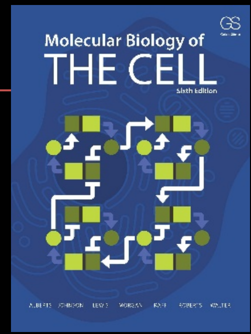
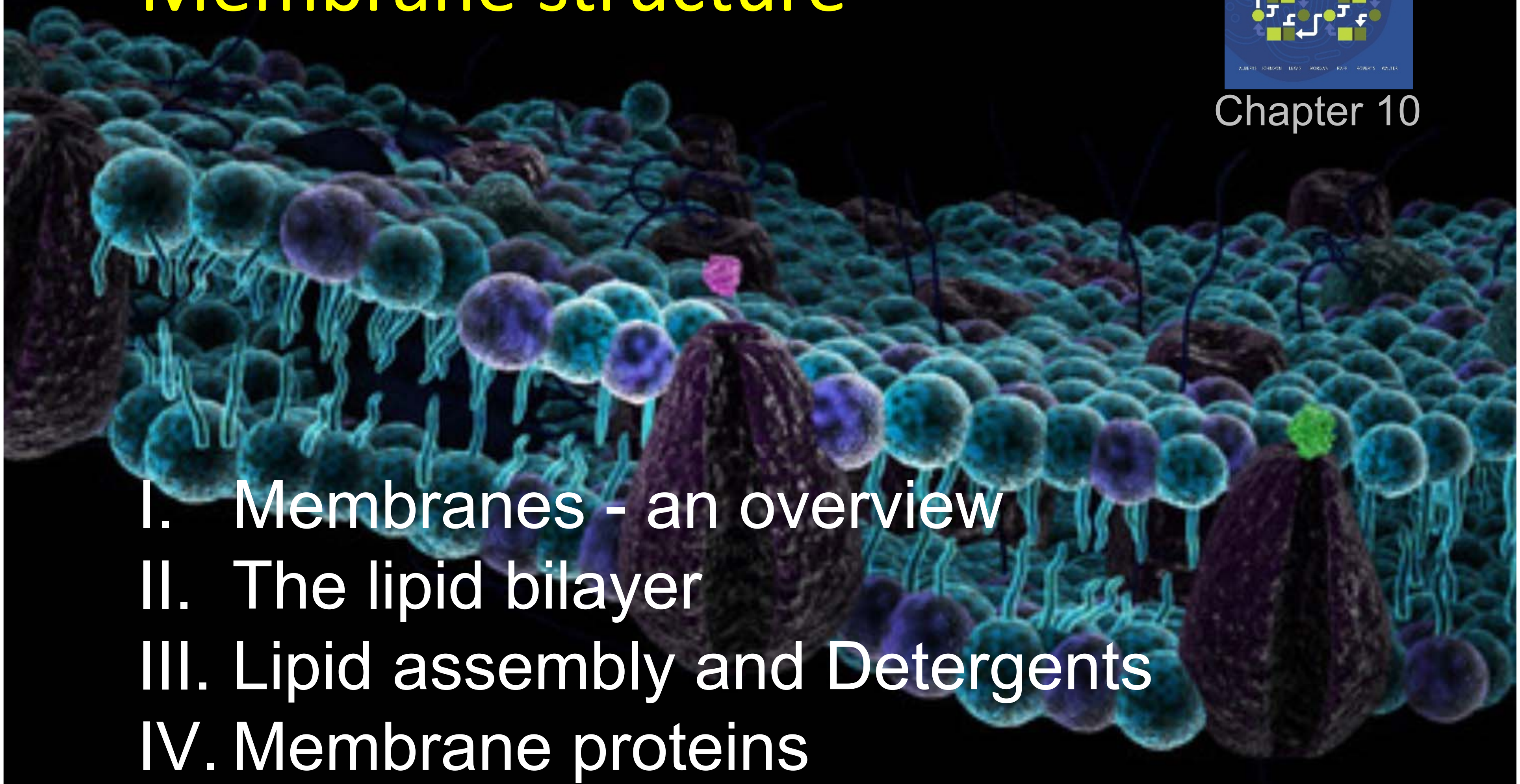
Lecture 4

Membrane structure



Chapter 10



I. Membranes - an overview
II. The lipid bilayer
III. Lipid assembly and Detergents
IV. Membrane proteins

1. General function of membranes

Membranes separate substances.

The limiting membrane of the cell is the plasma membrane (PM)

The PM separates the content of the cellfrom the environment

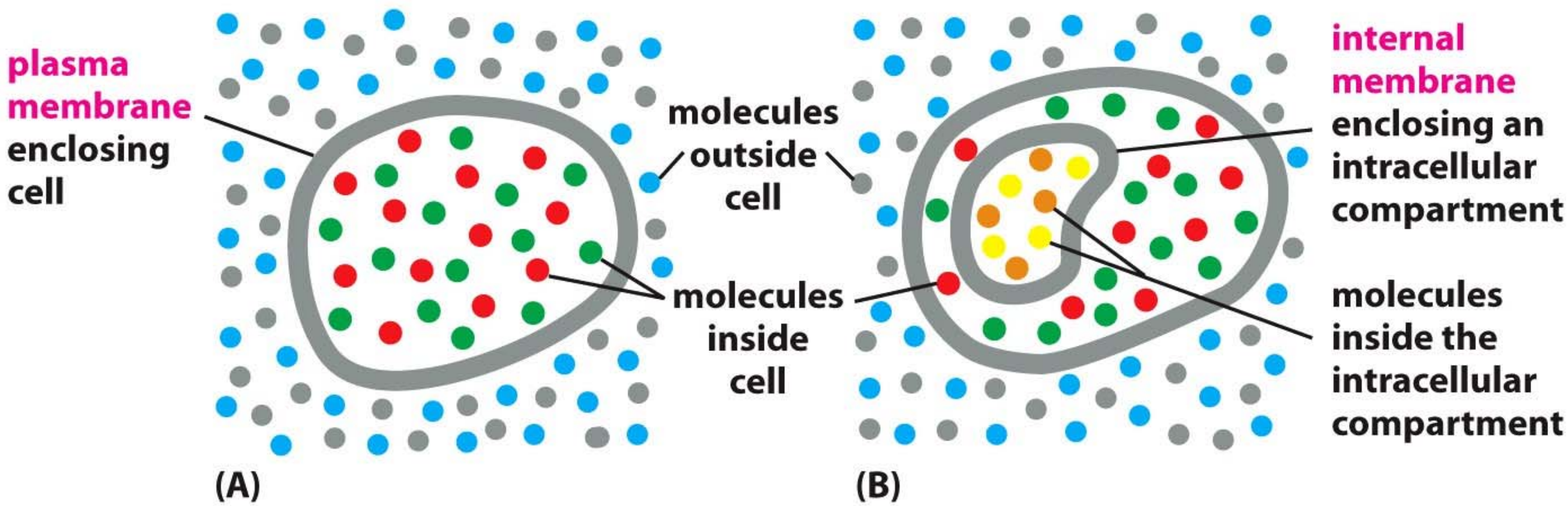


Figure 11-1 Essential Cell Biology 3/e (© Garland Science 2010)

Selective barriers allow the separation of content.....

Membranes of cells are different

Membranes are not always the same

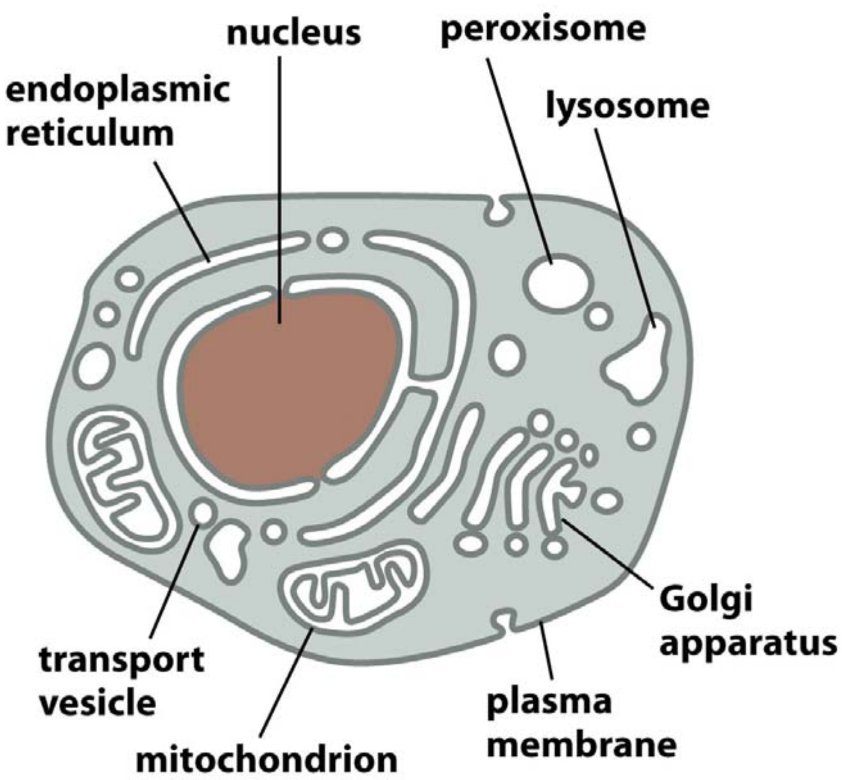


Figure 11-3 Essential Cell Biology 3/e (© Garland Science 2010)

- nucleus
  (double membrane)
- mitochondria
  (double membrane)
- Plastids (chloroplasts, etc.)
  (double membrane)

- endoplasmic reticulum
  (single membrane)
- Golgi apparatus
  (single membrane)
- peroxisome, lysosomes, endosomes, vesicles…
  (single membrane)

The plasma membrane has many functions

Features & function:

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

Functions of plasma membrane include:

- Compartmentalization (relatively impermeable barrier)
- Scaffolding (transmembrane protein connect extracellular matrix or adjacent cells to cytoskeleton)
- Gatekeeper, highly selective (selectively let some materials in and secret others out)
- Senses outside signals (receptors on the membrane signal to other proteins inside the cell)
- Energy transduction (establish ion gradients to drive ATP synthesis , or produce and transmit electric signals)

Membranes are lipid **bilayers**, they consist of **lipid and protein**

Membrane surrounds cells and
membranes surrounds organelles within the cell

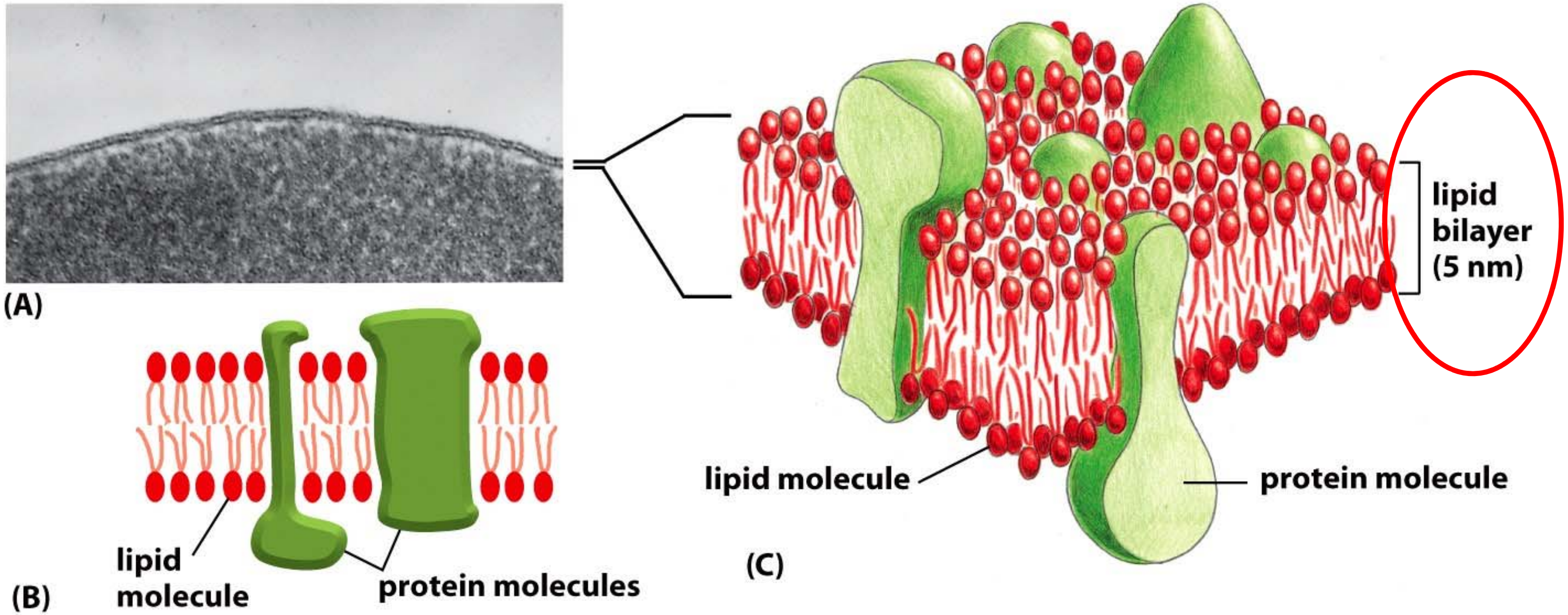


Figure 11-4 Essential Cell Biology 3/e (© Garland Science 2010)

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

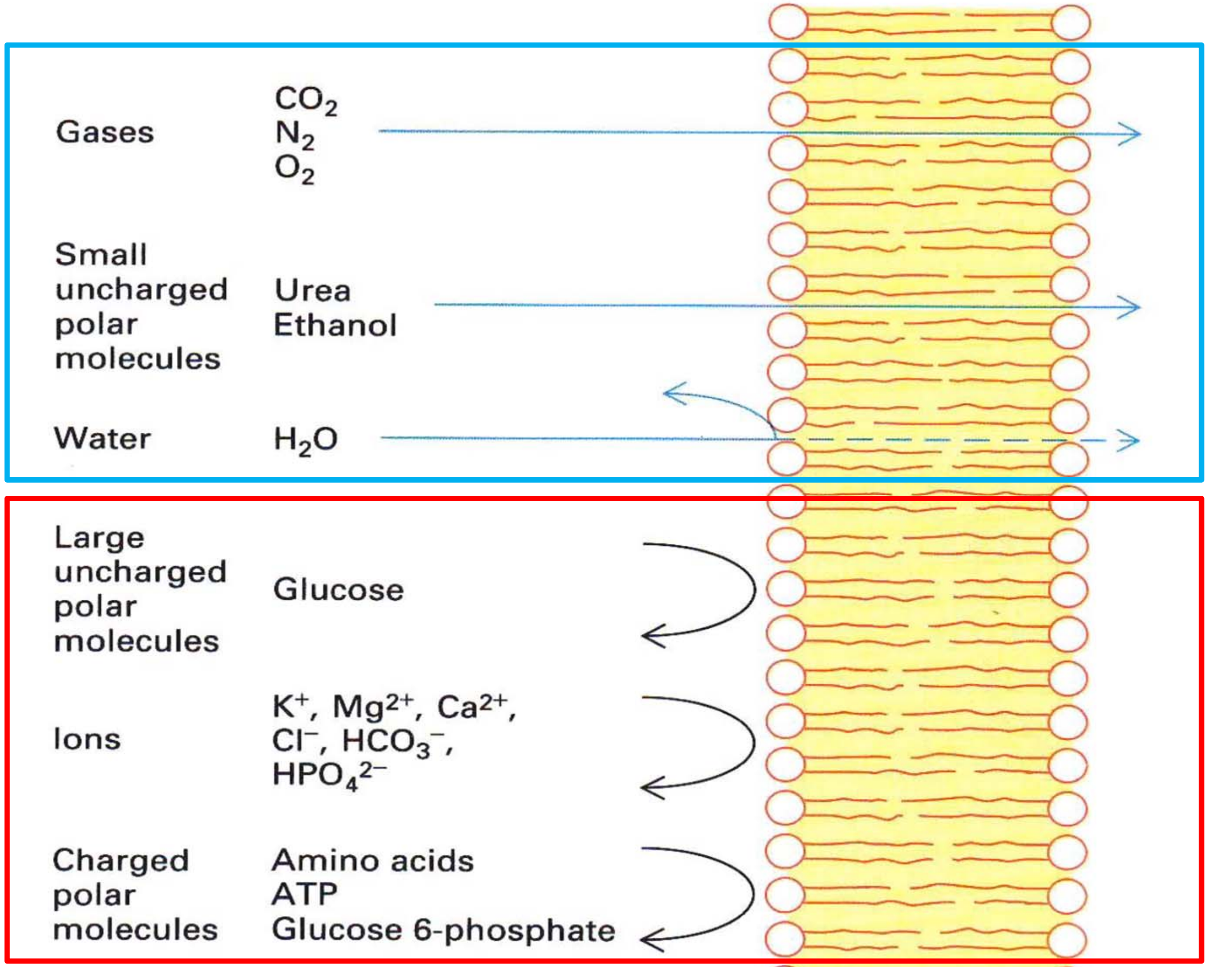
Lipid bilayer is about 5nm thick !!!

The permeability of membranes

Permeability greatly differs for different substances:

permeate

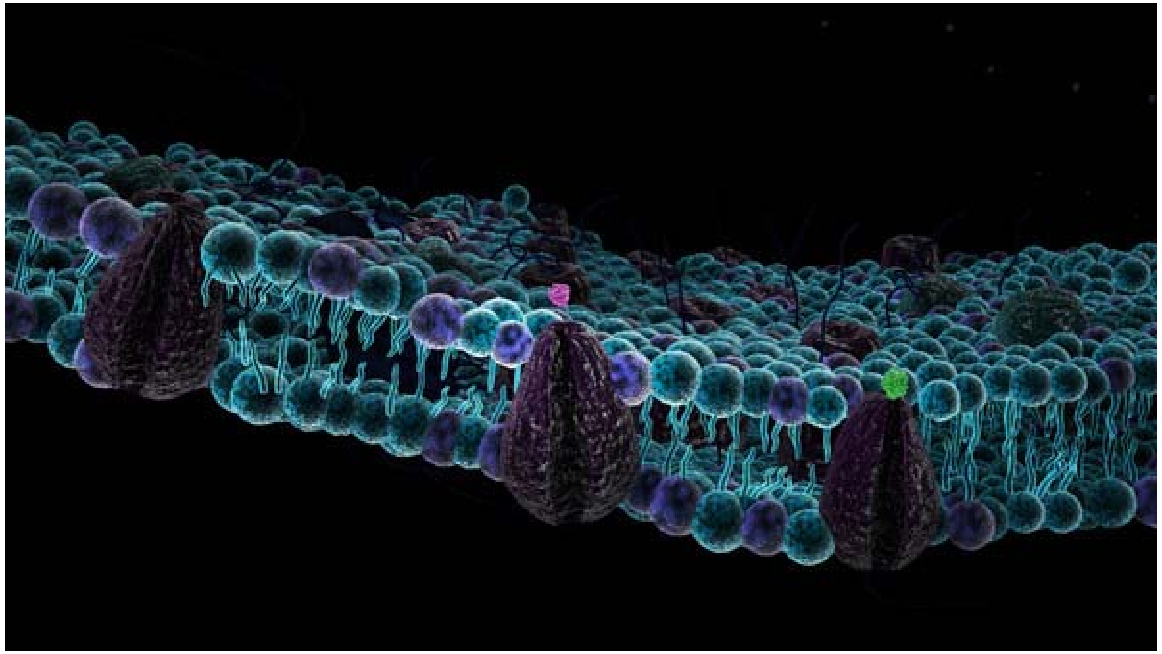
don’t permeate



Which molecules can pass and which molecules can#t?

Surface of membrane is not smooth and flat…

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



2. The lipid bilayer

A. Lipid composition of membranes
  - Phospholipids
  - Sterols
  - Glycolipids

B. Asymmetric distribution of lipids between lipid leaflets of a membrane

C. Concept of “lipid raft” domains

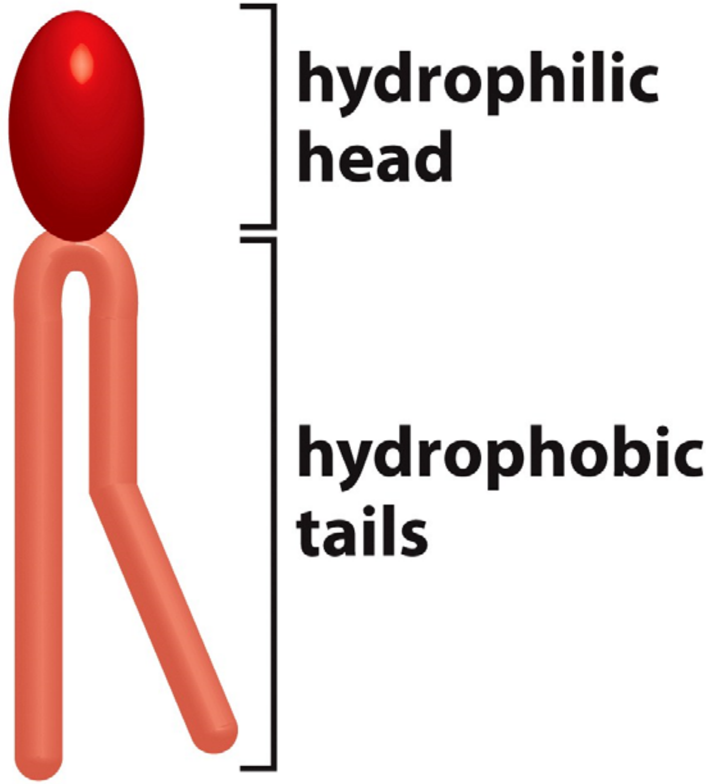
D. Motions of lipid molecules

E. Phase transition

F. Lipid storage in cells

A. Lipid composition: Lipids? What is a lipid?

Lipids are amphipathic molecules



“water-loving”

“water-fearing”

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

Major types of lipids

Membranes contain different types of lipids

There are **three major types of** lipids:

- **Phospholipids** (main components)
- **Sterols**
- **Glycolipids**

Table 10–1 Approximate Lipid Compositions of Different Cell Membranes

LIPID	PERCENTAGE OF TOTAL LIPID BY WEIGHT					
	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM	*E. COLI* BACTERIUM
Cholesterol	17	23	22	3	6	0
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

Phospholipids

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

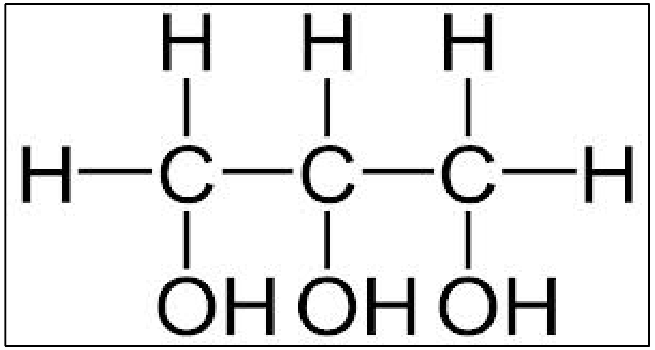
Lipid composition defines the properties of membranes (viscosity, curvature…)

Phospholipids:

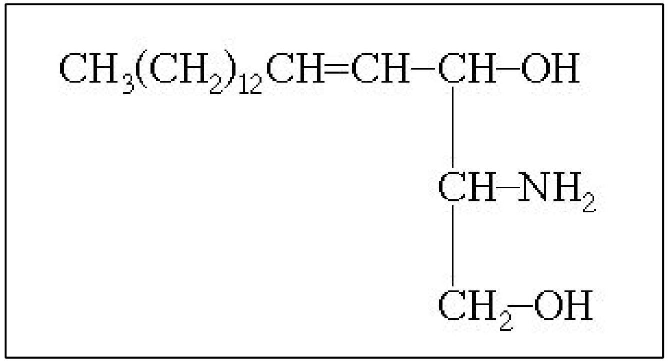
Two major groups of phospholipids, based on the backbone:

1. Phosphoglycerides: **glycerol** as backbone; main phospholipids
2. Sphingomyelins: **sphingosine** as backbone

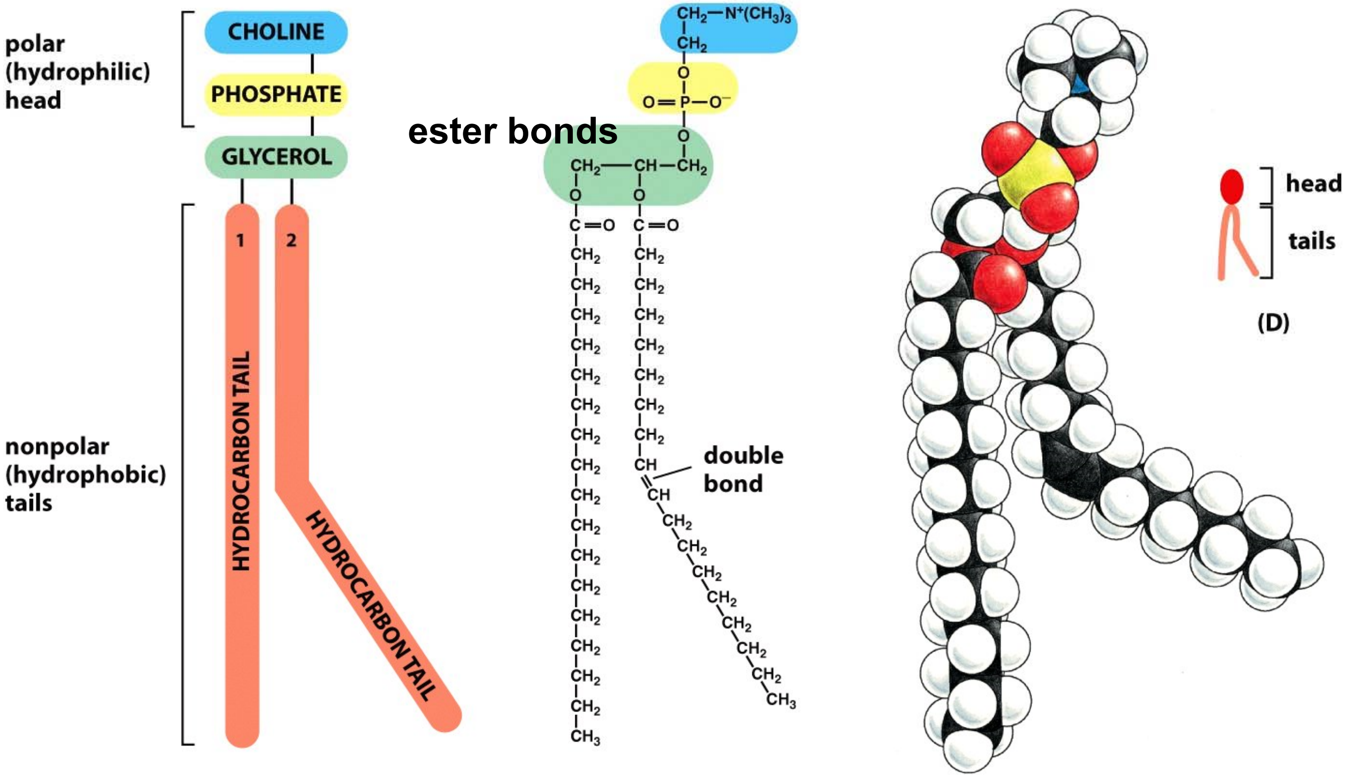
Glycerol



Sphingosine

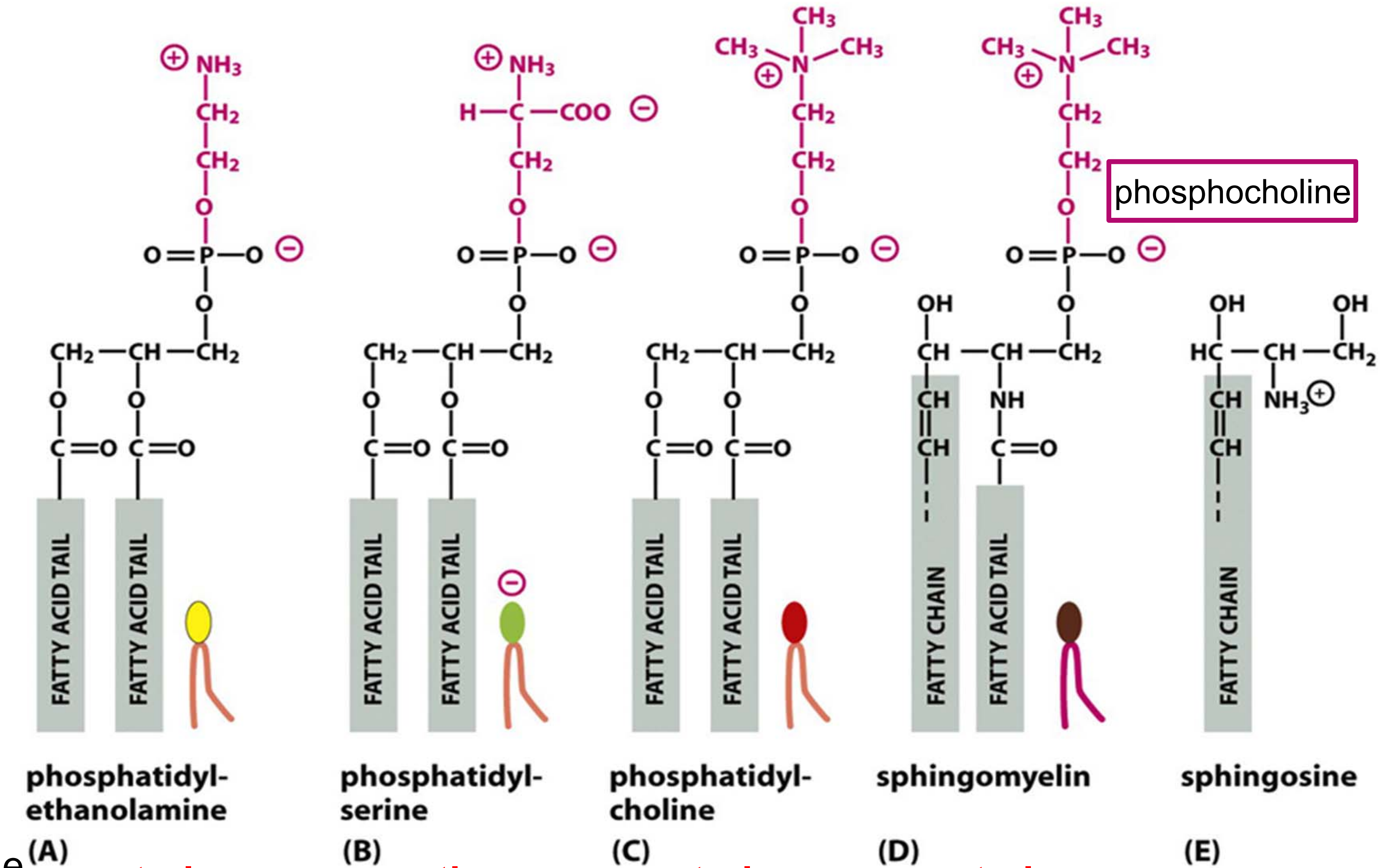


The phosphoglyceride molecule



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) The ***cis*-double bonds** create **kinks** in the tail, and **make the lipid more fluid**.

Four major types of phospholipids of the PM



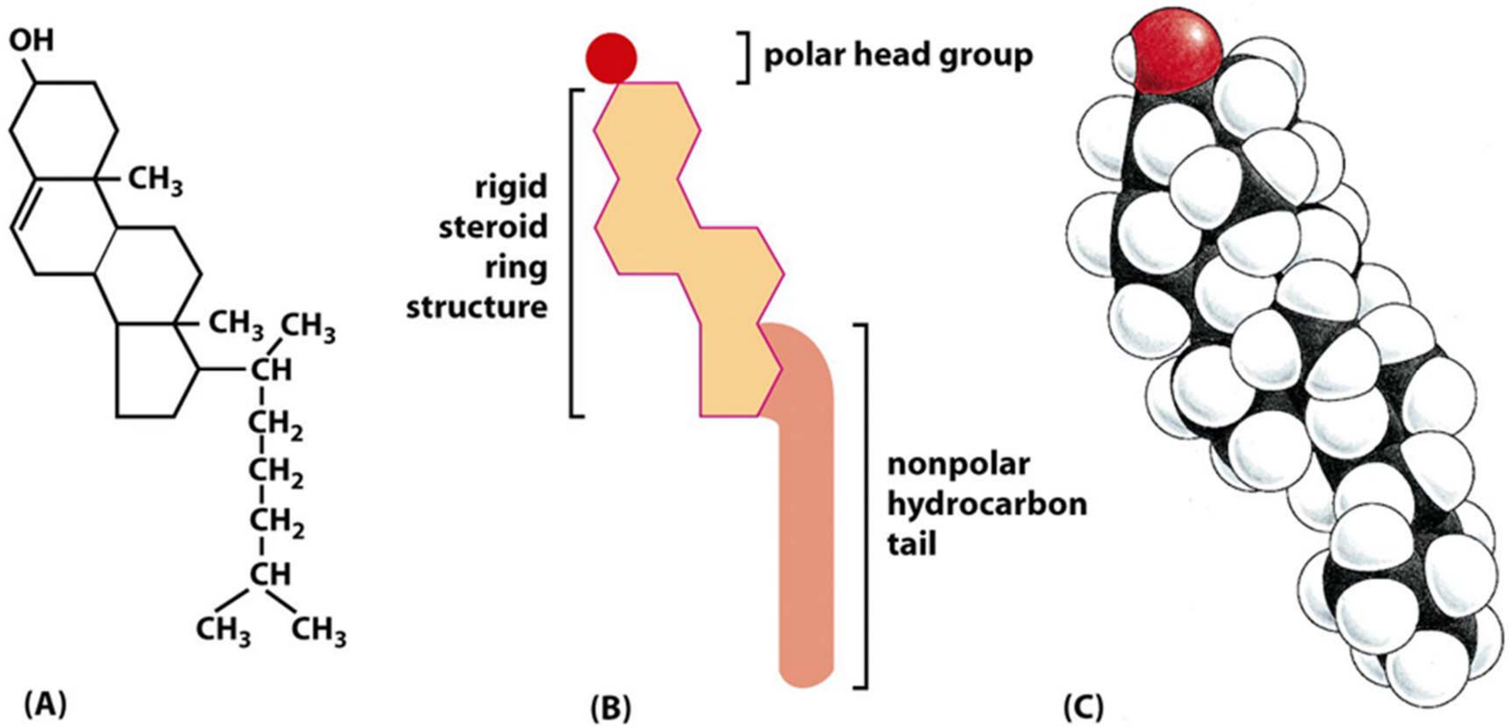
Charge state : **neutral** **negative** **neutral** **neutral**

Phosphatidyethanolamine, phosphatidylserine, phosphatidylcholine and sphingomyelin make up to 50% of the mass of all lipids in a mammalian cell!

Sterols

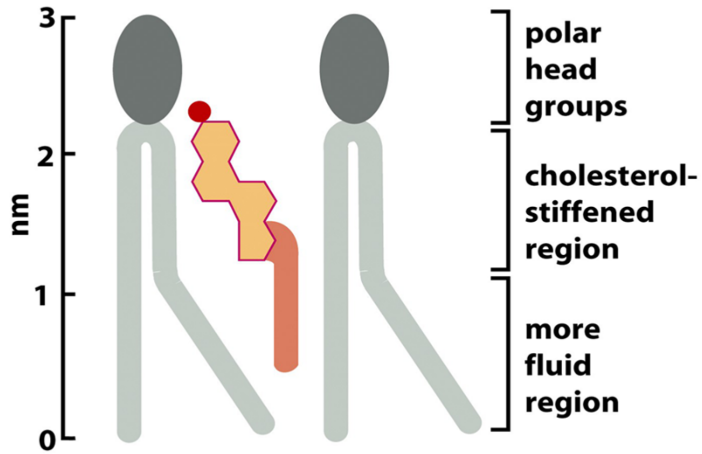
In animals: cholesterol
In plants: phytosterol
In fungi: ergosterol

All sterols have the similar 4-ring isoprenoid structure



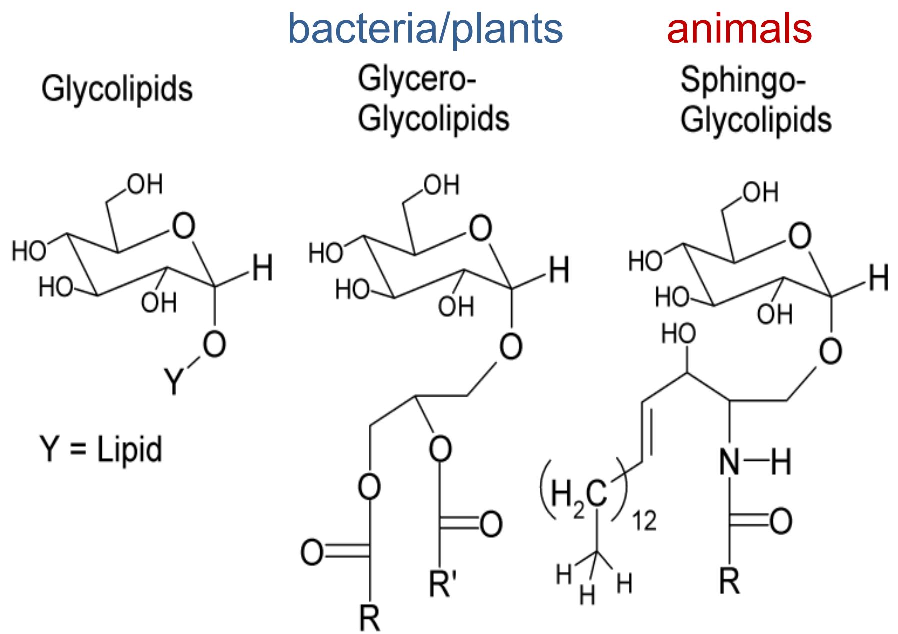
Cholesterol - regulates membrane fluidity !!!

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

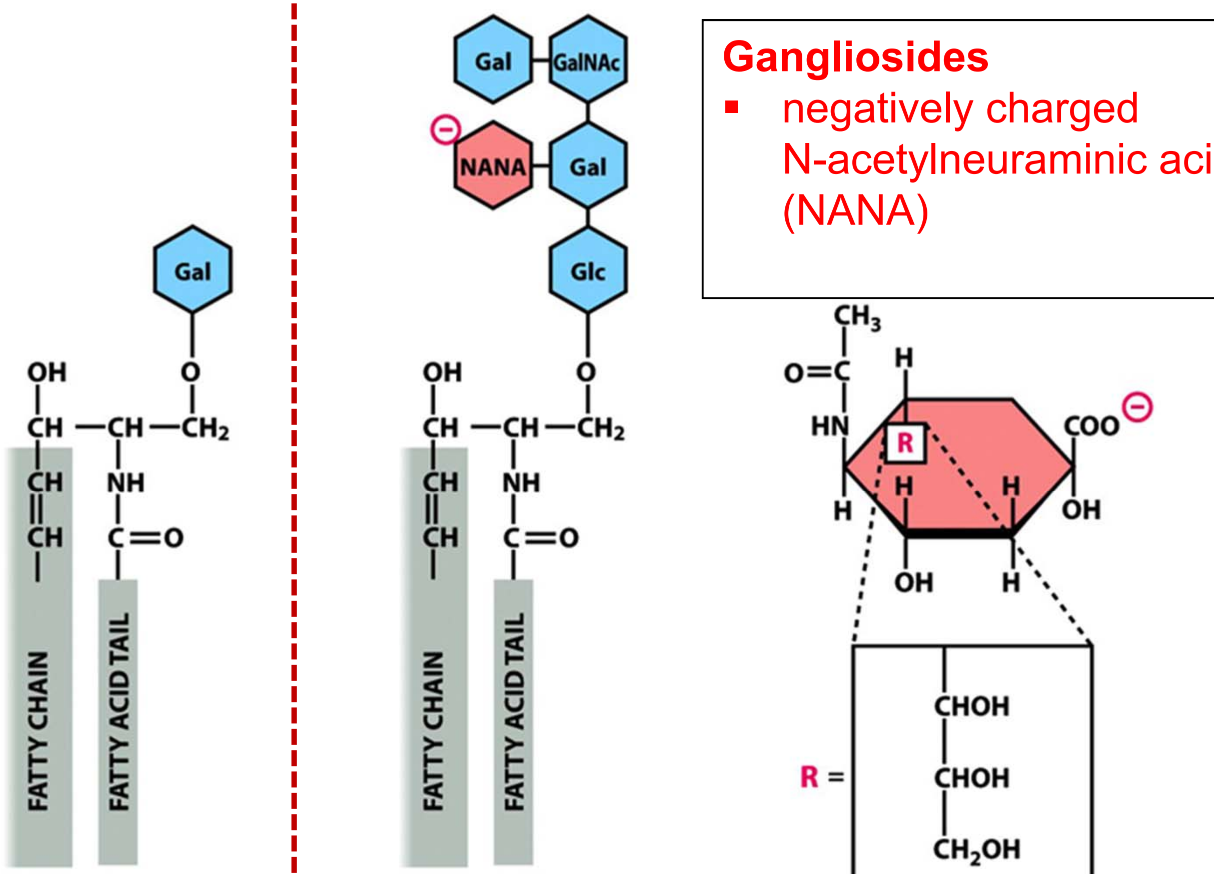


Glycolipids: sugar-containing lipids

- Present in relatively small amounts (less than 5%)
- Occur mainly in nerve cells.
- They are present on the **surface** of all **plasma membranes** (PM)
- They locate **in the outer** -non-cytosolic **monolayer** of the PM
- Sugar part projects **always outside** on the cell surface
  (Can you imagine why?)



Glycolipids: sugar-containing lipids



(A) galactocerebroside
neutral

(B) G_{M1} ganglioside
negative charge

(C) sialic acid (NANA)
negative charge

Gangliosides
- negatively charged N-acetylneuraminic acid (NANA)

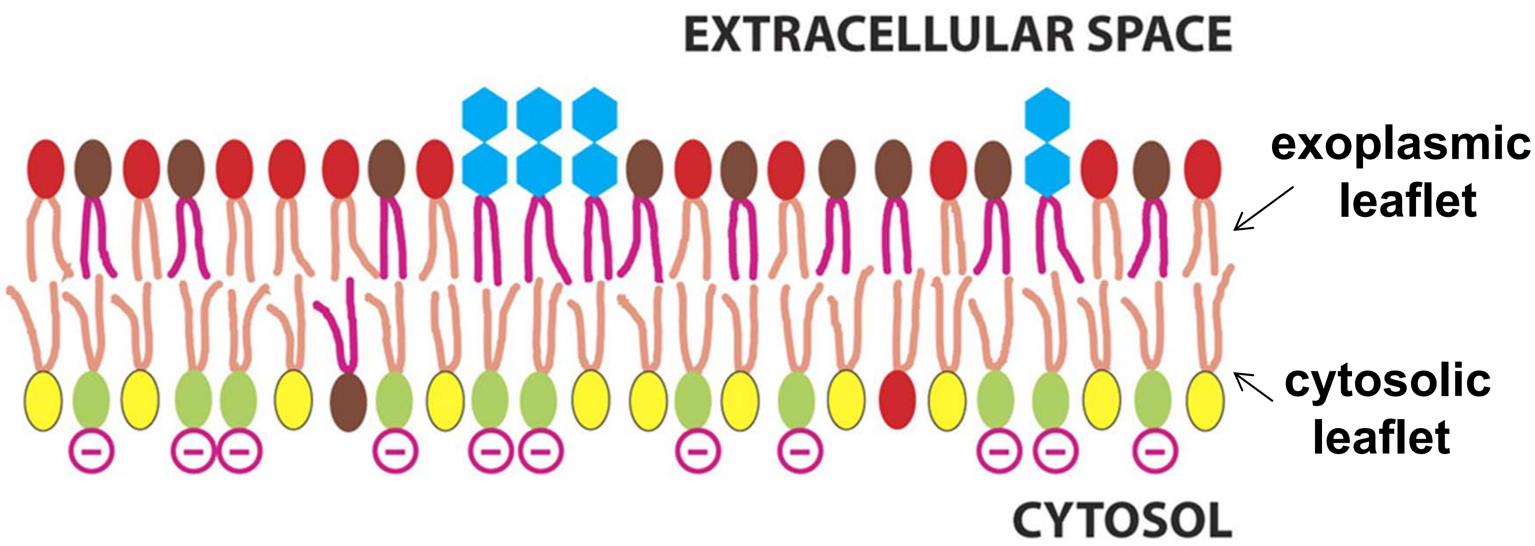
Glycolipids: Function in the cell

- Help to **protect** the cell surface/membrane against harsh conditions (pH, proteolytic enzymes)
- Function in cell **recognition** processes
- Charged glycolipids (Gangliosides) influence **electrical field** of the membrane
- Provide **entry points for toxins**:
    - Cholera toxin binds to and enters **only cells** that **have G_{m1}** on their surface (e.g. intestinal epithelial cells)
    - Cholera toxin causes **prolonged increase** of **cyclic AMP (cAMP)**, which causes efflux of **Na^+** and **water into the intestine**

Always remember function/effects of drugs and toxins !!!

B. Asymmetric lipid distribution in membrane leaflets

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



Knowledge about the distribution of lipids is crucial to understand cellular functions !!!

Asymmetrical distribution of inositol phospholipids

Why is the asymmetrical distribution of phospholipids important?

The **phosphoinositides at the inner leaflet** are **important** for **signal transduction** processes:

They are **modified** (phosphorylated/dephosphorylated/cleaved) in the course of signal transduction either by a **kinases** <u>or</u> by **lipases**:

Option 1: **Phosphorylation** by a **kinase** creates binding sites for other proteins that relay the signal

Option 2: **Cleavage** by **lipase** results signaling molecules which relay the signal

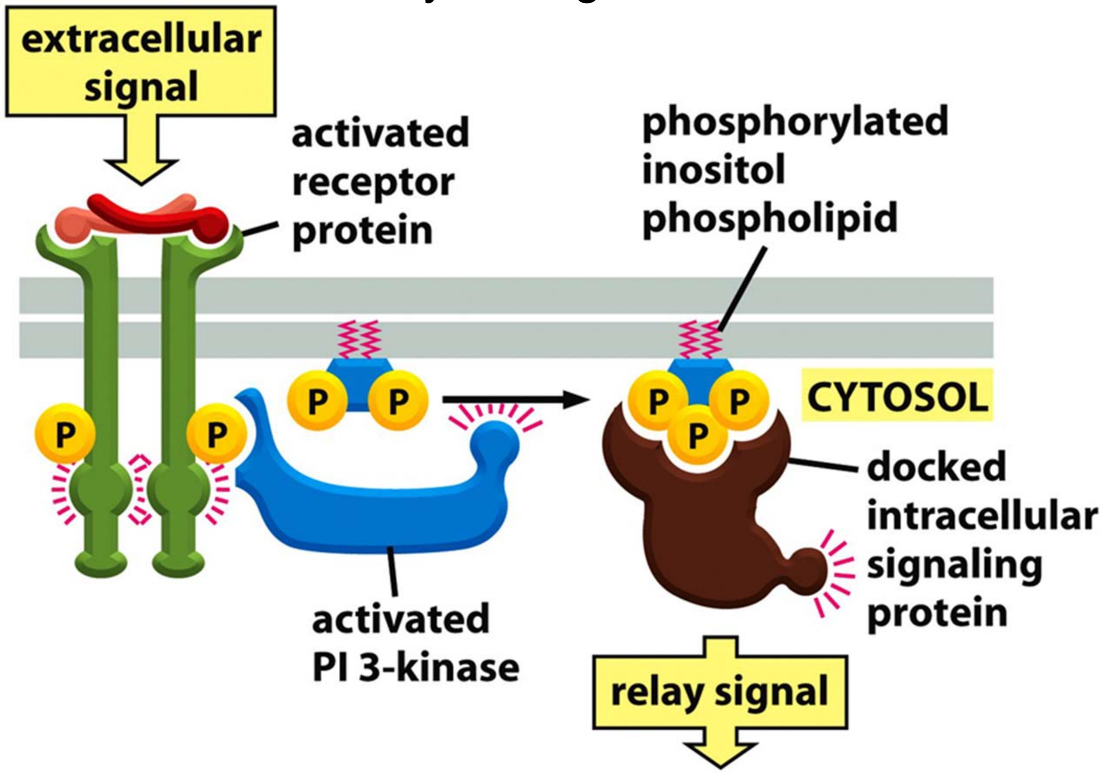
How does it work?

PRINCIPLE: Phosphorylation of PI creates membrane binding sites for other proteins

Asymmetrical distribution of phospholipids: PI

Phosphatidyl**i**nositol (**PI**) at the **inner/cytosolic** leaflet

Option 1: Phosphorylation of PI by **P**hospho**i**nositide **3**-**k**inase (**PI3K**) creates PI3P that binds other proteins that relay the signal…..

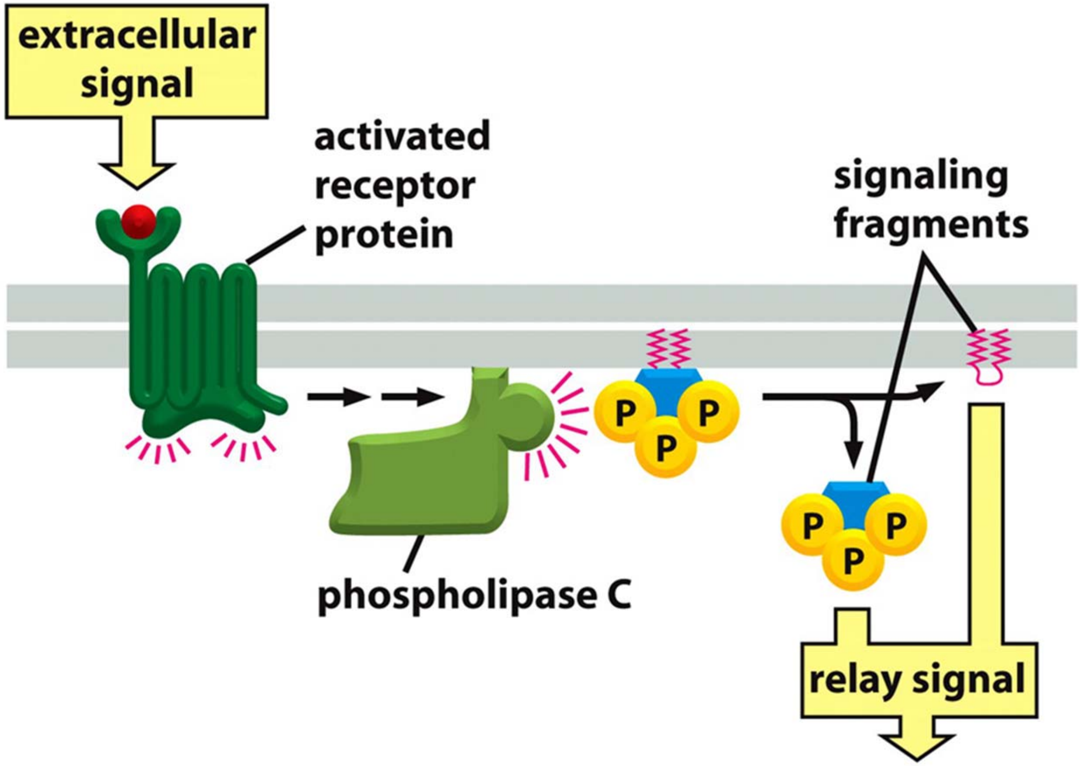


PRINCIPLE: Phosphorylation of PI creates membrane binding sites for other proteins that relay the signal…. Can also induce curvature and induce transport events…

Asymmetrical distribution of phospholipids: PI

Phosphatidyl**i**nositol (**PI**) at the **inner/cytosolic** leaflet

Option 2: **Cleavage** by **lipase** results in signaling molecules that relay the signal

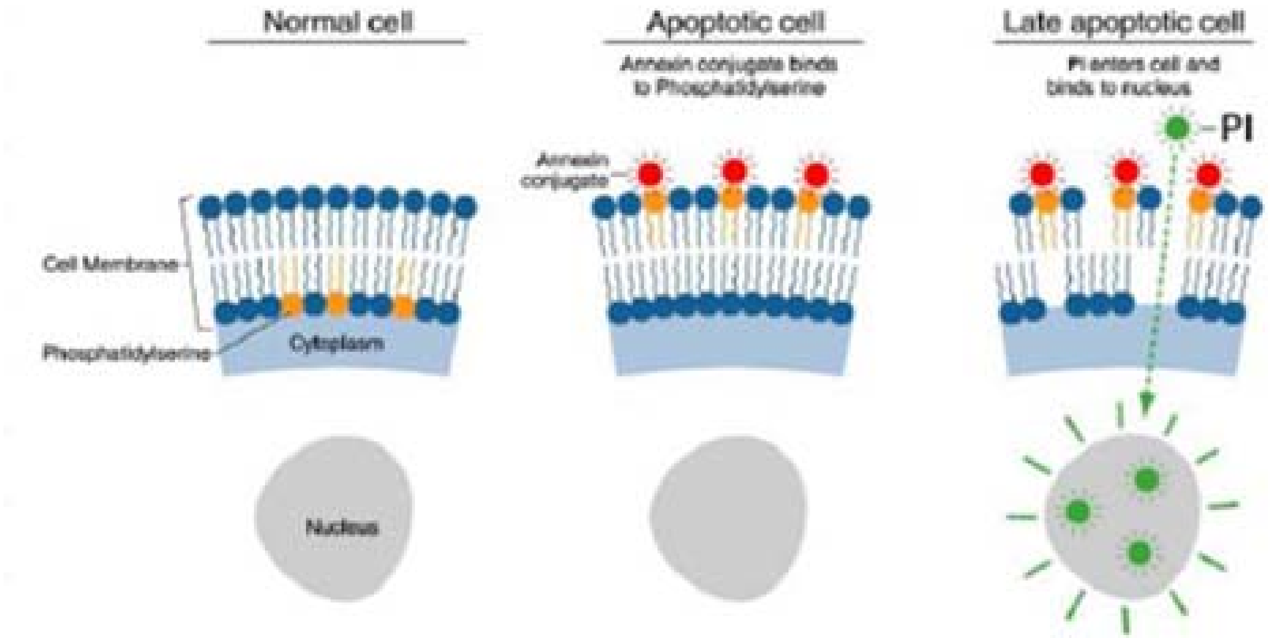


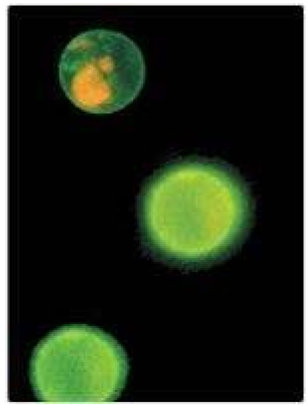
PRINCIPLE: An activated receptor recruits phospholipase C to the membrane, which cleaves phosphoinsoitides and cleaved (free) phosphoinositides relay the signal….

Asymmetrical distribution of phospholipids: PS

Phosphatidyl**s**erine (**PS**)at the inner leaflet of the PM

- Phosphatidylserine (PS) locates only on the cytosolic leaflet **but** when cells undergo programmed cell death (apoptosis), it is **translocated** to the **exoplasmic sideThere, it** can be detected by Annexin V labeling

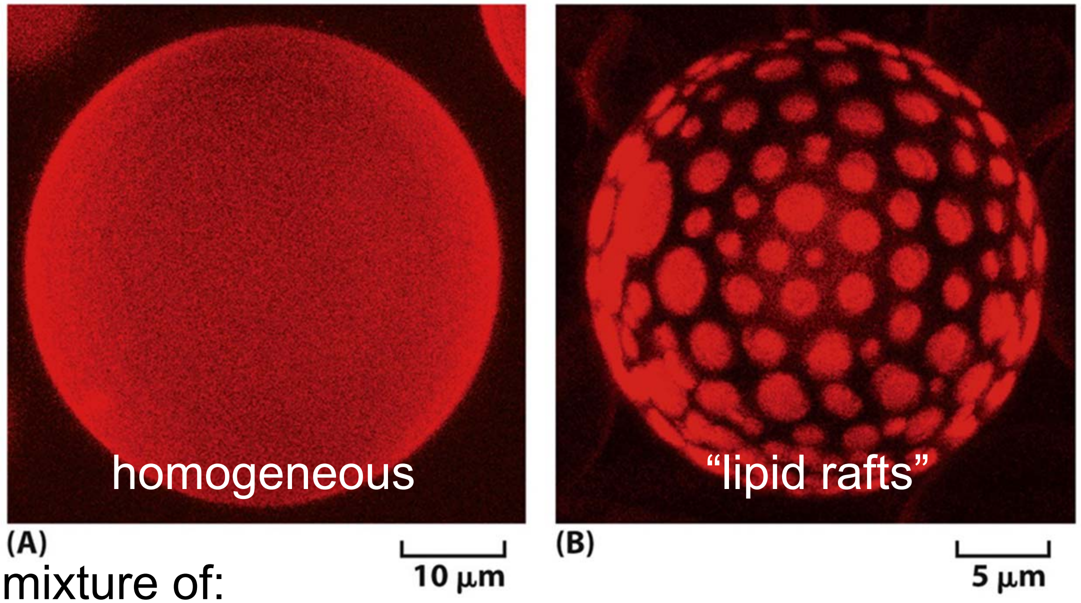




Method to identify early stages of programmed cell death (apoptosis)

C. Membranes can possess different domains: “lipid rafts”

- Lipids are **not always** homogeneously distributed
- “Lipid rafts” mainly consists od a mixture of **sphingomyelin** and **cholesterol, they are** thought to also **concentrate specific membrane proteins**



Liposome with mixture of:

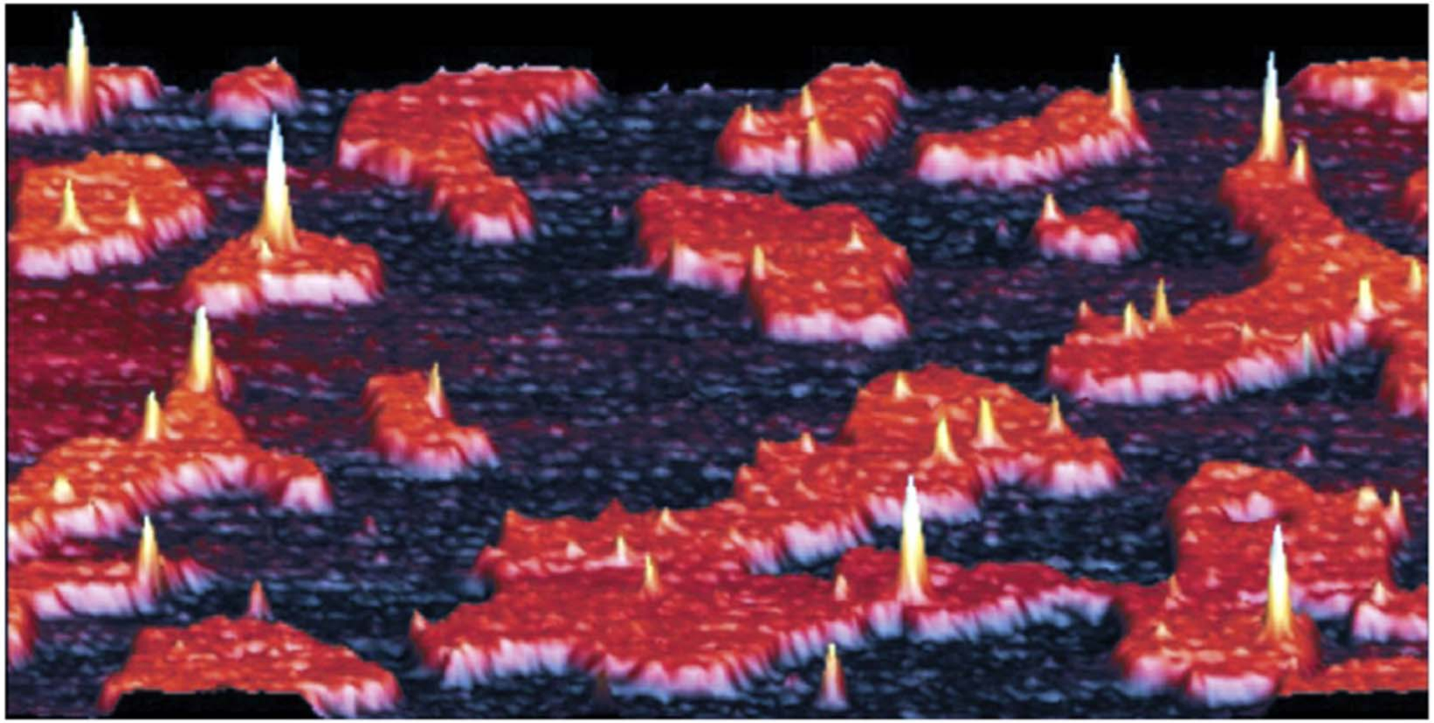
sphingomyelin: **phosphatidylcholine**
1: 1

cholesterol:sphingomyelin:phosphatidylcholin
1: 1 :1

This is an artificial bilayer….

Membrane protein locates in the lipid rafts

Lipid rafts can concentrate specific membrane domains



Atomic force microscopy of artificial membrane

500 nm

Yellow: membrane proteins
Red: “lipid rafts” (sphingomyelin and cholesterol)

Which lipids form “lipid rafts”?

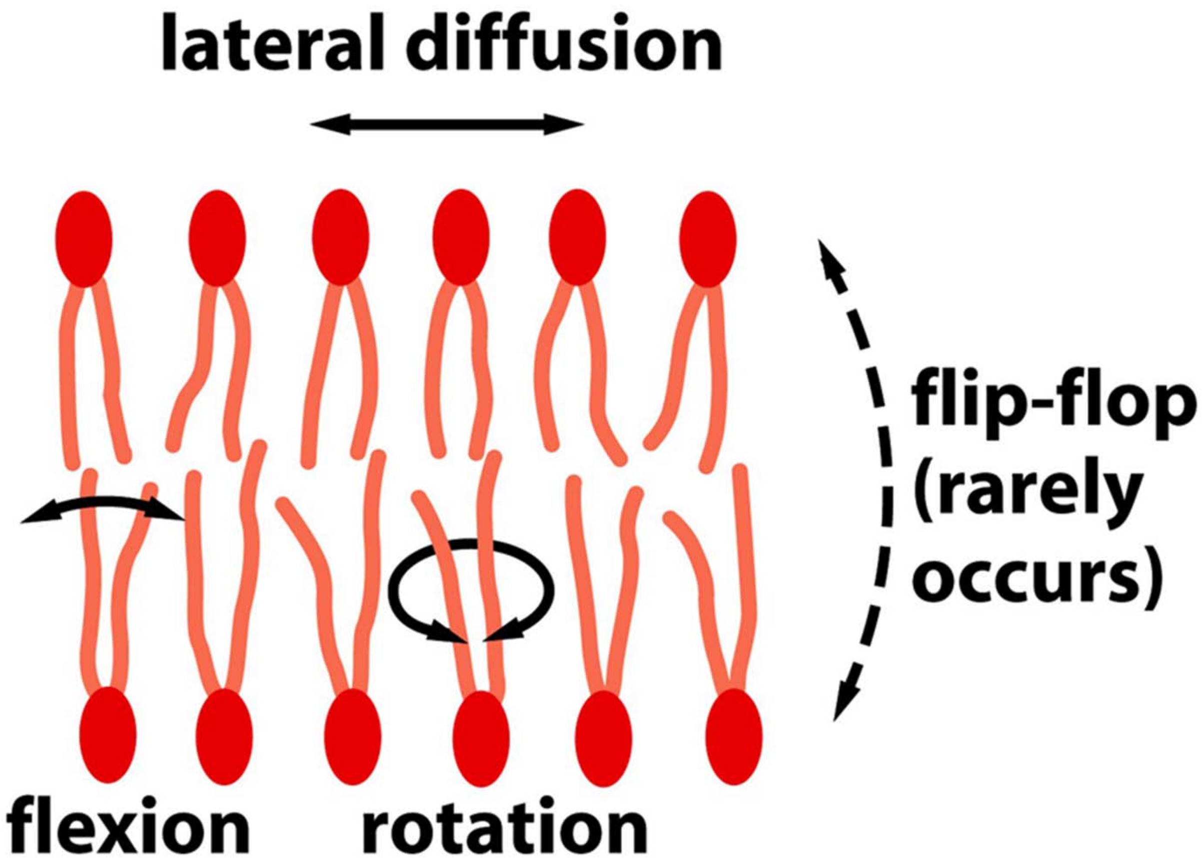
D. Motion of the lipid molecules

Lipids are highly dynamic

- **Rapidly** change their **lateral position** in the leaflet (analyzed by using FRAP)
- **Rotate** very **rapidly** along their long axis
- **Hydrophobic** tail is highly **flexible**
- **Flip-flop** (moving from one leaflet to the other leaflet) only in **very rarely**
- Flip-flop requires a special enzyme on membrane: **Phospholipid translocators** can **catalyze** the **rapid flip-flop**

What is FRAP ?

Modes and directions of motion for lipid molecules



The two-dimensional lipid bilayer is a fluid

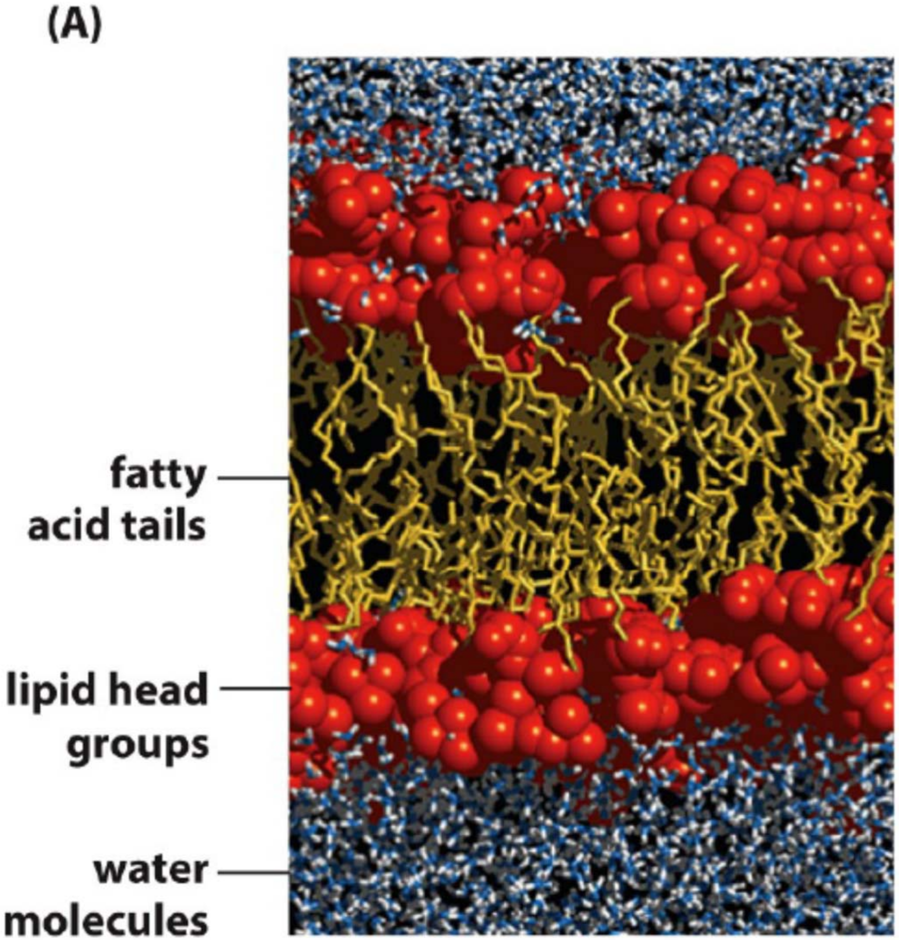


Figure 10-10 Molecular Biology of the Cell 6e (© Garland Science 2015)

The fluidity of a lipid bilayer…

… depends on its composition and temperature

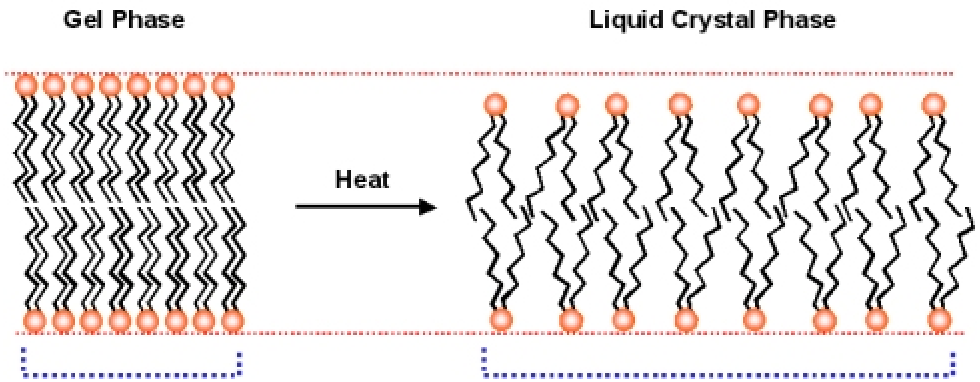
- **Short** hydrocarbon chains and **double** bonds **lower Tm**.
- Lower organisms can adjust lipid composition to keep membrane fluid at different environmental temperatures.
- **Cholesterol modulates** the property of membrane in two ways:
  - At **lower temperature/lower concentration**:
    it inserts into lipid molecules and prevents the tightening.
  - At **higher temperature/higher concentration**:
    it tightens the packing of the lipids

E. Phase transition of lipid membrane

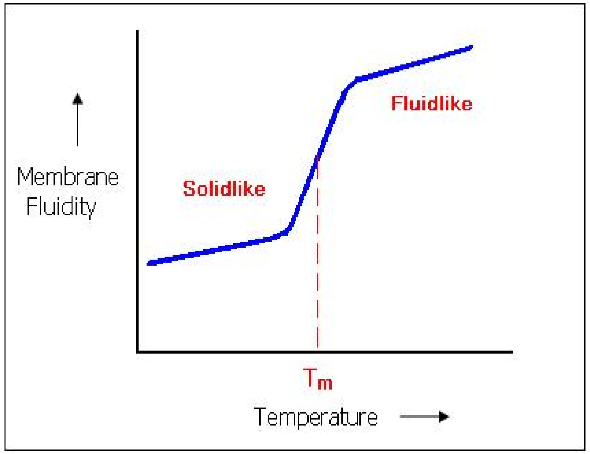
Membranes exist in different phases, dependent on the temperature

gel phase

lipid crystal phase



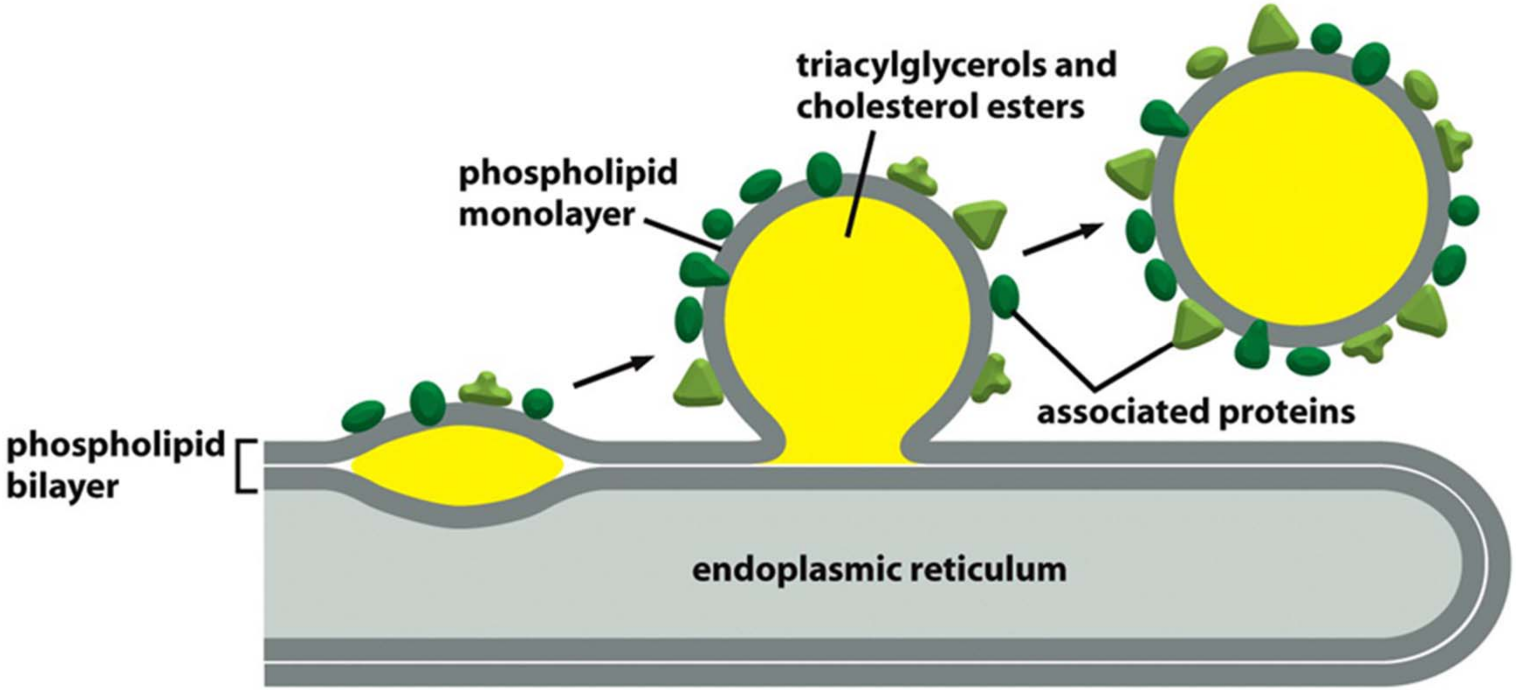
Membrane Fluidity vs. Temperature



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

F. Adipocyte-specialized cell for lipid storage

Lipid droplets are surrounded by a **lipid monolayer**

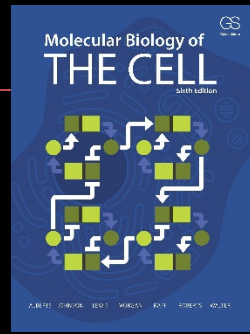


Lipid droplets:

- store neutral **triacylglycerides** and **cholesterol esters**
- They are surrounded by **monolayer** of phospholipids and a variety of proteins
- some are important for lipid metabolism.

Lecture 4

Membrane structure



Chapter 10

I. Membranes - an overview
II. The lipid bilayer
III. Lipid assembly and Detergents
IV. Membrane proteins

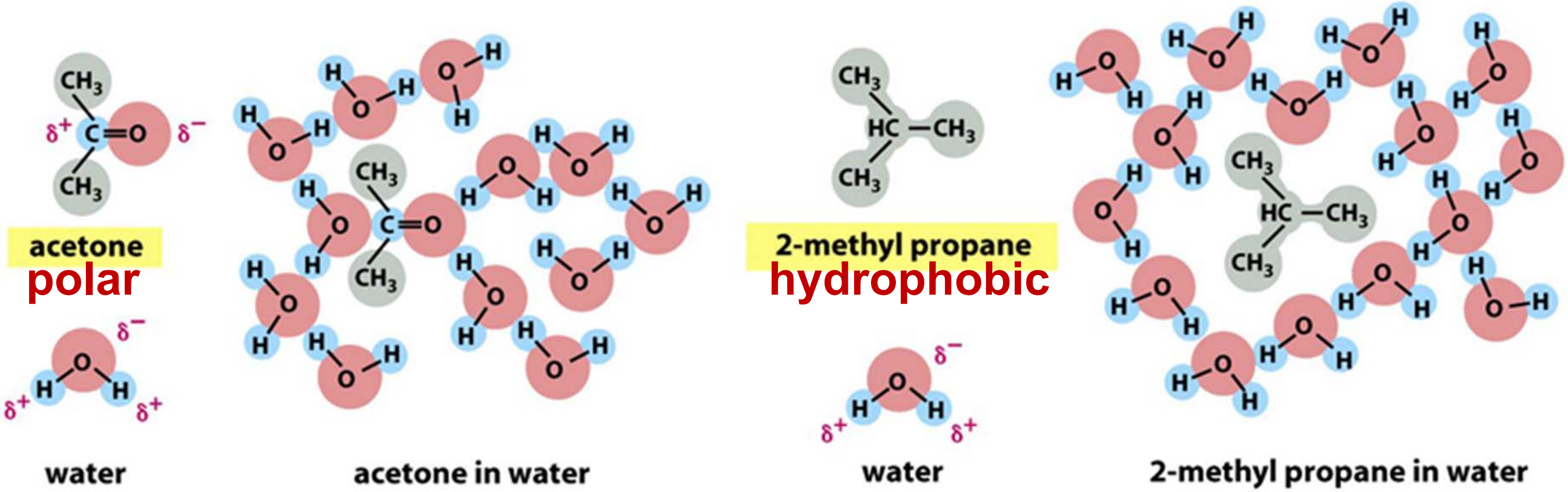
Lecture 4 Membrane structure

Outline

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

III. Lipid assembly

Why do hydrophobic molecules stay together?

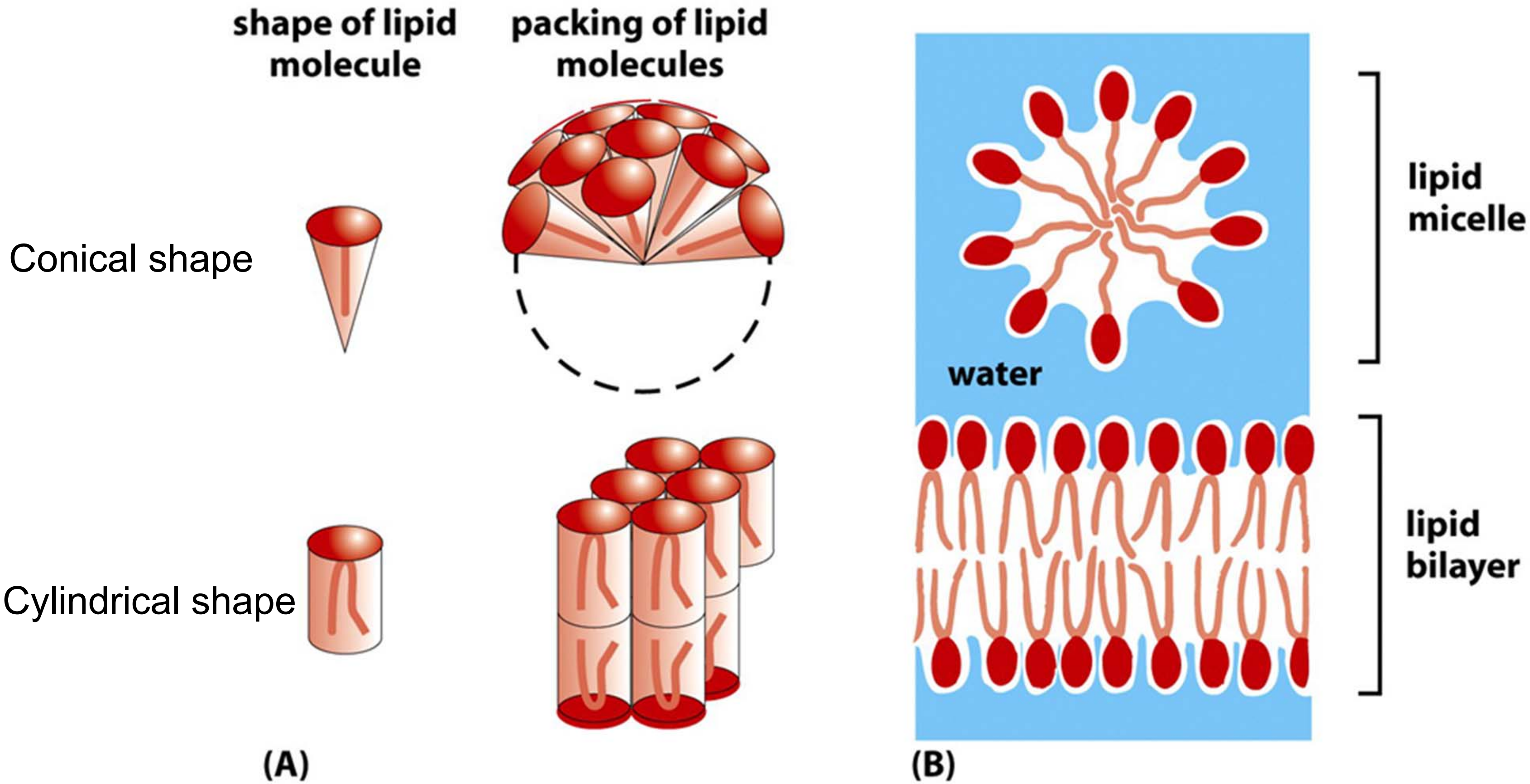


Acetone is **polar and forms electrostatic interactions**

Forces water into ice-like cages:
This causes increase in order, so multiple hydrophobic molecules stay together to minimized the increase in free energy

Formation of micelles and lipid bilayers

Micelles or lipid bilayer form spontaneously in aqueous solution



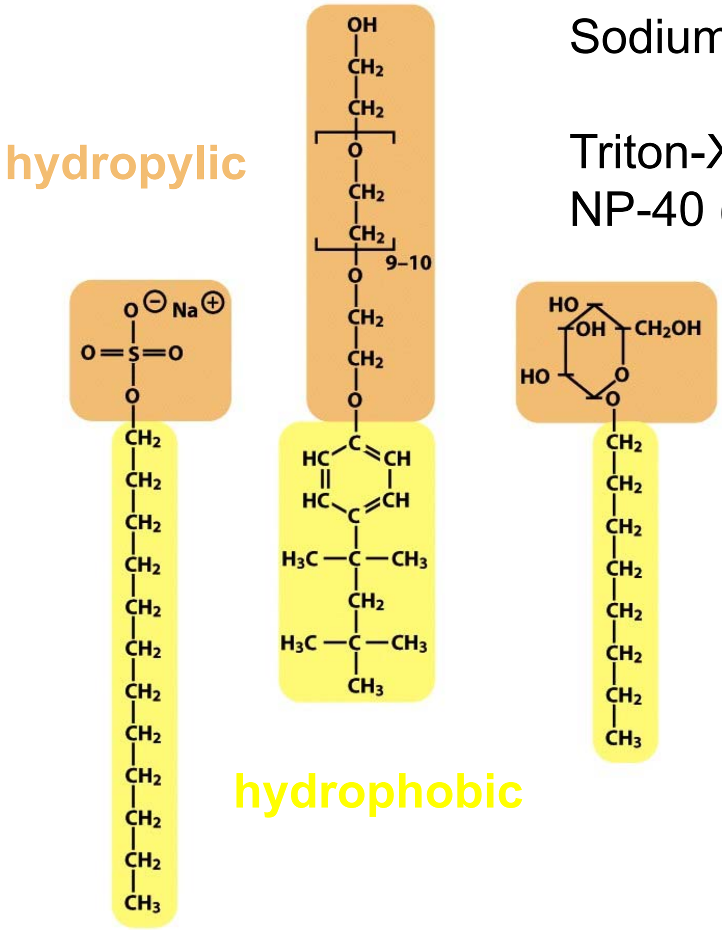
Micelle or bilayer depends on the shape of the molecule: conical versus cylindrical…

Detergents in membrane studies

Features of detergents:

- Small **amphiphilic** molecules of **variable** structure.
- **Better soluble** in water than lipids.
- Divided into **two** major groups:
  - **ionic** detergents
  - **non-ionic** detergents.
- The **hydrophobic part intercalate** into **hydrophobic parts** of **lipids** and of **transmembrane proteins**.
- The **polar group** brings **lipids or proteins into aqueous face** and make them soluble.

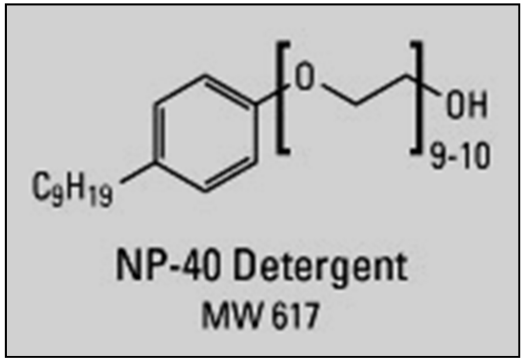
The structure of some common detergents



sodium dodecyl sulfate (SDS) | Triton X-100 | β-octylglucoside

Sodium dodecyl sulfate (SDS) (**ionic detergent**)

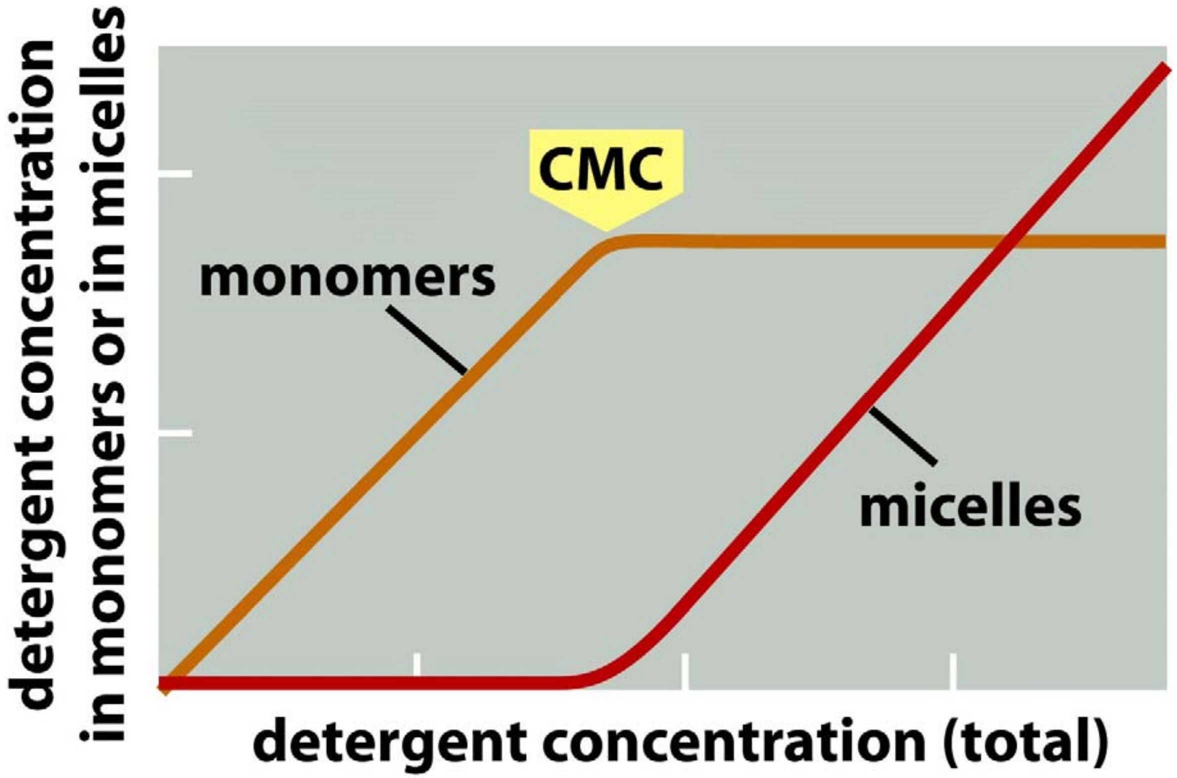
Triton-X-100 (**non-ionic** detergent)
NP-40 (nonidet-P-40) (**non-inonic** detergent)



Detergents are essential to solubilize membrane proteins

Detergents form micelles

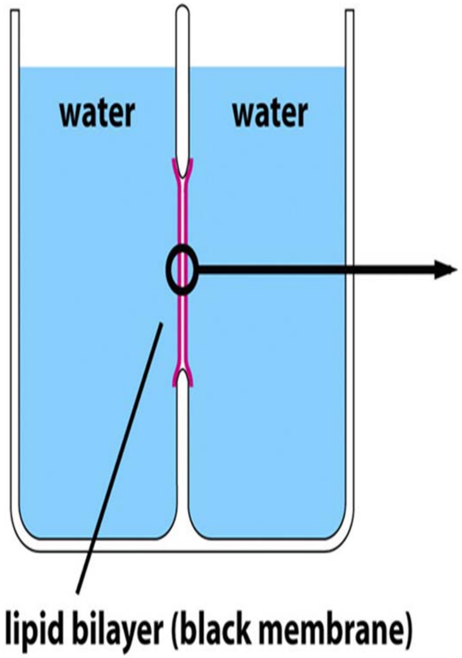
<u>C</u>ritical **<u>m</u>**icelle **<u>c</u>**oncentration (**CMC**) is the cncentrations at which detergents aggregate to **form micelles**

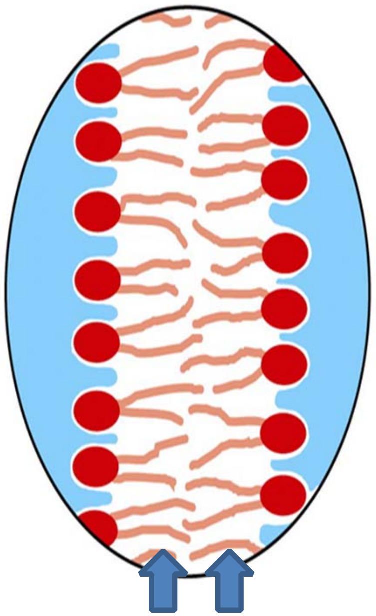


Black membranes and liposomes

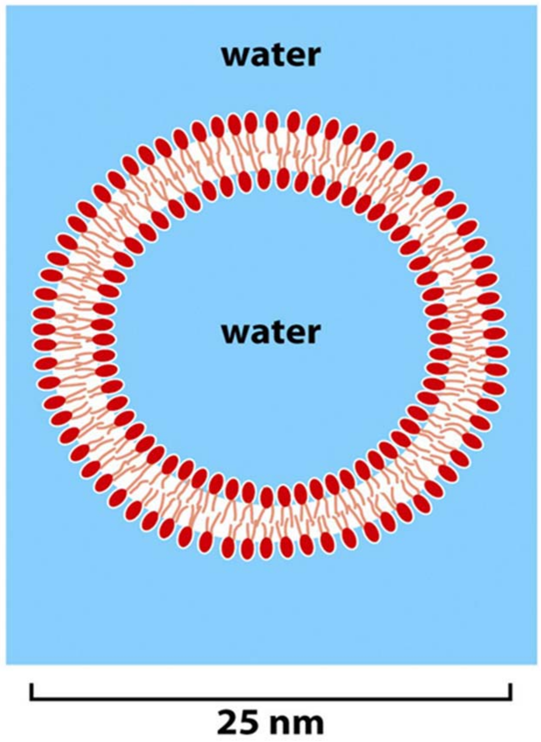
Black membranes are **planar lipid bilayers** (they appear black when they separate two aqueous compartments)

Liposomes are **spherical lipid bilayers**





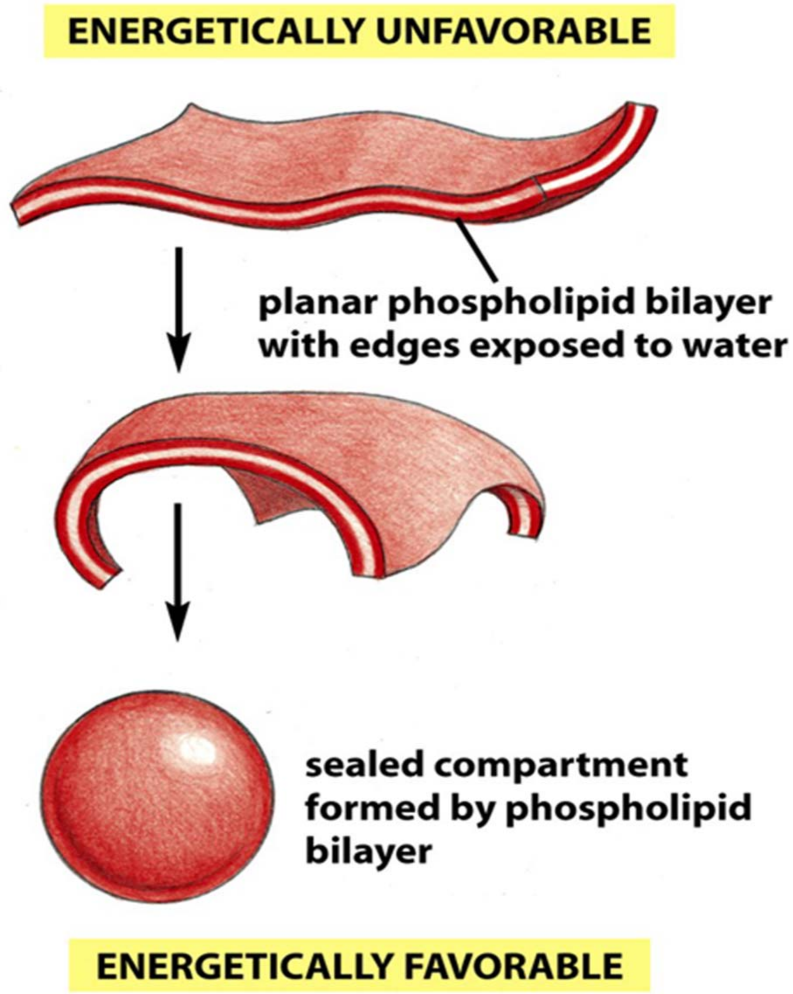
Each monolayer is a leaflet



WHAT is the difference between a liposome and a micelle?

How does a spherical lipid bilayer form?

Spontaneously

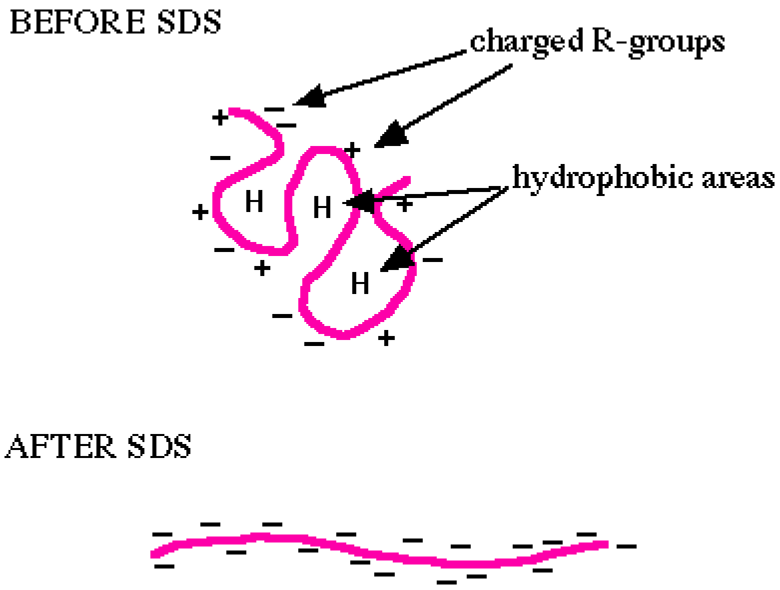


Edges are hydrophobic, what do hydrophobic domains in an aqueous environment?

Detergents in membrane studies

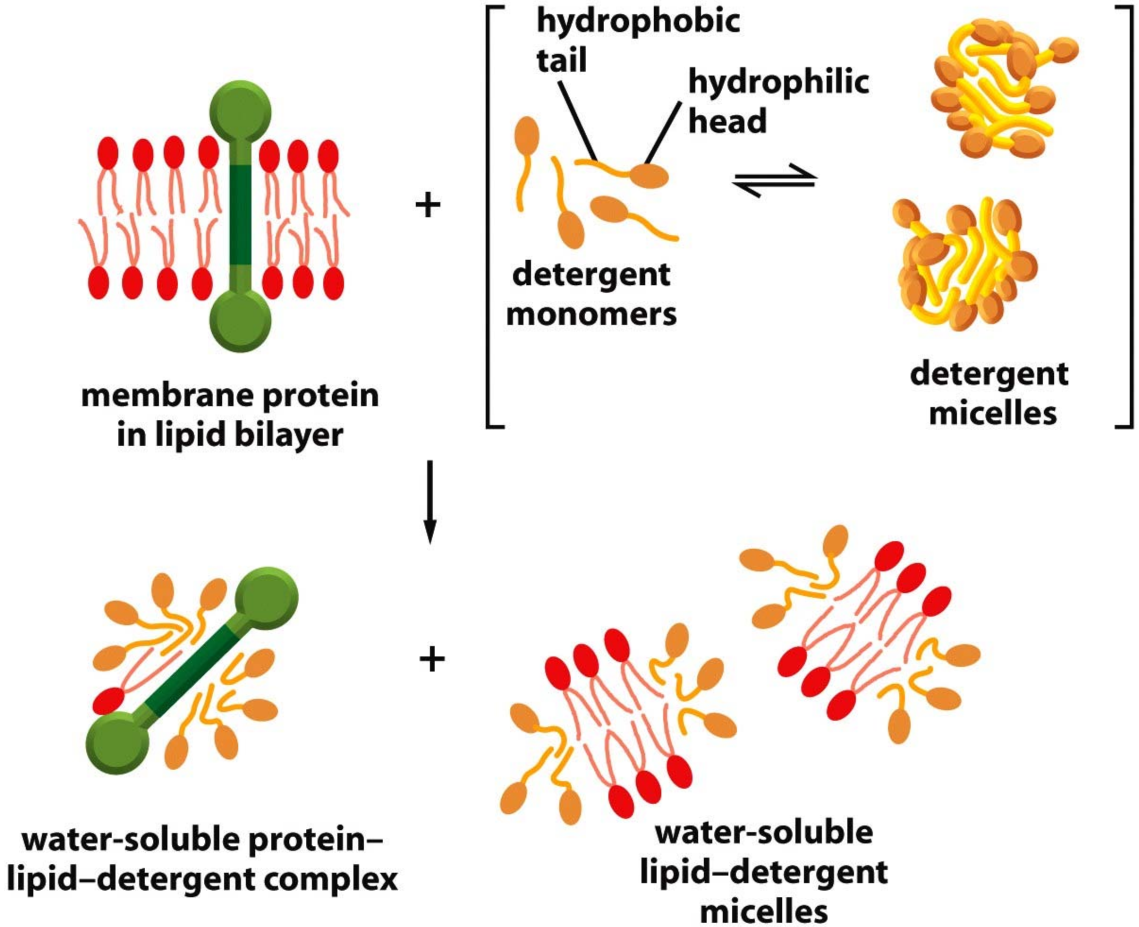
- Small amphiphilic molecules of variable structure.
- More soluble in water than lipids.
- Divided into ionic and non-ionic detergents.
- The hydrophobic part intercalate into hydrophobic parts of lipids and transmembrane proteins.
- The polar group bring lipid or protein into aqueous face and make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

SDS- solubilization of protein

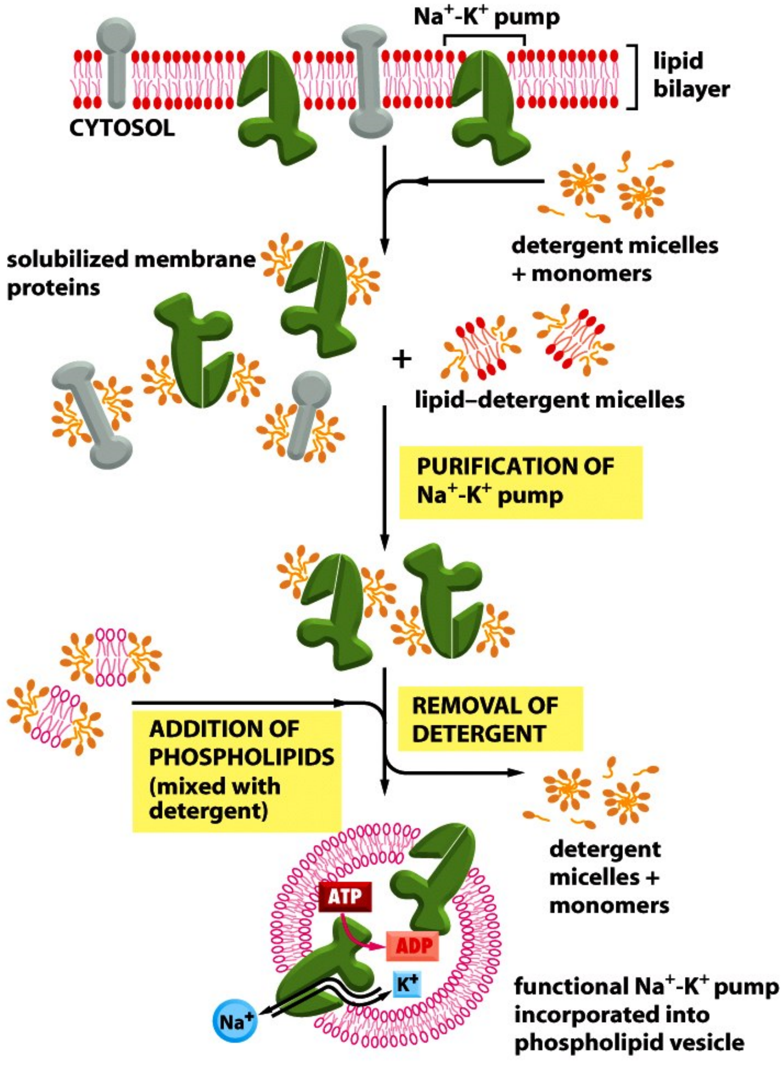


SDS fully denature protein.
Cover protein molecules with negative charges.
Dependent on the protein molecular weight, proportional amount of SDS are covered on protein.

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



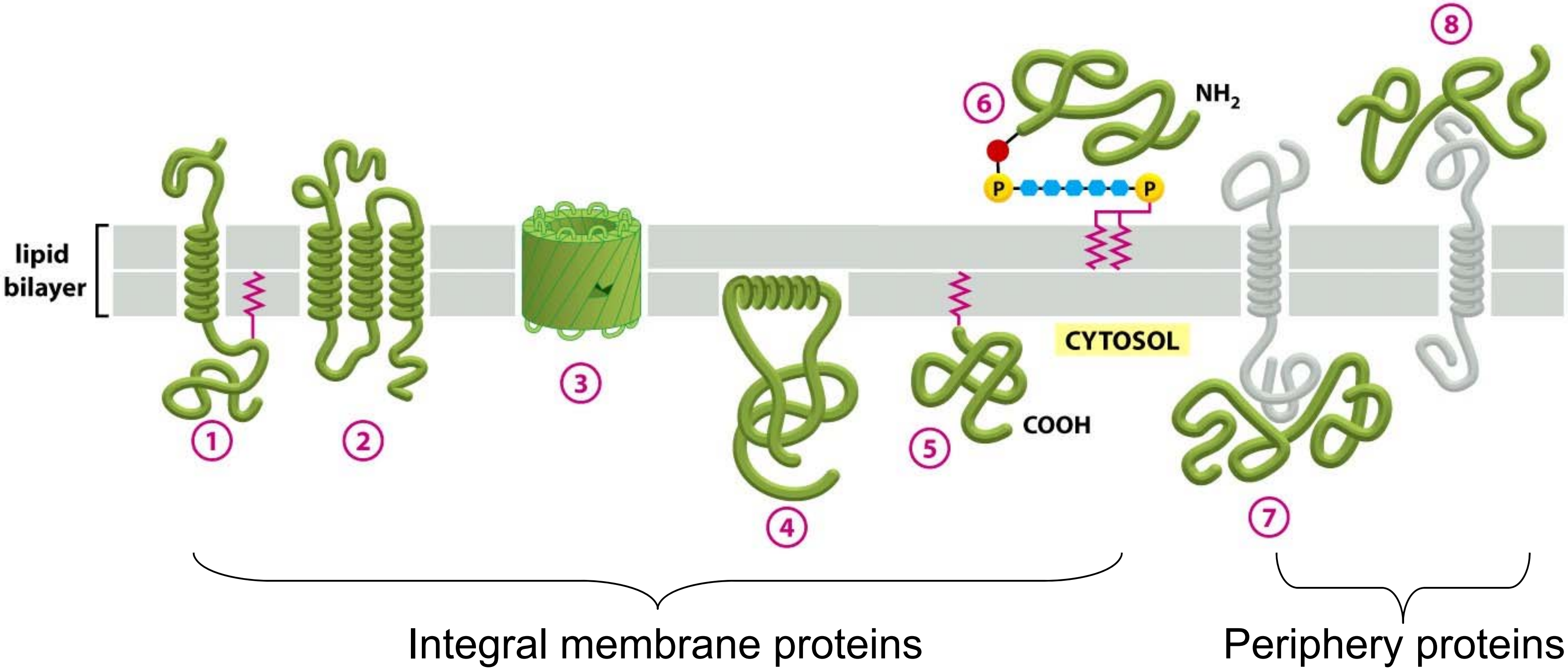
How to use detergent to study membrane proteins



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 proteins
  b. covalently tethered protein:
    - covalently linked to membrane phospholipids or glycolipids

- Many integral proteins are glycoproteins
  covalently linked via Asn (asparagine), Ser (serine), or Thr (threonine) to sugars

Integral membrane proteins

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

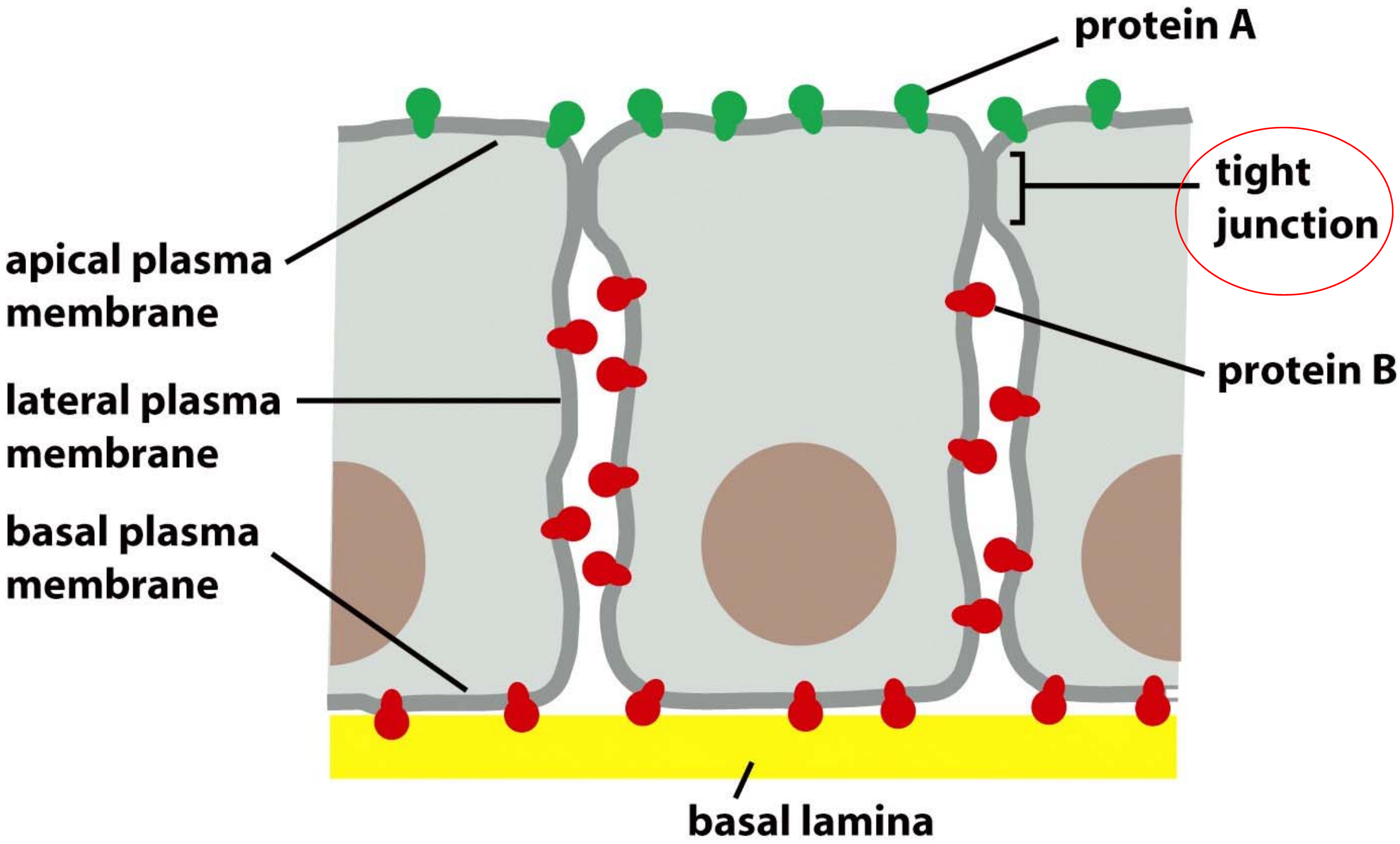
c. Lipid–linked proteins are made in cytosol as soluble protein, after lipid linkage, it is targeted to lipid membrane.

d. Glycosylphosphatidylinositol (GPI) anchor is made as transmembrane protein, after cleavage of transmembrane domain, it is linked by GPI anchor and targeted to membrane.

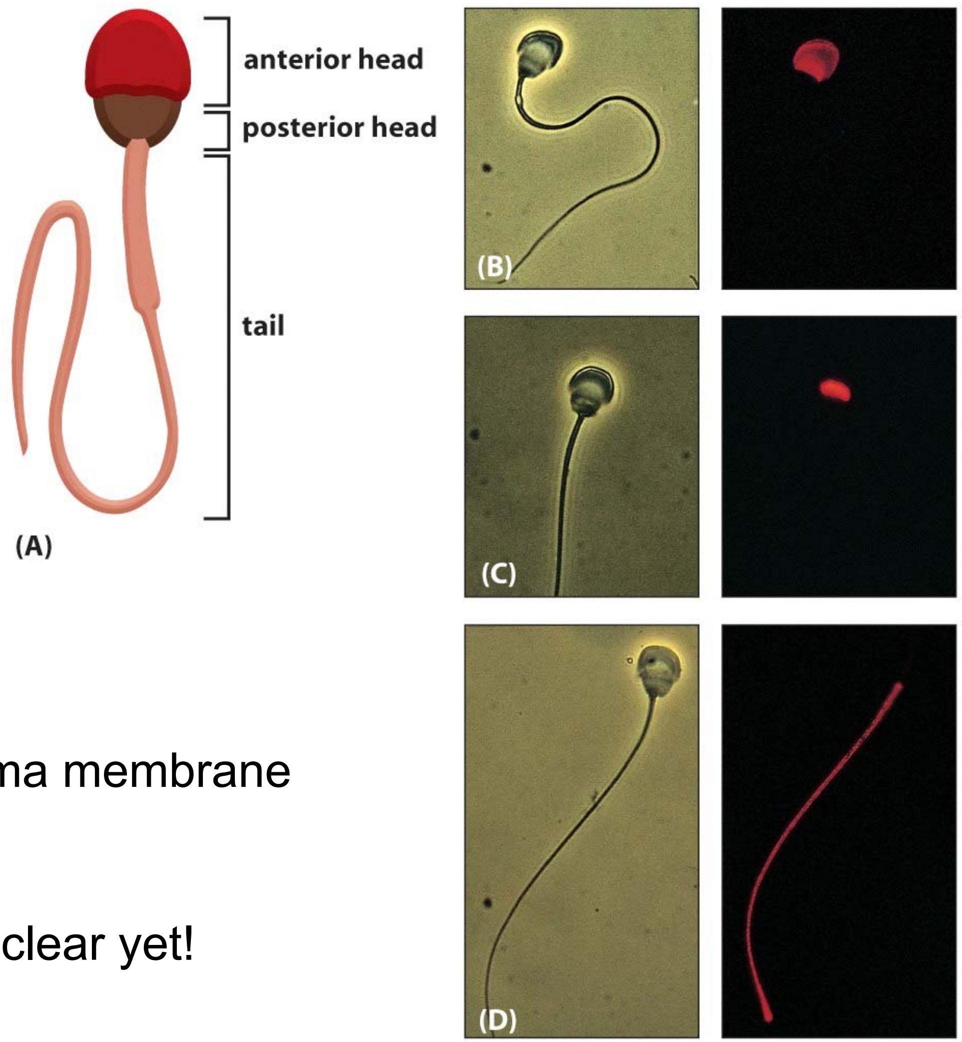
Membrane protein asymmetry

- 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
(Restriction of proteins/lipids in specific domains)



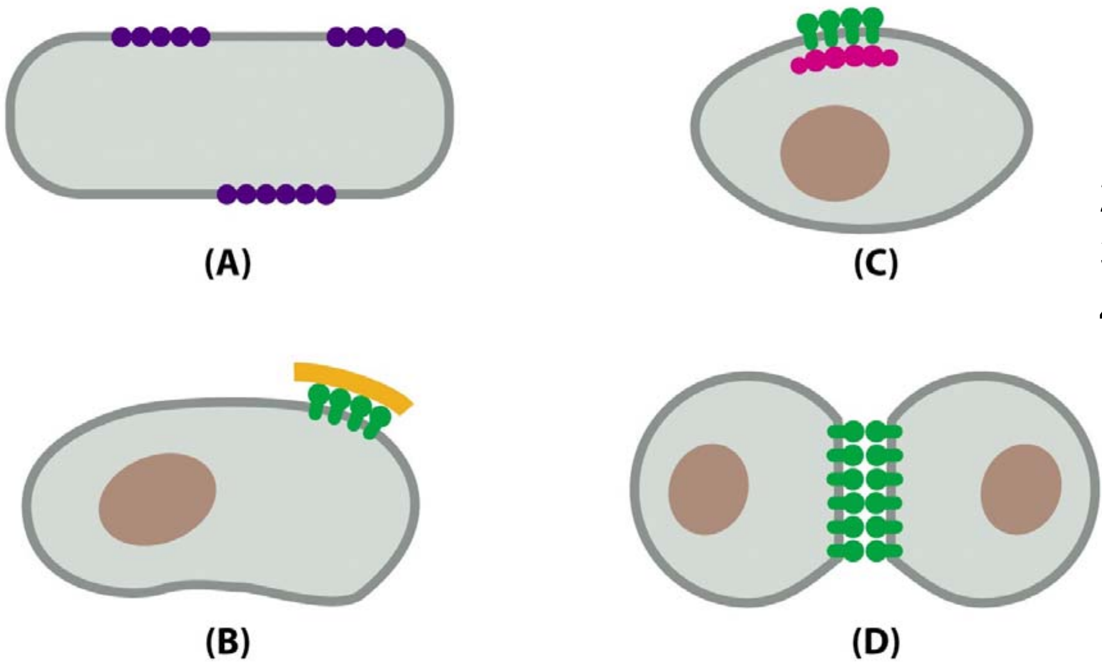
Three domains in plasma membrane of a guinea pig sperm



Sperm cell:
continuous plasma membrane

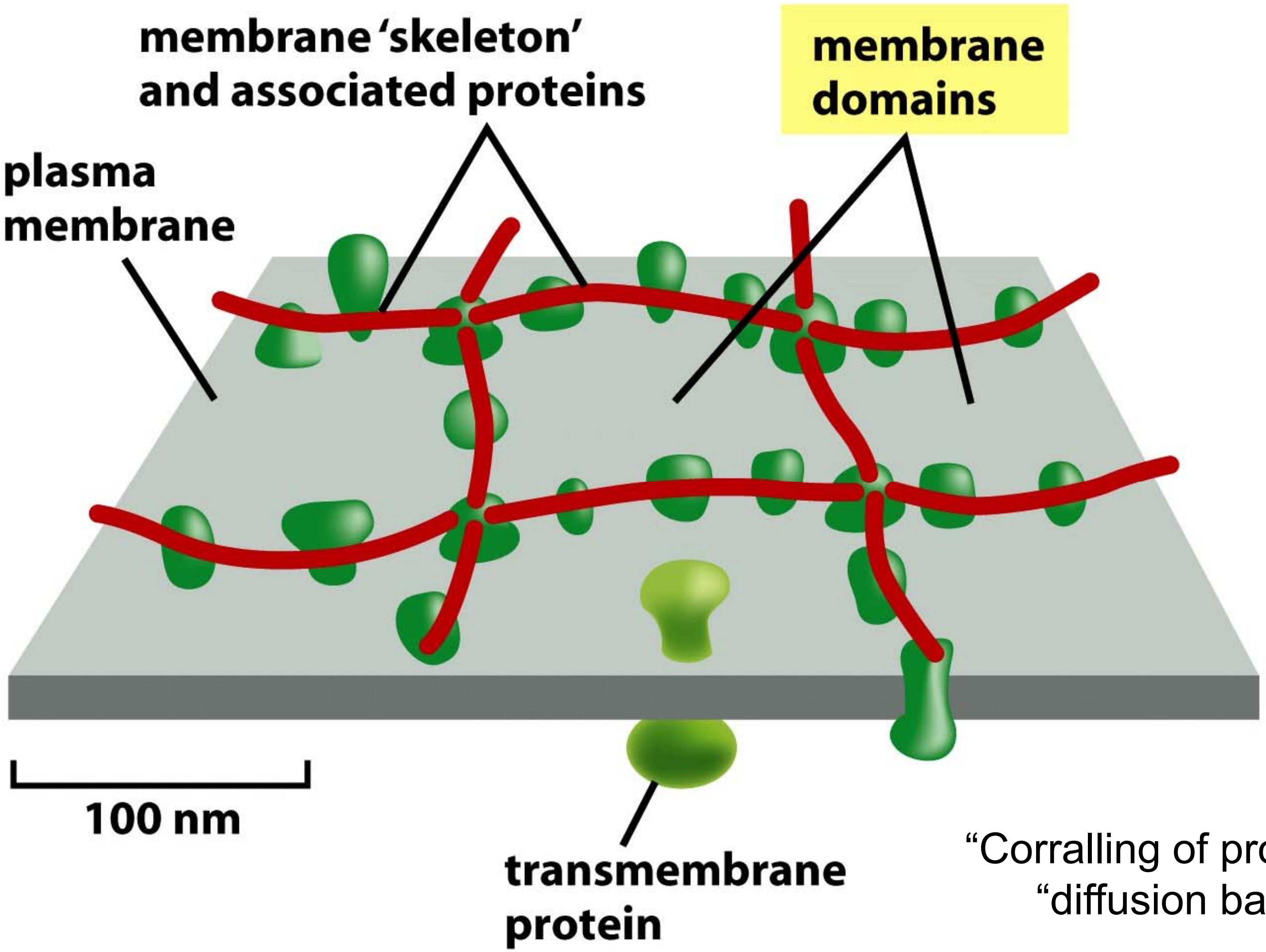
Mechanism: not clear yet!

How membrane protein forms domains?

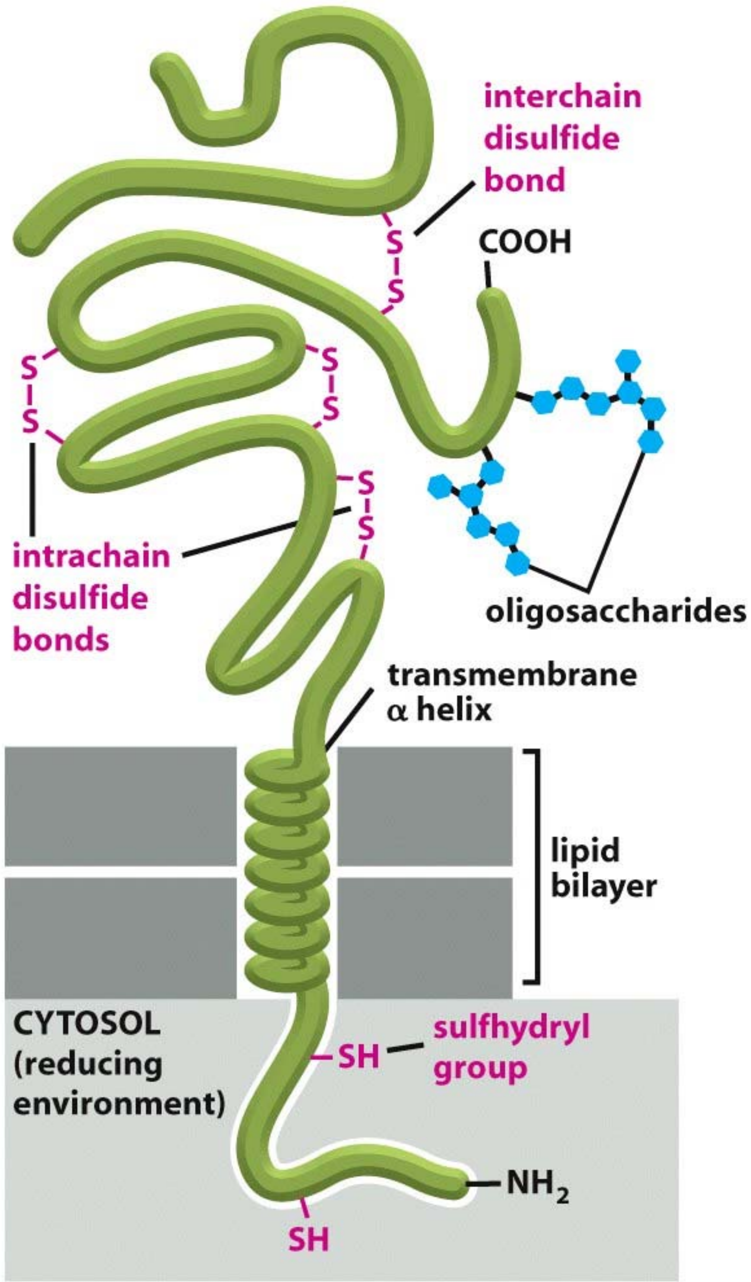


1. self-assemble into aggregates
2. Tethered by outside molecules
3. Tethered by inside molecules
4. Confined by cell-cell junctions

Example: Cytoskeleton network restricts membrane protein diffusion



"Corralling of proteins as "diffusion barrier"

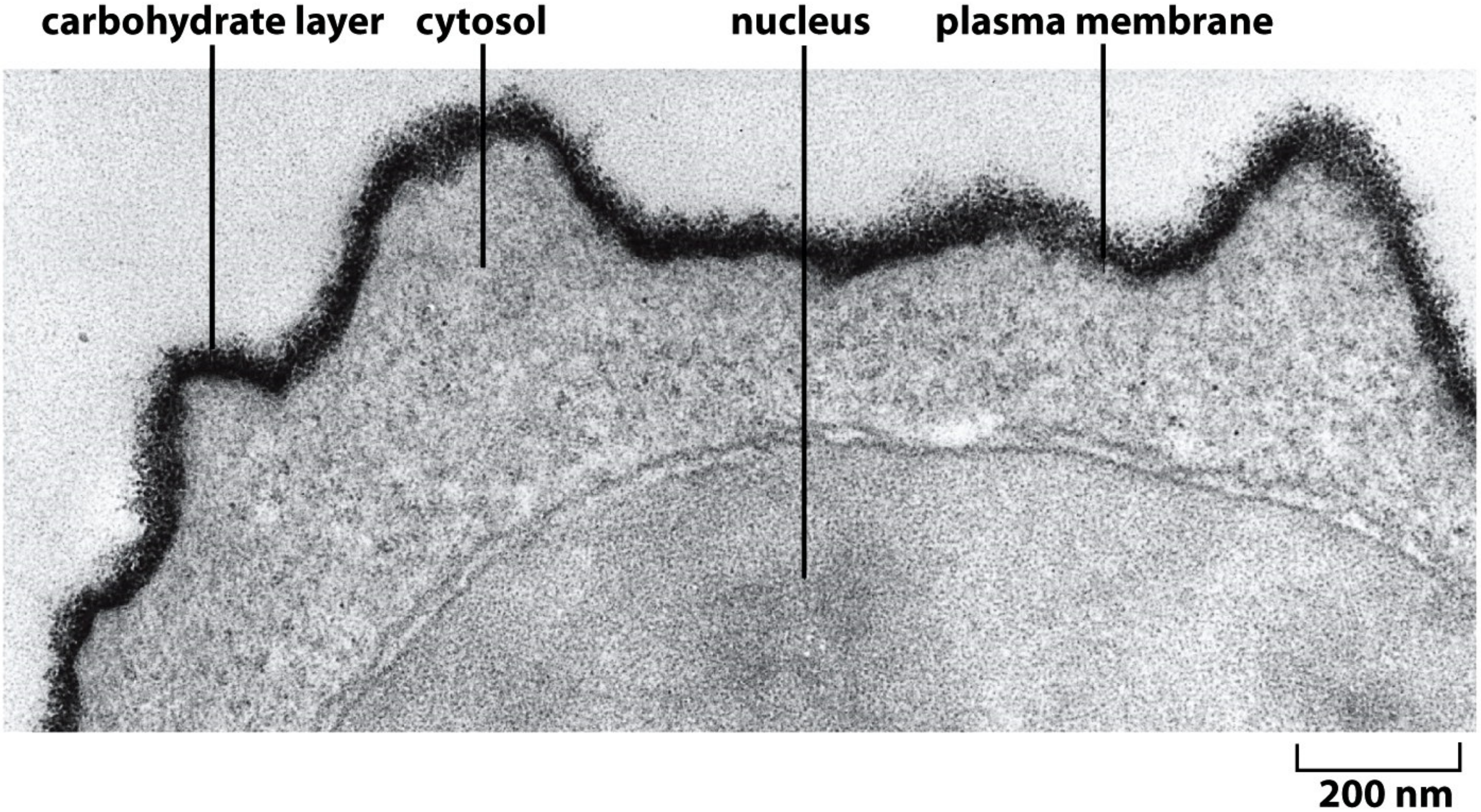


- Oligosaccharide chains are diverse and are on the exoplasmic side of membrane proteins.
- Due to reducing cytosolic environment, rare disulfide bonds form, in contrast, extensive disulfide bonds form in exoplasmic side for membrane proteins to stabilize the protein.

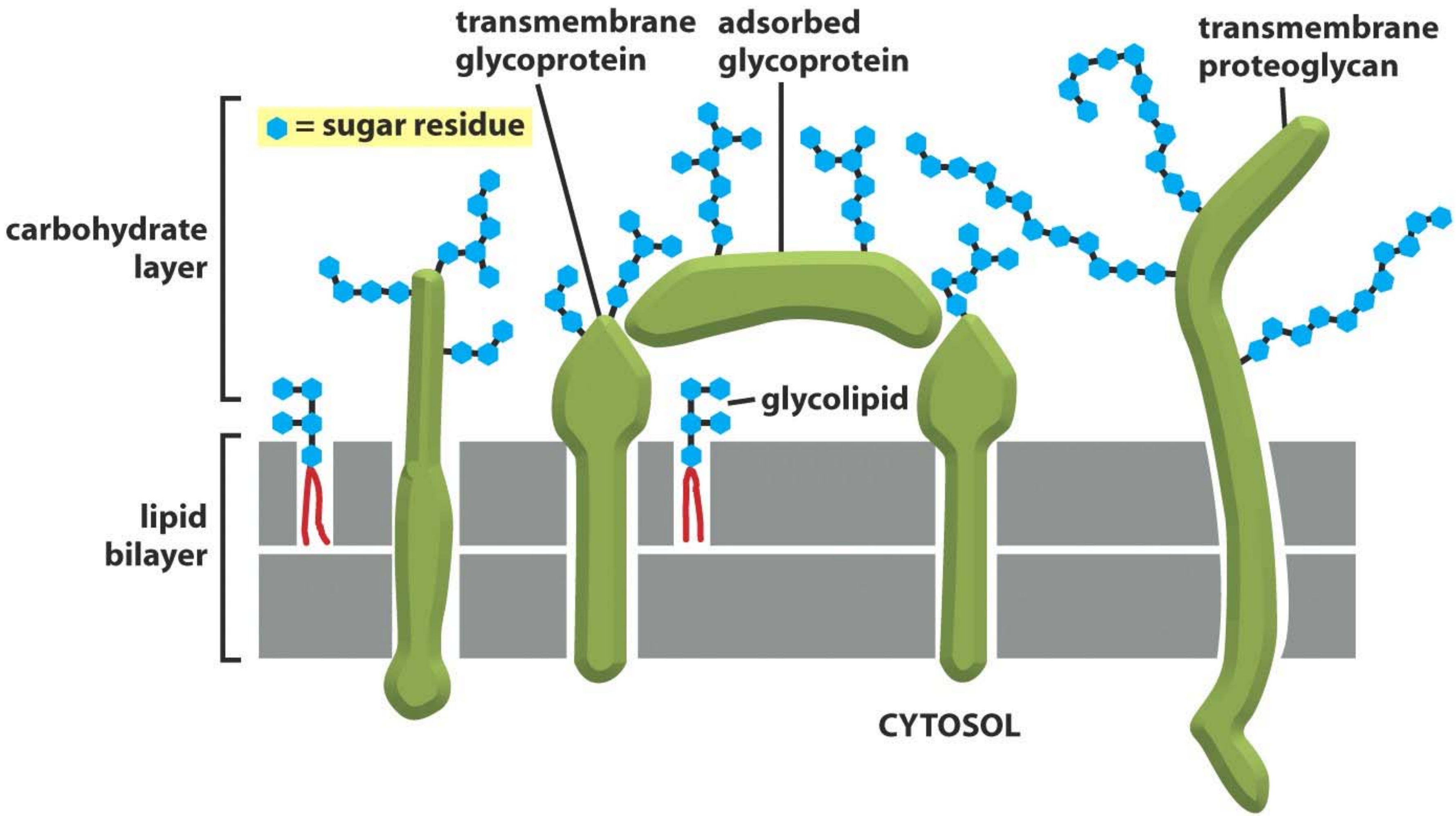
Glycoproteins

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

Carbohydrate layer by ruthenium red stain



Types of glycosylation



Membrane protein mobility

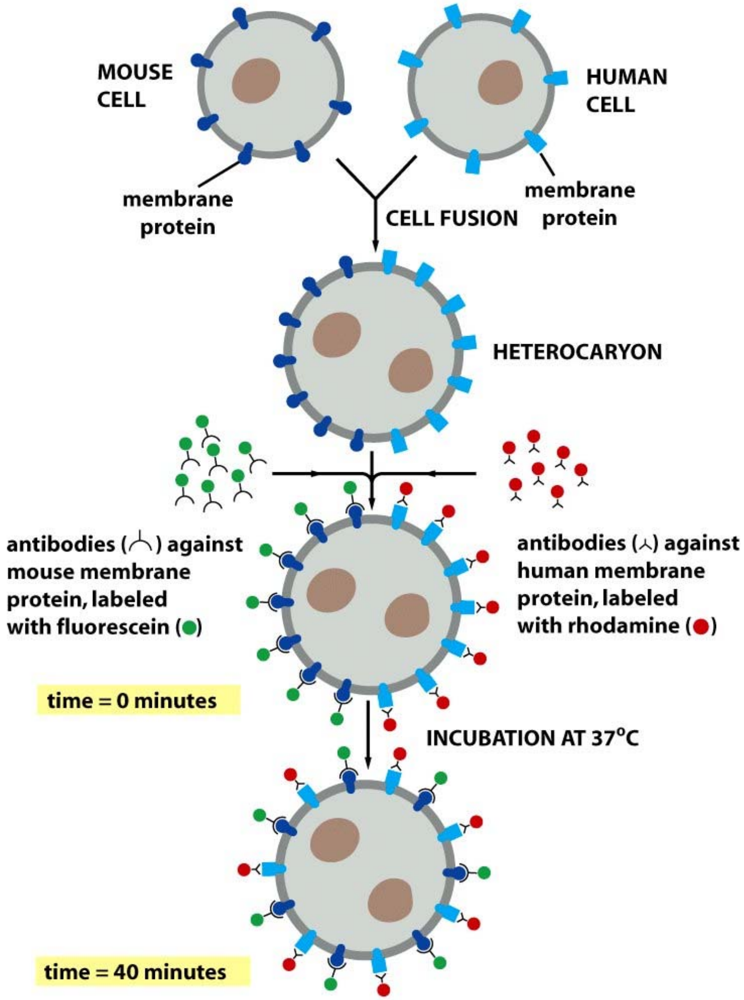
- **Rotational** mobility
- **Lateral** diffusion
- Protein mobility vary greatly.

  (1). Some proteins are free to move.

  (2). Others may be tethered to structures in the cytoplasm or extracellular spaces

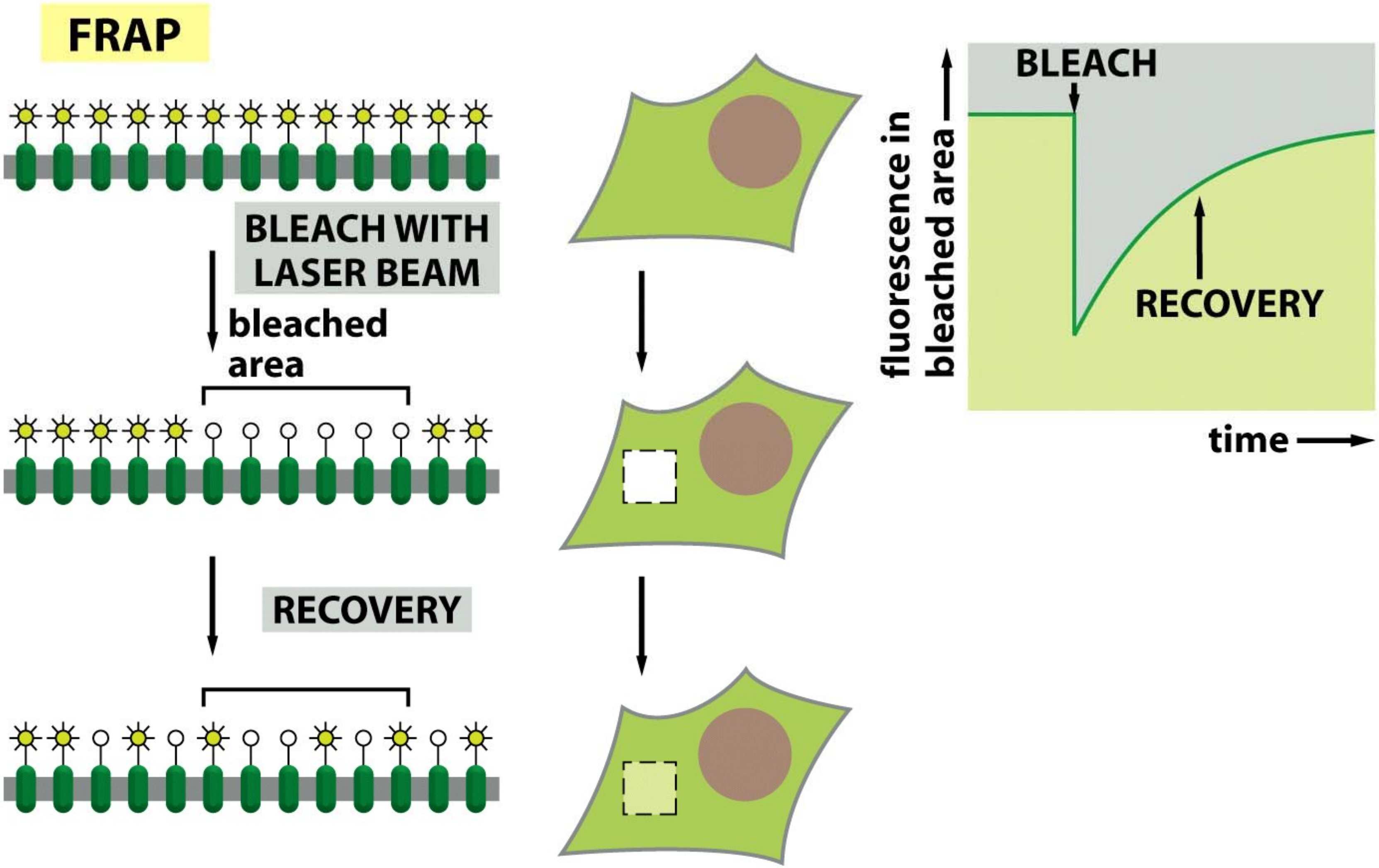
  (3). Some types of cell junctions (e.g., tight junctions) can restrict protein movements to a specific membrane domain.

Example 1: membrane protein has lateral diffusion

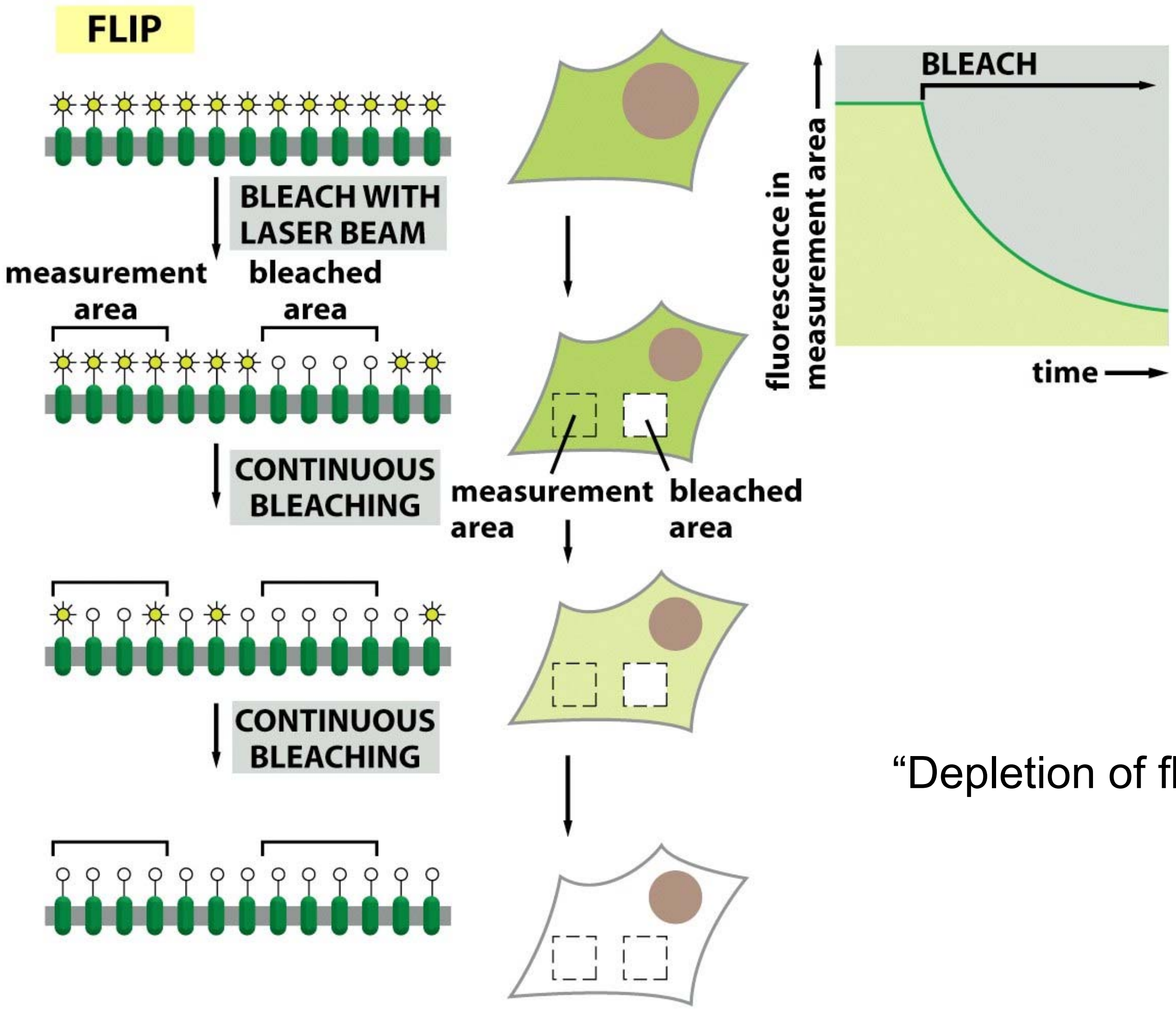


Immediately after fusion:
Proteins are separated and mix after time by diffusion

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

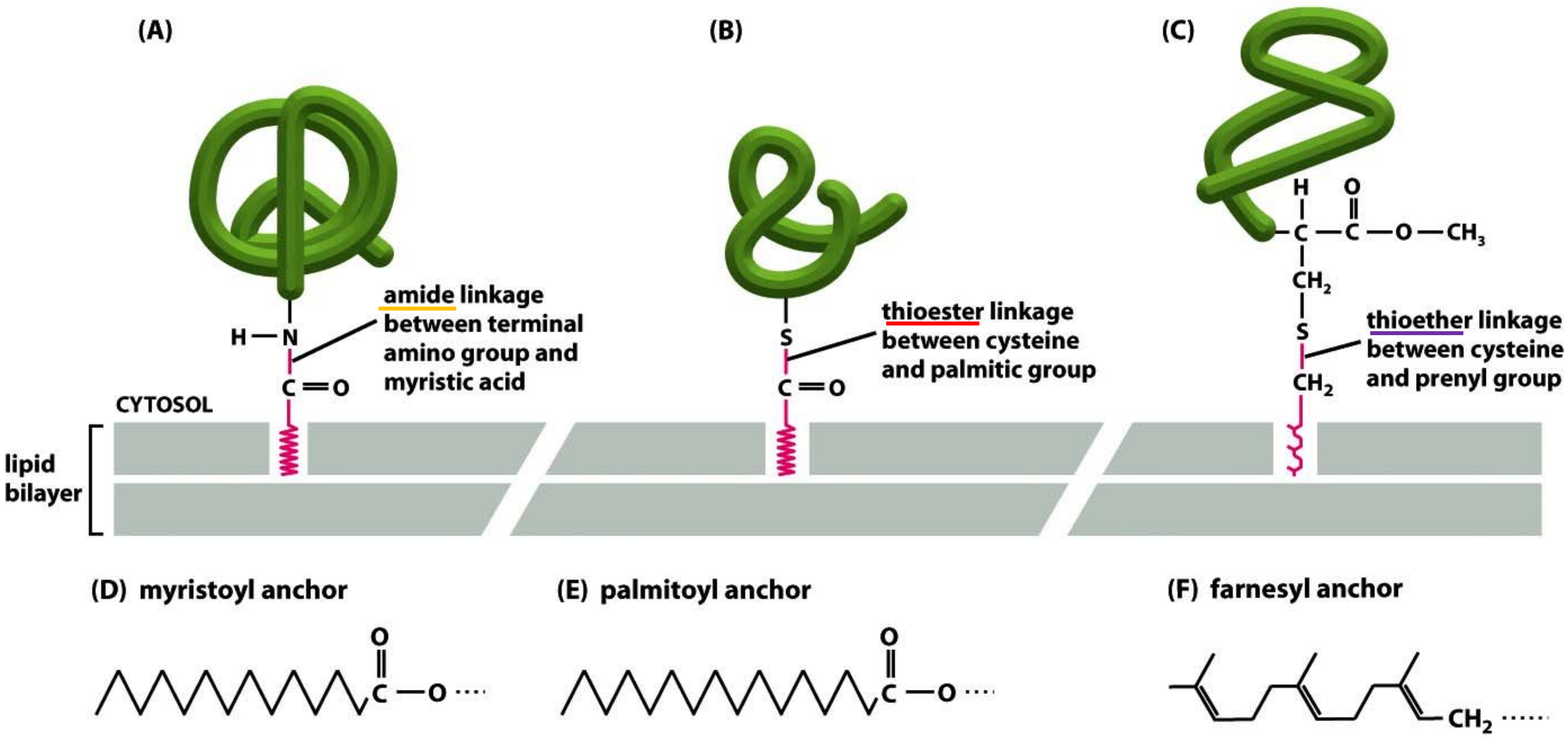


Example 3: Fluorescence loss in photobleaching (FLIP) to detect membrane protein diffusion



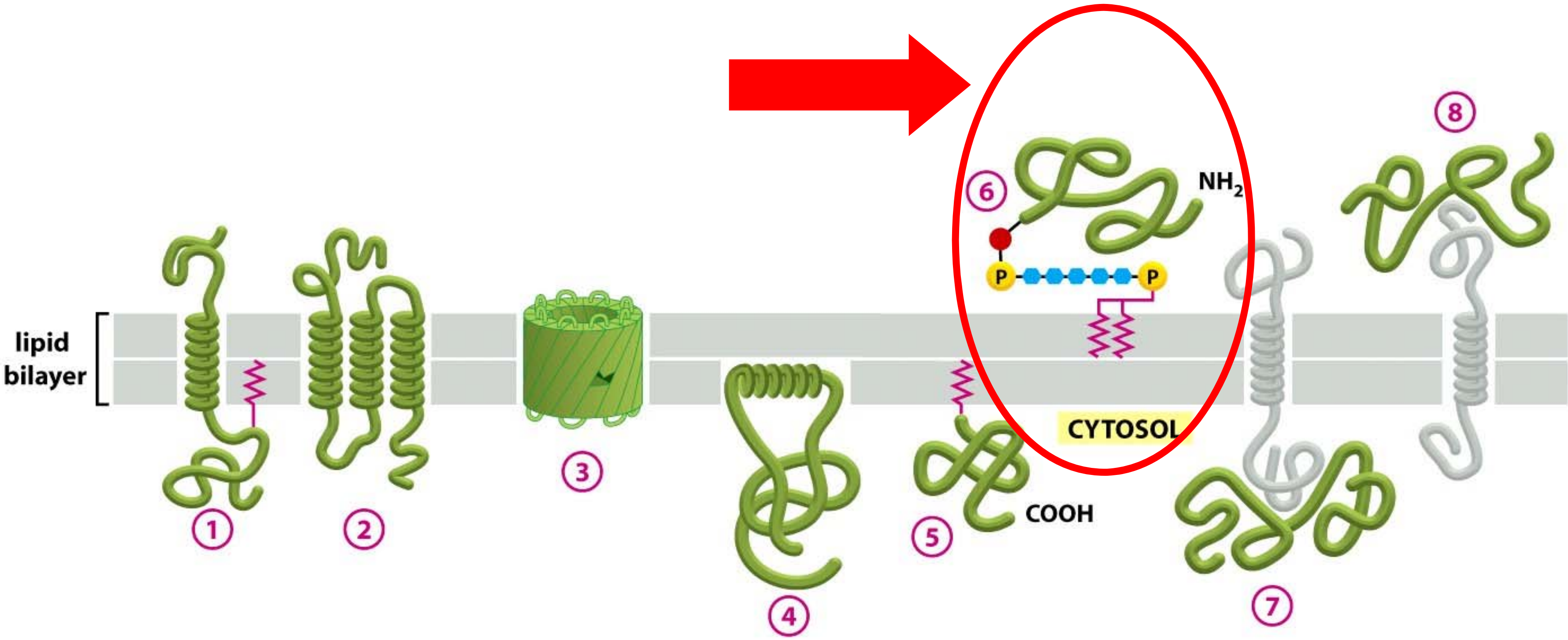
"Depletion of fluoresce"

Ways for membrane proteins to covalently attach to membrane lipid



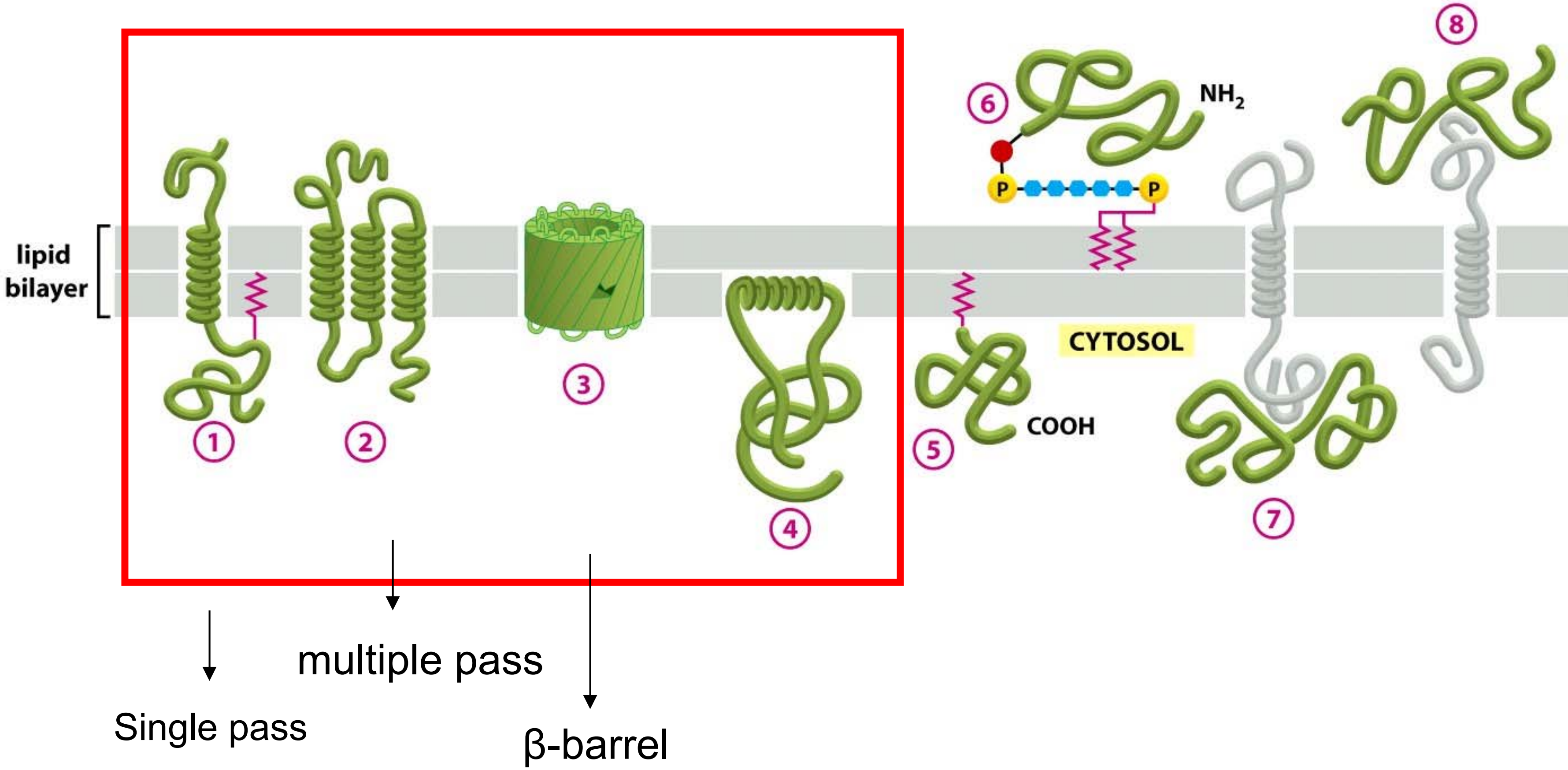
14-carbon (saturated) 16-carbon (saturated) 15-carbon (unsaturated)

Glycosylphosphatidylinositol(GPI) anchor

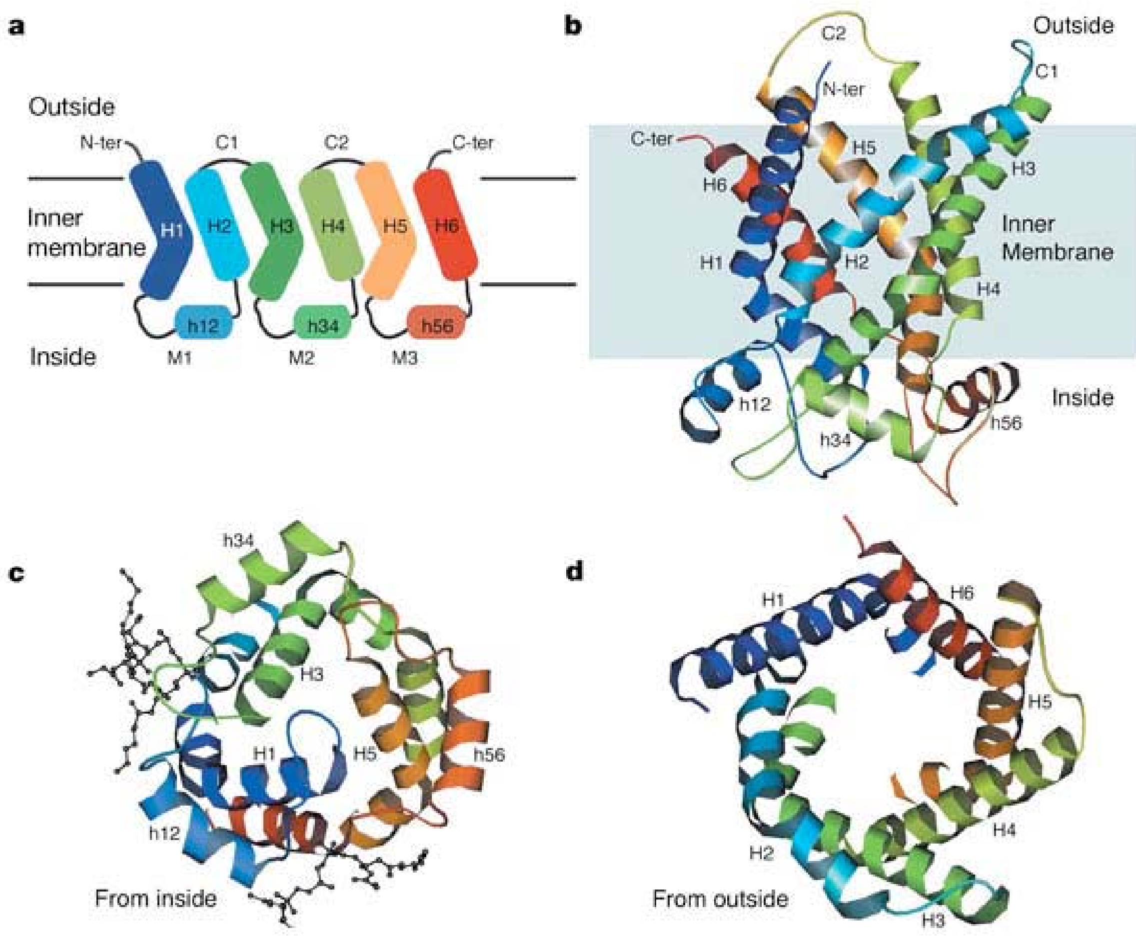


GPI anchored protein can be recognized by phosphatidylinositol-specific phospholipase C
And be cleaved off from the membrane.

Membrane protein topology



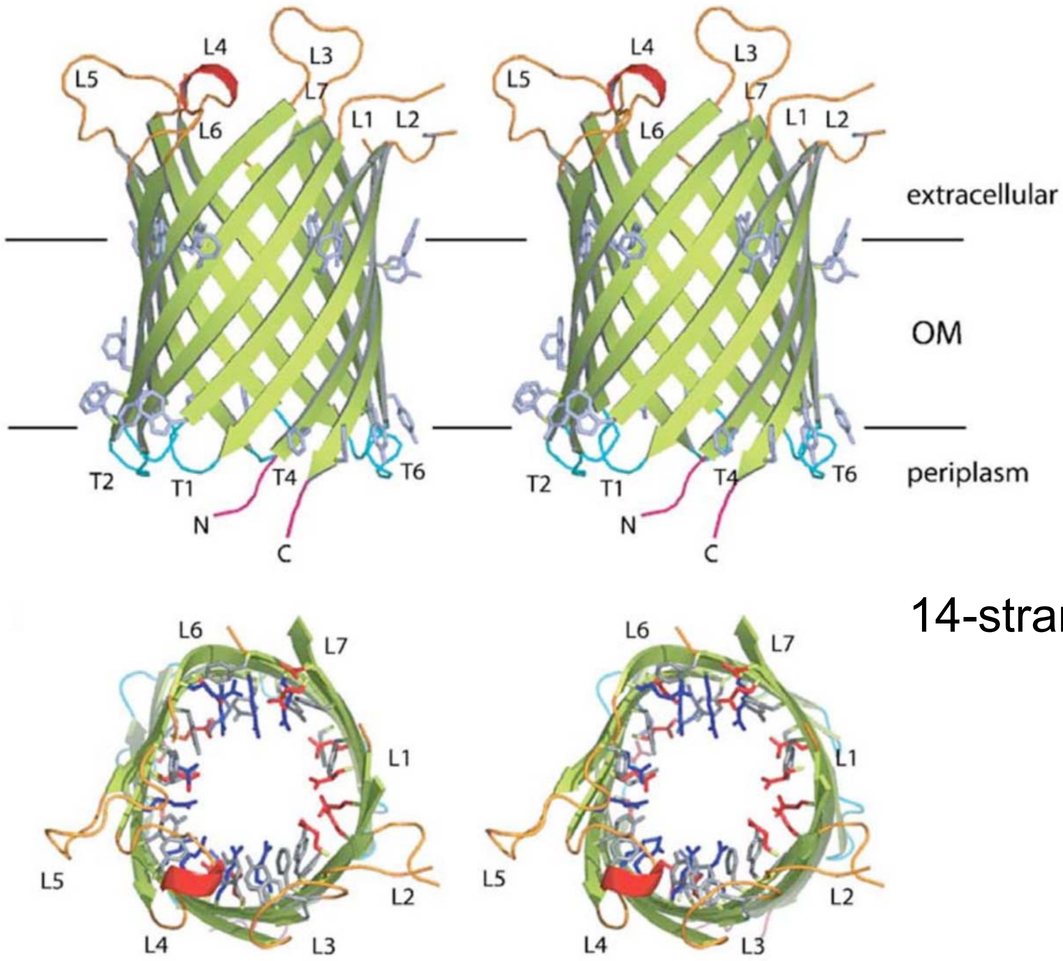
Example 1: mitochondria ATP/ADP carrier
(multiple pass transmembrane protein)



6 α-helices form a compact transmembrane domain

Pebay-Peyroula et al, Nature 2003

Example 2: outer membrane protein G
(β-barrel transmembrane protein)

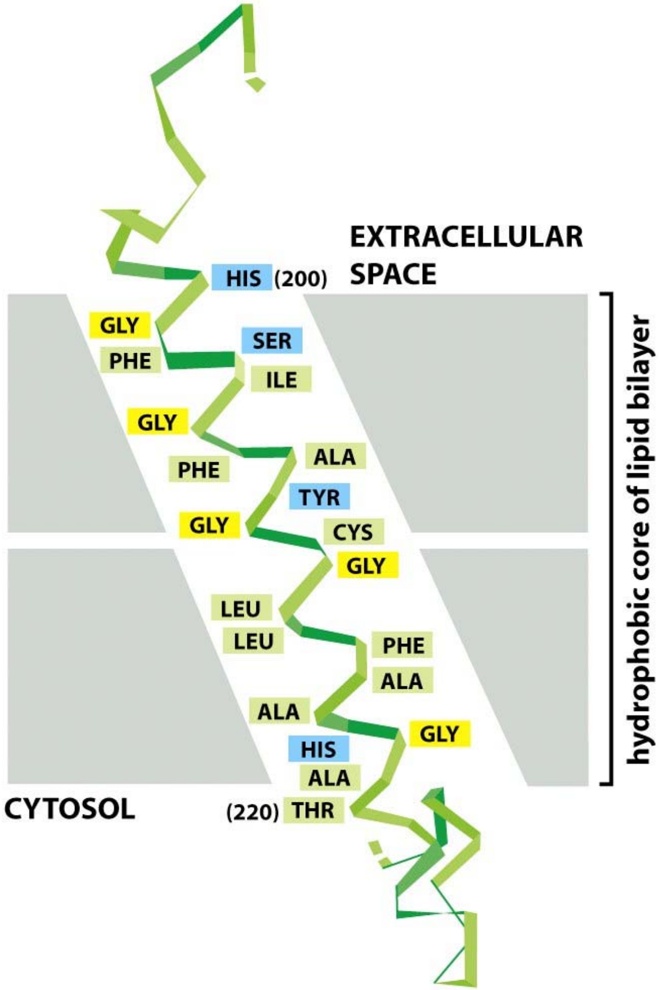


14-stranded β-barrel

Subbarao and van den Berg, JMB 2006

Transmembrane α-helix

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



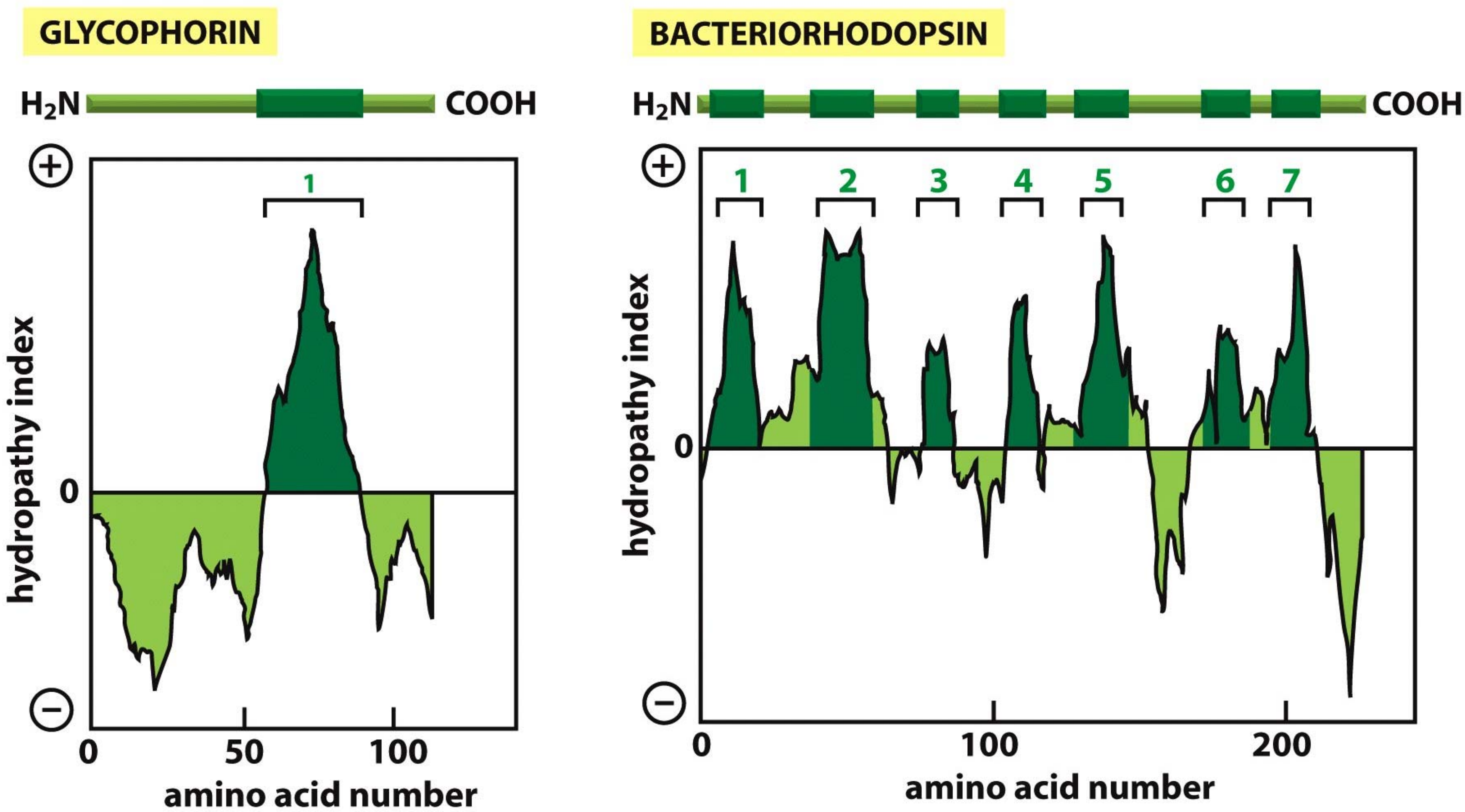
Hydrophobic amino acids:
green and yellow

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

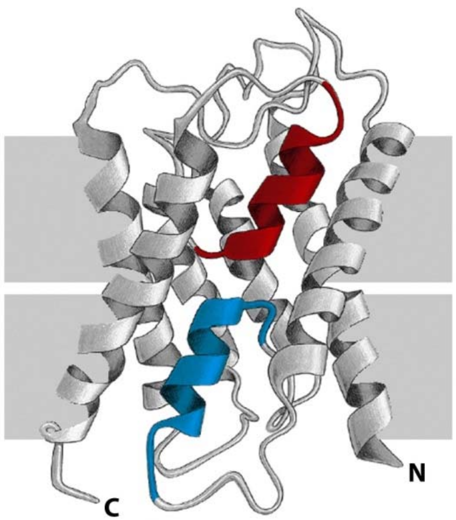


Single pass

7 passes

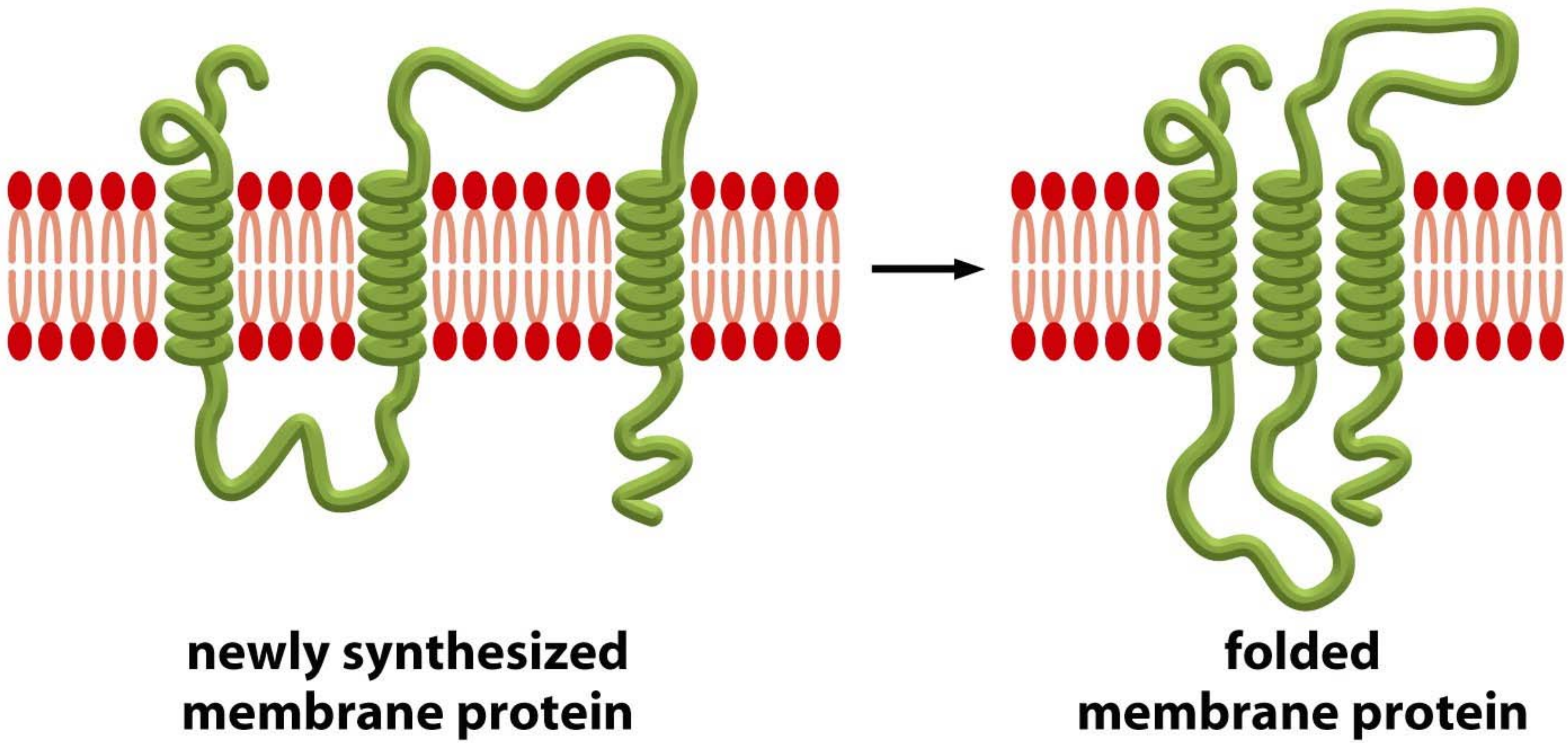
Some transmembrane protein regions can't be predicted by hydropathy plots, these includes…

- 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.

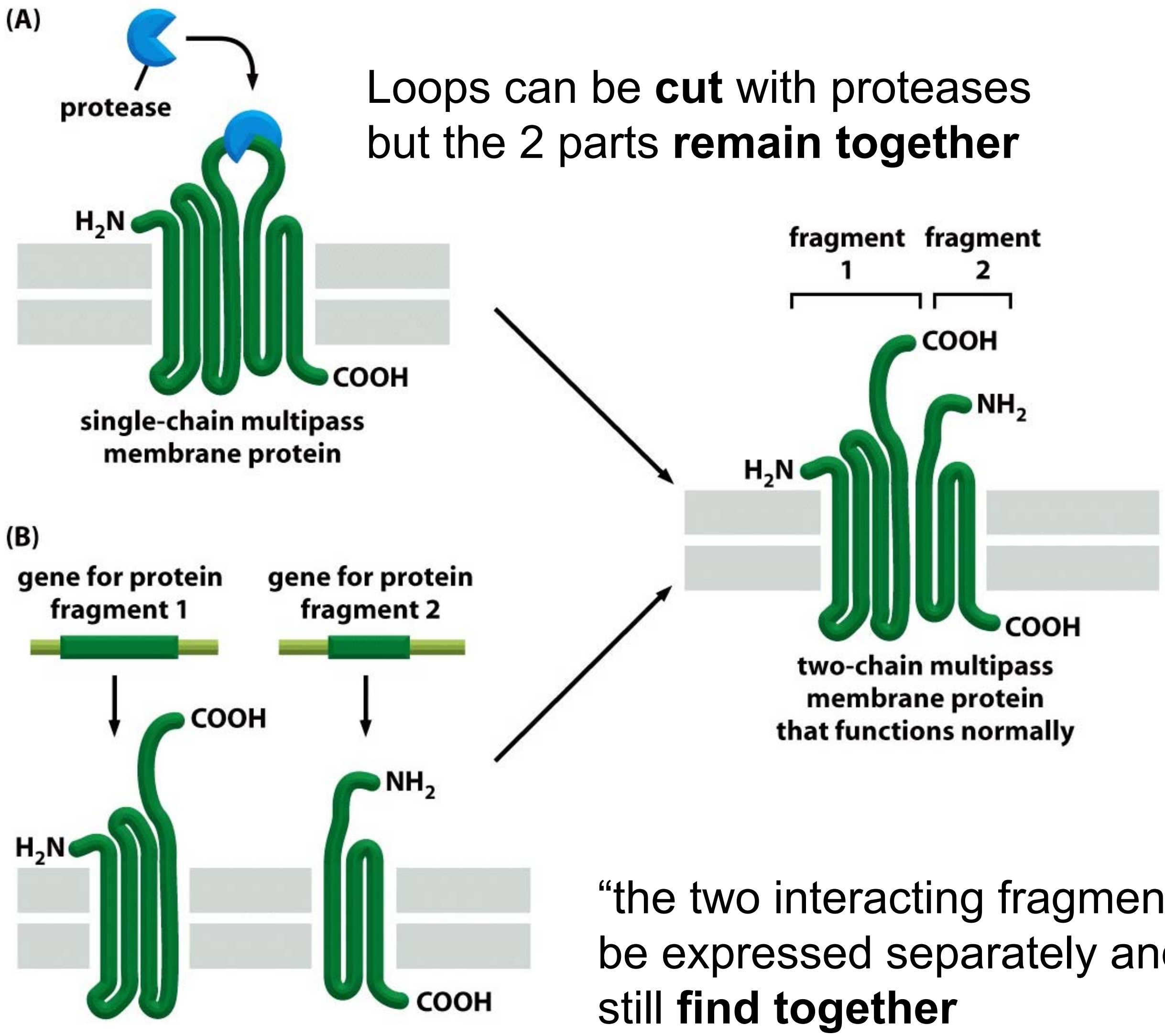


Colored **two α-helices** in aquaporin water channel **are buried** at an interface formed by protein-protein interactions, they are not hydrophobic.

Transmembrane α-helix has specificity for its interaction partners



Transmembrane α-helix has specificity for its interaction partners



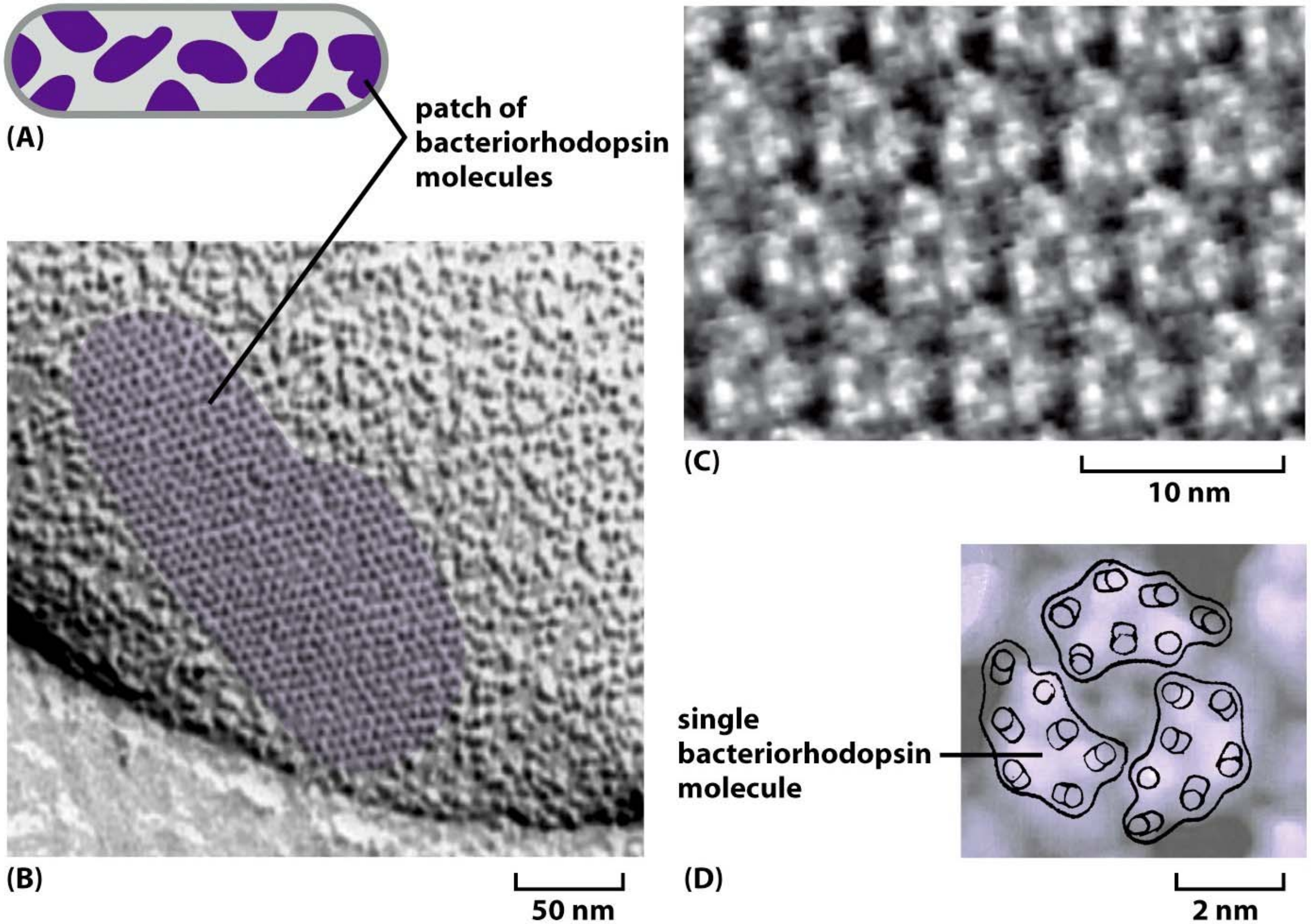
Loops can be **cut** with proteases but the 2 parts **remain together**

“the two interacting fragments can be expressed separately and will still **find together**

Bacteriorhodopsin-the first membrane transport protein whose structure was determined

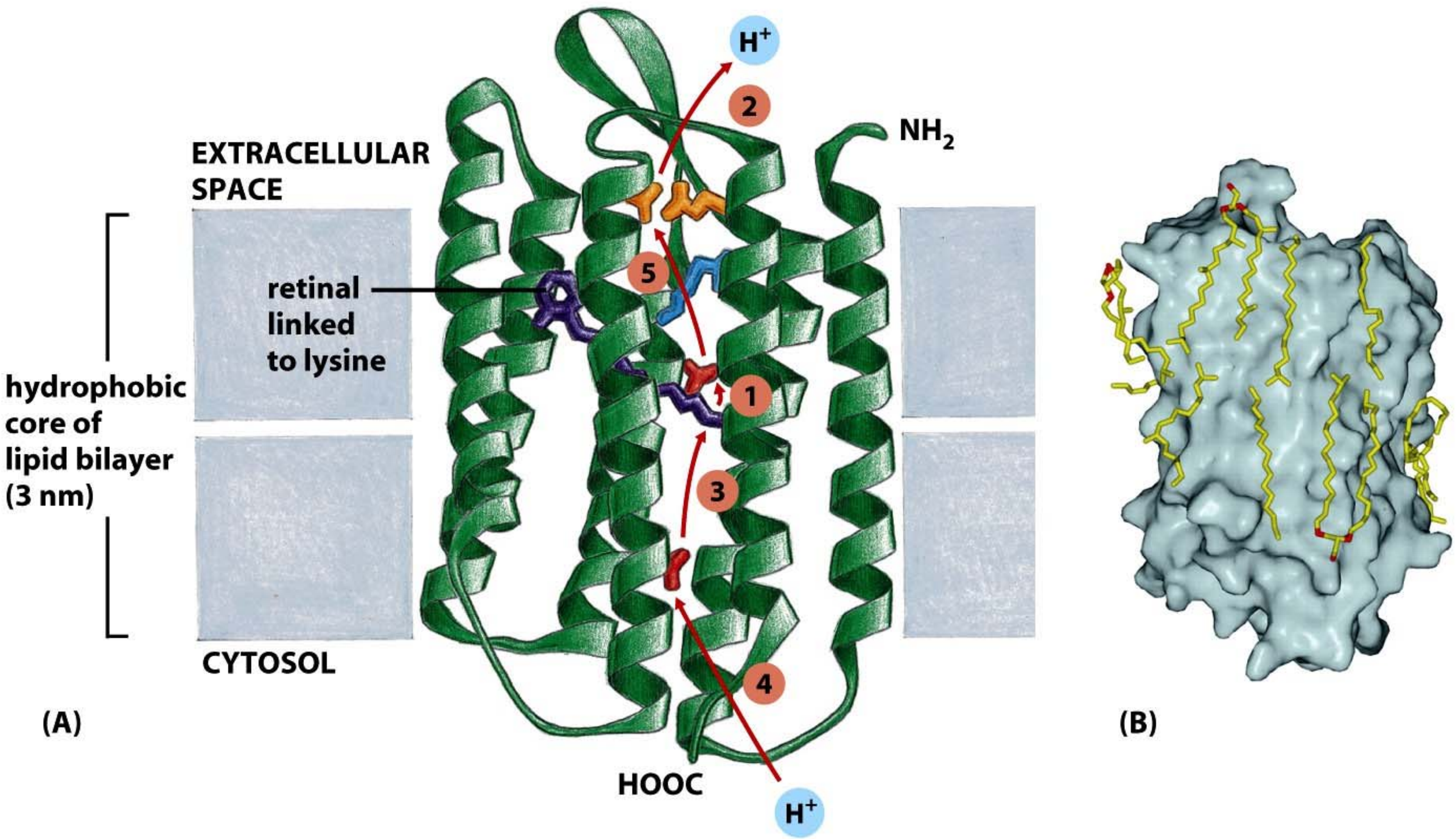
- Exist in the plasma membrane of archaean *Halobacterium salinarum* who lives in sea water
- Pump protons in the presence of sunlight and set up proton gradients across the membrane.
- Use the proton gradients to harvest ATP or other energy requiring activities.

Bacteriorhodopsin

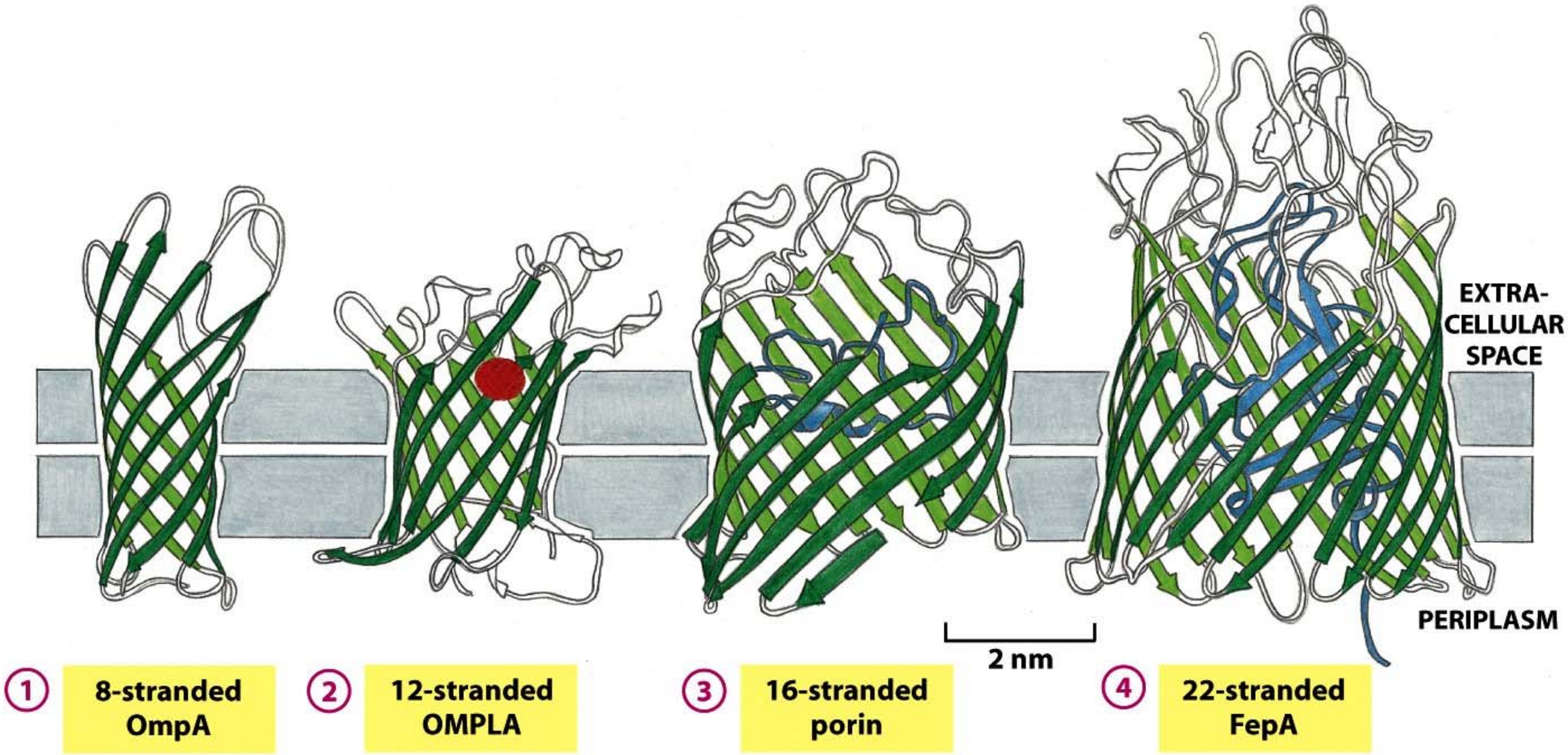


3 monomeric Bacteriorhodopsin **Each** has **7-passes** Transmembrane Domains.

3-dimentional structure of bacteriorhodopsin



Different types of β-barrel



Receptor
(ligand binding)

Lipase
(active center, red)

Pore
inner loops (blue)
regulate size

Transporter
(filled with an entire protein)

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