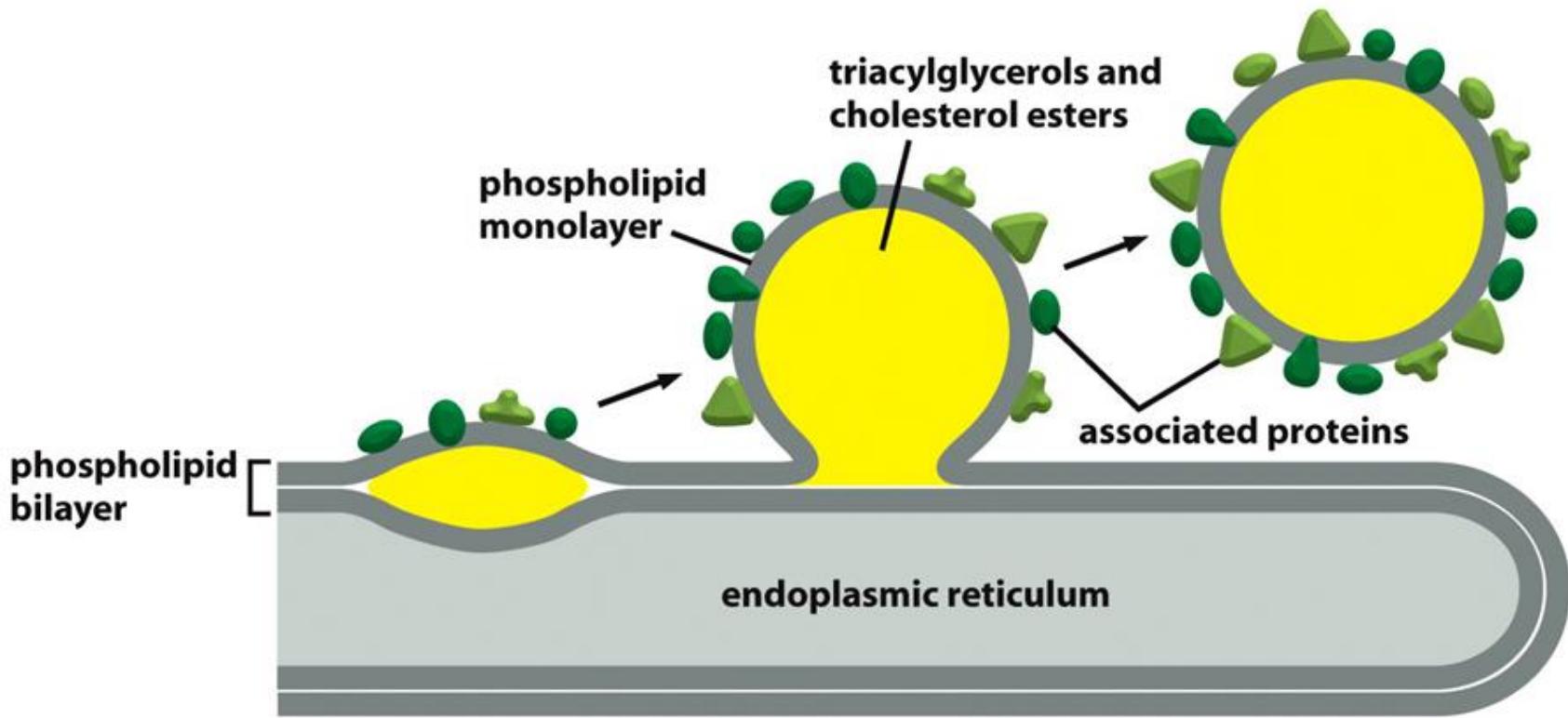


Membrane Fluidity vs. Temperature



With an increase in temperature, the sharp transition is made from a more rigid membrane to a more fluid one.

9. Adipocyte-specialized cell for lipid storage

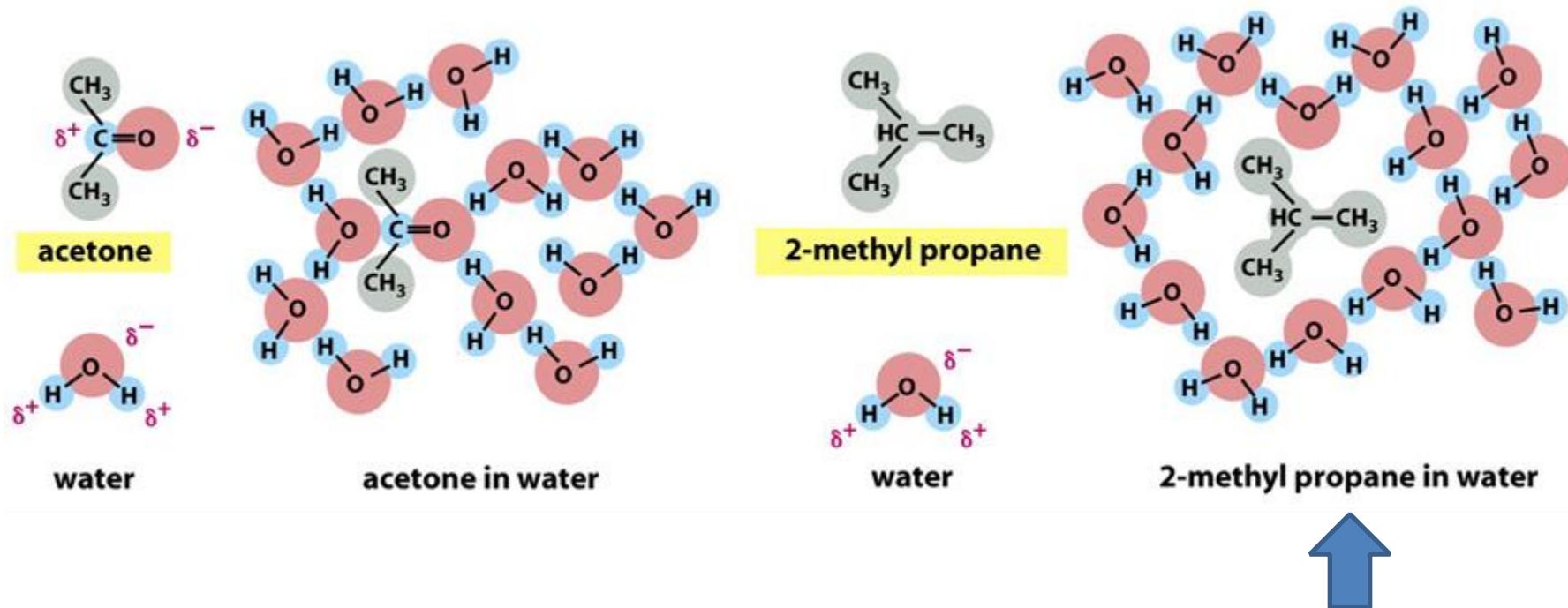


Lipid droplets: store neutral triacylglyceride and cholesterol esters

They are surrounded by monolayer of phospholipids and a variety of proteins, some are important for lipid metabolism.

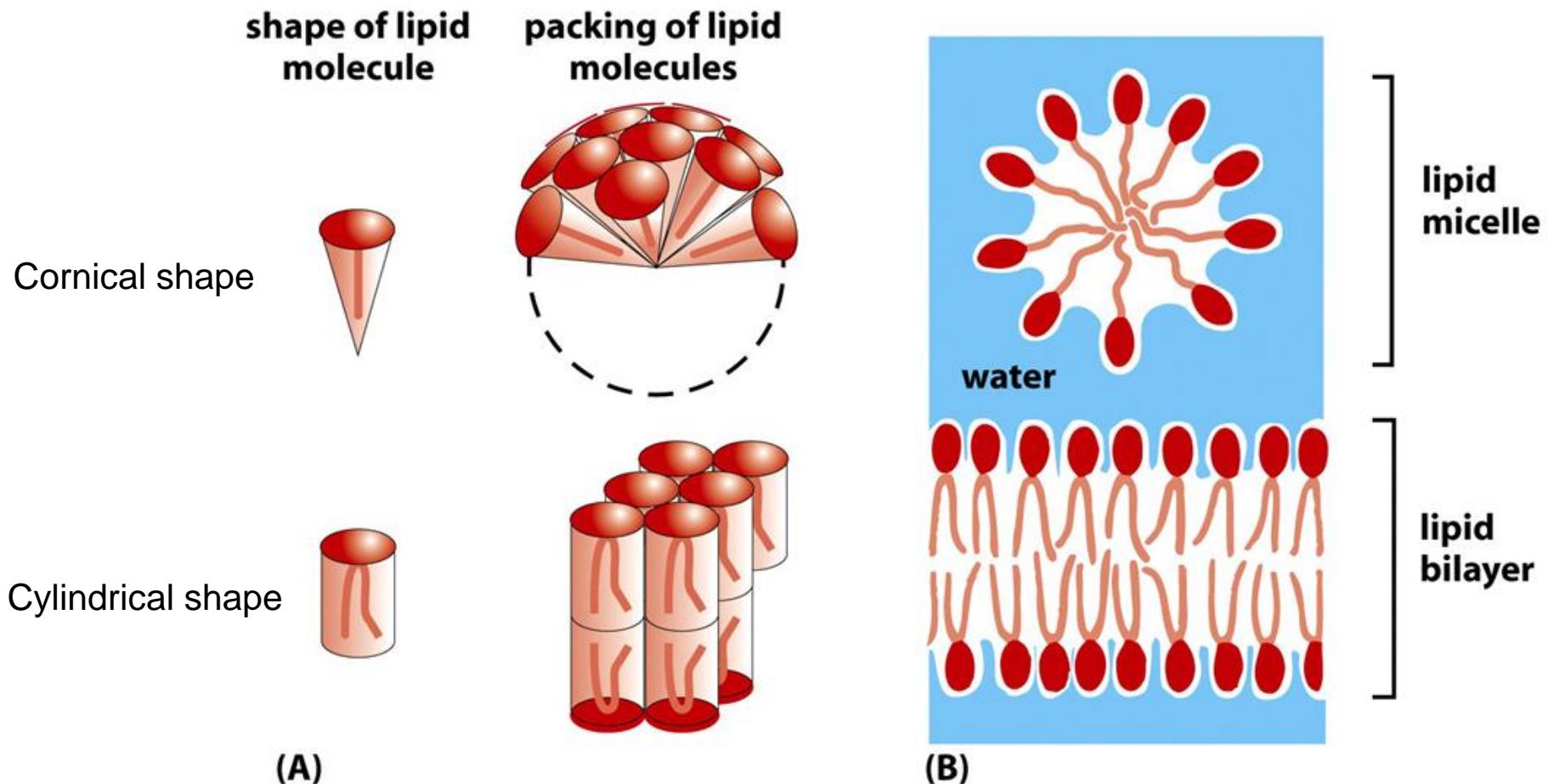
III. Lipid assembly

Why do hydrophobic molecules stay together?

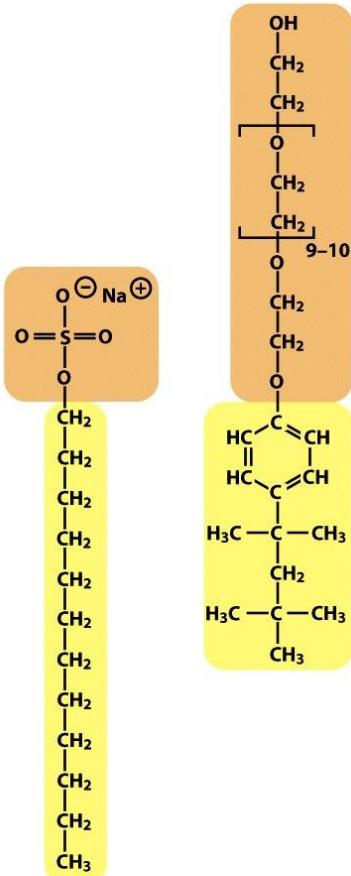


Ice-like cages: This causes increase in order so multiple hydrophobic molecules stay together to minimized the increase in free energy

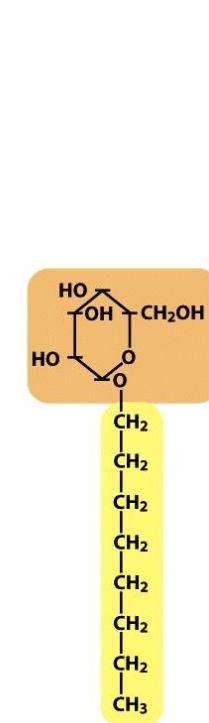
micelle or lipid bilayer forms in aqueous solution



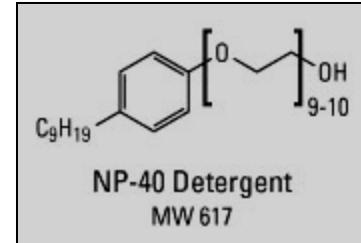
The structure of some common detergents



**sodium dodecyl
sulfate
(SDS)**



Triton X-100



β-octylglucoside

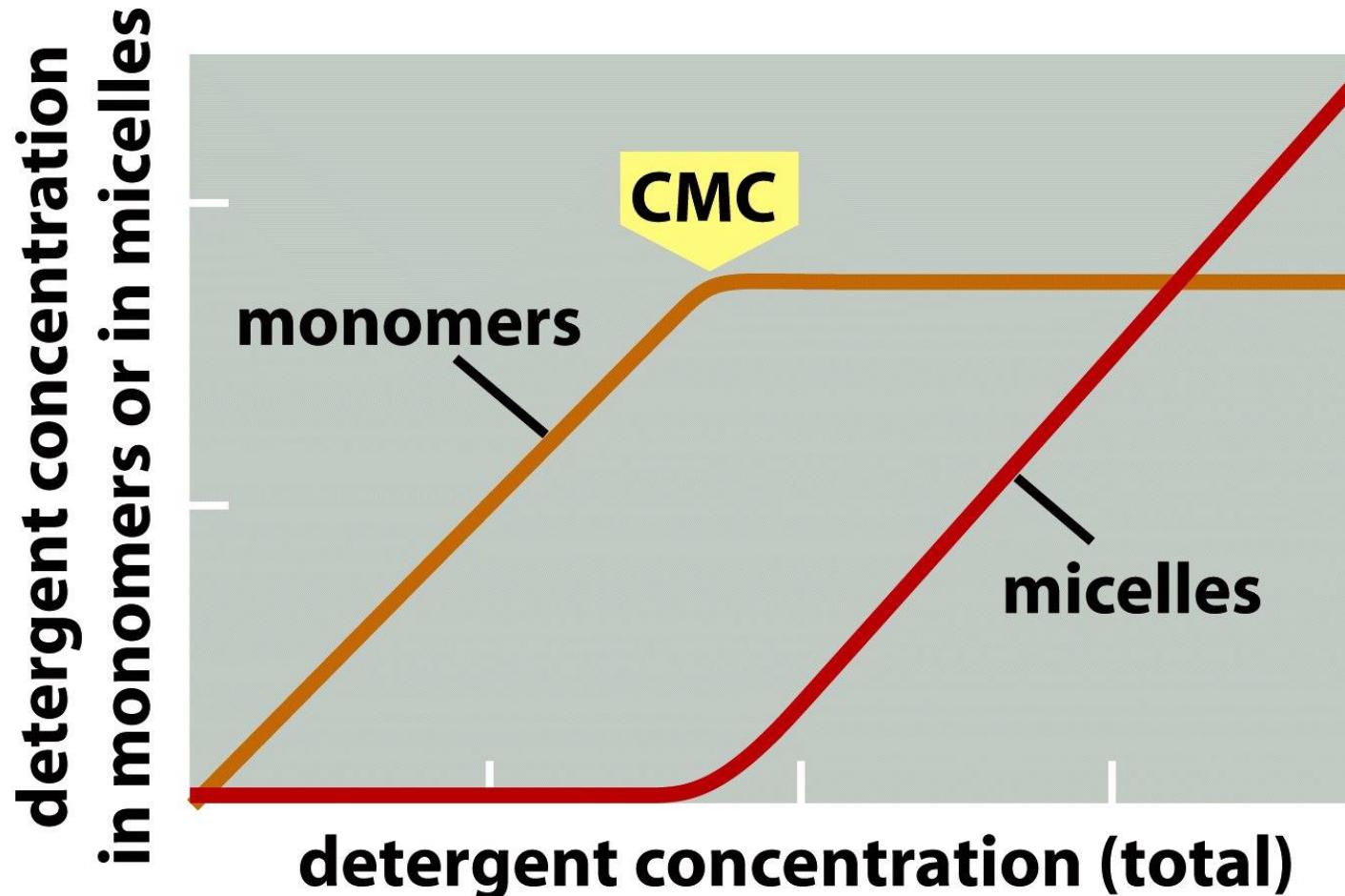
Sodium dodecyl sulfate (SDS)- ionic detergent

Triton-X-100 – non-ionic detergent

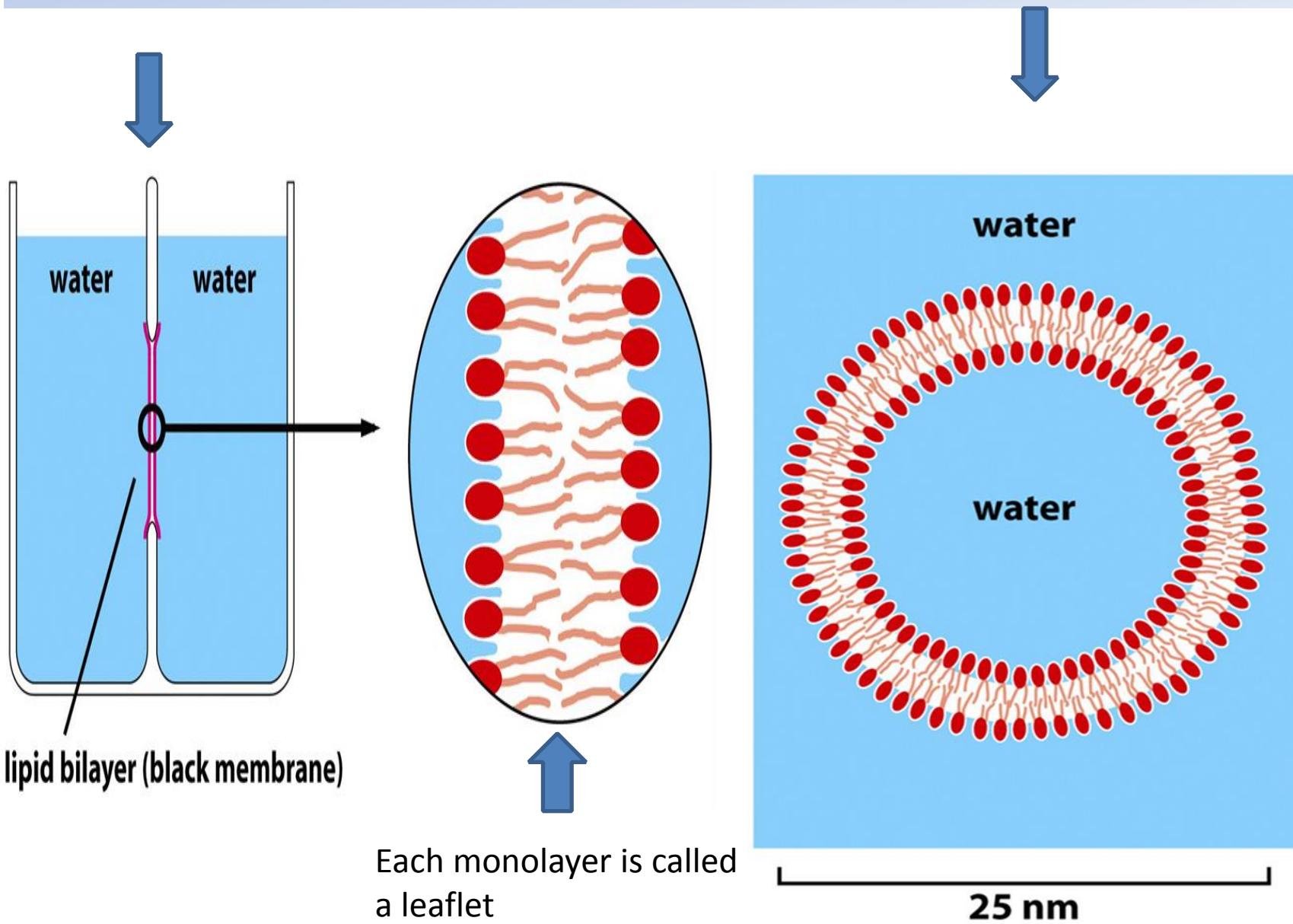
NP-40 (nonidet-P-40)- non-ionic detergent

Critical micelle concentration (CMc)

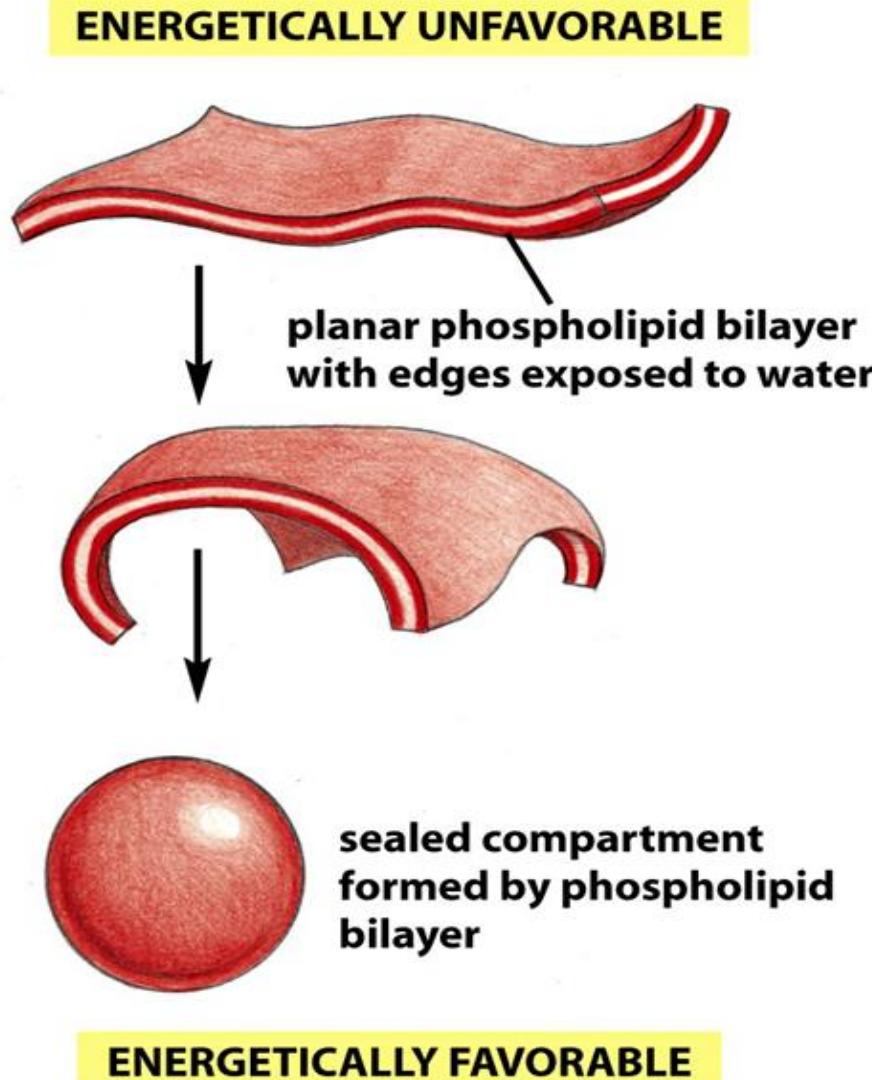
- Concentrations at which detergents aggregate to form micelles



Black membrane and liposome



How does a spherical lipid bilayer form?

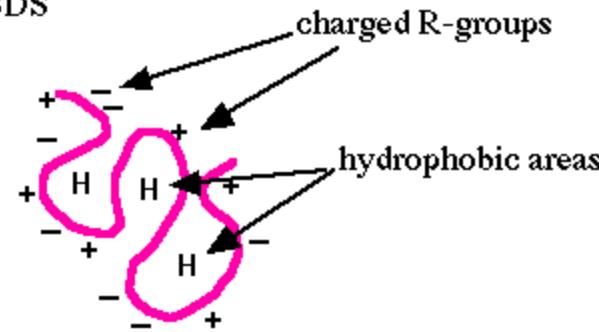


Detergents in membrane studies

- Small amphiphilic molecules of variable structure.
- More soluble in water than lipids.
- Divided into ionic and non-ionic detergents.
- The hydrophobic part intercalate into hydrophobic parts of lipids and transmembrane proteins.
- The polar group bring lipid or protein into aqueous face and make them soluble.

SDS- solubilization of protein

BEFORE SDS



AFTER SDS

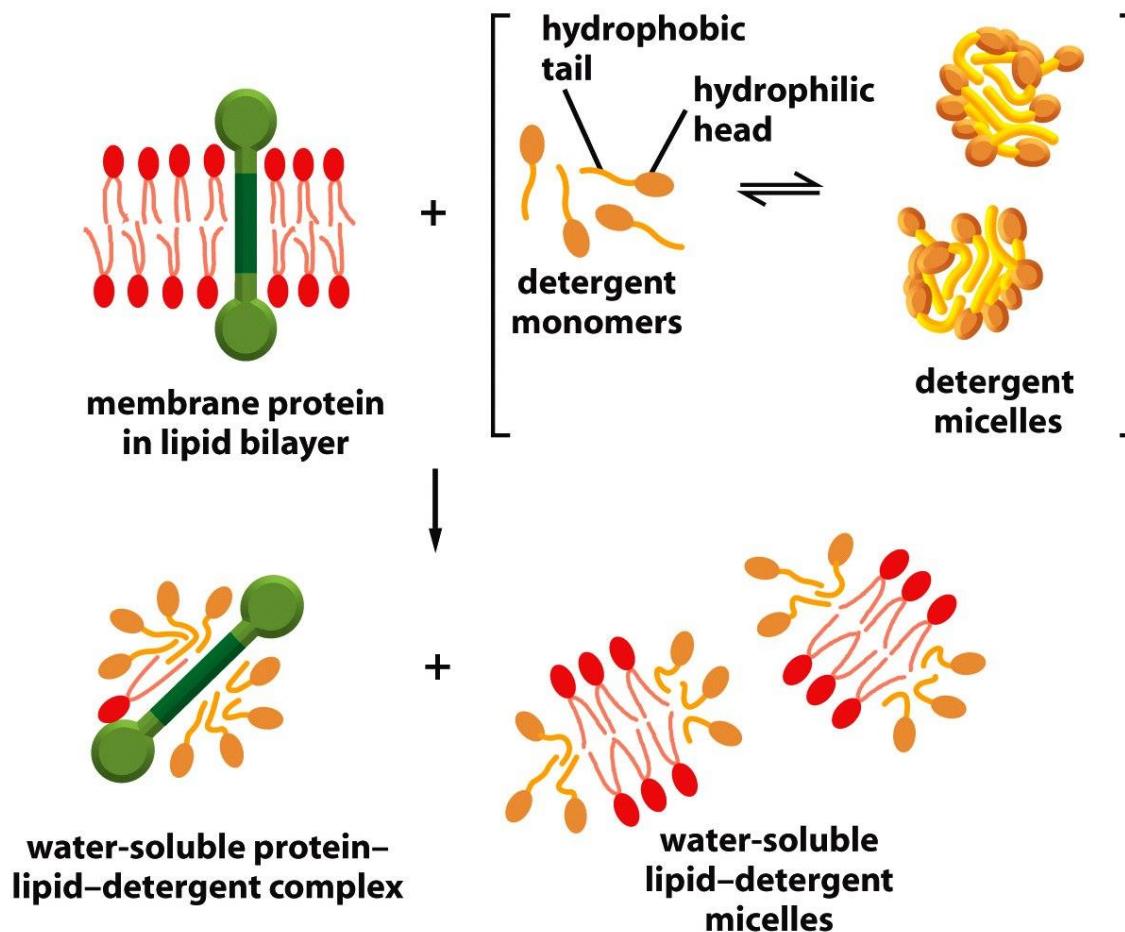


SDS fully denature protein.

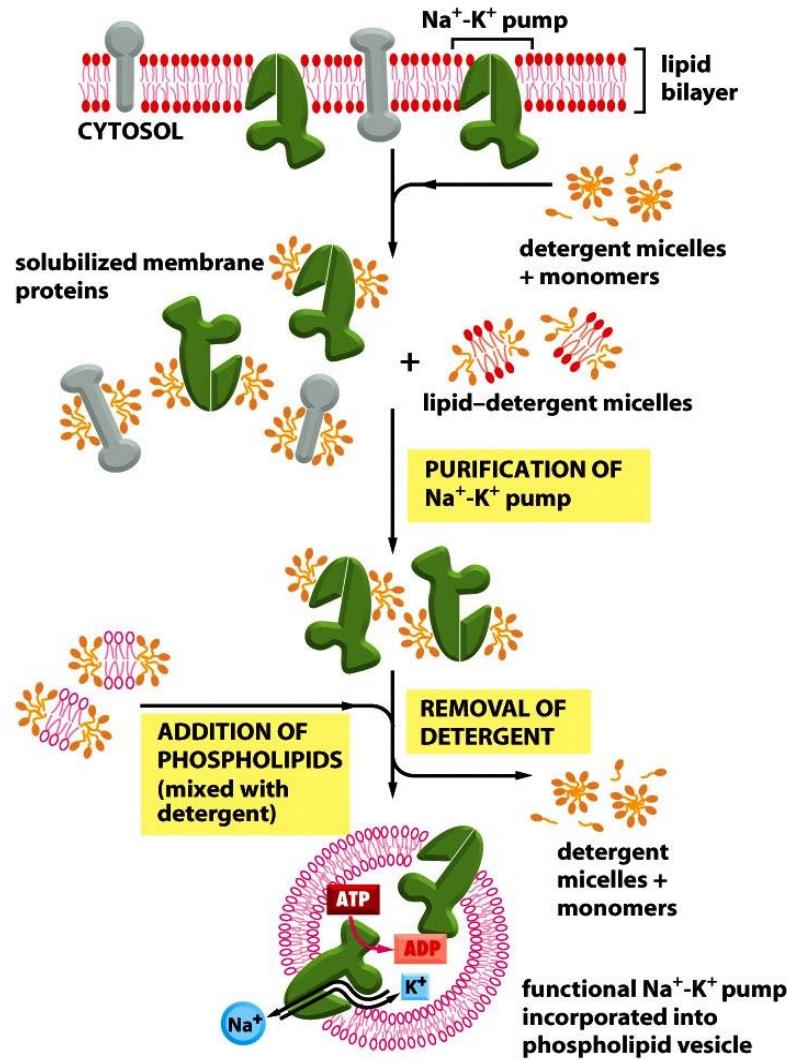
Cover protein molecules with negative charges.

Dependent on the protein molecular weight, proportional amount of SDS are covered on protein.

Non-ionic detergent does not denature protein, it only solubilize membrane components



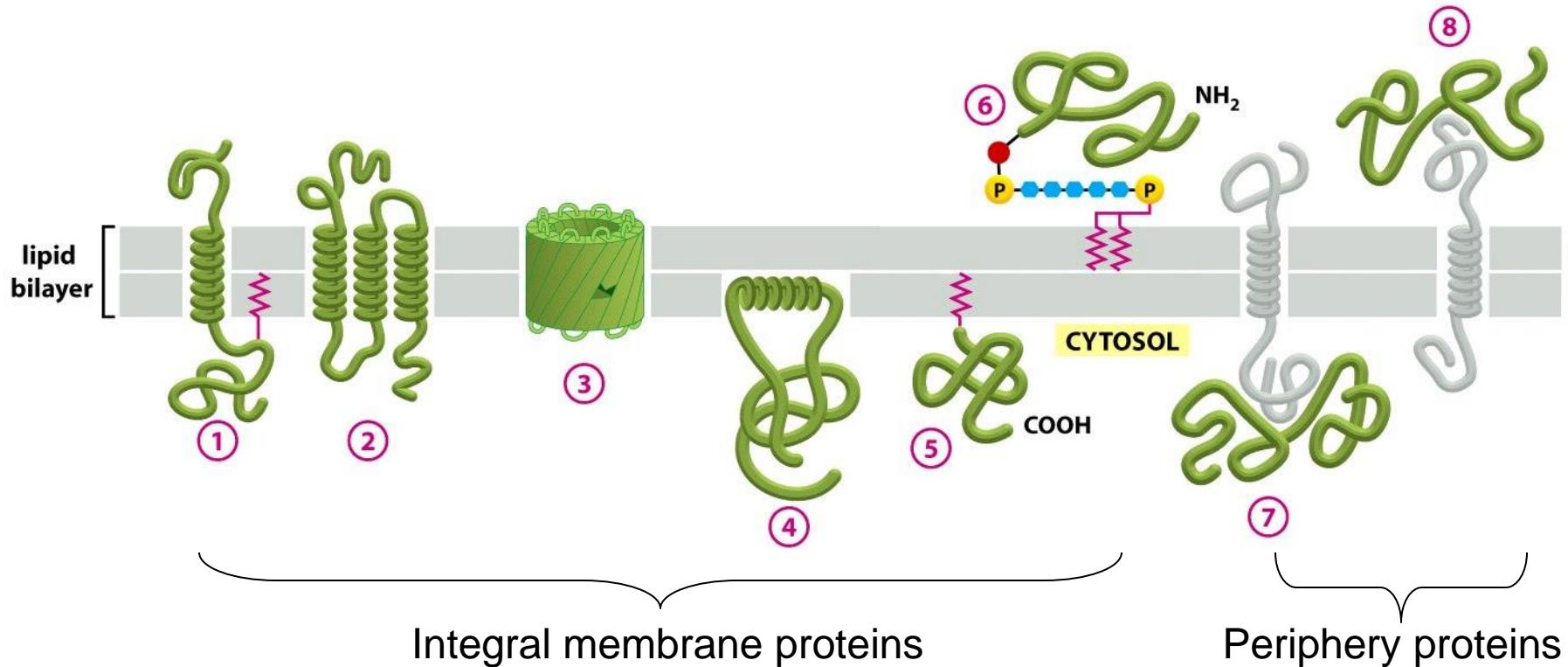
How to use detergent to study membrane proteins



IV. Membrane proteins

- Different types of membrane proteins :
 1. Peripheral membrane protein
 2. Integral membrane protein:
 - a. Transmembrane protein
 - b. Covalent linkage by lipid groups or insertion of hydrophobic regions into lipid bilayer.

Types of membrane proteins



Membrane proteins

Generally constitute half of total membrane mass.

But the amount varies in different type of membrane:

- a. in myelin membrane: <25% of membrane mass is protein
- b. in inner membrane of mitochondria and chloroplast: ~75% of membrane

Function of membrane proteins:

1. Transport
2. Enzyme
3. Receptor sites
4. Intercellular junctions
5. Cell-cell recognition
6. Cytoskeletal and extracellular matrix attachment

Facts of membrane proteins

- Membrane proteins account for ~30% of genome in living organisms.
- Many membrane-embedded receptors, transporters, and ion channels are important therapeutic targets.
- There are over 17,000 structures of water-soluble proteins, but only ~150 unique structures of membrane proteins.

History of membrane protein structure determination

1984 Photosynthetic reaction centre, Deisenhofer et al, *JMB* 1984

1990 Bacteriorhodopsin, Henderson et al, *JMB* 1990

1992 Porin (beta-barrel), Weiss & Schulz, *JMB* 1992

1998 K⁺ channel, Doyle et al, *Science* 1998

2000 Rhodopsin, Palczewski et al, *Nature* 2000

Categories of membrane proteins

- **Integral proteins** (not released by harsh salt concentrations or extreme pH, which would change the ionic interactions between proteins or protein/polar groups of lipid)
- **Peripheral membrane proteins** (released by the above-mentioned conditions)

Periphery membrane proteins

- Do not penetrate the phospholipid bilayer and do not covalently link to other membrane components, but form ionic links to membrane structures
- Dissociation does not disrupt membrane integrity
- Located on both extracellular and intracellular sides of the membrane
- Often link membrane to non-membrane structures
- Synthesis of peripheral proteins:
 - a. cytoplasmic (inner) side: made in cytoplasm
 - b. extracellular (outer) side: made in ER and exocytosed

Integral membrane proteins

- Penetrate the bilayer or span the membrane entirely, can only be removed from membranes by disrupting the phospholipid bilayer
- Two types:
 - a. transmembrane protein
 - b. covalently tethered proteincovalently linked to membrane phospholipids or glycolipids
- Many integral proteins are glycoproteins
covalently linked via Asn, Ser, or Thr to sugars

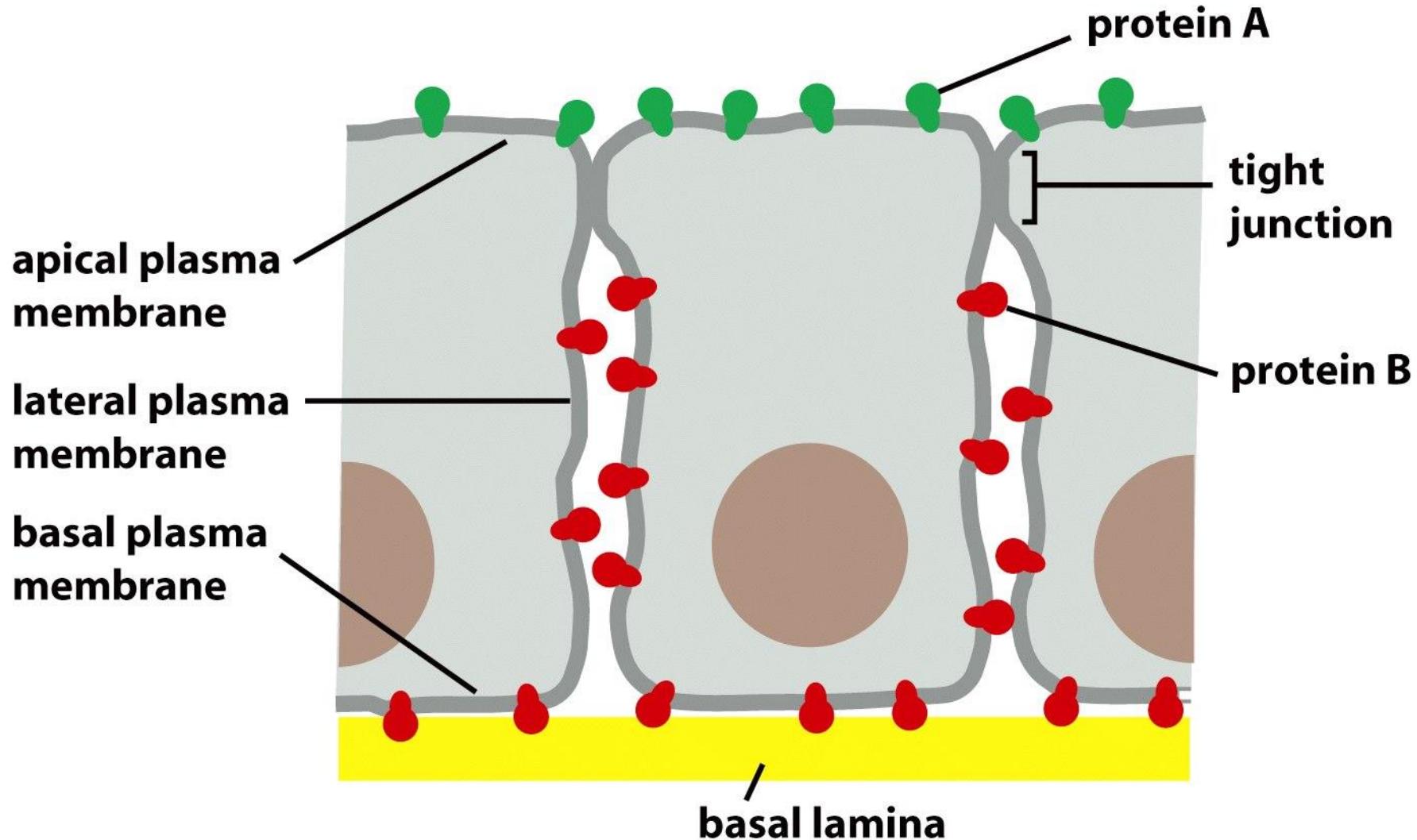
Synthesis of integral proteins:

- a. occurs in the **rough endoplasmic reticulum**
- b. many integral proteins are glycoproteins
 - (1) glycosylation begins in lumen of ER
 - (2) carbohydrates are modified in Golgi apparatus
- c. Lipid-linked proteins are made in cytosol as soluble protein, after lipid linkage, it is targeted to lipid membrane.
- d. Glycosylphosphatidylinositol(GPI) anchor is made as transmembrane protein, after cleavage of transmembrane domain, it is linked by GPI anchor and targeted to membrane.

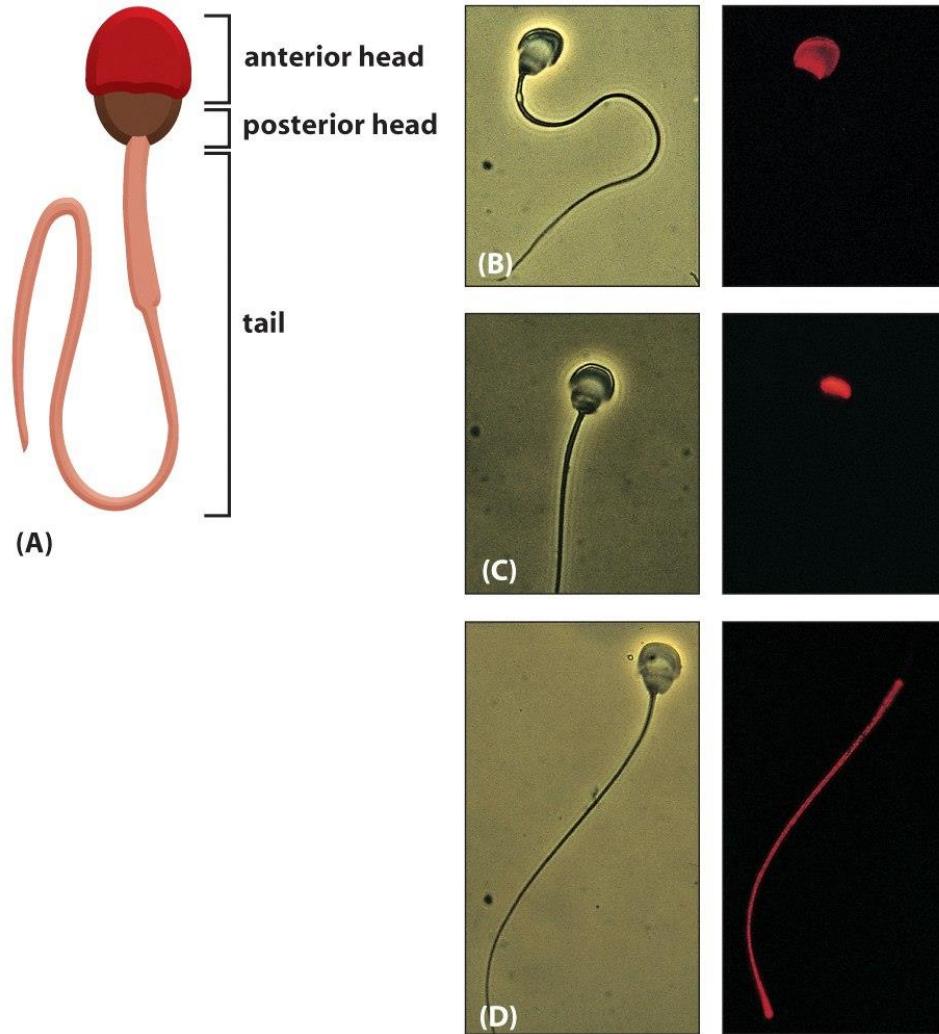
Membrane protein asymmetry

- Each type has a unique conformation and orientation
- Flip-flop of proteins does not occur
- Conformational changes of protein can occur
- Proteins are confined to specific domains
- Carbohydrates of glycoproteins are always at outer surface, as well as disulfide bond

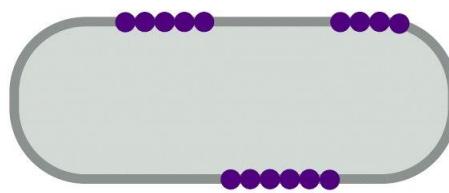
Specific domains in membrane proteins



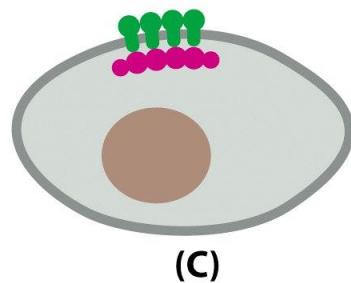
Three domains in plasma membrane of a guinea pig sperm



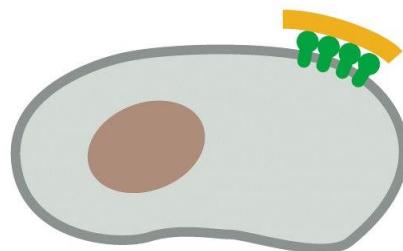
How membrane protein forms domains?



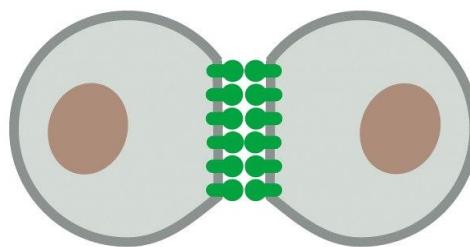
(A)



(C)



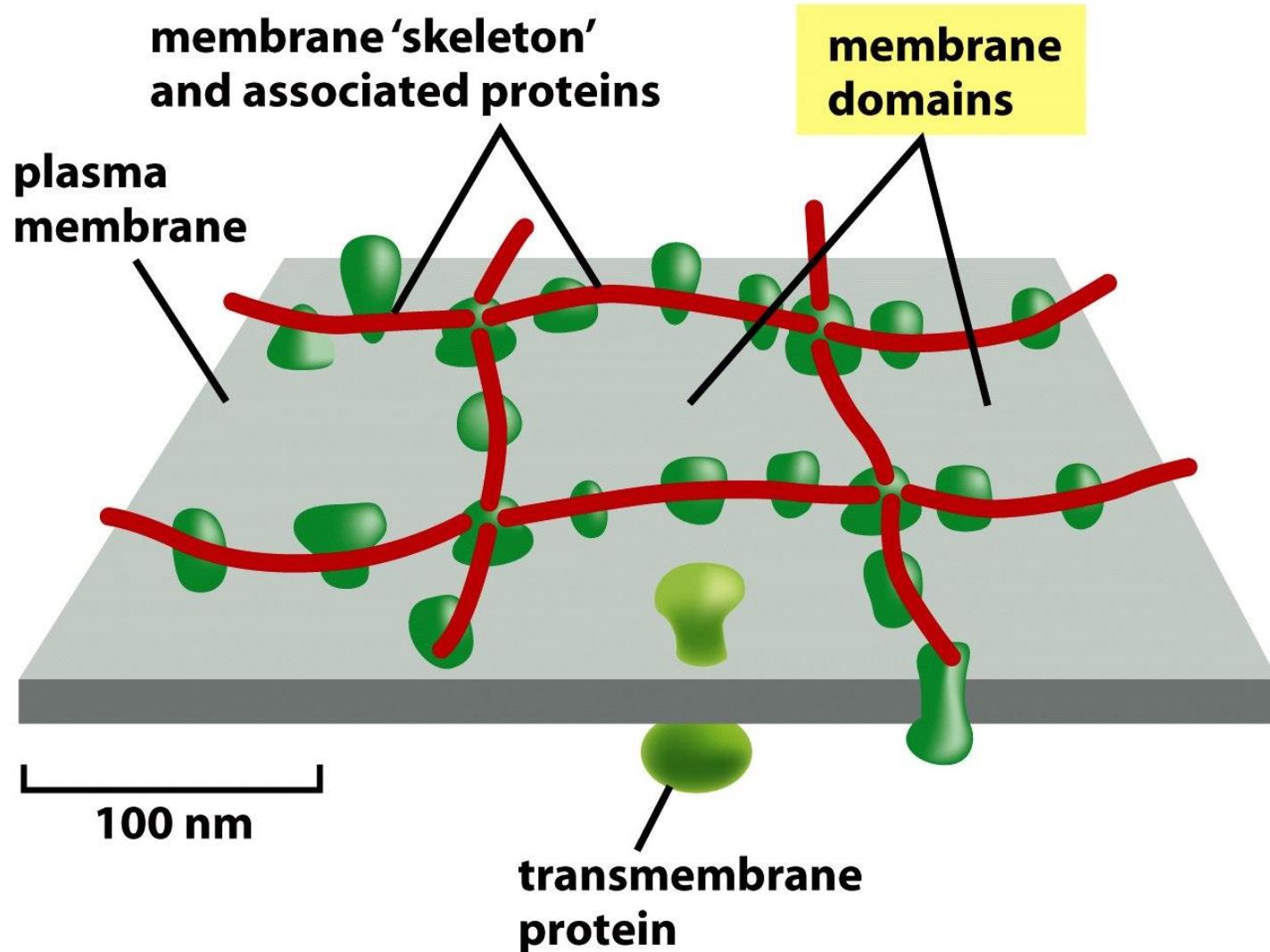
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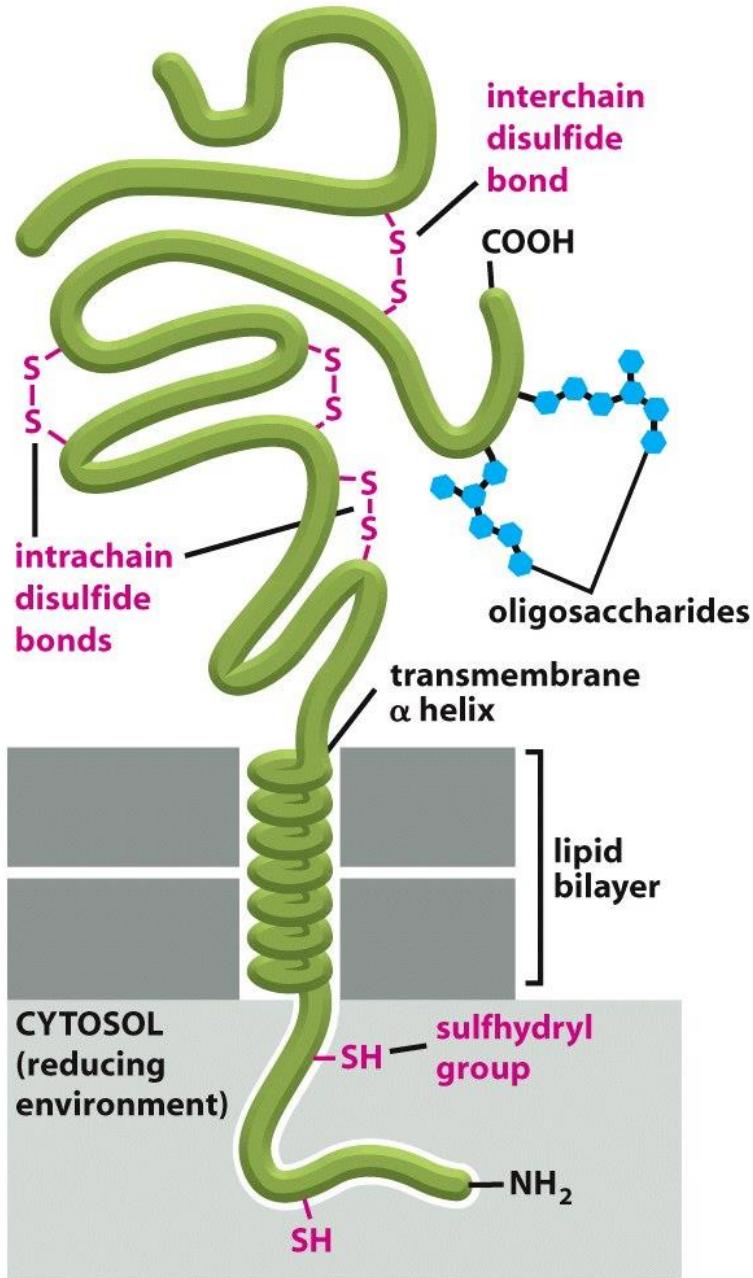


(D)

1. self-assemble into aggregates
2. Tethered by outside molecules
3. Tethered by inside molecules
4. Confined by cell-cell junctions

Example: Cytoskeleton network restricts membrane protein diffusion



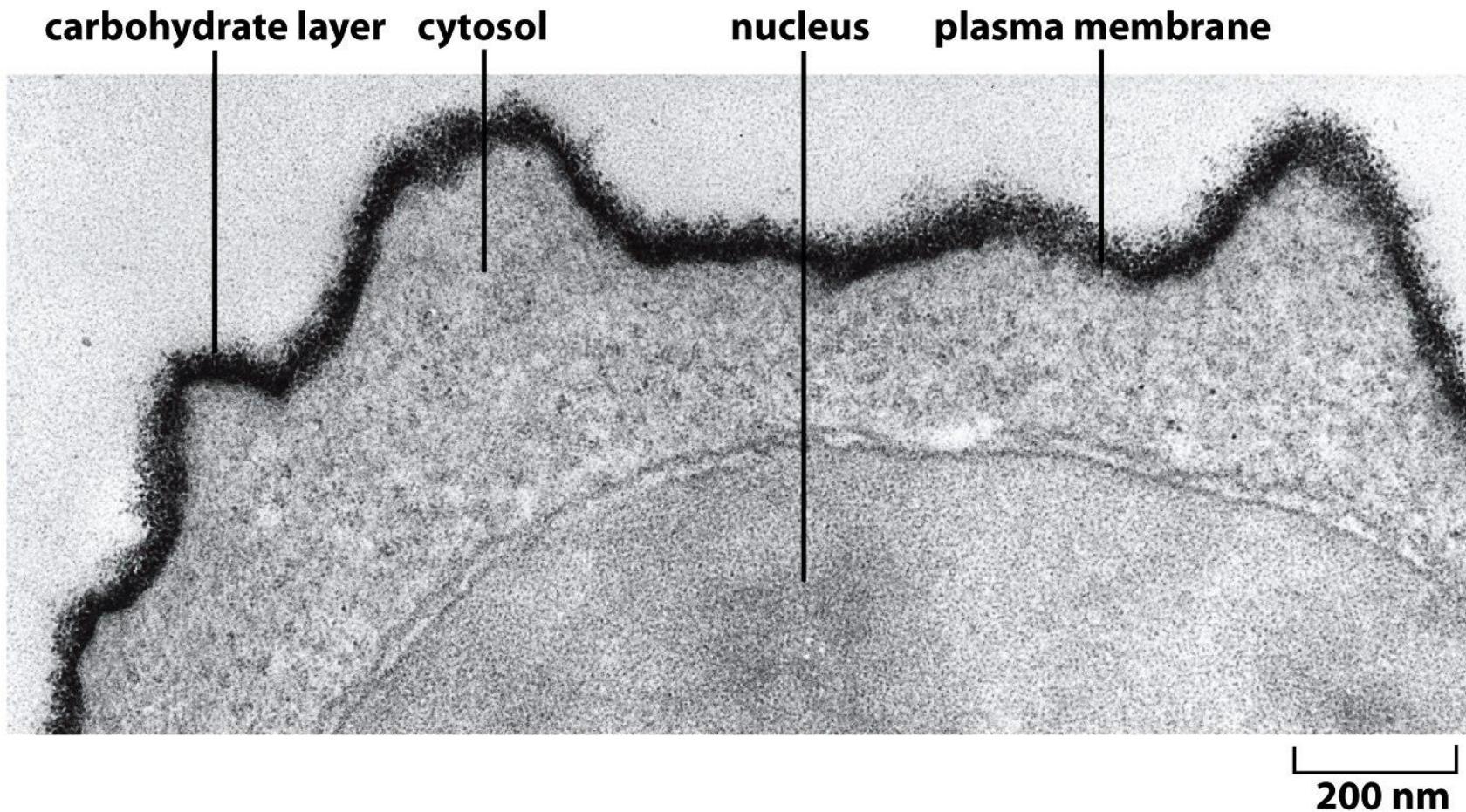


Oligosaccharide chains are diverse
In exoplasmic side of membrane
Proteins.
Due to reducing cytosolic environment,
Rare disulfide bonds form, in contrast,
Extensive disulfide bonds form in
Exoplasmic side for membrane proteins
To stabilize the protein.

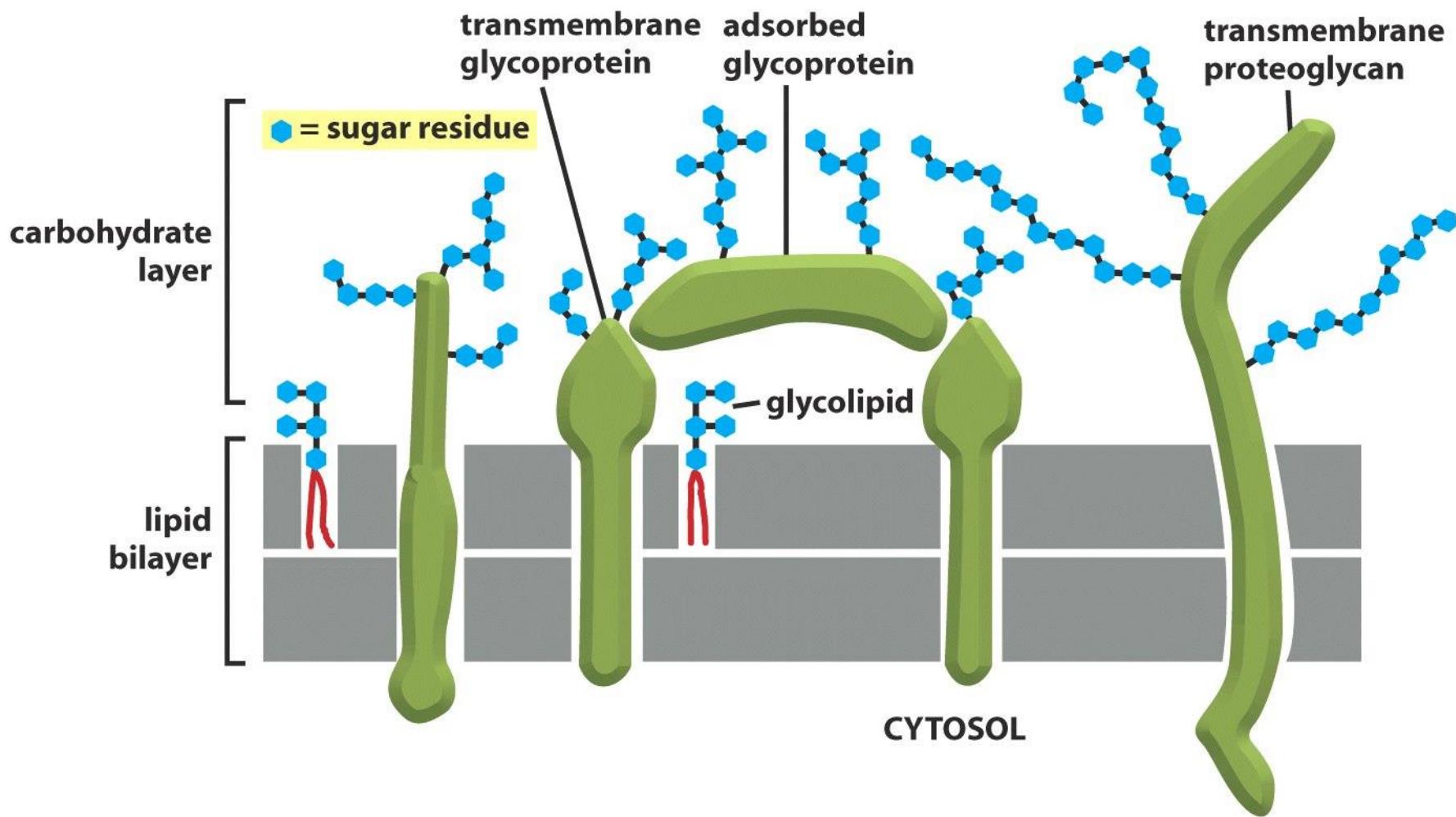
Glycoproteins

- Most plasma membrane proteins are glycosylated.
- Glycocalyx: carbohydrate rich zone in cell surface.
- Lectins: carbohydrate-binding proteins can be used to label carbohydrate layer.
- Functions: mediate cell-cell adhesion
 - protect against mechanical and chemical damage.
 - keep cell at appropriate distance, etc.

Carbohydrate layer by ruthenium red stain



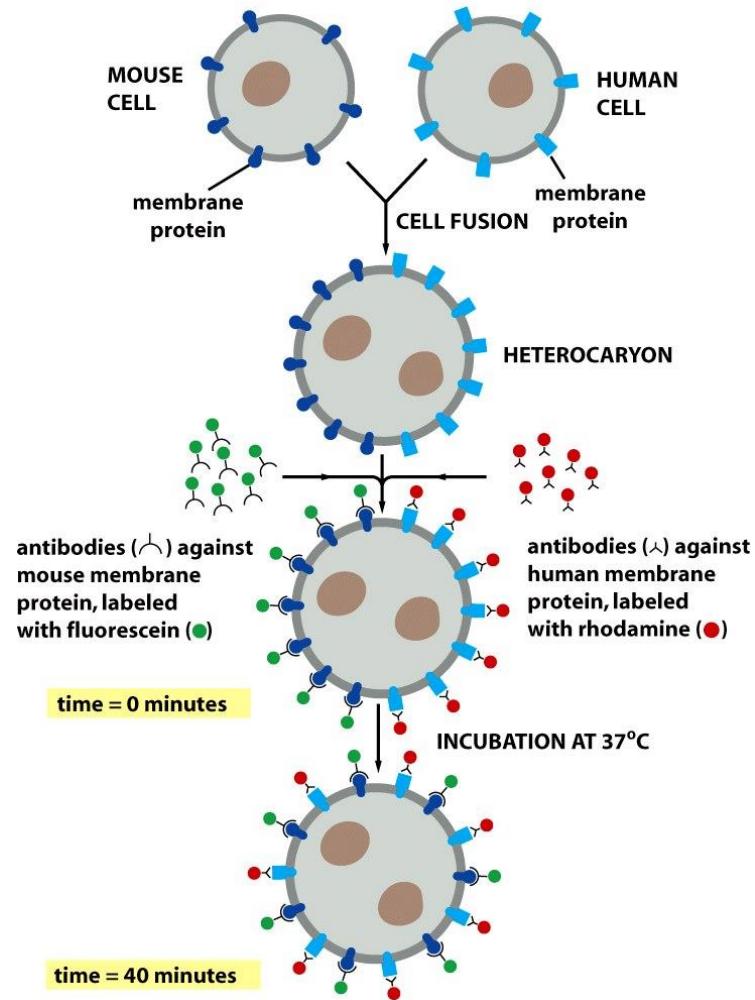
Types of glycosylation



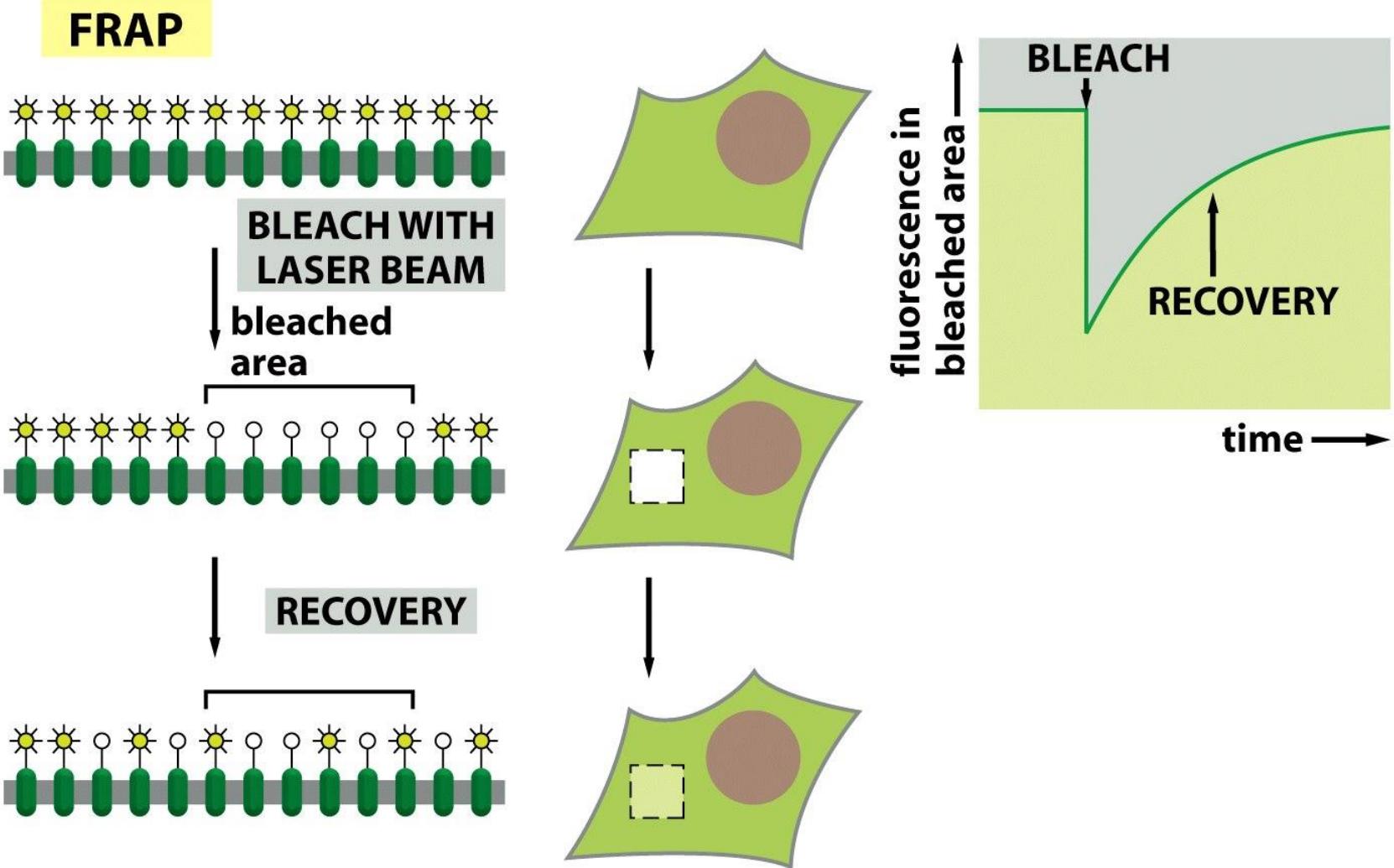
Membrane protein mobility

- Rotational mobility
- Lateral diffusion
- Protein mobility vary greatly.
 - (1). Some proteins are free to move.
 - (2). Others may be tethered to structures in the cytoplasm or extracellular spaces
 - (3). Some types of cell junctions (e.g., tight junctions) can restrict protein movements to a specific membrane domain.

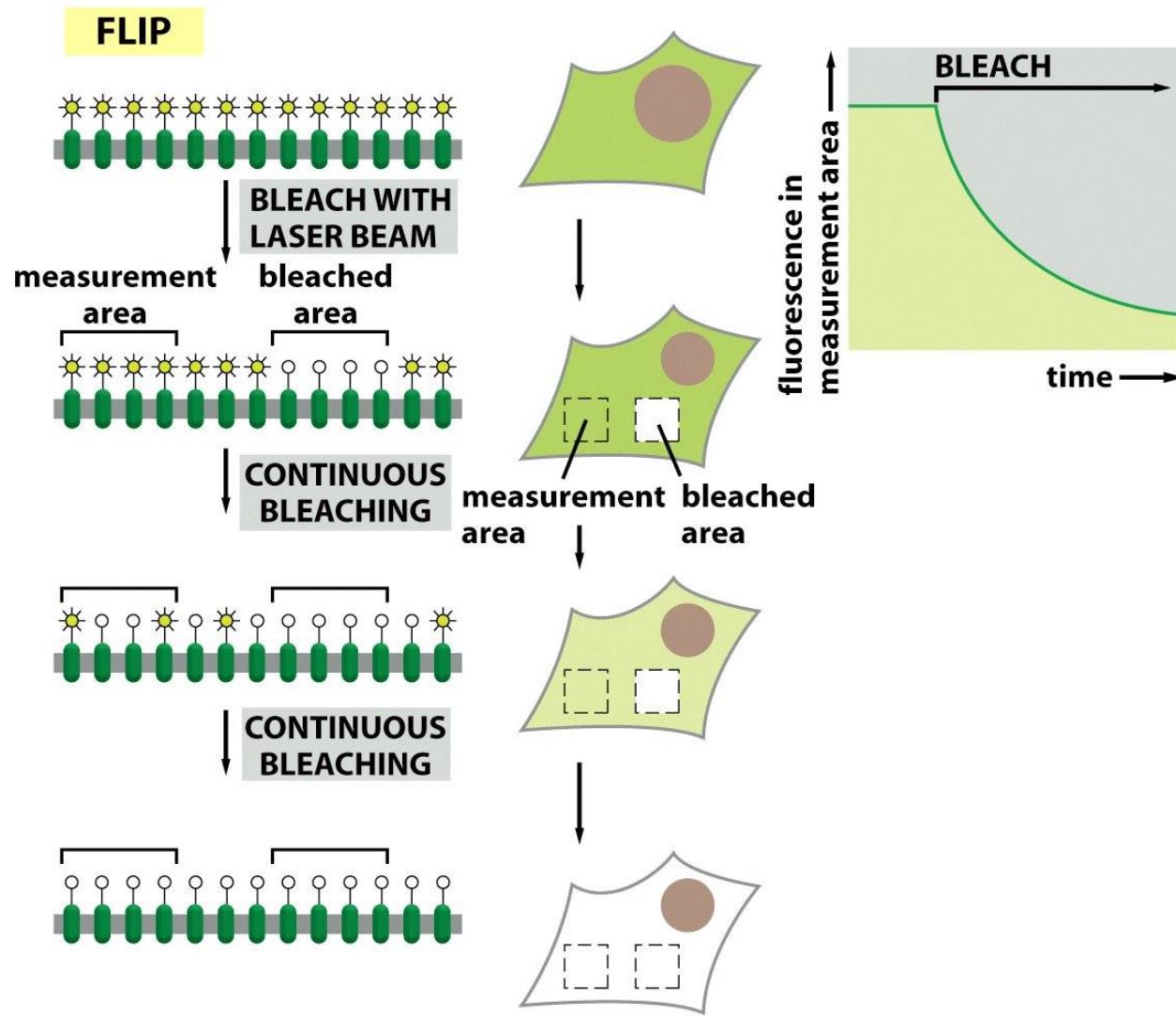
Example 1: membrane protein has lateral diffusion



Example 2: Fluorescence recovery after photobleaching to detect membrane protein diffusion



Example 3: Fluorescence loss in photobleaching (FLIP) to detect membrane protein diffusion

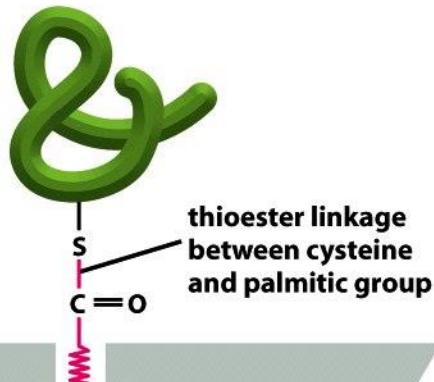


Ways for membrane proteins to covalently attach to membrane lipid

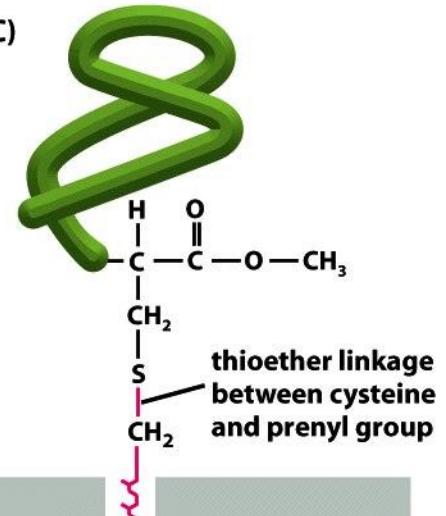
(A)



(B)



(C)



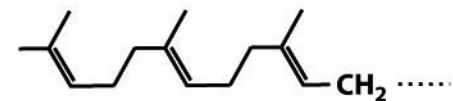
(D) myristoyl anchor



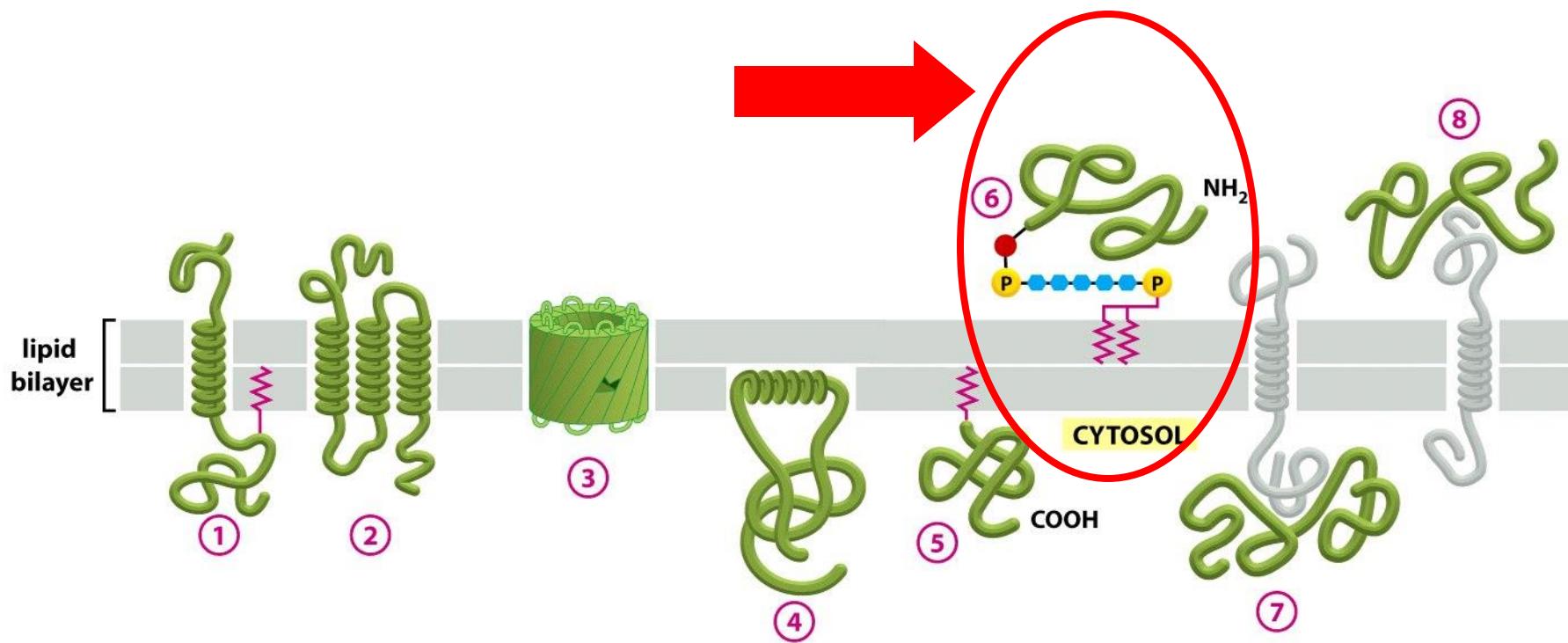
(E) palmitoyl anchor



(F) farnesyl anchor

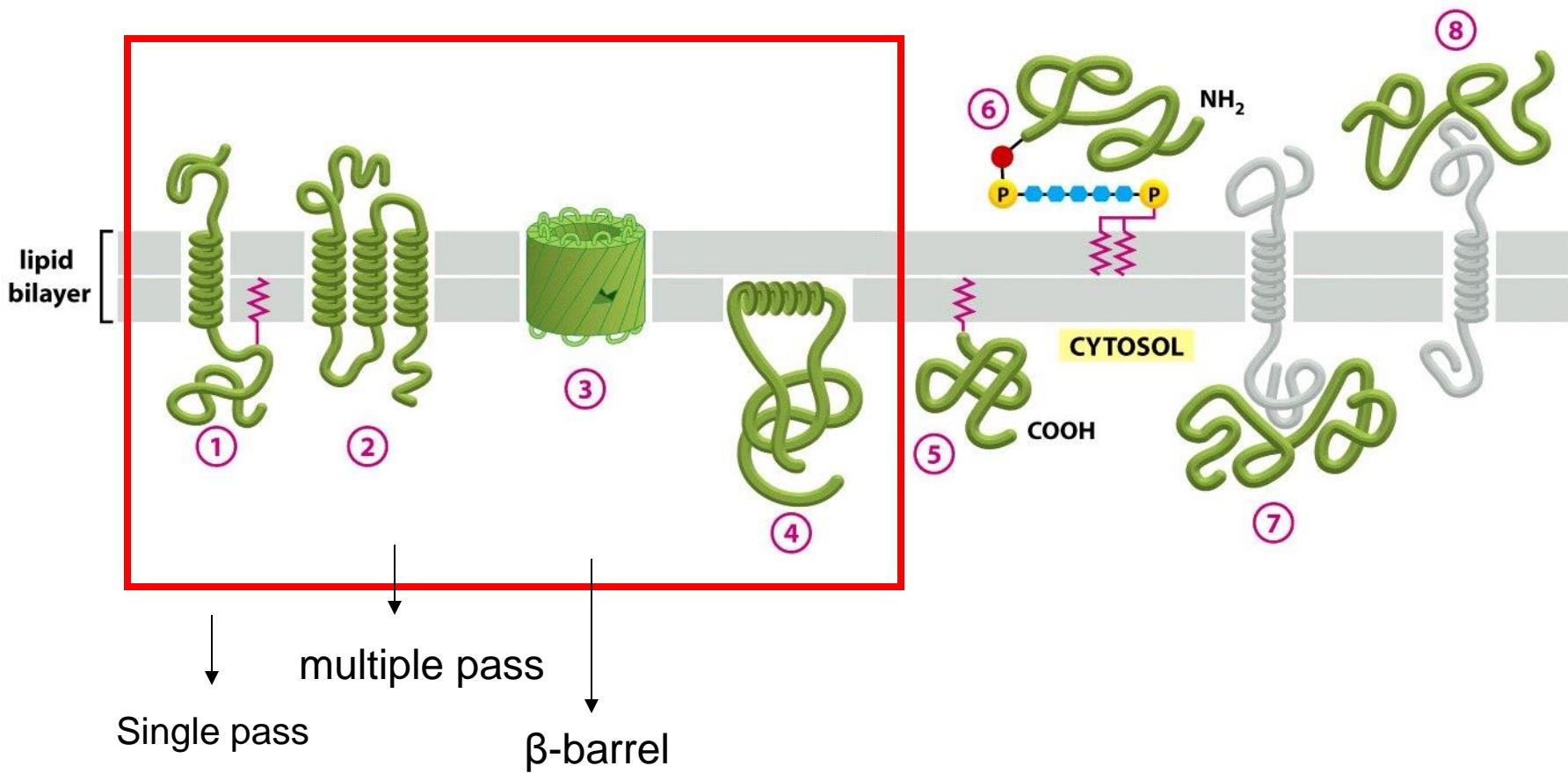


Glycosylphosphatidylinositol(GPI) anchor

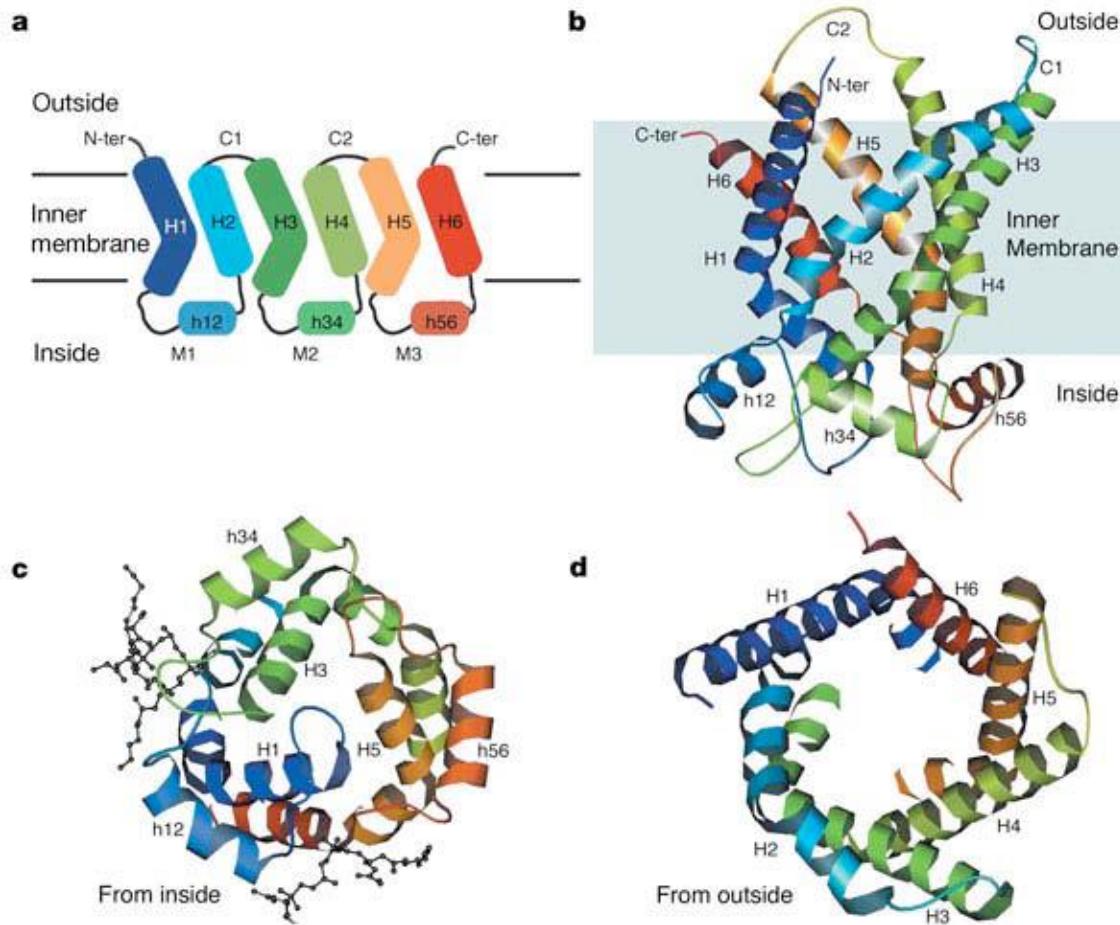


GPI anchored protein can be recognized by **phosphatidylinositol-specific phospholipase C** And be cleaved off from the membrane.

Membrane protein topology

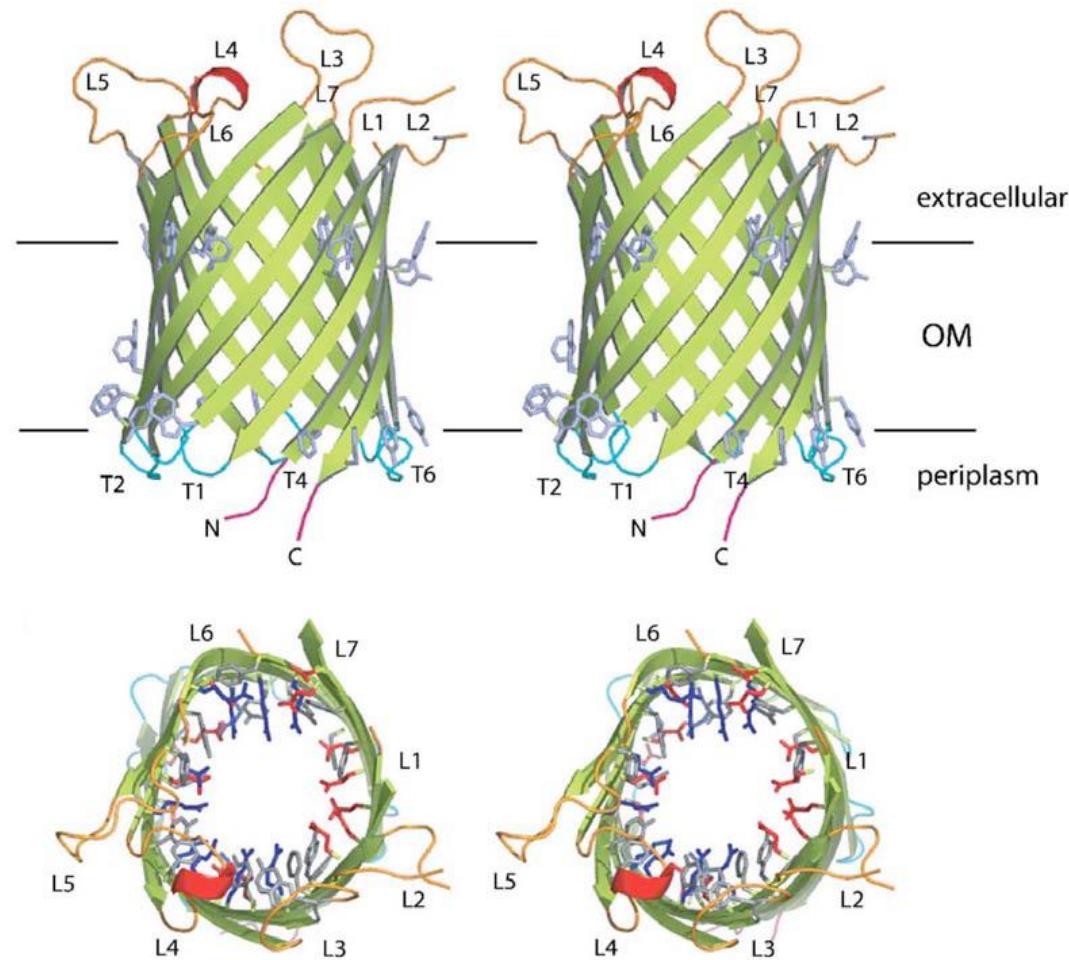


Example 1: mitochondria ATP/ADP carrier



Pebay-Peyroula et al, Nature 2003

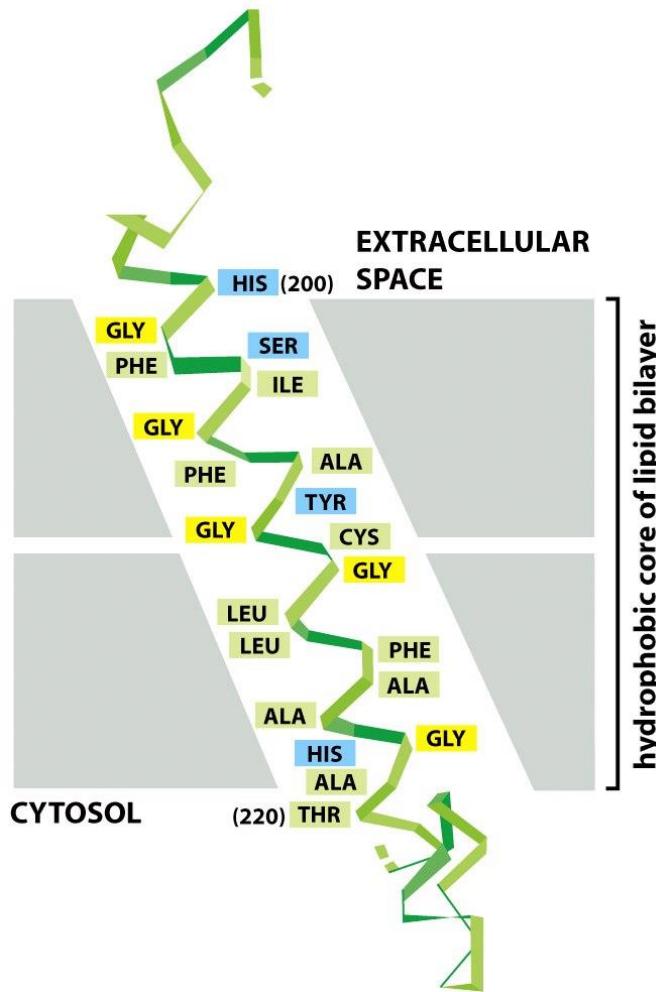
Example 2: outer membrane protein G



Subbarao and van den Berg, JMB 2006

Transmembrane α -helix

- A segment of 20-30 amino acids with a **high degree of hydrophobicity**



Hydrophobic amino acids:
green and yellow

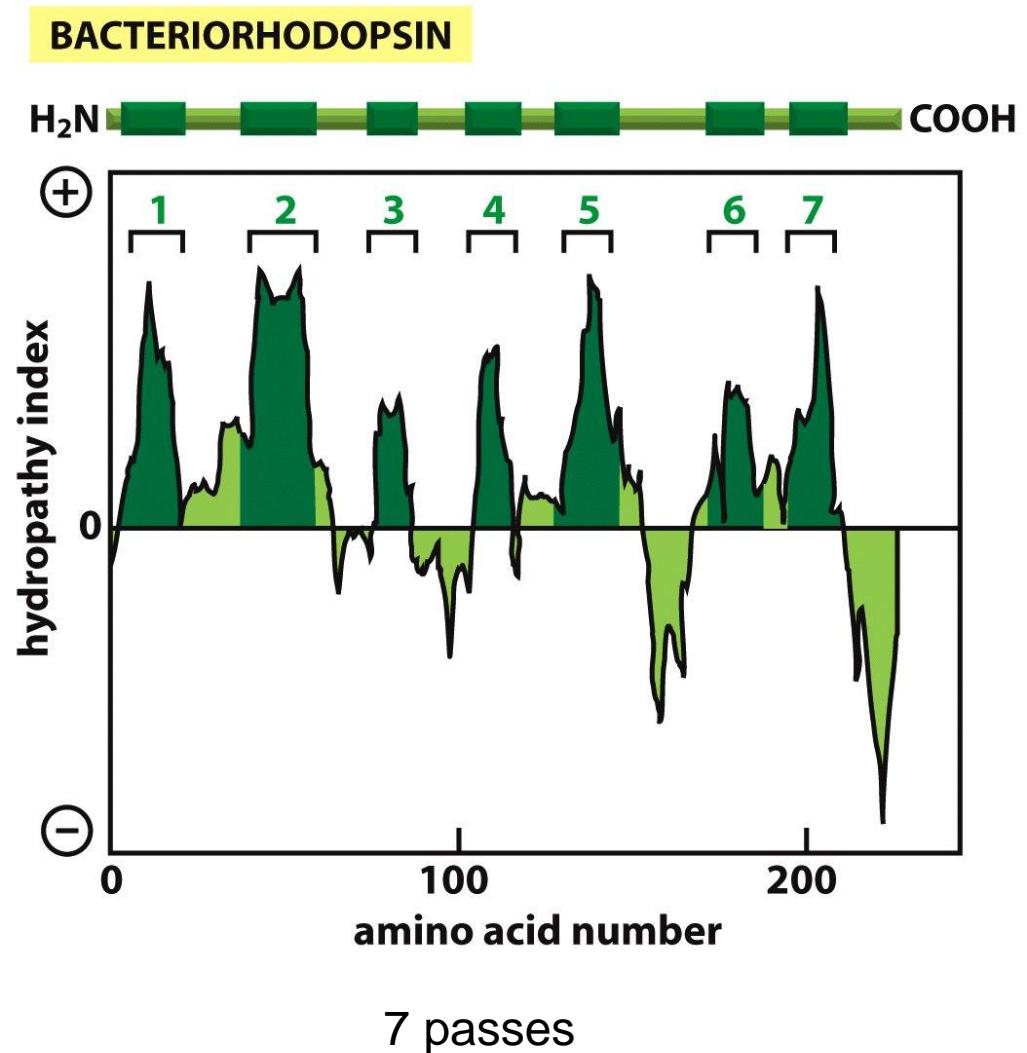
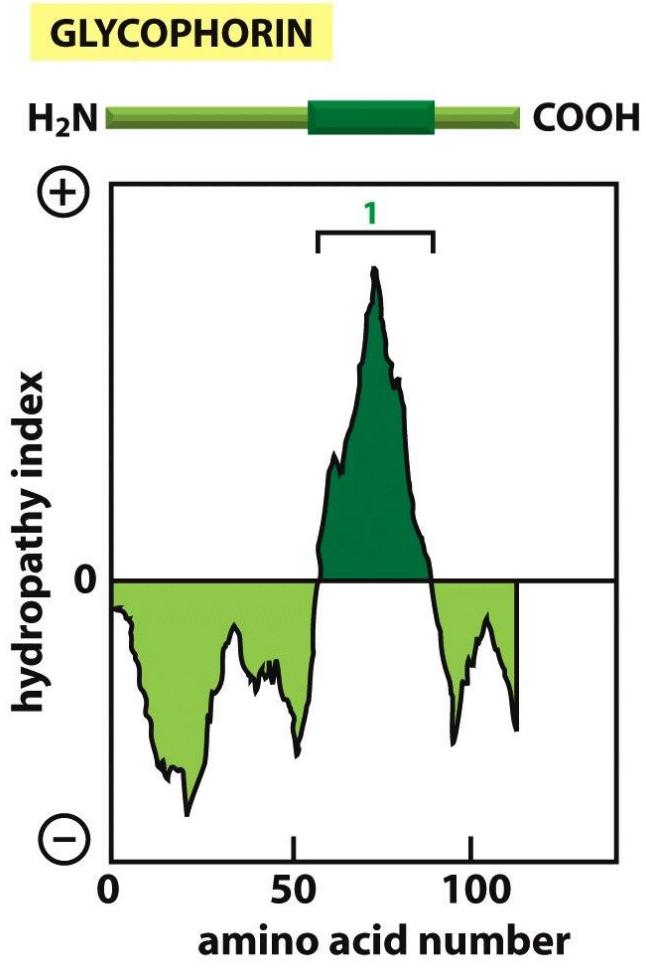
Hydrophobicity can be predicted by amino acid sequence

Hydrophobicity Scales

Kyte-Doolittle Hopp-Woods

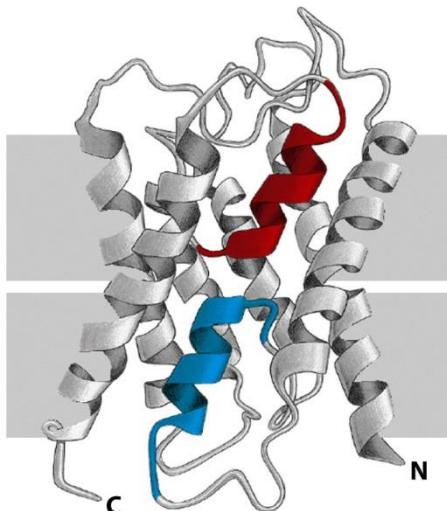
| | | |
|---------------|------|------|
| Alanine | 1.8 | -0.5 |
| Arginine | -4.5 | 3.0 |
| Asparagine | -3.5 | 0.2 |
| Aspartic acid | -3.5 | 3.0 |
| Cysteine | 2.5 | -1.0 |
| Glutamine | -3.5 | 0.2 |
| Glutamic acid | -3.5 | 3.0 |
| Glycine | -0.4 | 0.0 |
| Histidine | -3.2 | -0.5 |
| Isoleucine | 4.5 | -1.8 |
| Leucine | 3.8 | -1.8 |
| Lysine | -3.9 | 3.0 |
| Methionine | 1.9 | -1.3 |
| Phenylalanine | 2.8 | -2.5 |
| Proline | -1.6 | 0.0 |
| Serine | -0.8 | 0.3 |
| Threonine | -0.7 | -0.4 |

Hydropathy plots to predict transmembrane α -helix



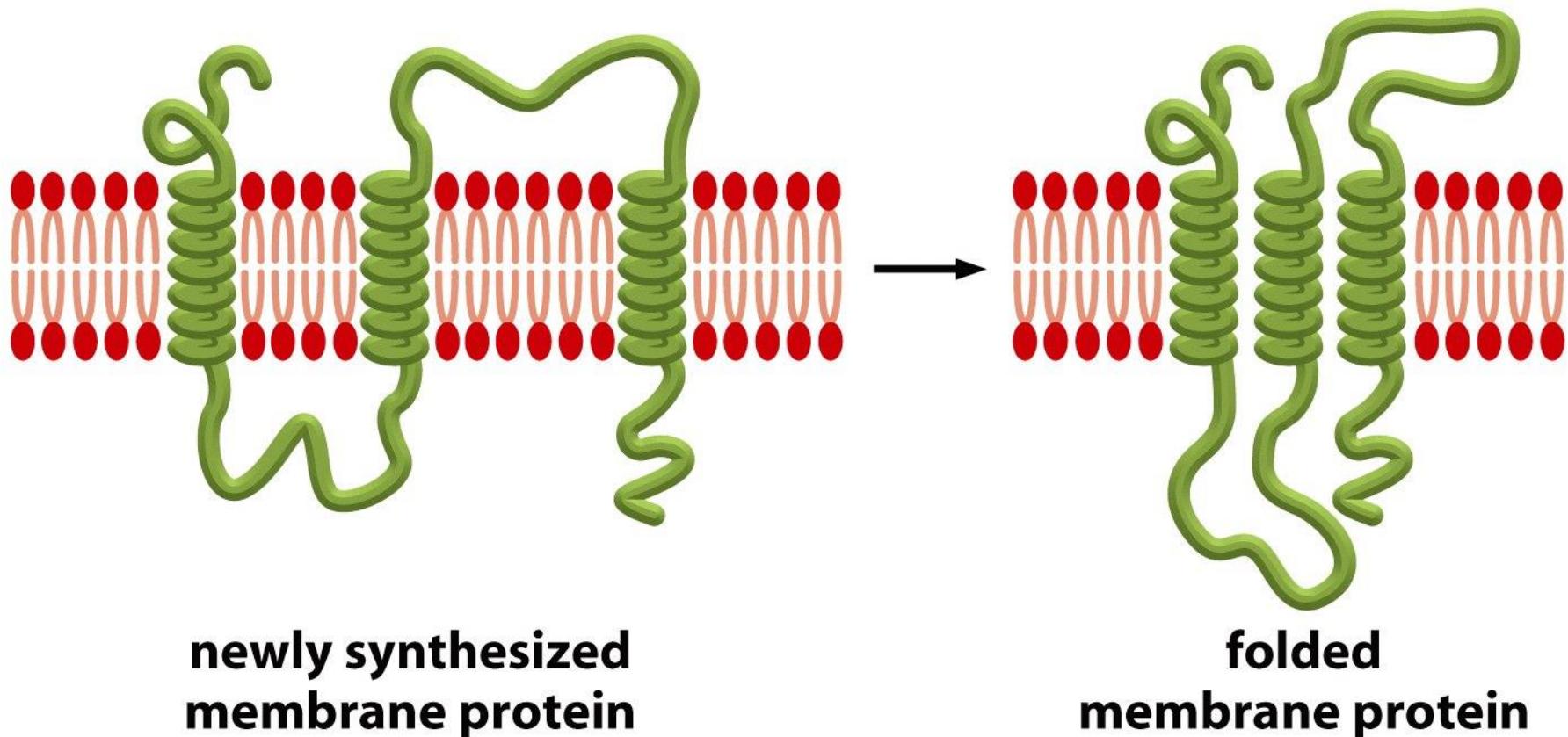
Some transmembrane protein regions can't be predicted by hydropathy plots, these includes...

- The β -barrels, as they are short and only every other amino acids is hydrophobic
- Membrane proteins who do not contact hydrophobic bilayer, but rather interact with other transmembrane proteins.

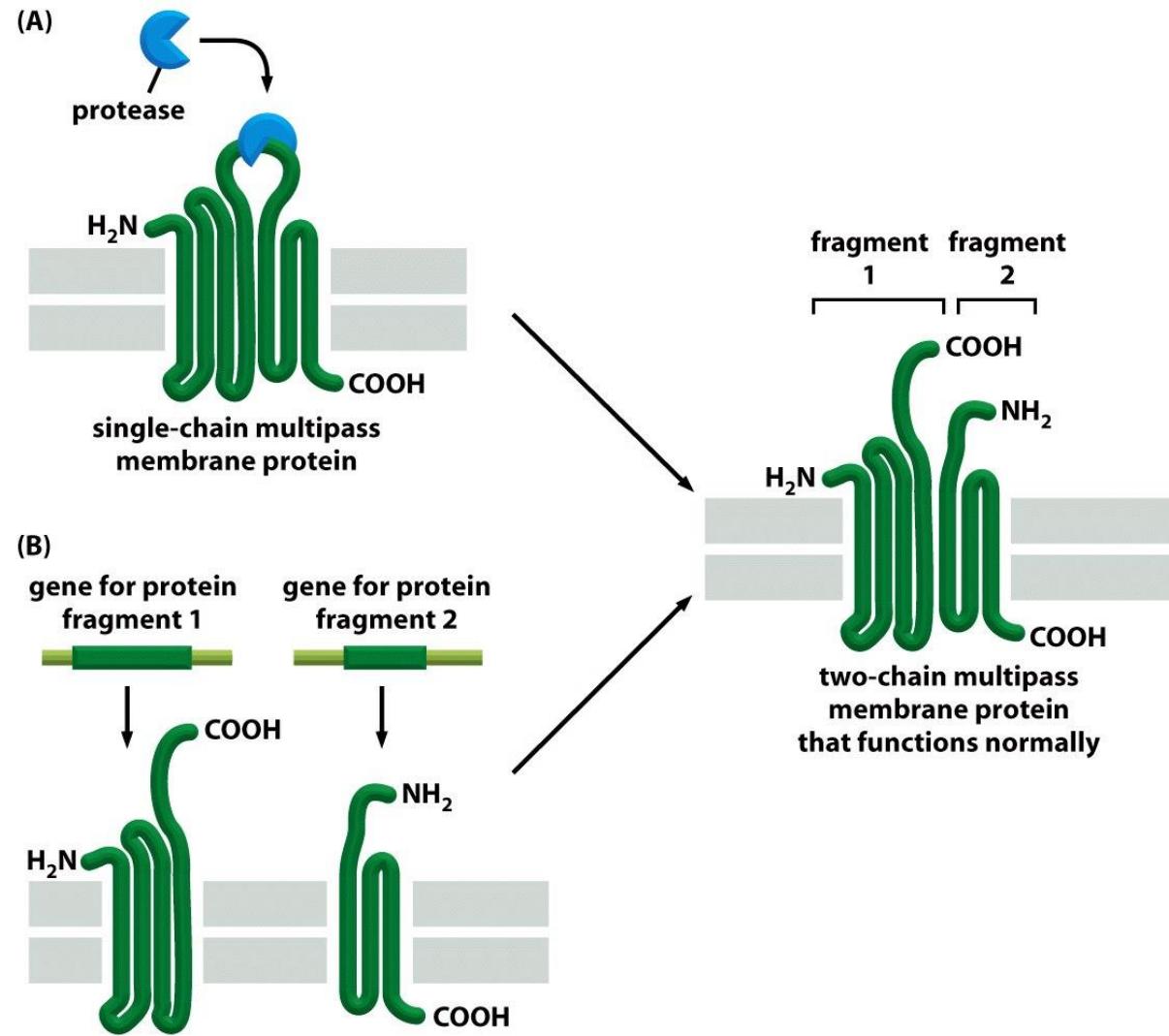


Colored two helices in aquaporin water channel are buried at an interface formed by protein-protein interactions, they are not hydrophobic.

Transmembrane α -helix has specificity for its interaction partners



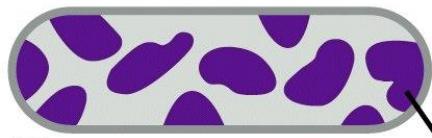
Transmembrane α -helix has specificity for its interaction partners



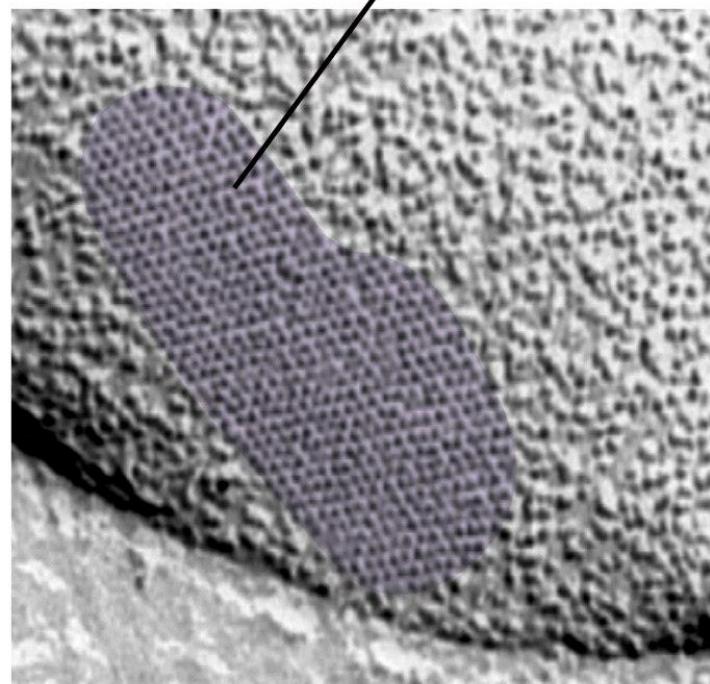
Bacteriorhodopsin-the first membrane transport protein whose structure was determined

- Exist in the plasma membrane of archaean *Halobacterium salinarum* who lives in sea water
- Pump protons in the presence of sunlight and set up proton gradients across the membrane.
- Use the proton gradients to harvest ATP or other energy requiring activities.

Bacteriorhodopsin

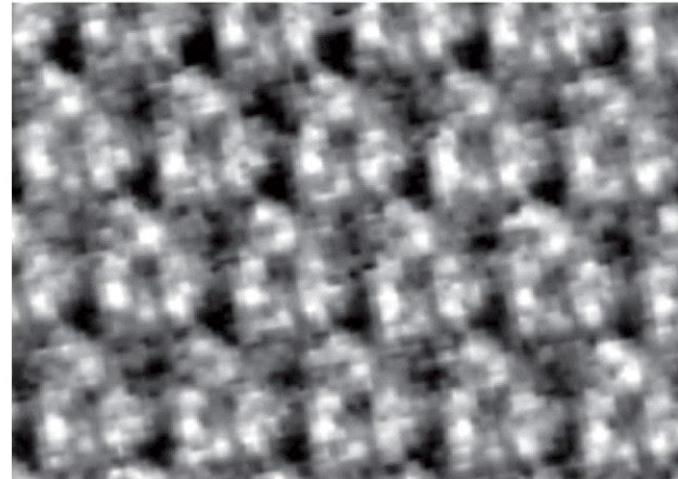


(A) patch of bacteriorhodopsin molecules



(B)

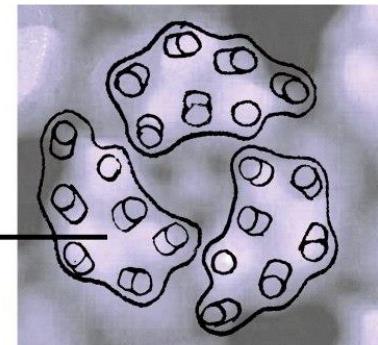
50 nm



(C)

10 nm

single bacteriorhodopsin molecule

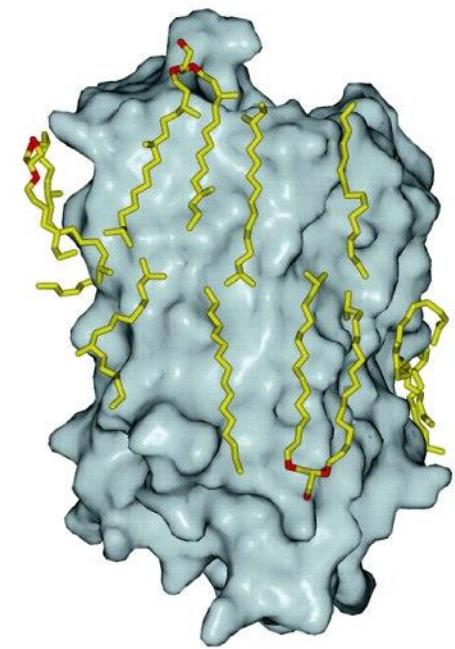
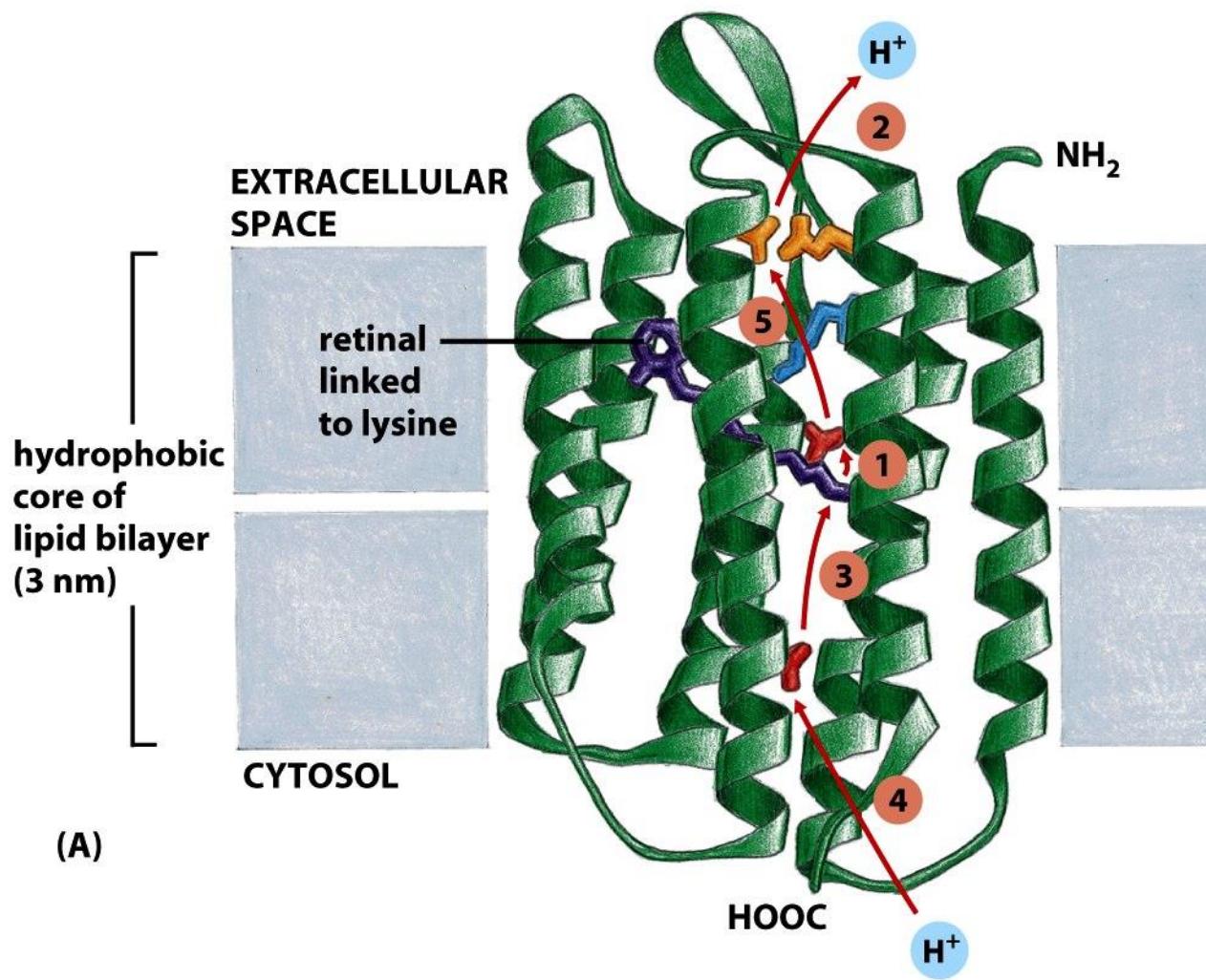


(D)

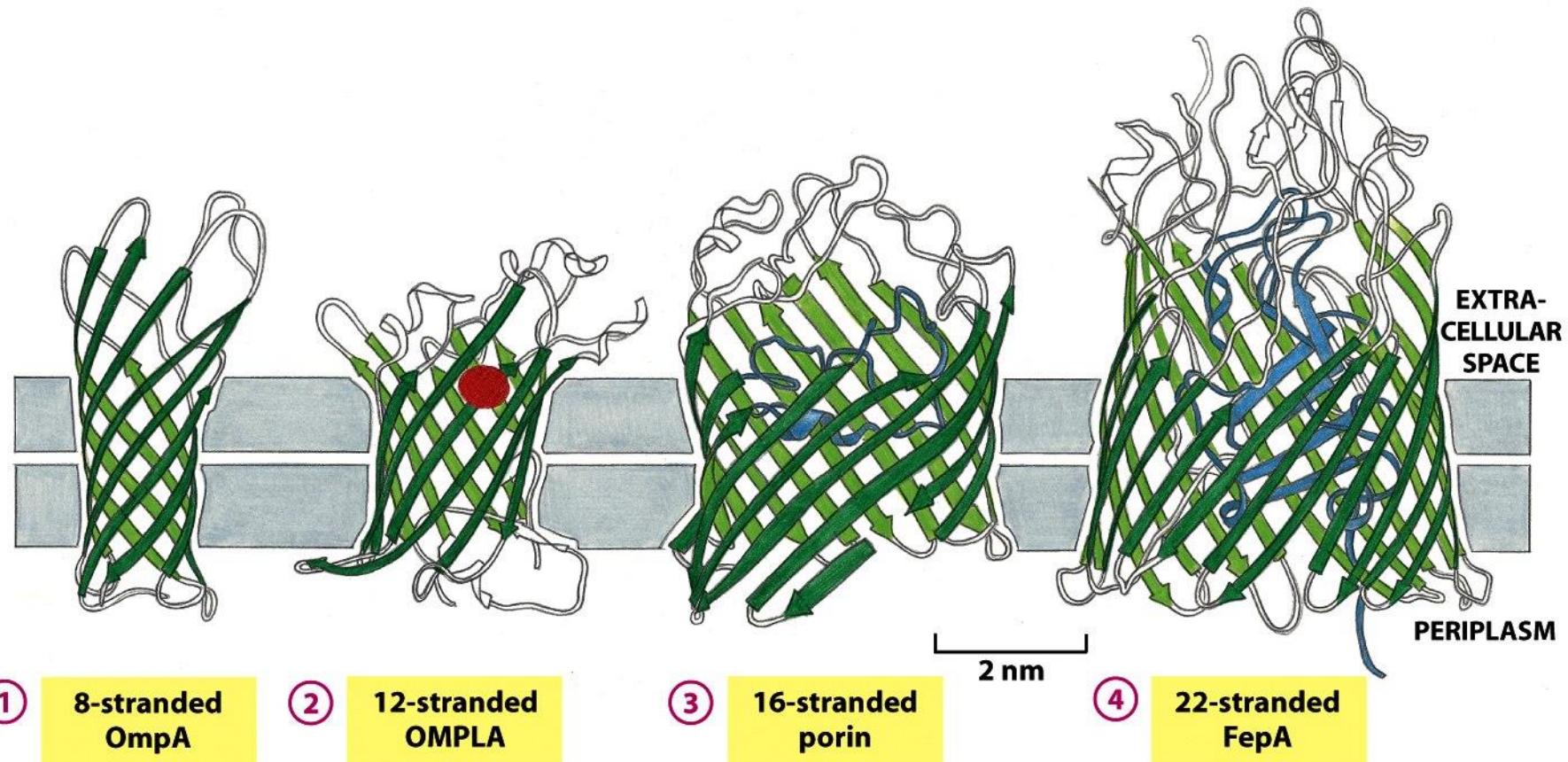
2 nm

3 monomeric Bacteriorhodopsin
Each has 7-passes Transmembrane Domains.

3-dimentional structure of bacteriorhodopsin



Different types of β -barrel



Transmembrane channel: β -barrel

- Relatively rigid, easy to be crystallized
- Conformational changes are less likely
- Abundant in outer membrane of mitochondria, chloroplast and bacteria.
- Most are **transport proteins**, such as porins, some are receptors and enzymes
- Inside barrel– polar amino acids
- Outside barrel- nonpolar amino acids
- Loops inside lumen ---selective molecules can pass