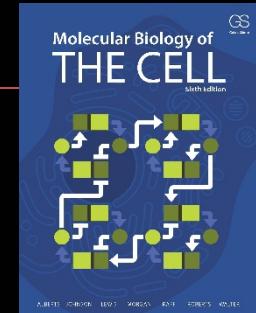
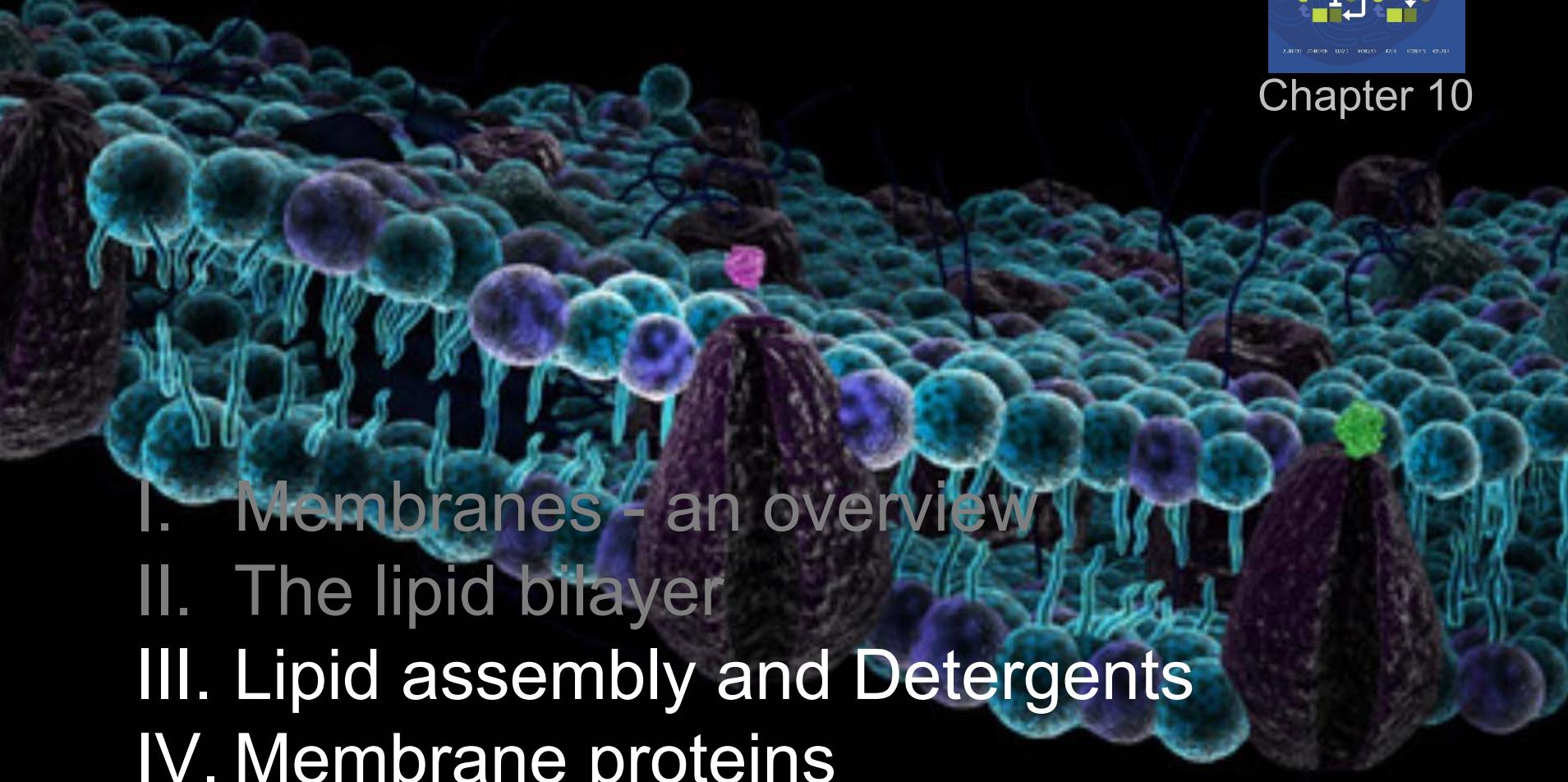


Lecture 4

Membrane structure

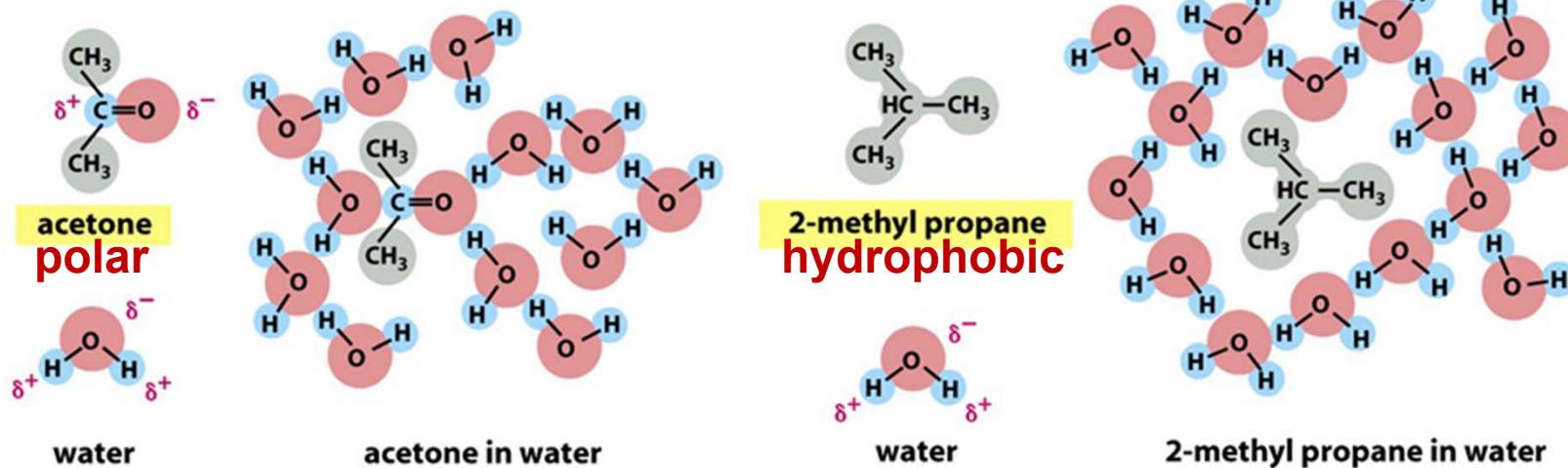


Chapter 10

- 
- A detailed 3D rendering of a cell membrane. The membrane is composed of a lipid bilayer where each lipid has a blue spherical head and two green wavy tails. Various proteins are embedded in the bilayer; some are purple spheres, others are larger and more complex structures like channels or pumps. A few proteins have long, thin, hair-like appendages extending from the membrane surface. The overall texture is bumpy and organic.
- I. Membranes - an overview
 - II. The lipid bilayer
 - III. Lipid assembly and Detergents
 - IV. Membrane proteins

III. Lipid assembly

Why do hydrophobic molecules stay together?

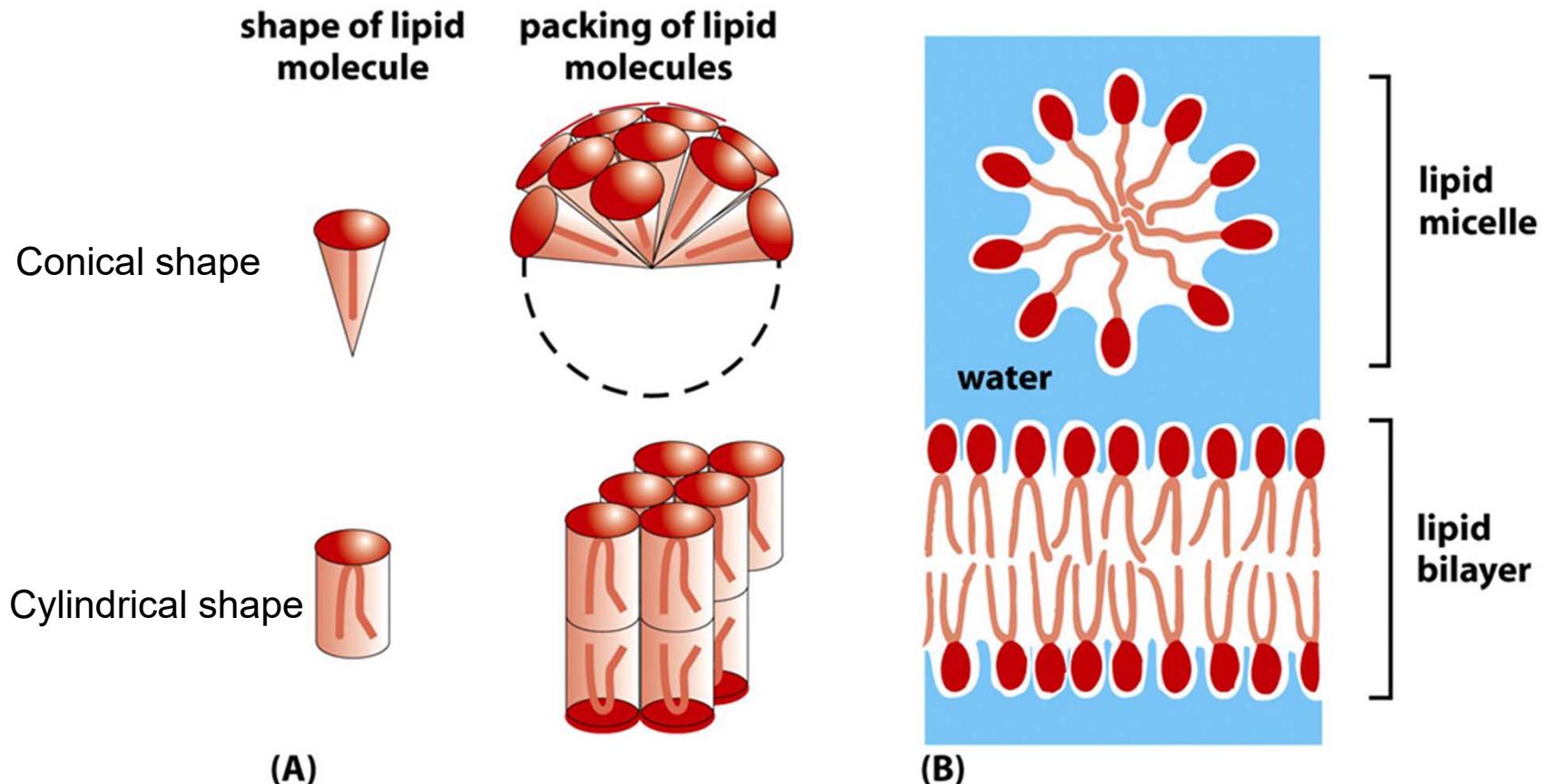


Acetone is polar and forms electrostatic interactions

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

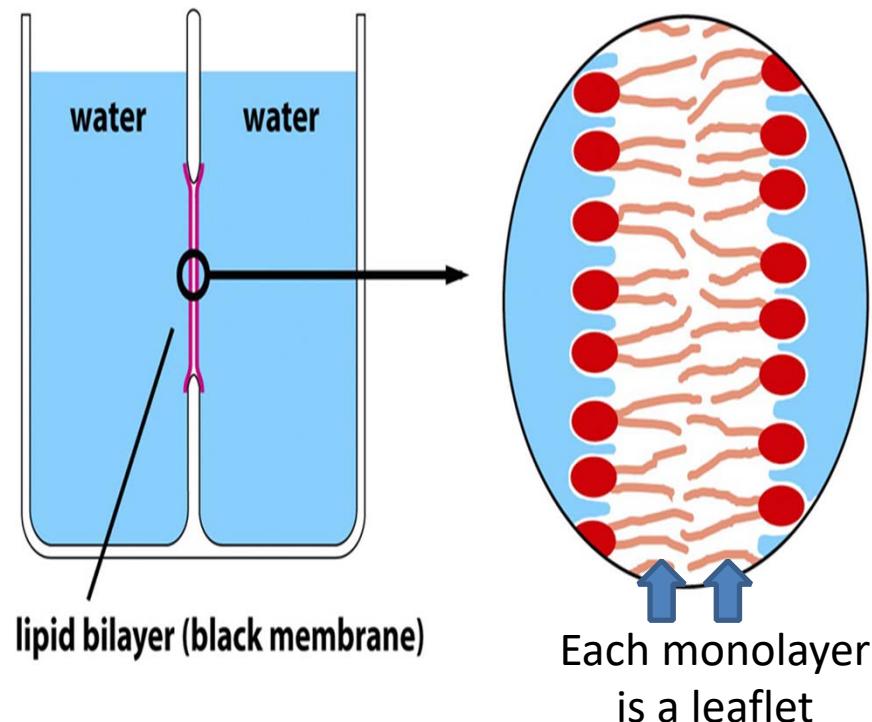
Micelles or lipid bilayer form spontaneously in aqueous solution



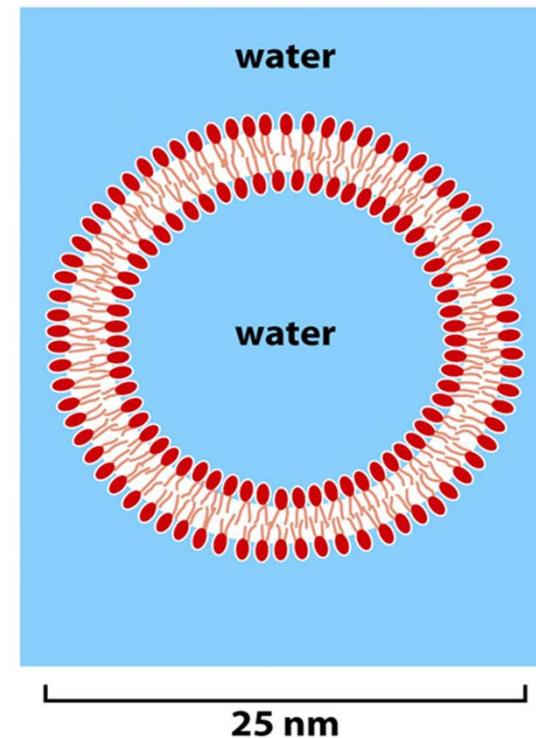
Micelle or bilayer depends on the shape of the molecule: conical versus cylindrical...

Black membranes and liposomes

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



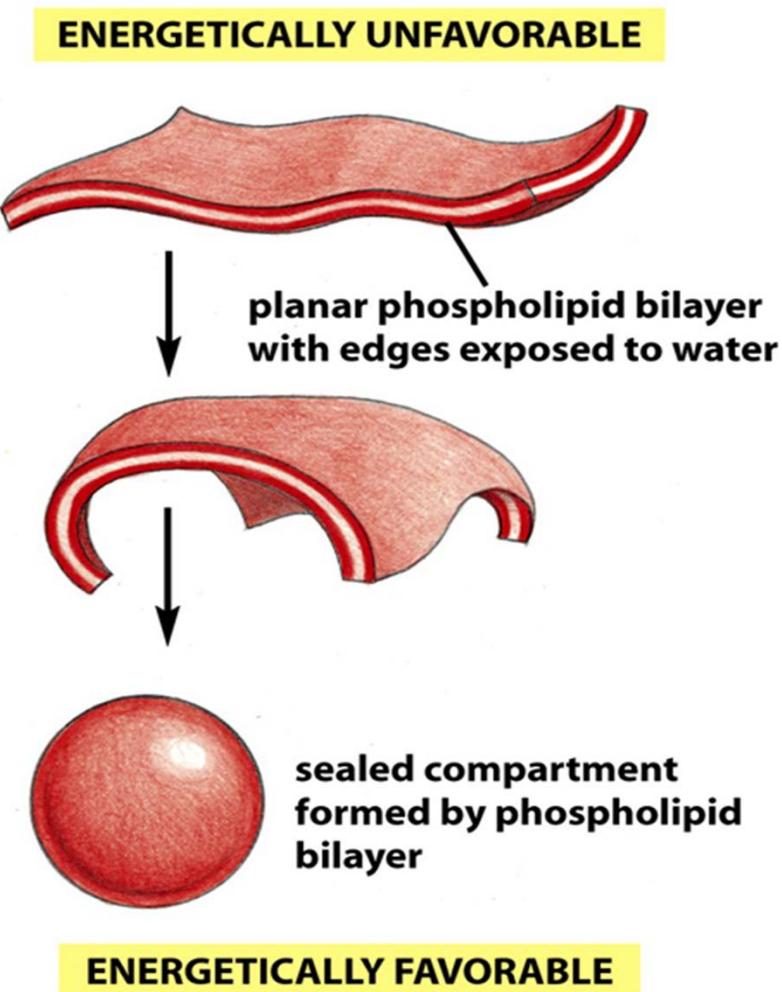
Liposomes are **spherical lipid bilayers**



WHAT is the difference between a liposome and a micelle?

How and why does a spherical lipid bilayer form?

Spontaneously!!!



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

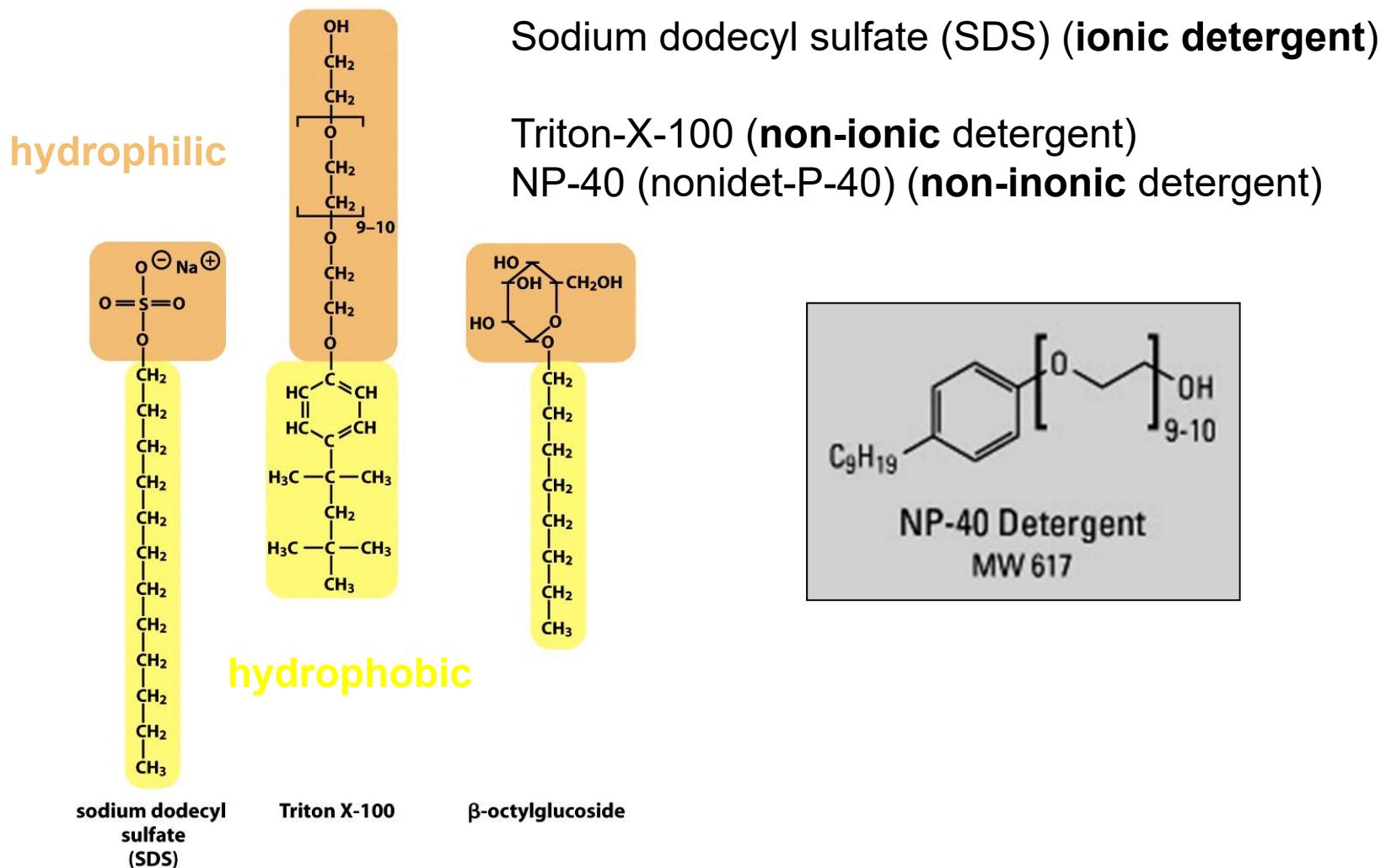
Detergents in membrane studies

What are detergents?

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.

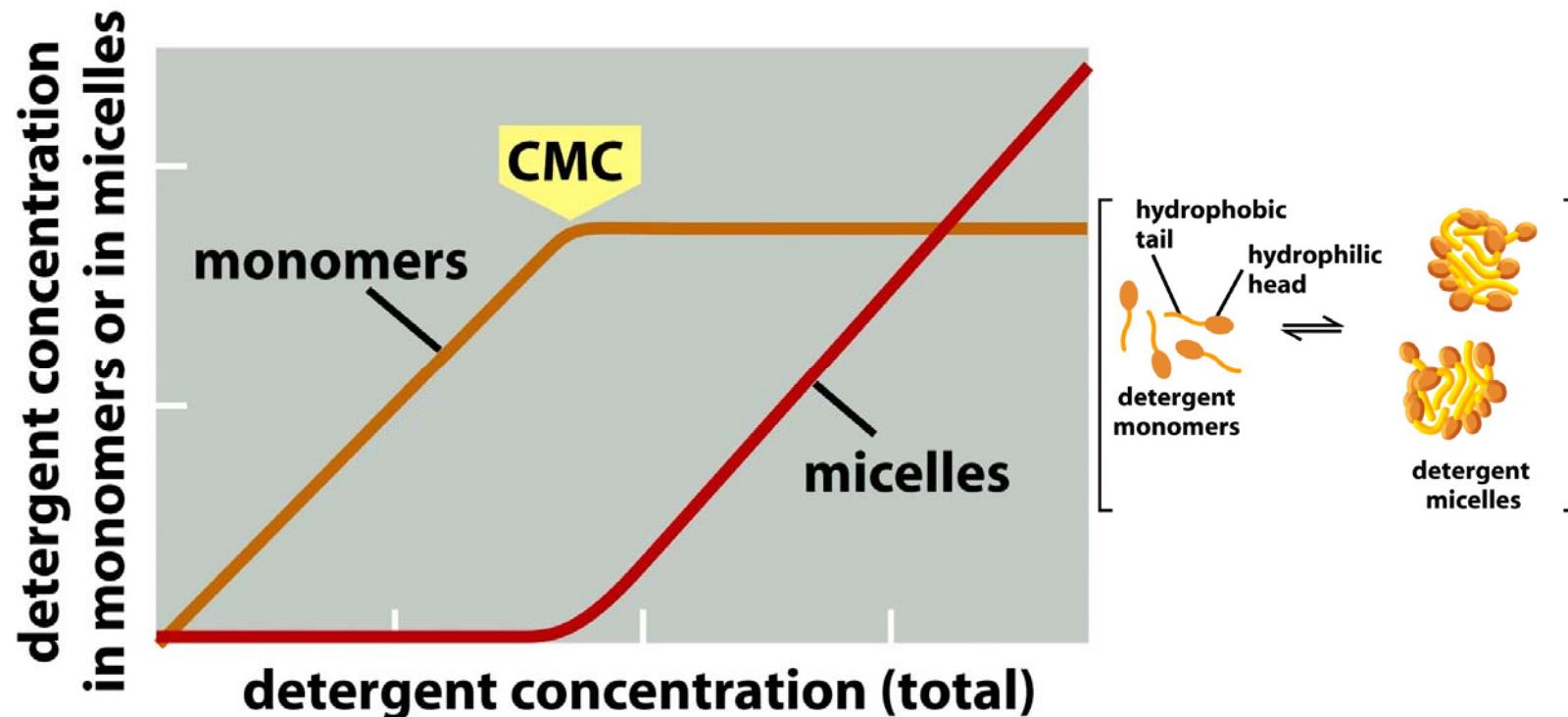
The structure of some common detergents



Detergents are absolute essential to solubilize membrane proteins

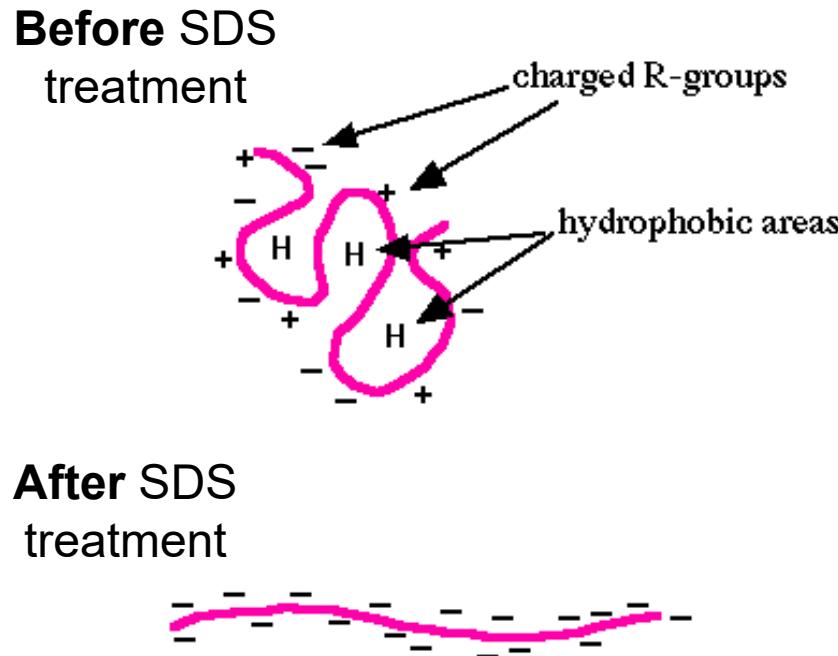
Detergents form micelles

Critical micelle concentration (**CMC**) is the concentrations at which detergents aggregate to **form micelles**



What is the critical micelle concentration and why is it important?

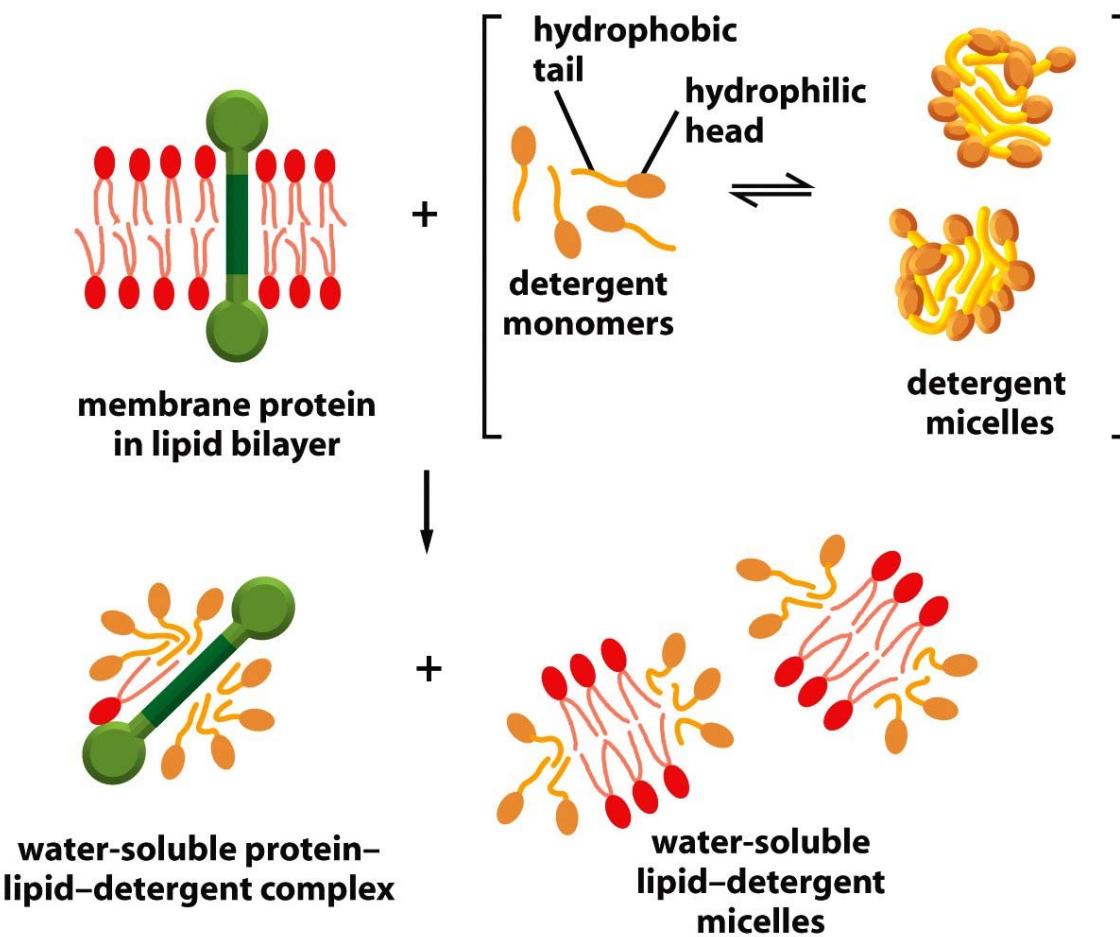
The usage of the ionic detergent SDS to solubilize proteins



- SDS fully denatures proteins
- SDS covers protein molecules with negative charges
- Charging the protein is essential for electrophoretic separation via SDS-polyacrylamide gel electrophoresis (**PAGE**)

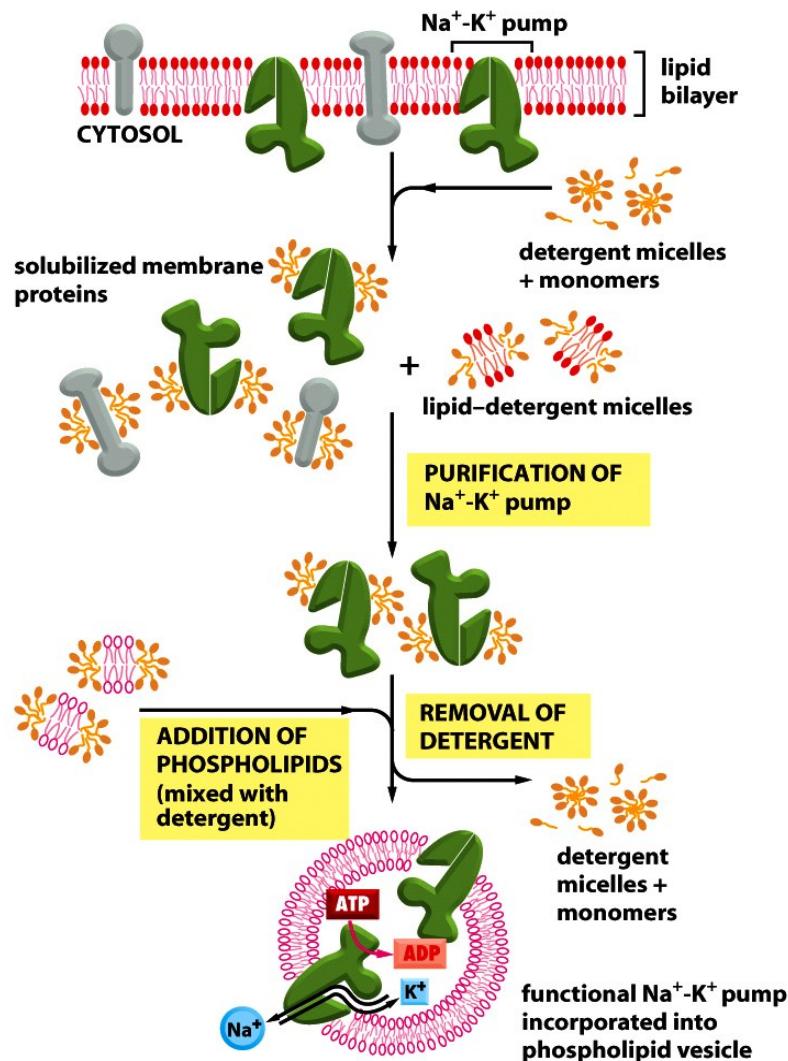
Non-ionic detergents do **not** denature proteins...

...they only solubilize membrane components



Remember the differences between ionic and nonionic detergents!

How to use detergent to study membrane proteins



- First, the membrane lipids are **substituted by the detergent** molecules
- Now, the detergent stabilizes protein structure (hydrophobic part).
- Next, the detergent is substituted by the added lipids
- The protein is then in a liposome and can be further analyzed

Isolation of membrane proteins...

Membrane proteins

Membranes separate substances.

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

The PM separates the content of the cell from the environment

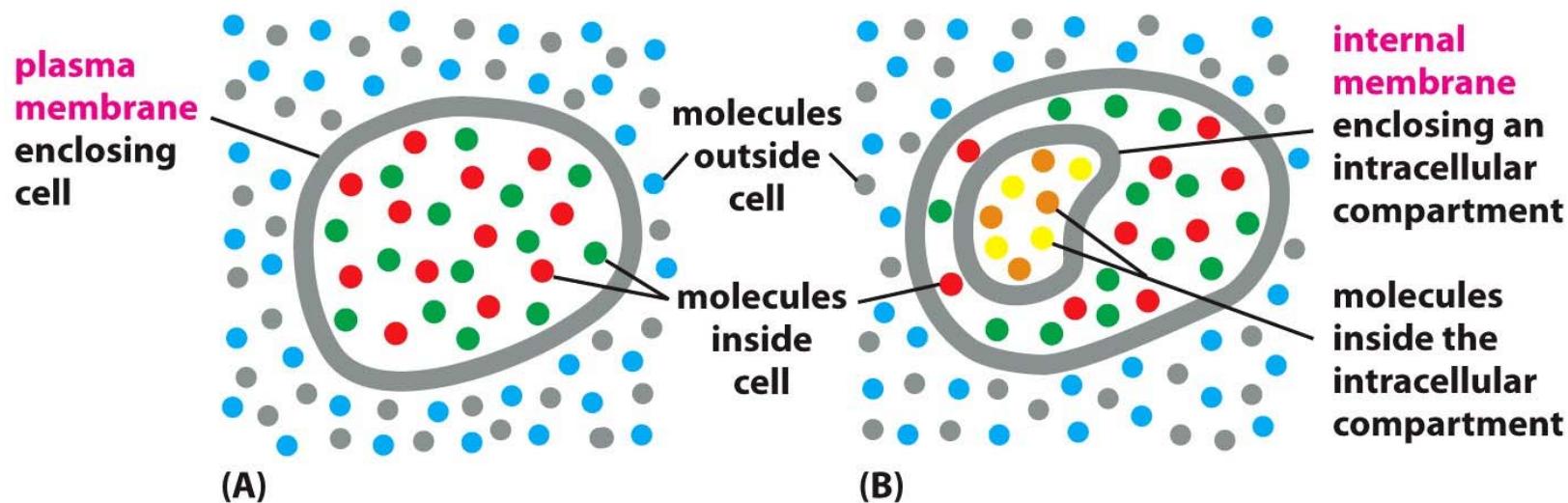


Figure 11-1 Essential Cell Biology 3/e (© Garland Science 2010)

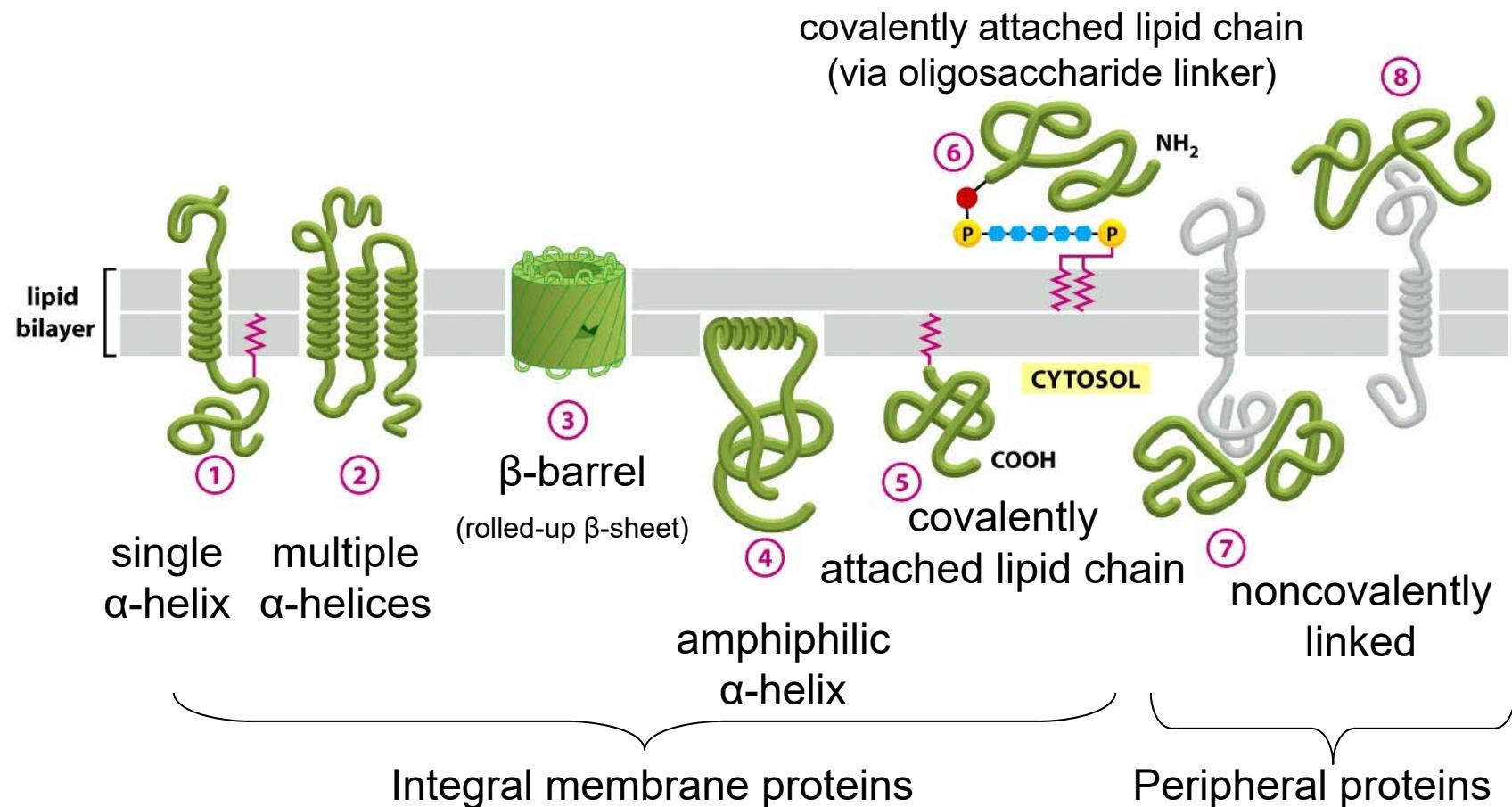
Selective barriers allow the separation of content....

IV. Membrane proteins - a simple overview

- There are **different types of membrane proteins** and they are classified according to the way how they are associated:
 - **Peripheral membrane proteins**
(only associated with the membrane or noncovalently linked with other membrane proteins)
 - **Integral membrane protein**
 - **Transmembrane protein**
(possess transmembrane domain/s)
 - **Covalently linked protein**
(linkage by lipid groups or insertion of hydrophobic regions into the lipid bilayer)

Types of membrane proteins

and their way of interacting with the lipid bilayer



Membrane proteins

General features:

- Generally constitute half of total membrane mass,
but the amount varies in different types of membranes:
 - a. in myelin membrane: **<25%** of membrane mass is protein
 - b. in inner membrane of mitochondria and chloroplast:
~75% of membrane mass
- Membrane proteins fulfill diverse functions:
 - Transport
 - Enzymatic activity
 - Receptors (signal perception/ligand binding)
 - Intercellular junctions
 - Cell-cell recognition
 - Attachment of cytoskeletal elements
and/or extracellular matrix attachment

Facts of membrane proteins

- Membrane proteins account for ~30% of the genome in most 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.

Structural analysis of membrane proteins is extremely difficult. Guess why that is...

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 and their properties

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

Peripheral membrane proteins...

Characteristic features:

- Do **not** penetrate the phospholipid bilayer
and do **not** covalently link to other membrane components,
but do form ionic links to membrane structures
- **Dissociation of peripheral membrane** proteins with chaotropic agents does **not** disrupt membrane integrity
- Can locate to **both**, extracellular and intracellular side of the membrane
- Often link membrane to non-membrane structures
- Synthesis of peripheral proteins at the plasma membrane (PM):
 - a. cytoplasmic (inner) side: synthesized in the cytoplasm
 - b. extracellular (outer) side: synthesized in ER and are delivered by exocytosis/secretion

Integral membrane proteins...

Characteristic features:

- 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:

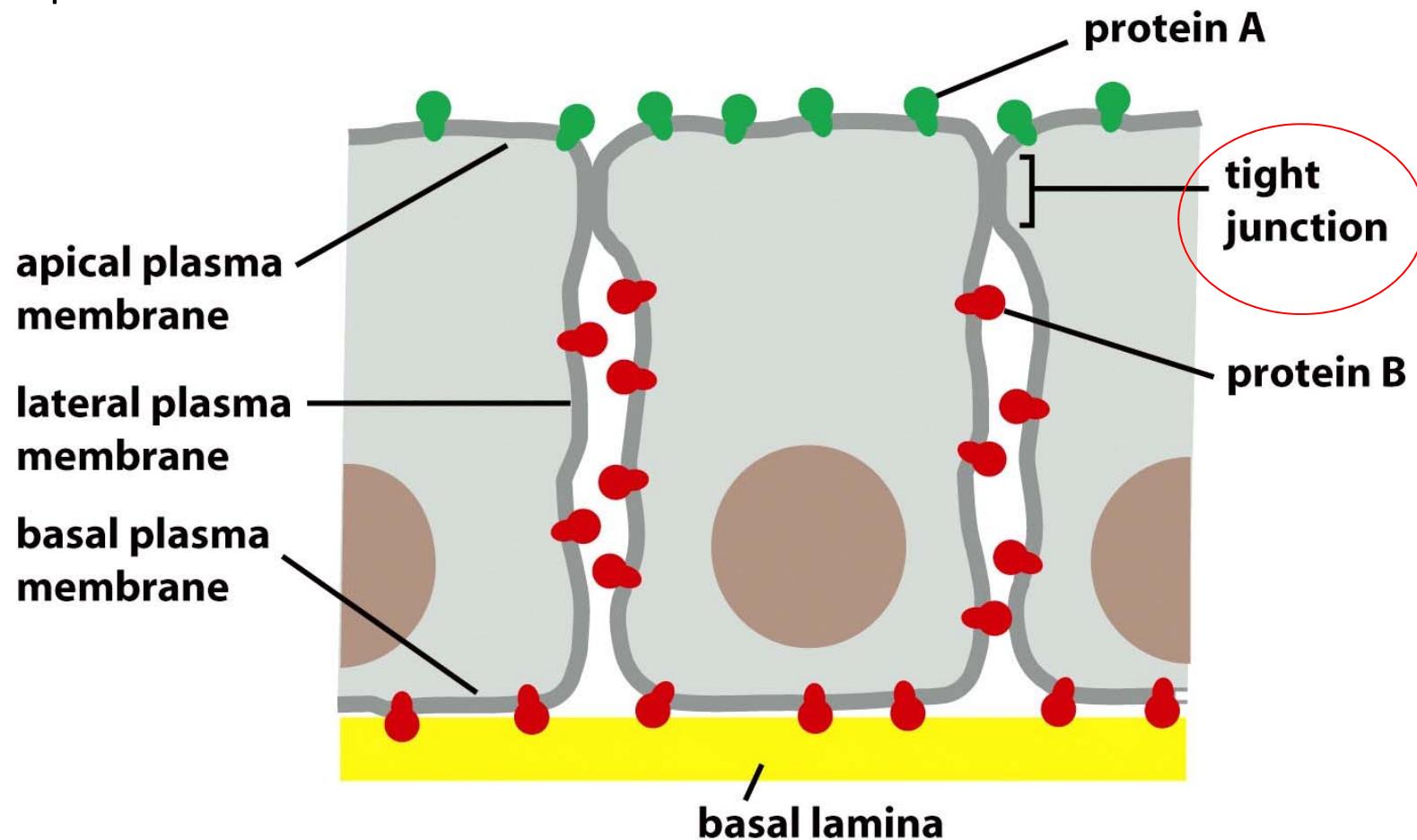
- Occurs in the (rough) endoplasmic reticulum (ER)
- Many integral membrane proteins are **glycoproteins**:
 - glycosylation begins in lumen of ER
 - carbohydrates are modified in Golgi apparatus
- **Lipid-linked proteins** are made in **cytosol as soluble protein first, it becomes a membrane protein after lipid linkage**
- **Glycosylphosphatidylinositol (GPI)** anchors are made as **transmembrane proteins**, 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 (change between the leaflets) of proteins does **not** occur
- Conformational changes of protein **can frequently occur** but that depends on protein function
- Proteins **can be** confined to specific domains
- **Carbohydrates of glycoproteins** are **always** at the **outer** surface
- **Disulfide bonds** are also **always** at the **outer** surface

Specific domains can contain specific membrane proteins

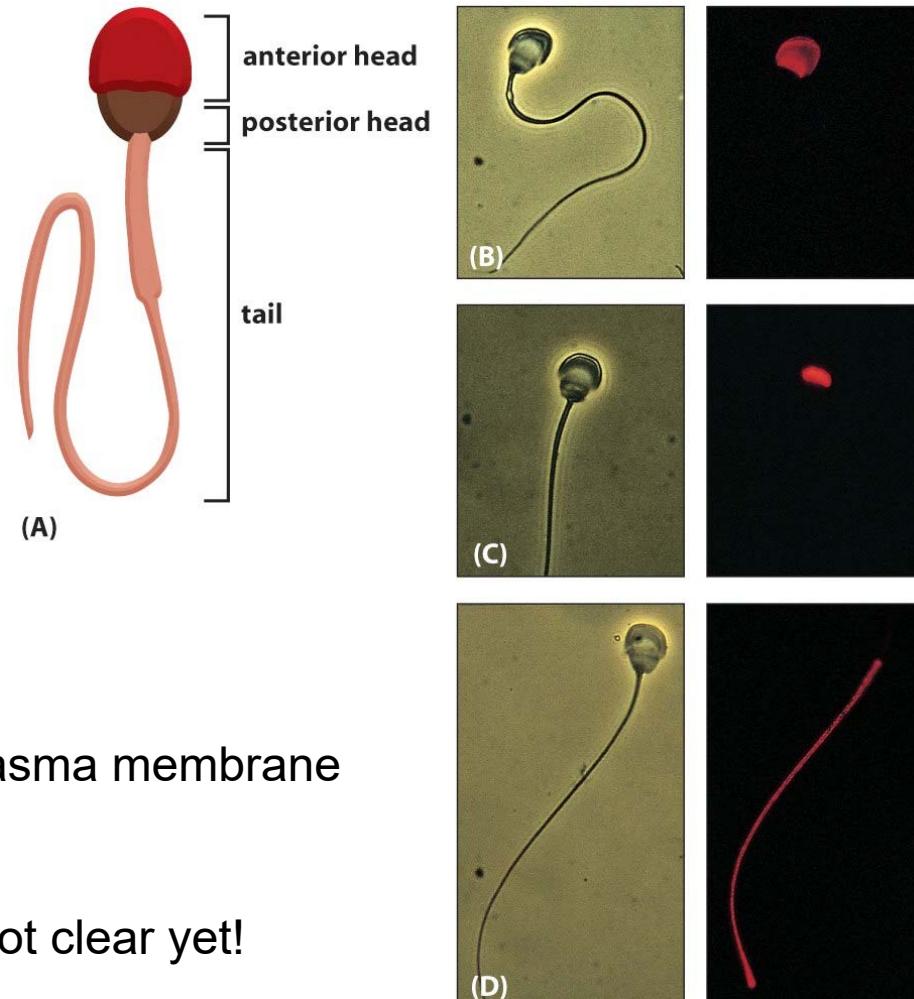
Restriction of proteins/lipids in specific domains
in epithelium cells



Tight Junctions: hold cells together and prevent diffusion of membrane proteins AND lipids to maintain distribution

Three domains in the PM: guinea pig sperm cell

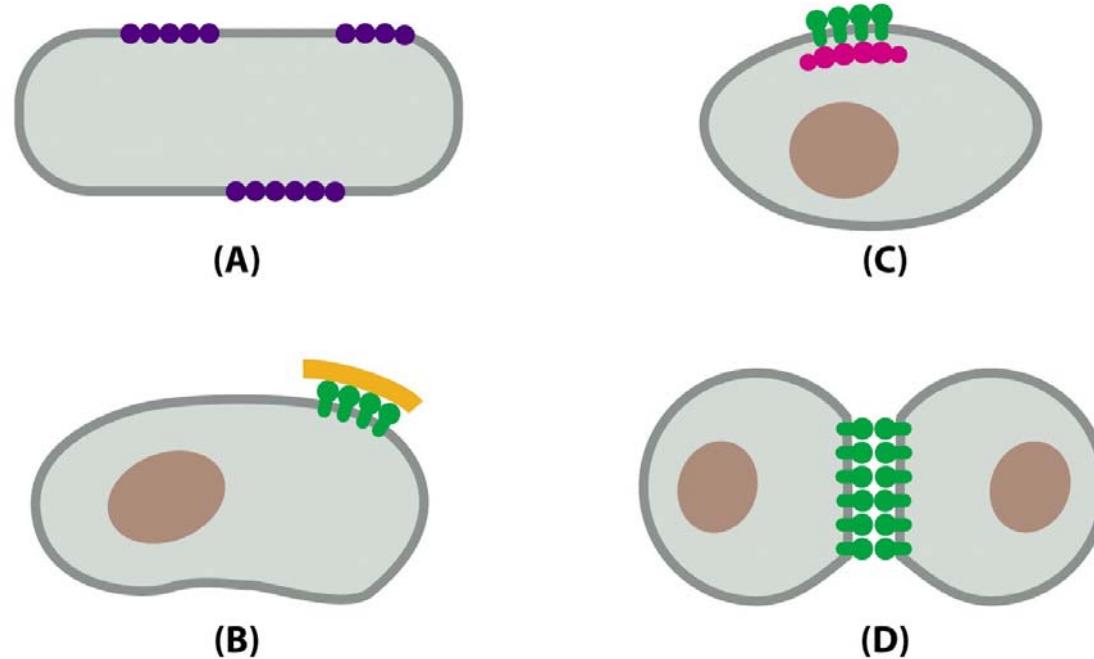
The plasmamembrane of a guinea pig sperm cell contains three different domains



Sperm cell:
continuous plasma membrane

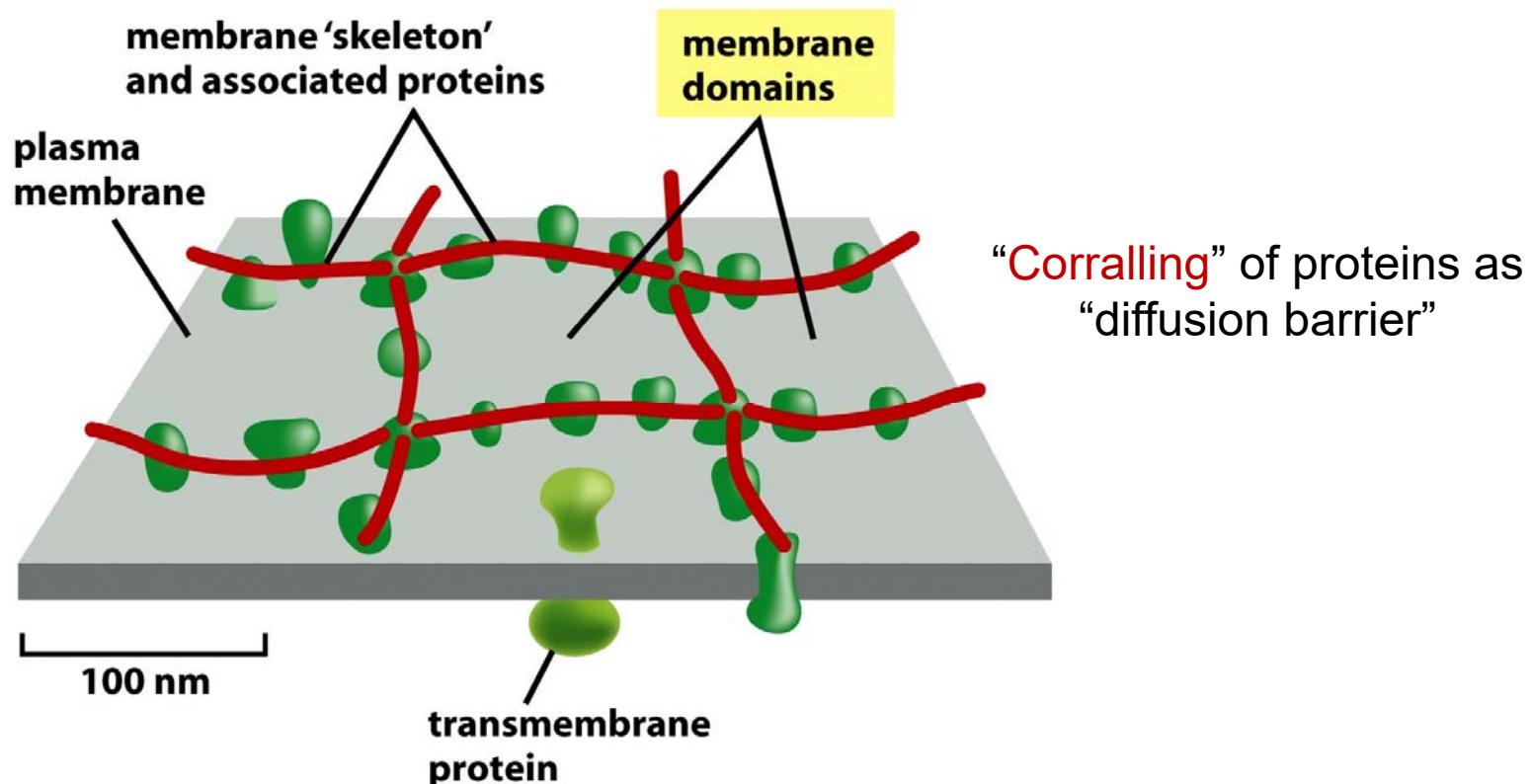
Mechanism: not clear yet!

How can membrane proteins form/accumulate in domains?



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

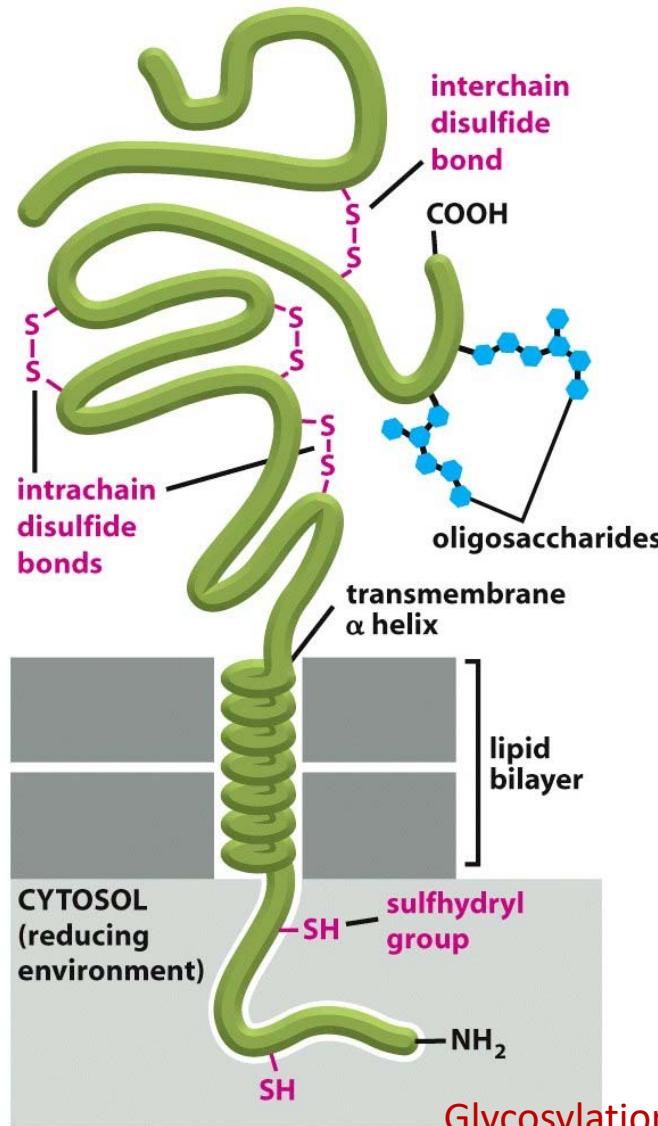
Cytoskeleton network restricts membrane protein diffusion



Membrane proteins can be kept in place by the cytoskeleton: **Corralling**
The attached **cytoskeleton** acts as a grid that prevents lateral diffusion!

Spectrin network (red) interacts with a **junctional complex** in red blood cells
Results: **Shaping of cells**: the **characteristic shape** of the **red blood cell**
Retention works best if membrane proteins have large **cytosolic domains**
and many PM proteins have that!

Post-translational modification of membrane proteins



- **Oligosaccharide** chains are **diverse** and are **always** on the **exoplasmic** side of plasma membrane proteins.
- Due to **reducing environment** of the cytosol, **disulfide bonds** form very rarely
- **Disulfide bonds** on membrane proteins **form** extensively in the **oxidizing environment in the ER** lumen and are therefore mainly found on the **exoplasmic** side.
- **Disulfide bonds** help to **stabilize/determine** the **structure** of proteins

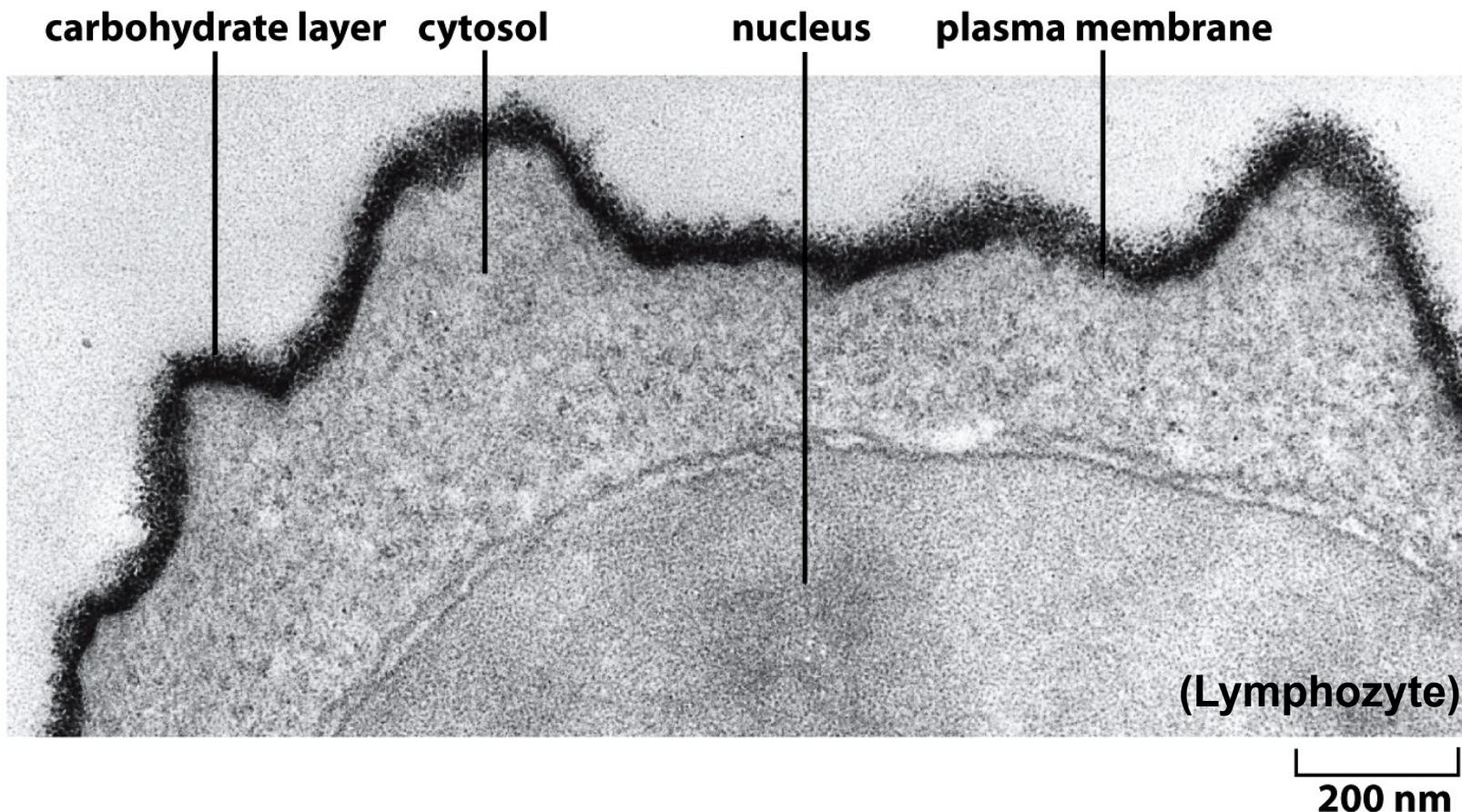
Glycosylations & disulfide bridges: always on the cell surface...

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 cells against mechanical and chemical damage
(keep cell at appropriate distance, etc.)
 - might serve as indicator for regulating their turn-over

Carbohydrate layer by ruthenium red stain

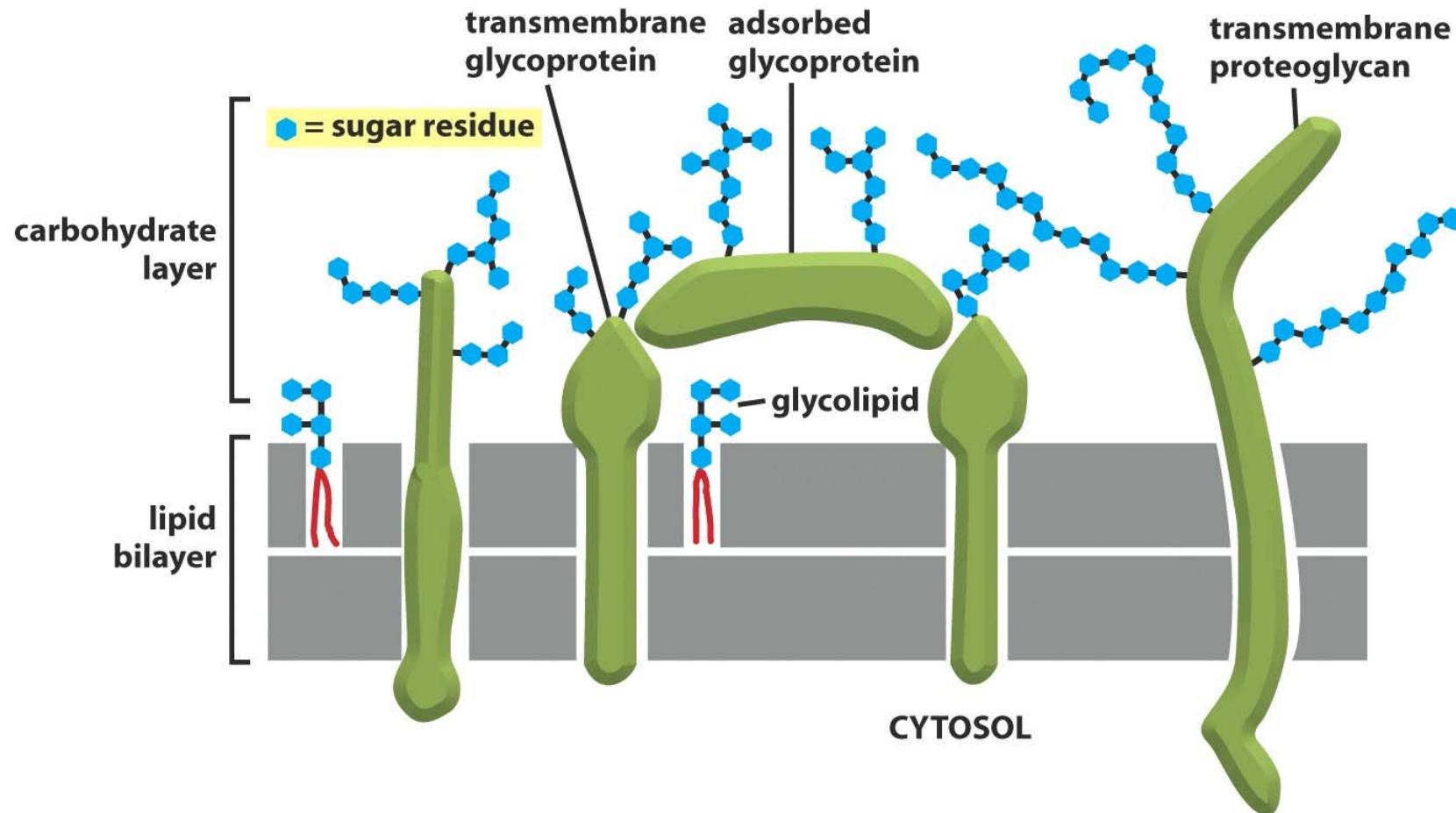
Carbohydrate layer (**Glycocalyx**) is made of **Glycoproteins** and **Glycolipids**



Carbohydrate layer or **Glycocalyx** is made of **Glycoproteins AND Glycolipids**

Different types of glycosylation

The oligosaccharide layer consists of glycoproteins & glycolipids



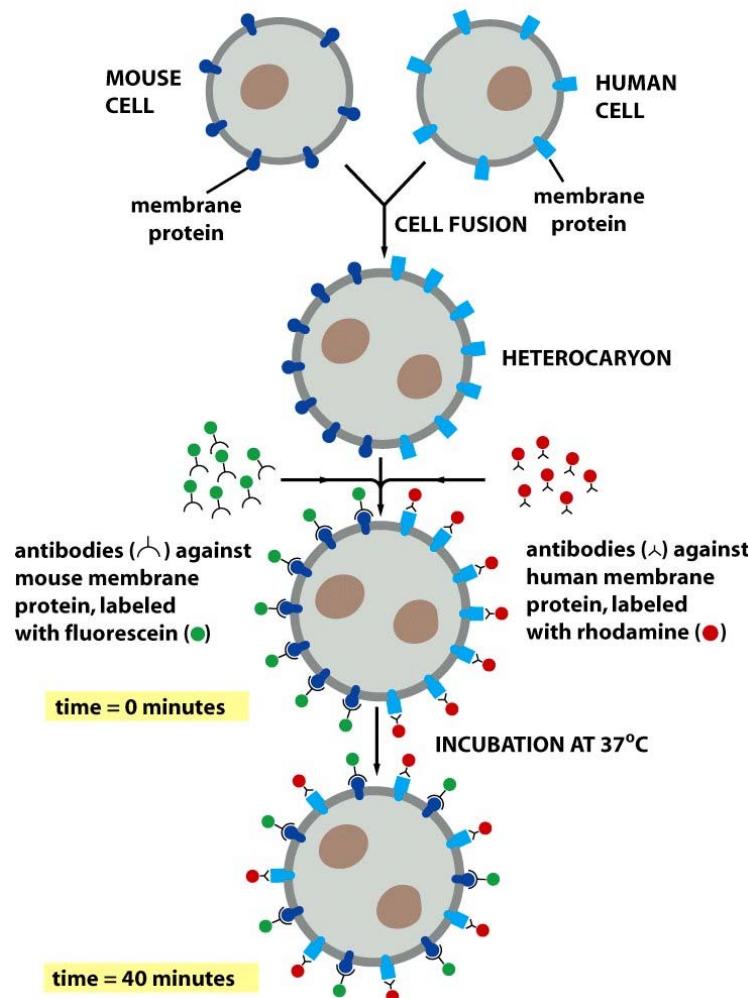
Mobility of membrane proteins

Membrane proteins move....

- **Rotational** mobility
- **Lateral** diffusion
- Protein mobility **varies** greatly:
 - **Some** proteins are **free to move**
 - **Others** are **restricted in mobility** either by:
 - **tethering to structures** in the **cytoplasm**
 - **tethering to extracellular structures**
 - Some types of **cell junctions** (e.g., tight junctions) can restrict protein movements to **keep them** in a specific membrane domain.

Mobility of membrane proteins by lateral diffusion

Example 1: Cell fusion experiment to detect lateral diffusion

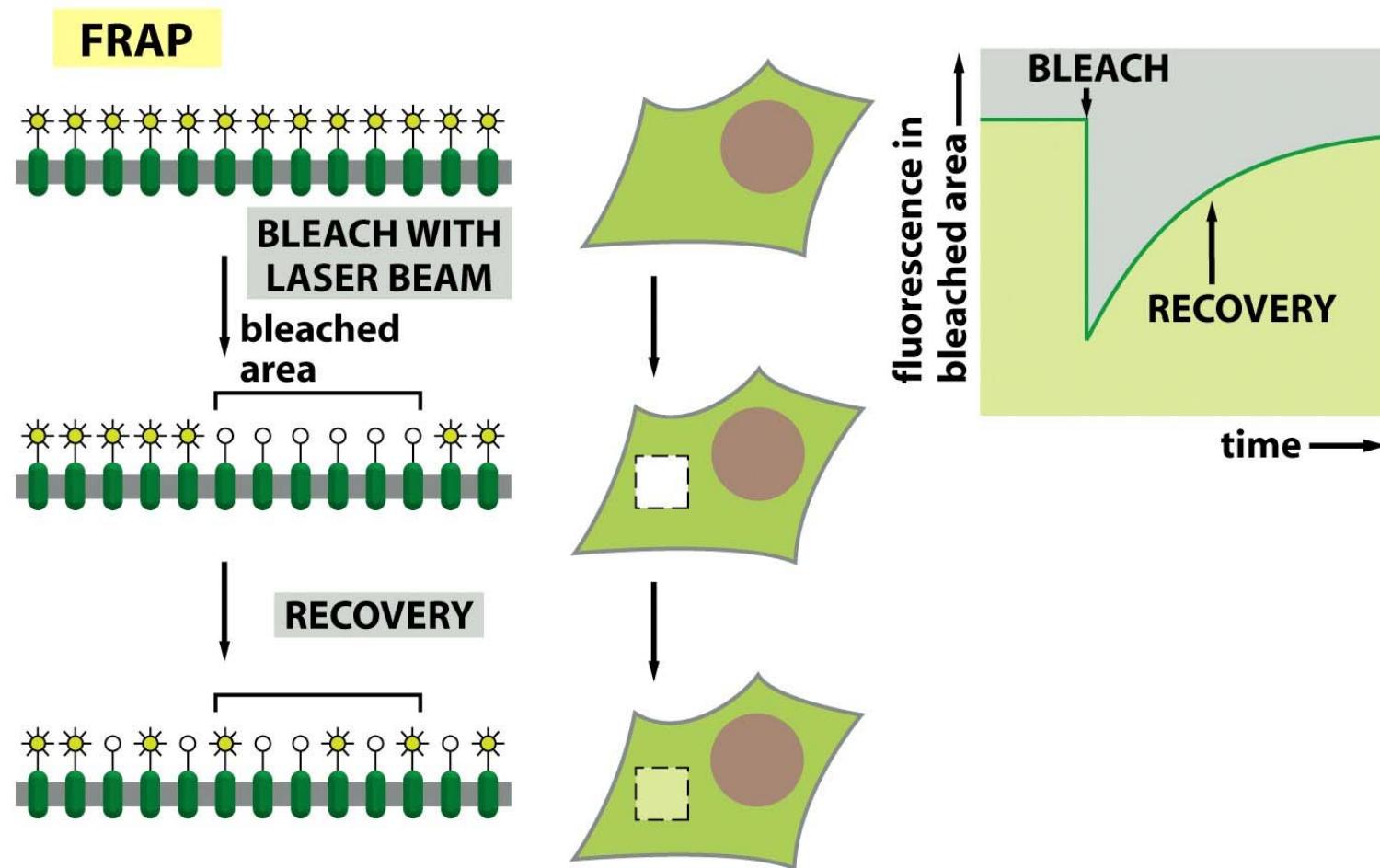


Immediately after fusion:
-Proteins are separated

After some time:
proteins are mixed by diffusion

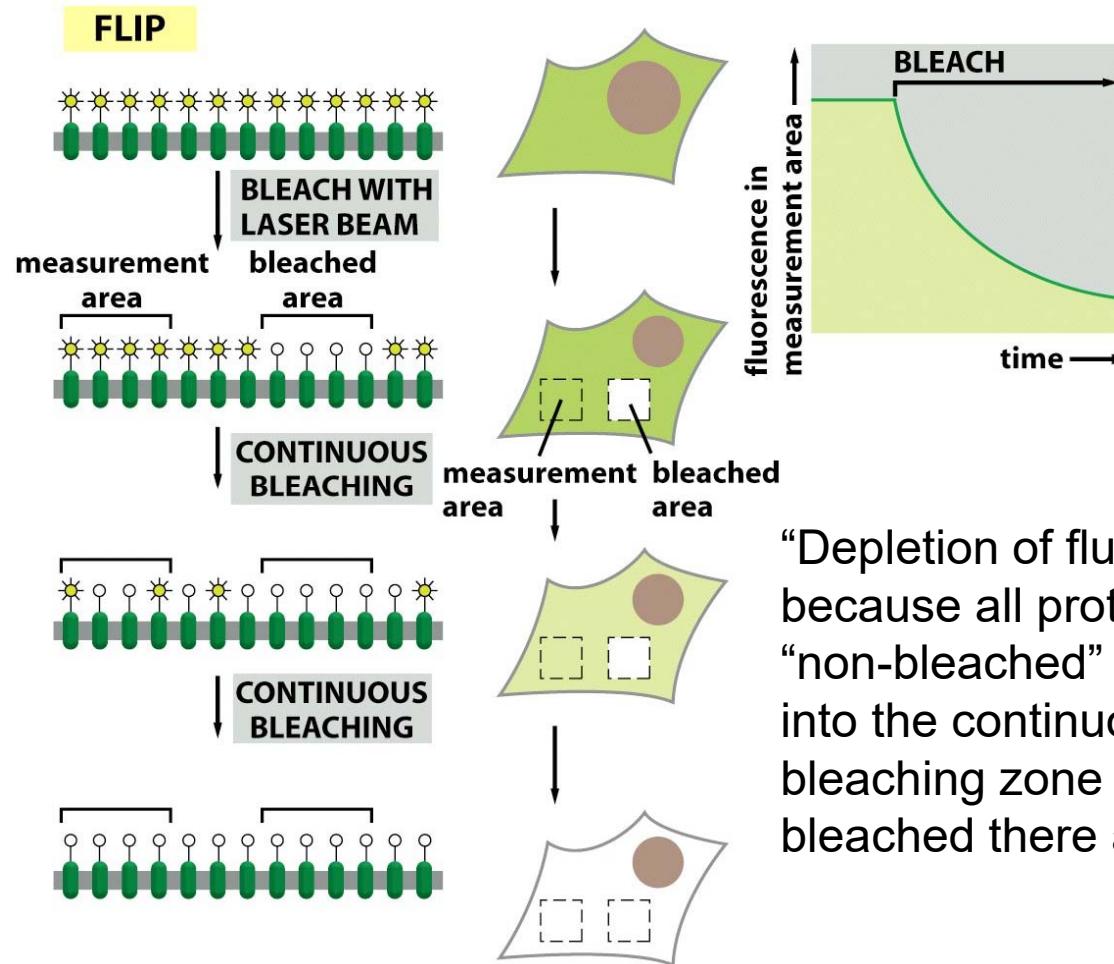
Mobility of membrane proteins by lateral diffusion

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



Mobility of membrane proteins by lateral diffusion

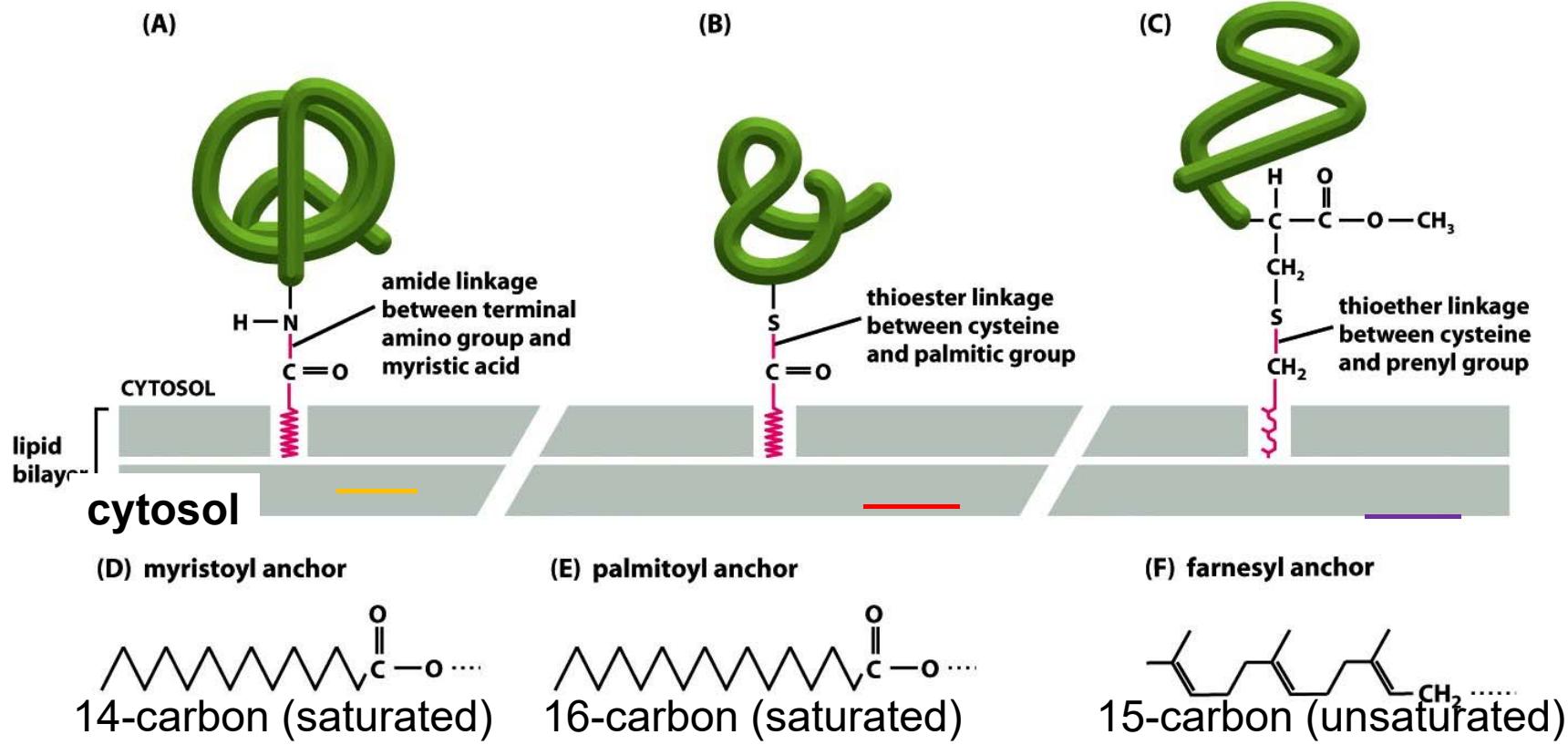
Example 3: Fluorescence loss in photobleaching (FLIP) to detect membrane protein diffusion



“Depletion of fluoresce”,
because all proteins from the
“non-bleached” zone diffuse
into the continuously/ongoing
bleaching zone and get
bleached there as well

Covalent attachment of membrane proteins to lipids

Ways for membrane proteins to covalently attach to membrane lipid



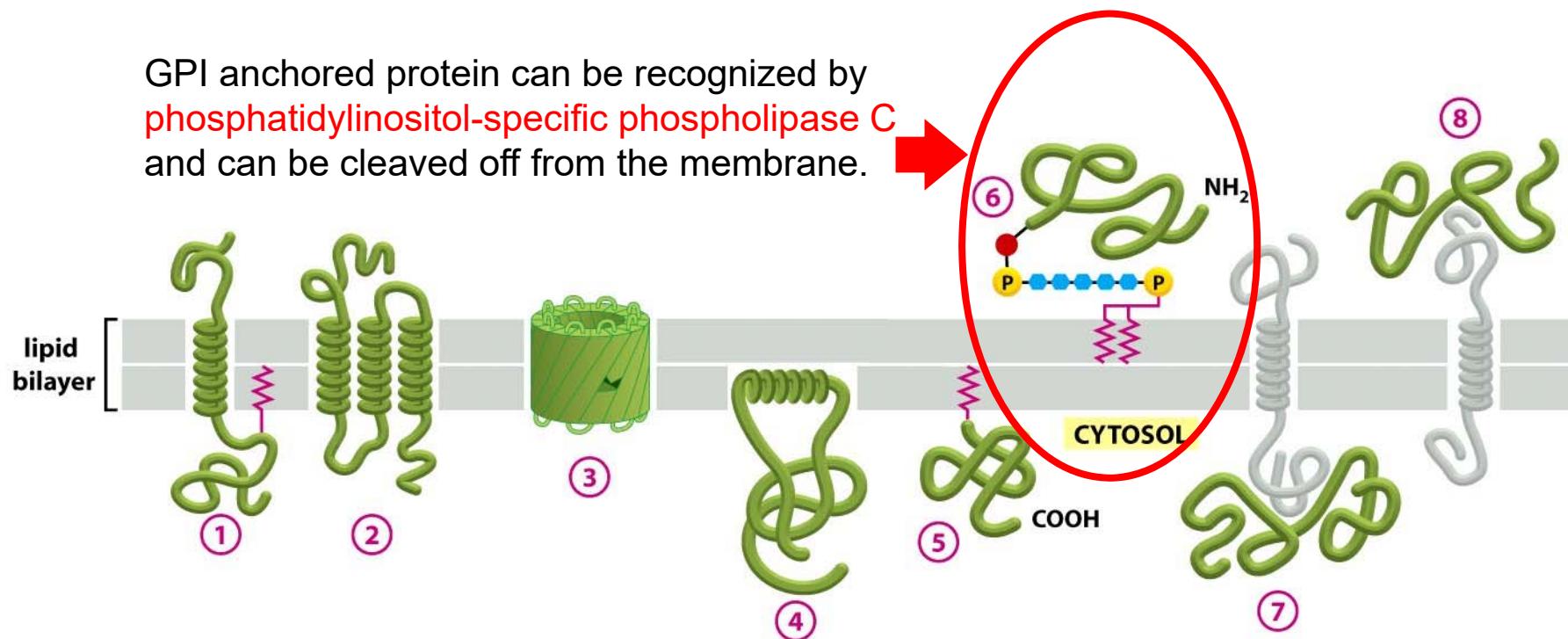
Addition of: FATTY ACIDS or PRENYL group

- A) Amide linkage (N-terminus)
- B) Thioester linkage at a given cysteine
- C) Thioether linkage (C-terminus)

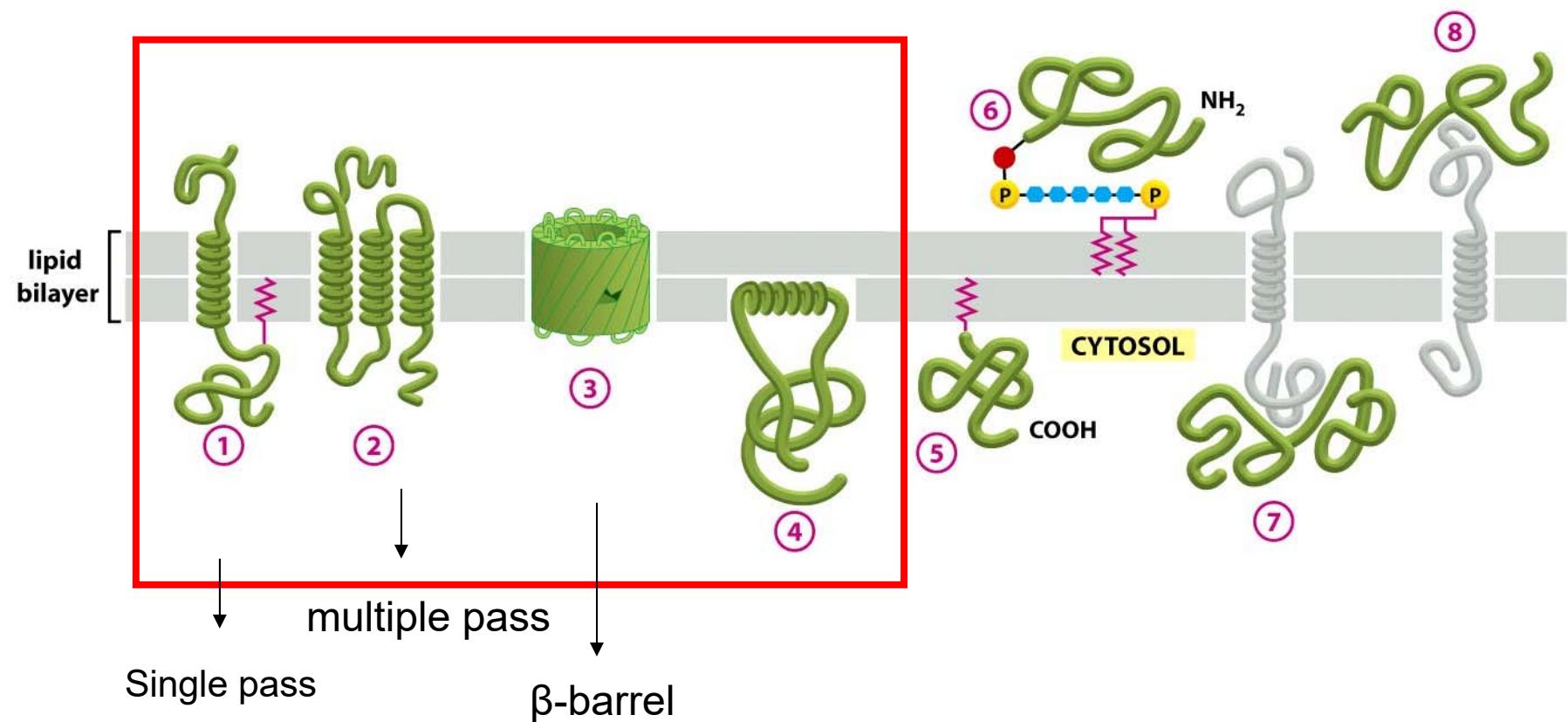
Glycosylphosphatidylinositol (GPI)-anchored Proteins

Discrimination between Glycosylated Transmembrane proteins and GPI-anchored proteins:
Digestion using phosphatidyl-inositol-specific phospholipase C

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

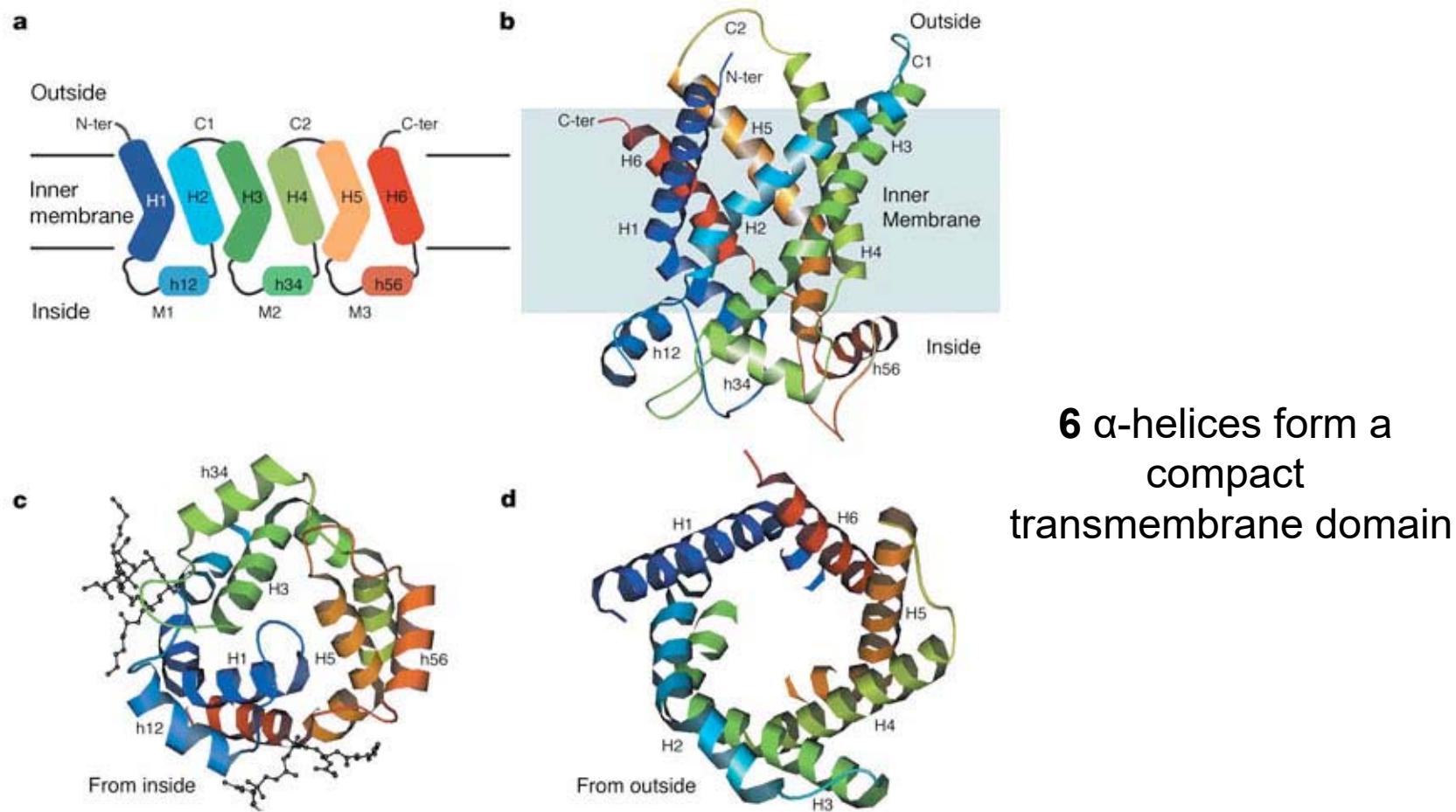


Topology of membrane proteins



Multiple pass transmembrane protein with 6 α -helices

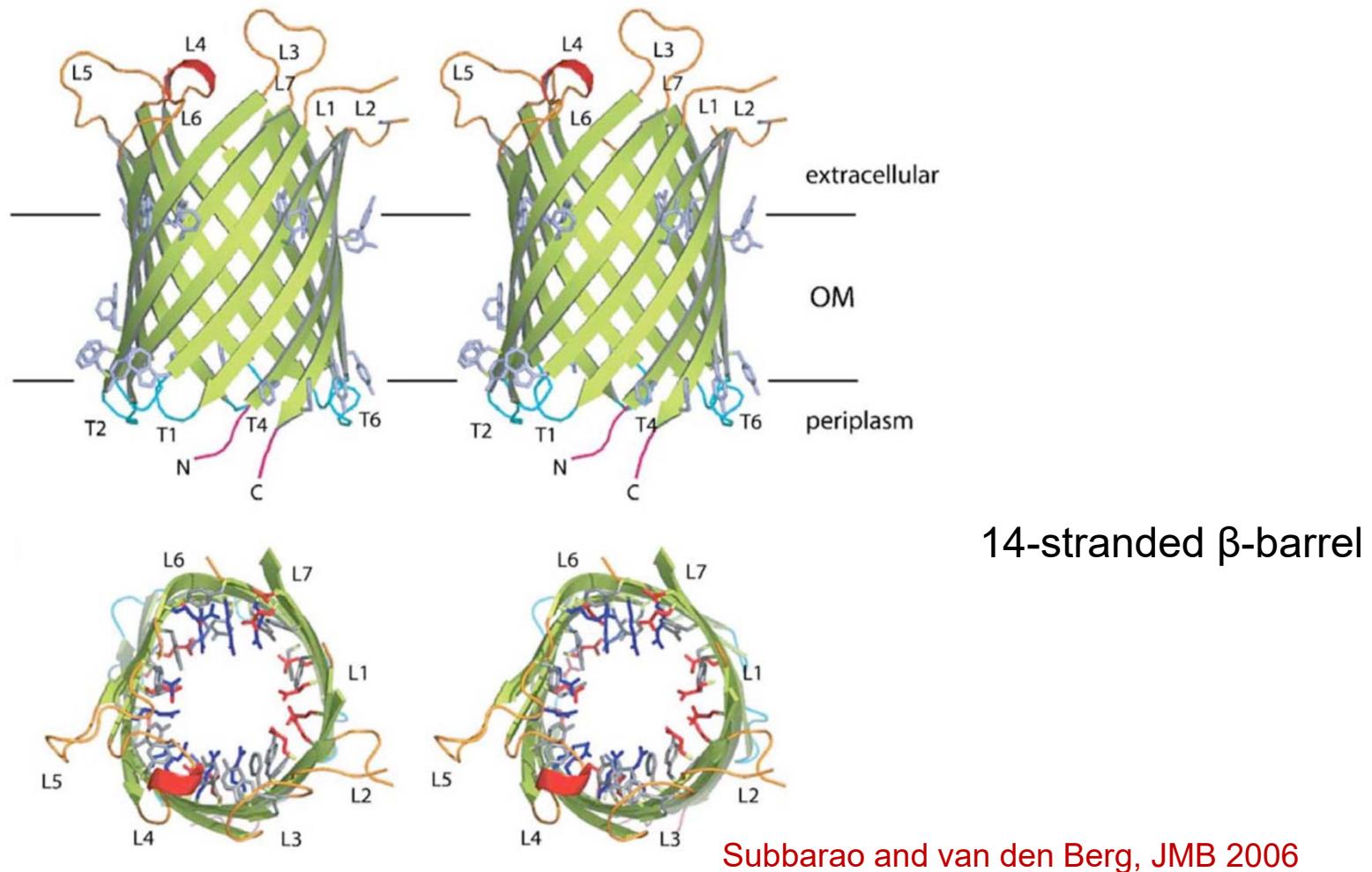
Example 1: mitochondria ATP/ADP carrier
(multiple pass transmembrane protein)



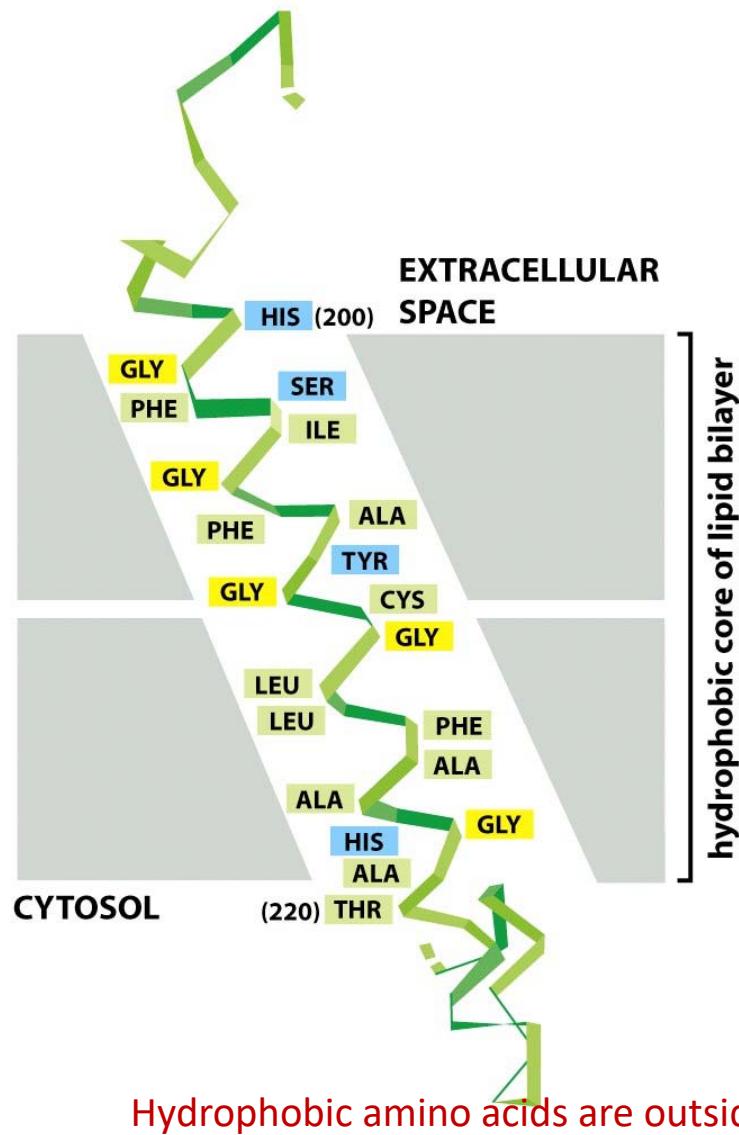
Pebay-Peyroula et al, Nature 2003

β -barrel transmembrane protein: 14-stranded β -barrel

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



Transmembrane α -helices



Transmembrane α -helices

- A segment of 20-30 amino acids with a **high degree of hydrophobicity**
- Hydrophobic amino acids are outside, facing the fatty-acid chains of the membrane

Hydrophobic amino acids:
green and yellow

Hydrophobicity is predicted based on the amino acid sequence

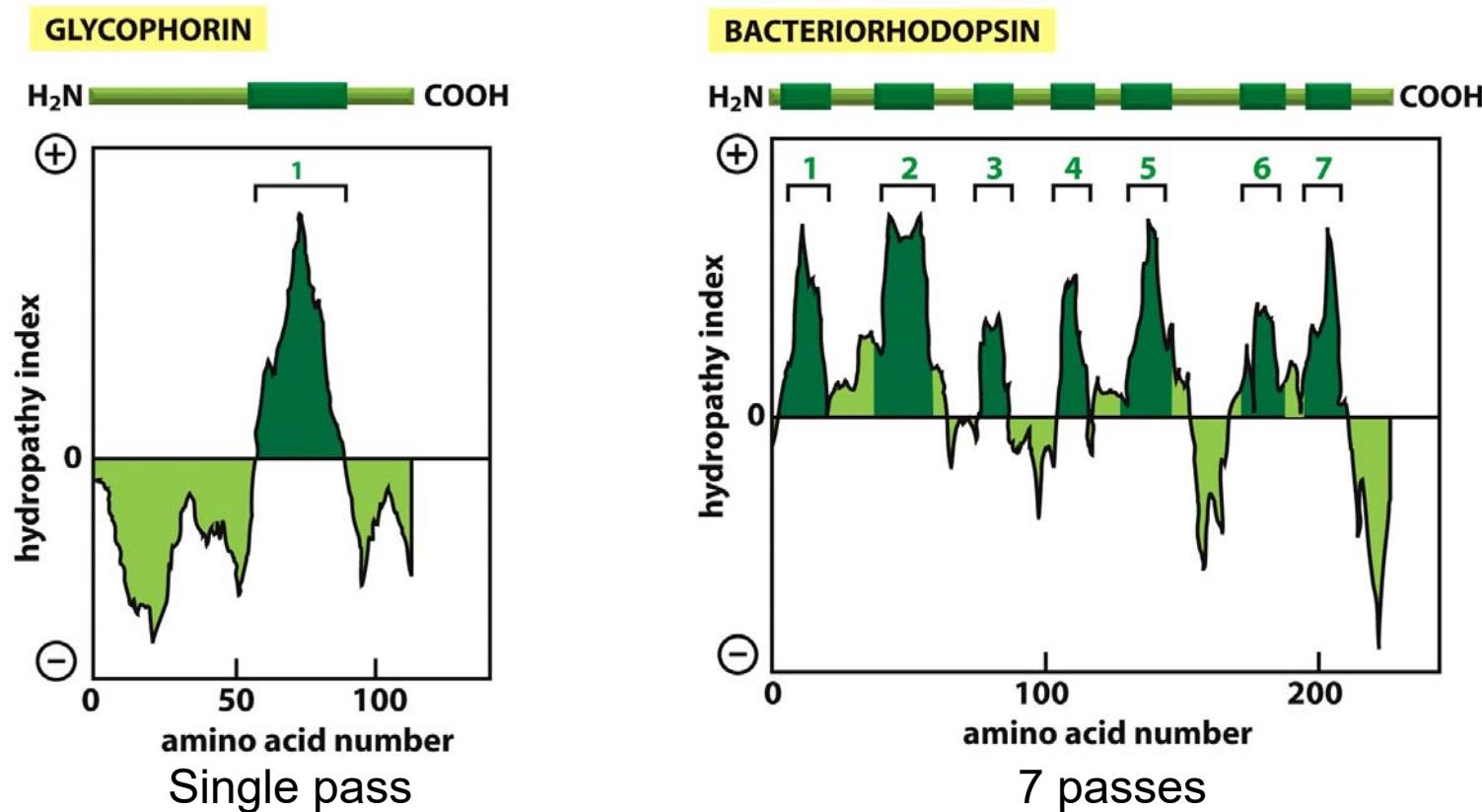
Hydrophobicity Scales

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 visualize hydrophobicity and hydrophilicity

Hydropathy plots to predict transmembrane α -helices this is important for the topology prediction of a transmembrane protein



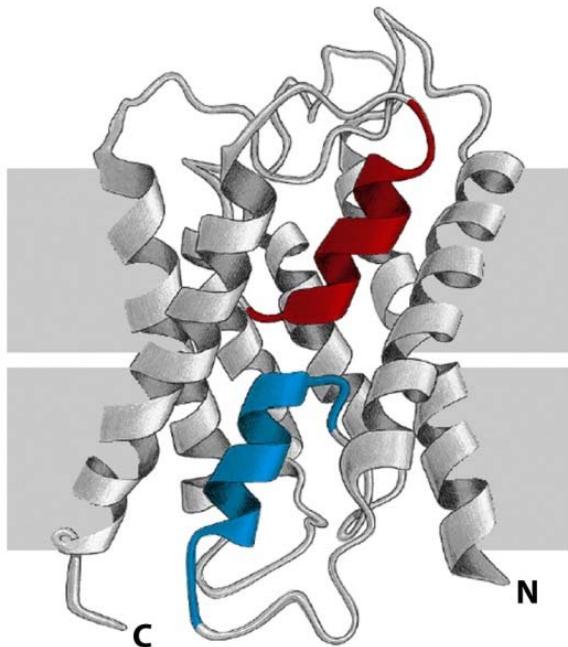
Hydropathy index:

Positive value indicates: free energy is required for transfer to water

Not all membrane interacting regions can be predicted..

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**.

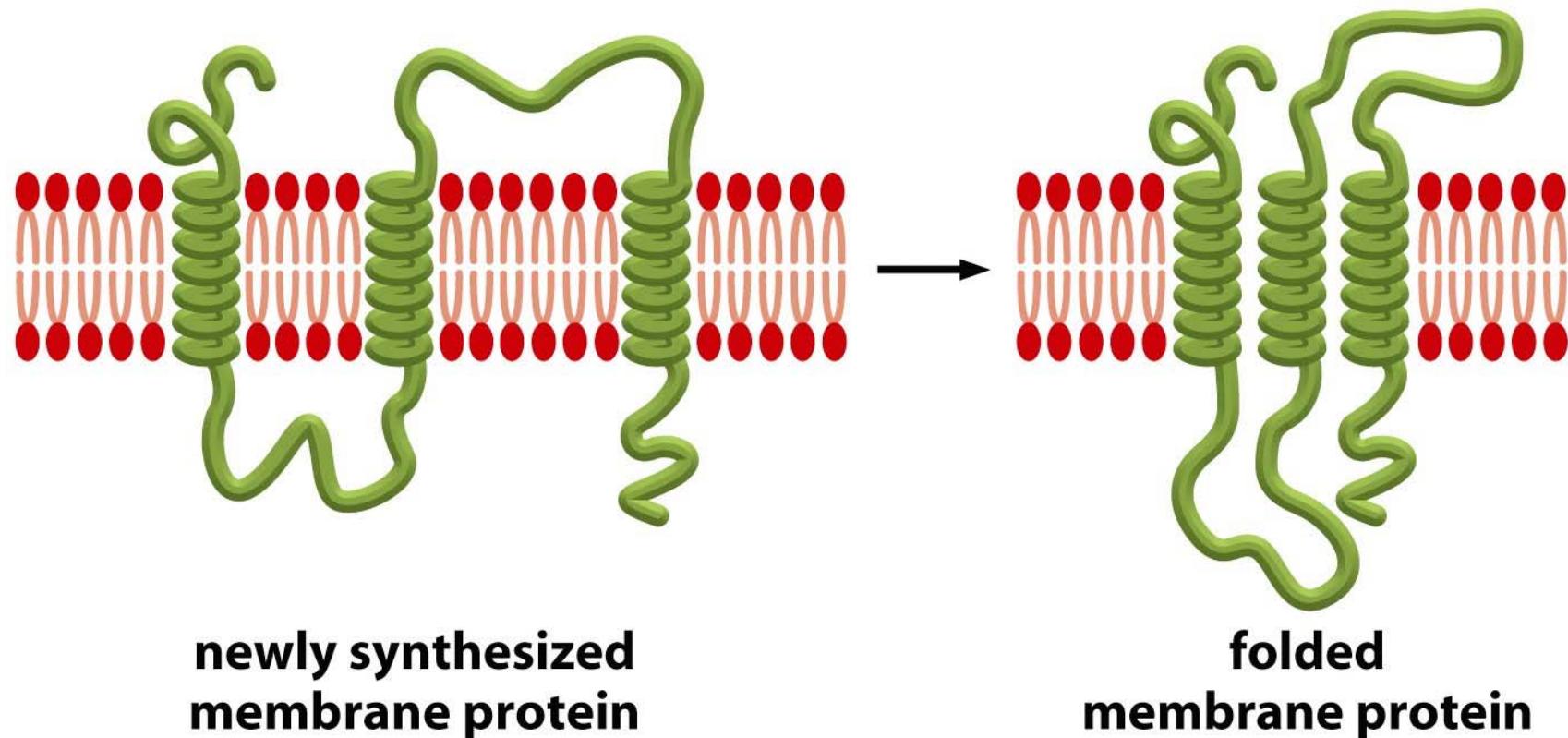


Colored **two α -helices** in a aquaporin water channel **are buried** at an interface formed by protein-protein interactions, they are **not hydrophobic**.

How unfair is that for biochemists...

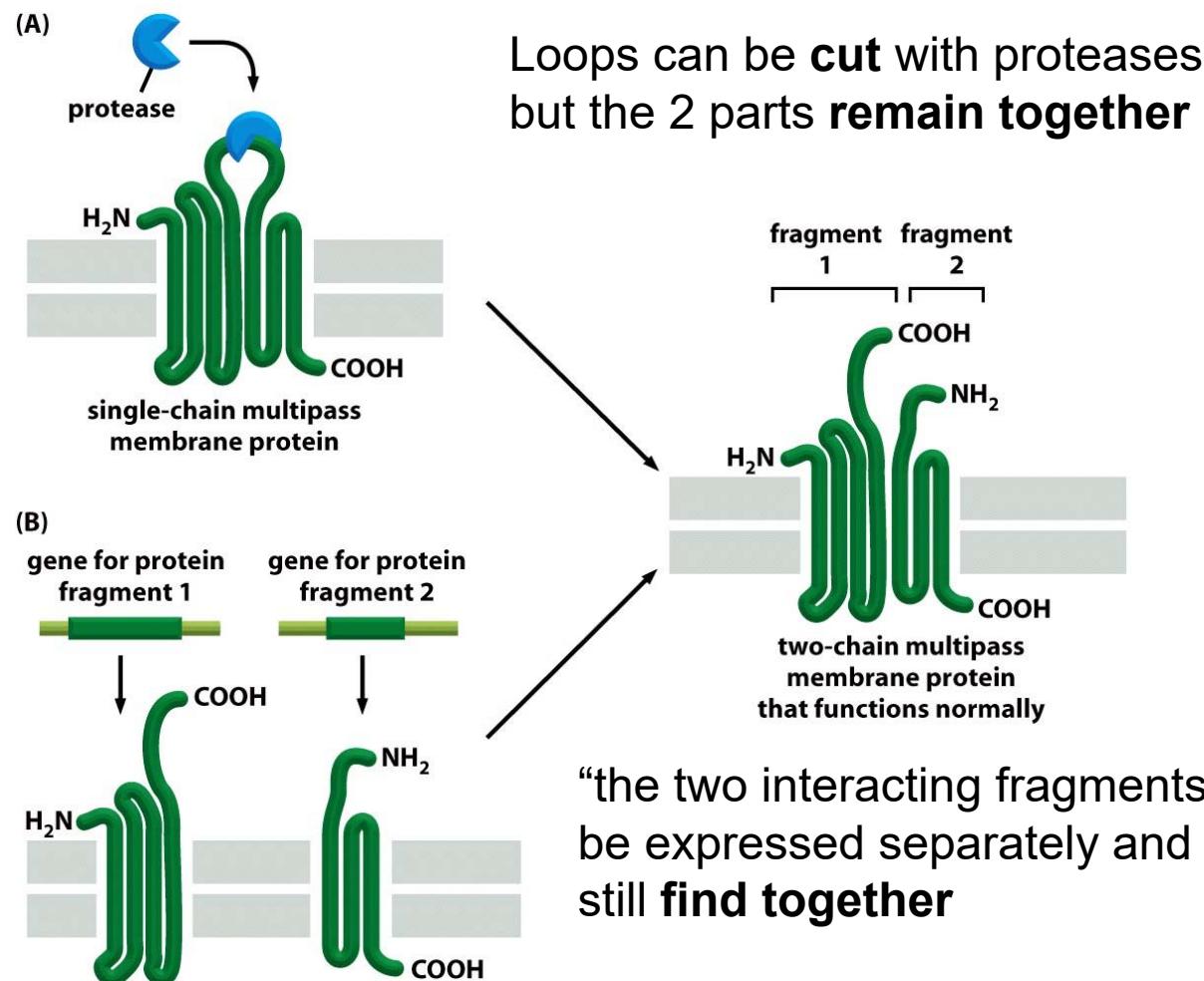
Specific interaction between TMDs

Transmembrane α -helices have specificity for its interaction partners



Interaction between the α -helices is stable

Transmembrane α -helix has specificity for its interaction partners



Bacteriorhodopsin:

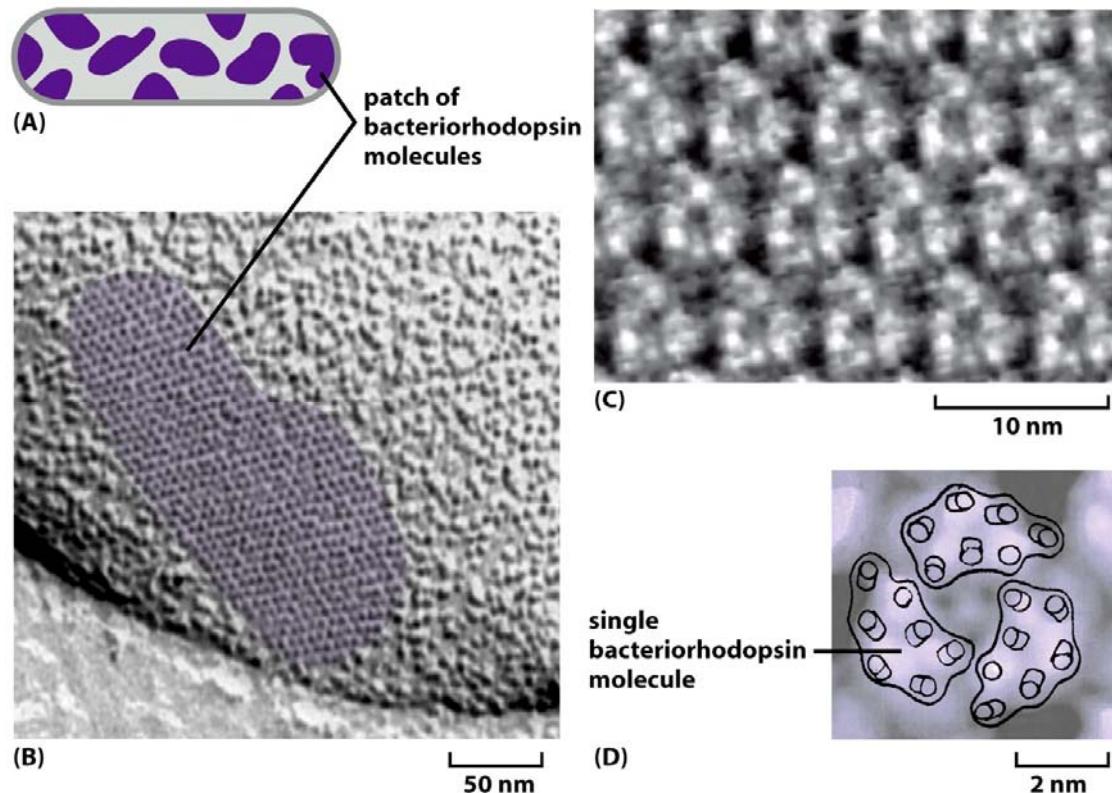
The first membrane transport protein whose structure was determined

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

Bacteriorhodopsin: light-driven proton pump !!! – WOW!

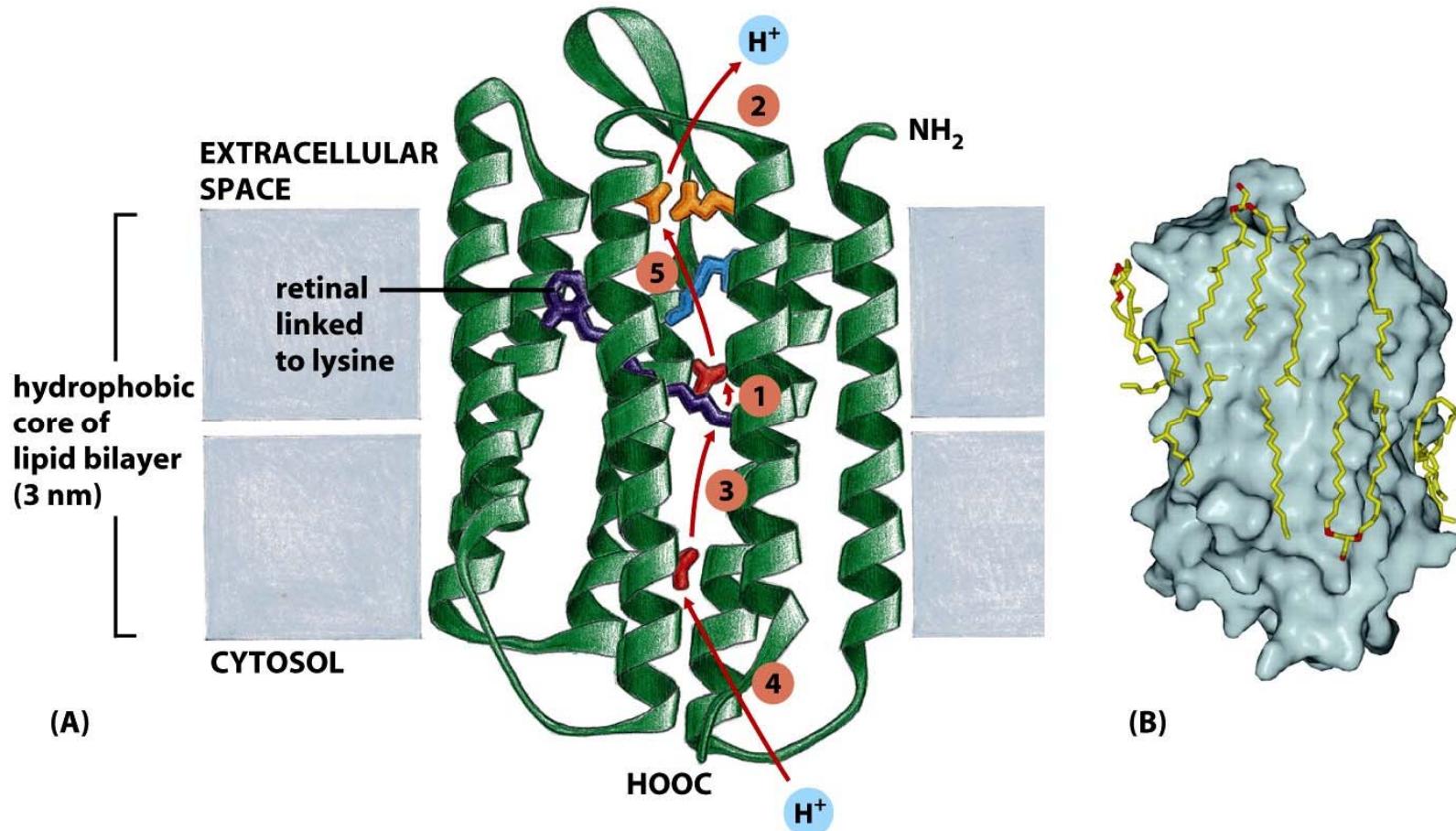
Bacteriorhodopsin

The first cristalized membrane protein



Bacteriorhodopsin:
3 monomers
Each monomer has
7 transmembrane
Domains (TMDs)

3-dimentional structure of bacteriorhodopsin



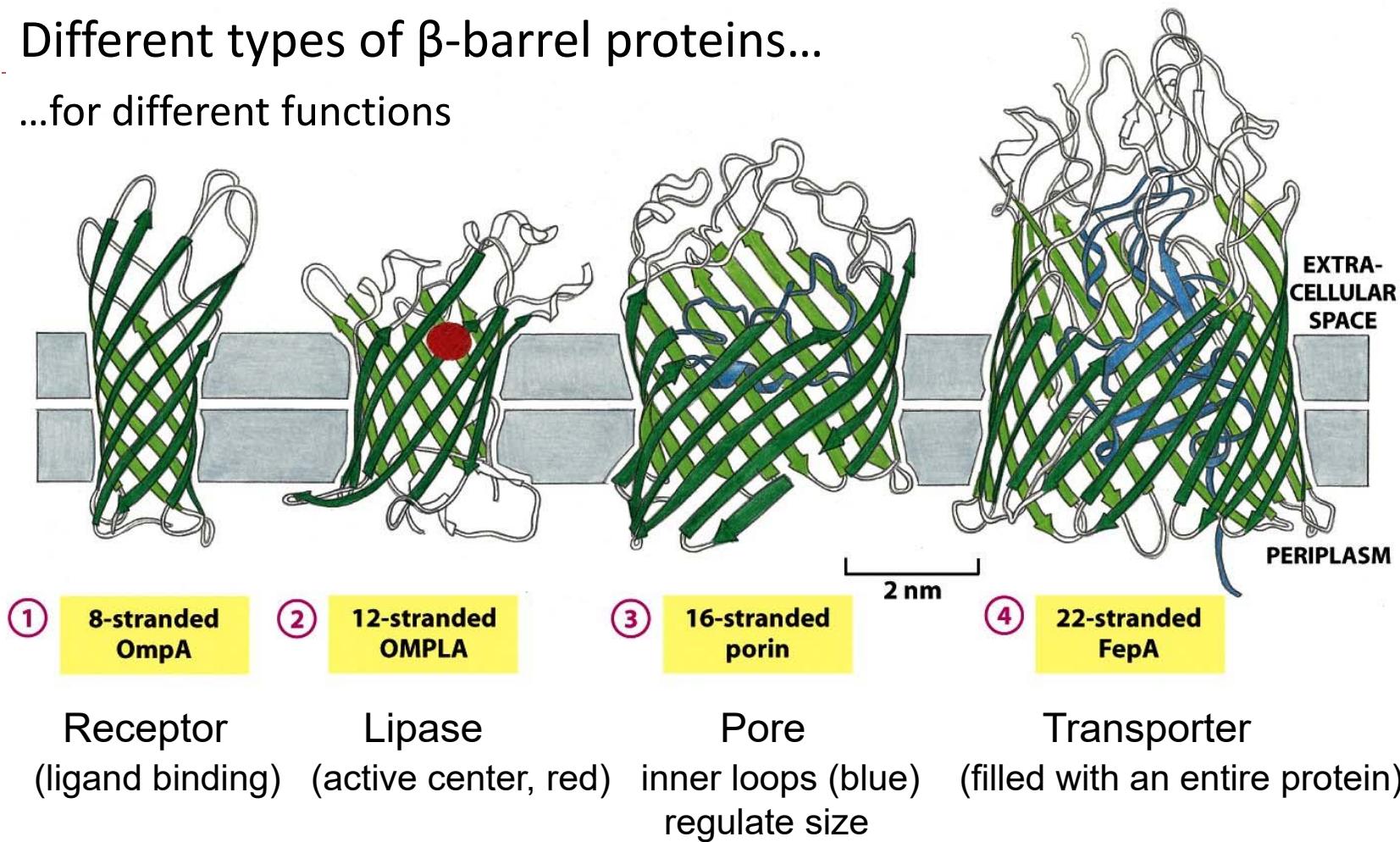
Bacteriorhodopsin: light-driven proton pump

Retinal: chromophore (light absorbing group) – linked to a lysine

Each molecule can pump several hundred H^+ per second

Different types of β -barrel proteins...

...for different functions



Some β -barrels form large Transmembrane Channels:

Found in: outer membrane of mitochondria and plastids

Number of β -sheets: 8-22

Transmembrane channels: β -barrel proteins

Features:

- Relatively **rigid** structures,
conformational changes are **less likely** to occur
- Relatively “**easy**” to crystallize for structure determination
- **Abundant** in **outer membranes** of
mitochondria, chloroplast and **bacteria**.
- Most β -barrel proteins have a “**transporting function**” e.g. **porins**
but there are also receptors and enzymes
- **Inside barrel: polar amino acids**
- **Outside barrel: nonpolar amino acids**
- **Loops inside lumen** confer **selectivity**: **only selected molecules can pass**