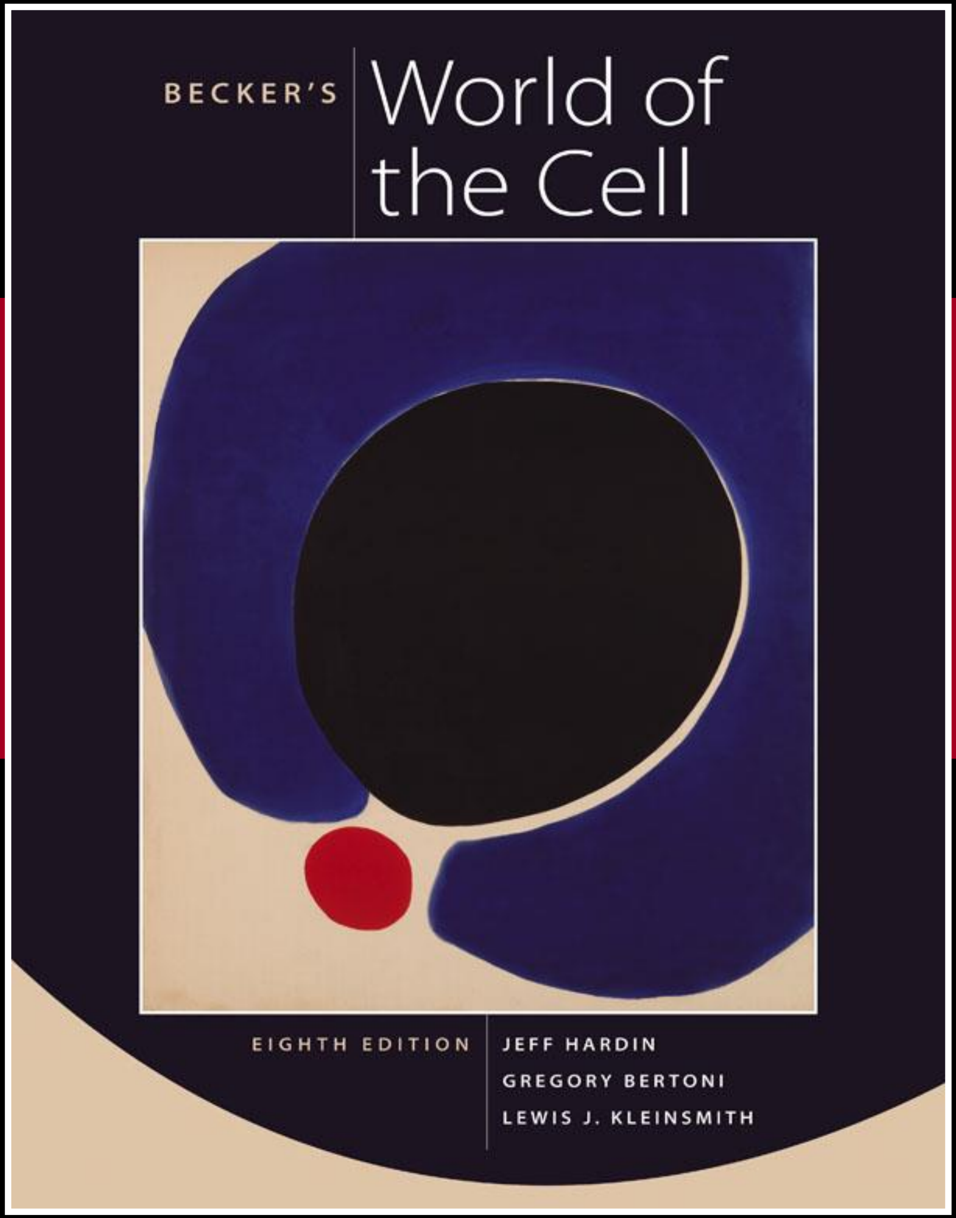


Chapter 7

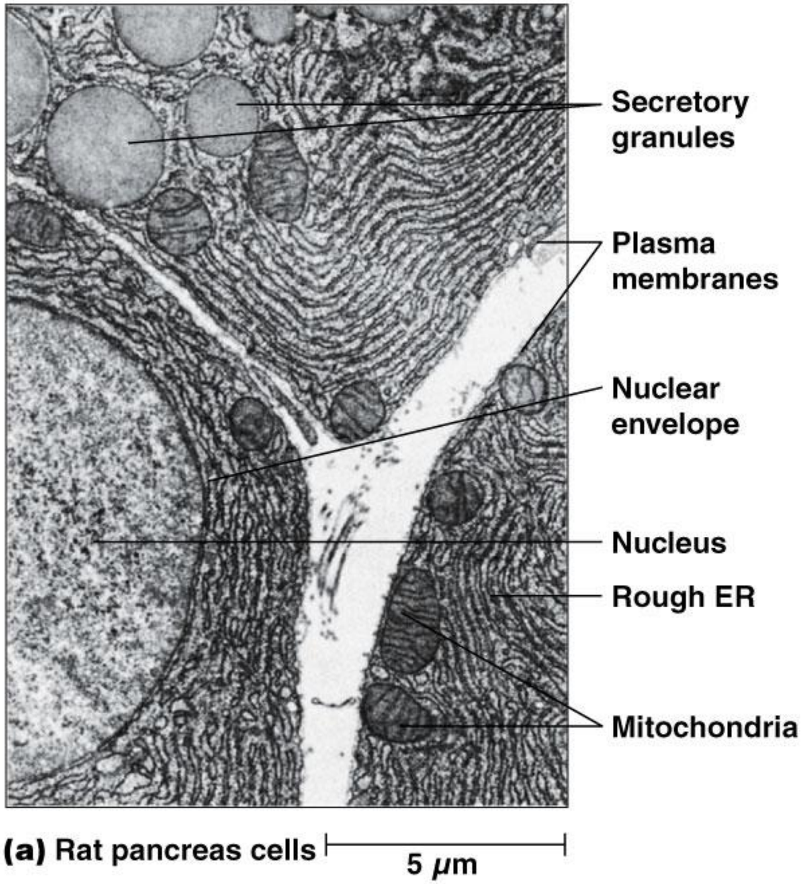
Membranes: Their Structure, Function, and Chemistry

Lectures by
Kathleen Fitzpatrick
Simon Fraser University

Membranes: Their Structure, Function, and Chemistry

- **Membranes** define the boundaries of a cell, and its internal compartments

- Membranes play multiple roles in the life of a cell



(a) Rat pancreas cells

The Functions of Membranes

1. Define boundaries of a cell and organelles and act as permeability barriers

2. Serve as sites for biological functions such as electron transport

3. Possess transport proteins that regulate the movement of substances into and out of cells and organelles

4. Contain protein molecules that act as receptors to detect external signals

5. Provide mechanisms for cell-to-cell contact, adhesion, and communication

Membranes Define Boundaries and Serve as Permeability Barriers

- Membranes separate the interior and exterior of cells and organelles

- They **are effective permeability** barriers due to their hydrophobic interior

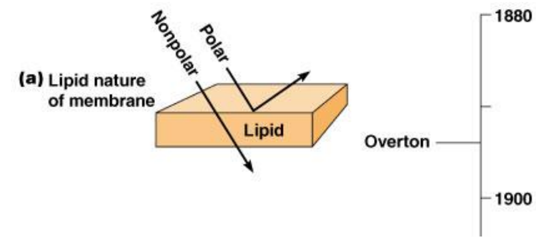
- The **plasma membrane** surrounds the whole cell, whereas **intracellular membranes** *compartmentalize* functions within the cell

Membranes Are Sites of Specific Proteins and Therefore of Specific Functions

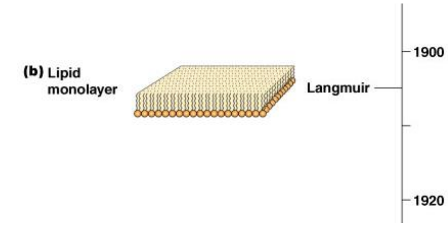
- Membranes are associated with specific functions because the molecules responsible for the functions are embedded in or localized on membranes

Models of Membrane Structure: An Experimental Approach

Overton and Langmuir: Lipids Are Important Components of Membranes



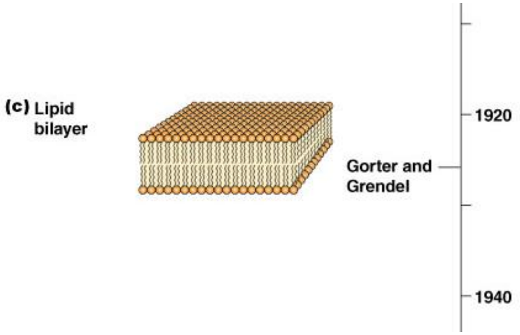
- In the 1890s Overton observed the easy penetration of **lipid-soluble** substances into cells and concluded that the cell surface had some kind of lipid "coat" on it



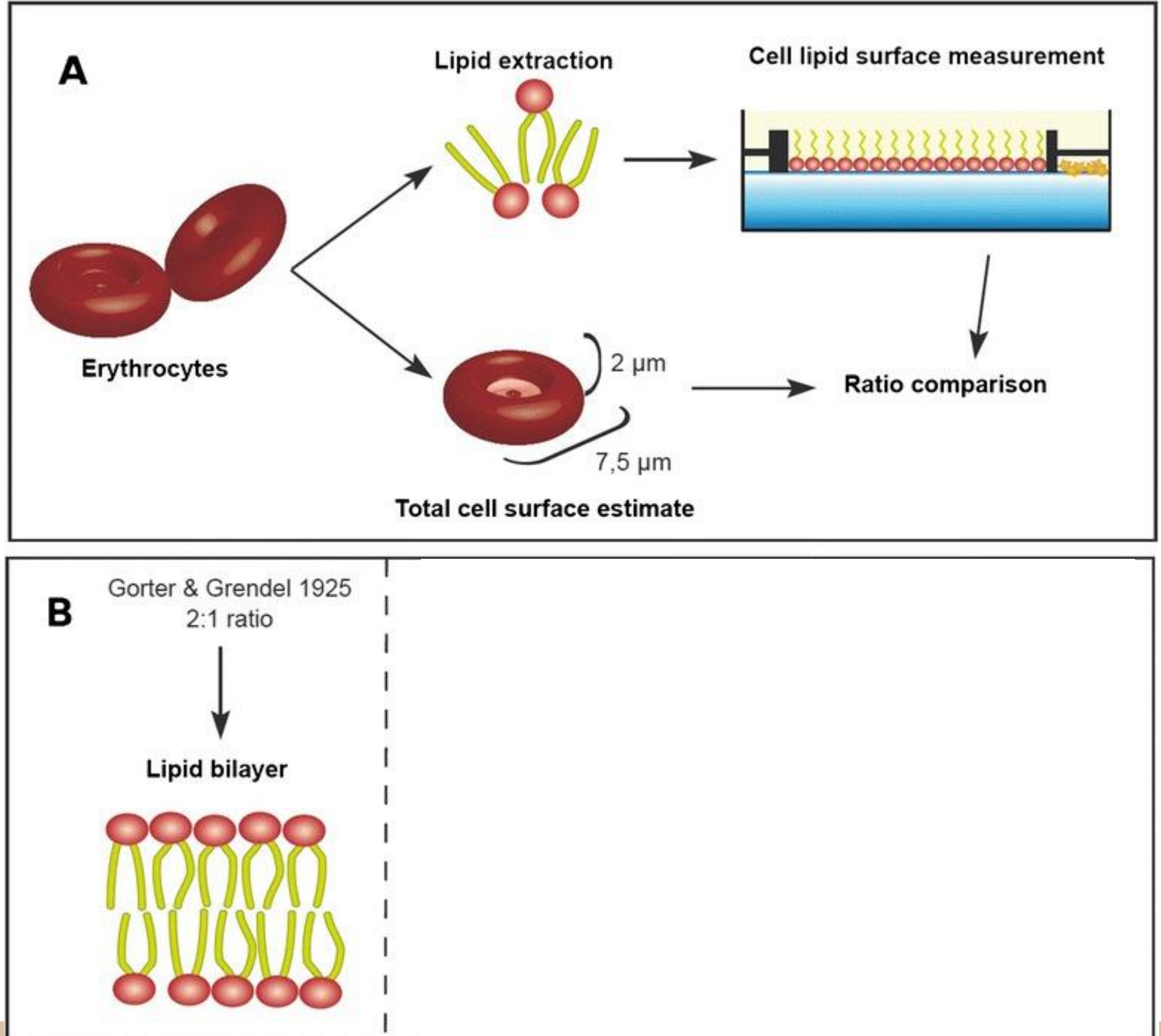
- Langmuir studied phospholipids and found that they were *amphipathic* and reasoned that they must ***orient (trtyb, twjh)*** on water with the hydrophobic tails away from the water

Gorter and Grendel: The Basis of Membrane Structure Is a Bilayer

- In 1925, these two physiologists extracted lipids from red blood cells and spread the lipids in a monolayer on a water surface

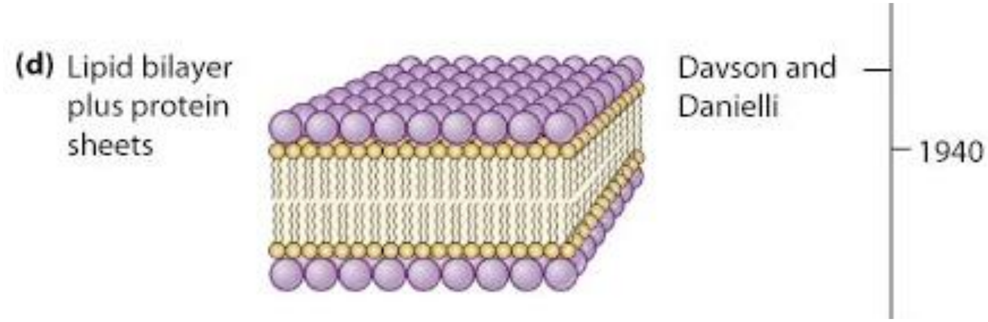


- The film on the water was twice the surface area of the blood cells, suggesting that lipids on the cell surface consisted of two layers
- They suggested that the most favorable structure would be a **lipid bilayer**, with the nonpolar regions of the lipids facing inward. Their data was wrong but the conclusion was correct!!


A
Lipid extraction
Cell lipid surface measurement
Erythrocytes
2 µm
7,5 µm
Ratio comparison
Total cell surface estimate
B
Gorter & Grendel 1925
2:1 ratio
Lipid bilayer

Davson and Danielli: Membranes Also Contain Proteins

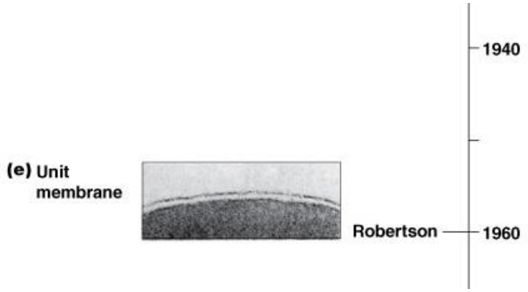
- Davson and Danielli showed that the bilayer alone could not account for all properties of membranes, especially
  - surface tension
  - solute permeability
  - electrical resistance



- They suggested that proteins are present in membranes, as thin sheets, coating the lipids

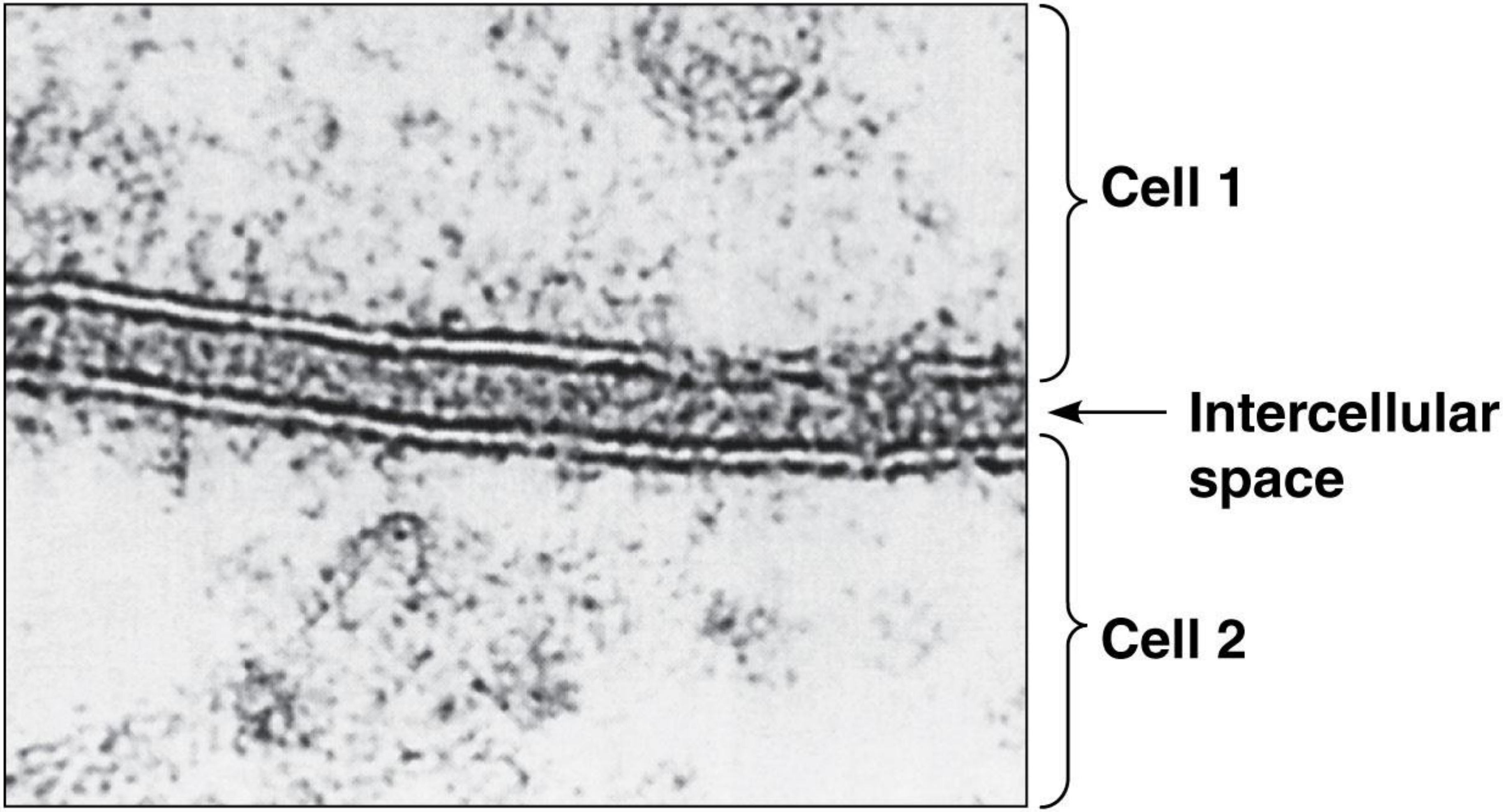
Robertson: All Membranes Share a Common Underlying Structure

- Using electron microscopy, biologists could verify the presence of membranes around cells and organelles



- A trilaminar structure, visible under the TEM, was observed for all membranes, leading to the suggestion of a common membrane structure, called the *unit membrane*

Figure 7-4



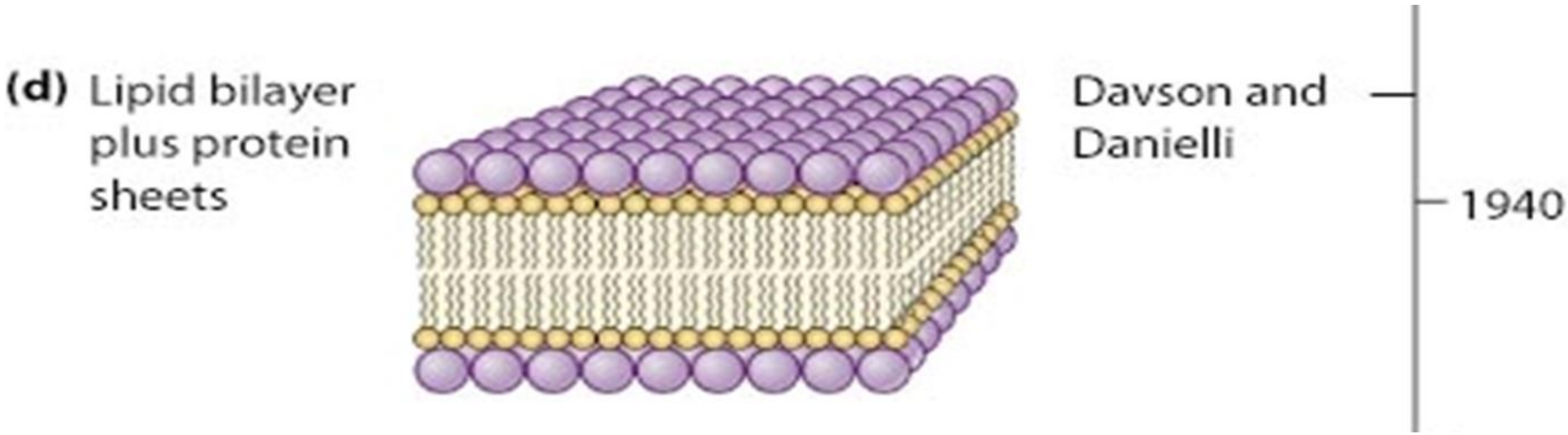
Further Research Revealed Major Shortcomings of the Davson–Danielli Model

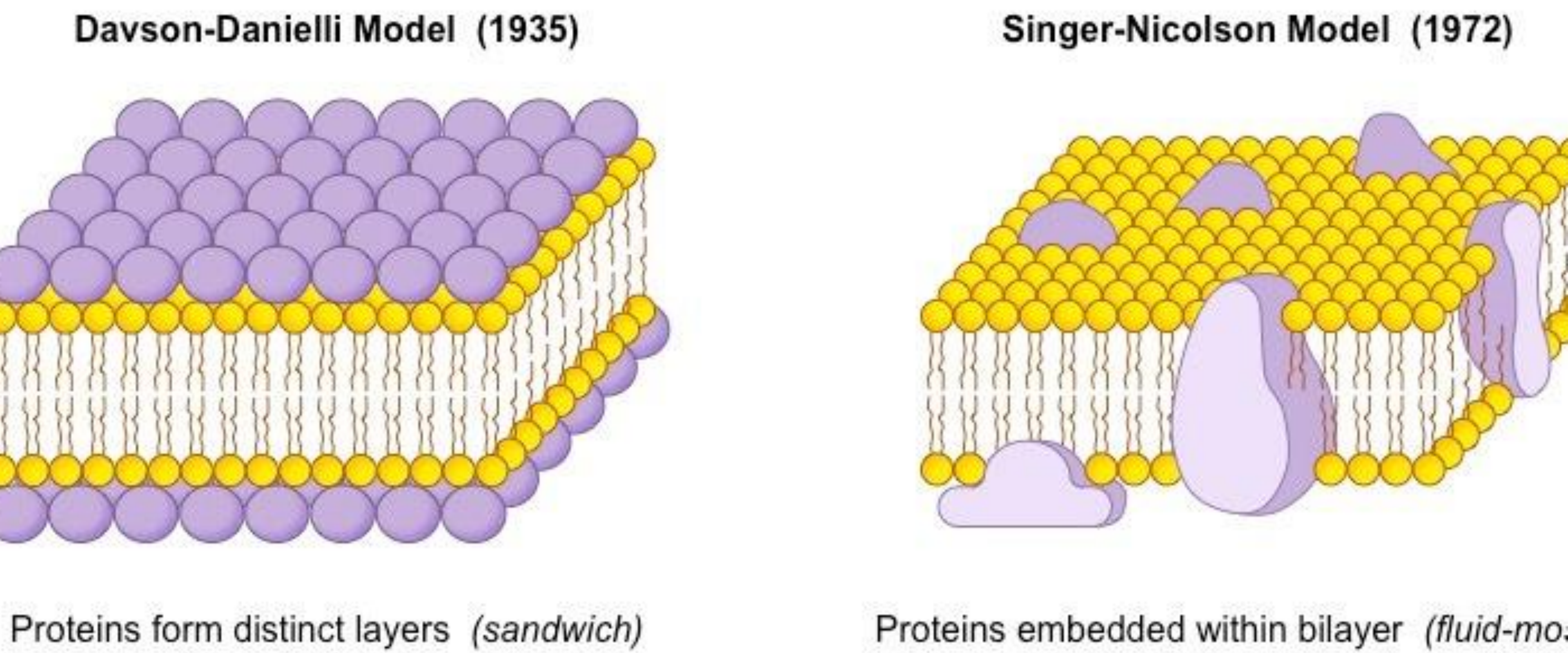
- Electron microscopy revealed that there was not enough space to either side of the bilayer for an additional layer of protein

- The Davson–Danielli model also did not account for the chemical distinctiveness of particular types of membranes, especially the protein/lipid ratio

- Membranes are susceptible to digestion by *phospholipases*, suggesting that membrane lipids are exposed

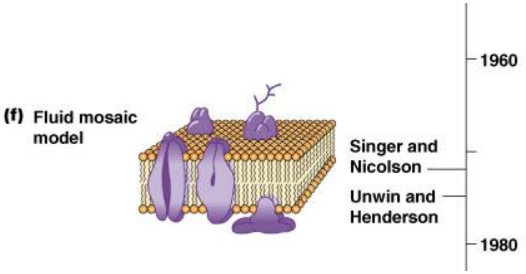
- Scientists were unable to isolate "surface" proteins from membranes unless organic solvents or detergents were used


(d) Lipid bilayer plus protein sheets
Davson and Danielli
1940


Davson-Danielli Model (1935)
Singer-Nicolson Model (1972)
Proteins form distinct layers (sandwich)
Proteins embedded within bilayer (fluid-mo

Singer and Nicholson: A Membrane Consists of a Mosaic of Proteins in a Fluid Lipid Bilayer

- The **fluid mosaic bilayer** model accounts for all the inconsistencies with previous models
- The model has two key features
  - A *fluid* lipid bilayer
  - A *mosaic* of proteins attached to or embedded in the bilayer



Three classes of membrane proteins

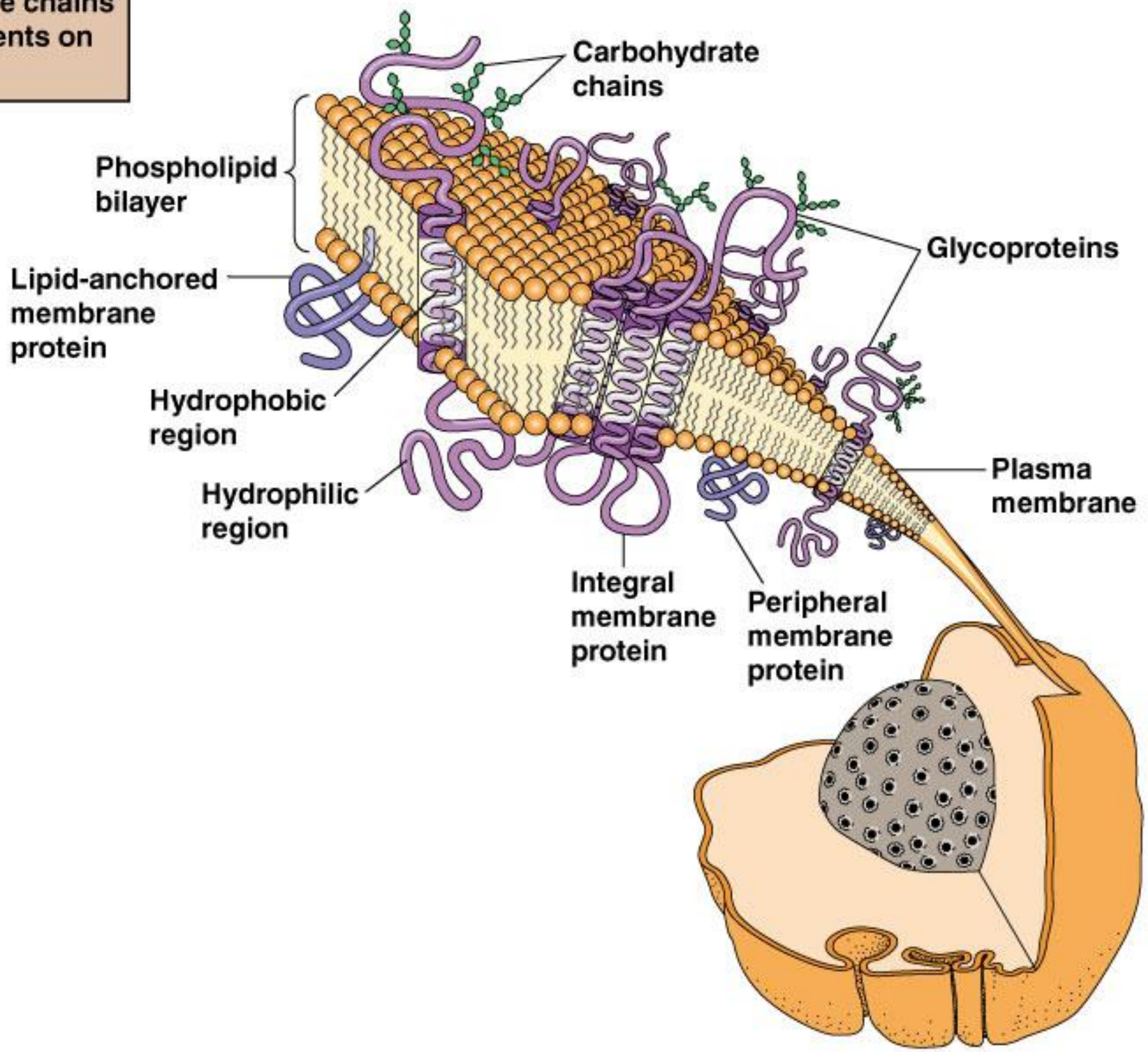
- 1. *Integral membrane proteins* are embedded in the lipid bilayer due to their hydrophobic regions

- 2 *Peripheral proteins* are hydrophilic and located on the surface of the bilayer

- 3. *Lipid-anchored proteins* are hydrophilic and attached to the bilayer by covalent attachments to lipid molecules embedded in the bilayer

Figure 7-5B

(b) An integral membrane protein with multiple α-helical transmembrane segments is shown below. Many integral membrane proteins of the plasma membrane have carbohydrate side chains attached to the hydrophilic segments on the outer membrane surface.



The fluid nature of the bilayer

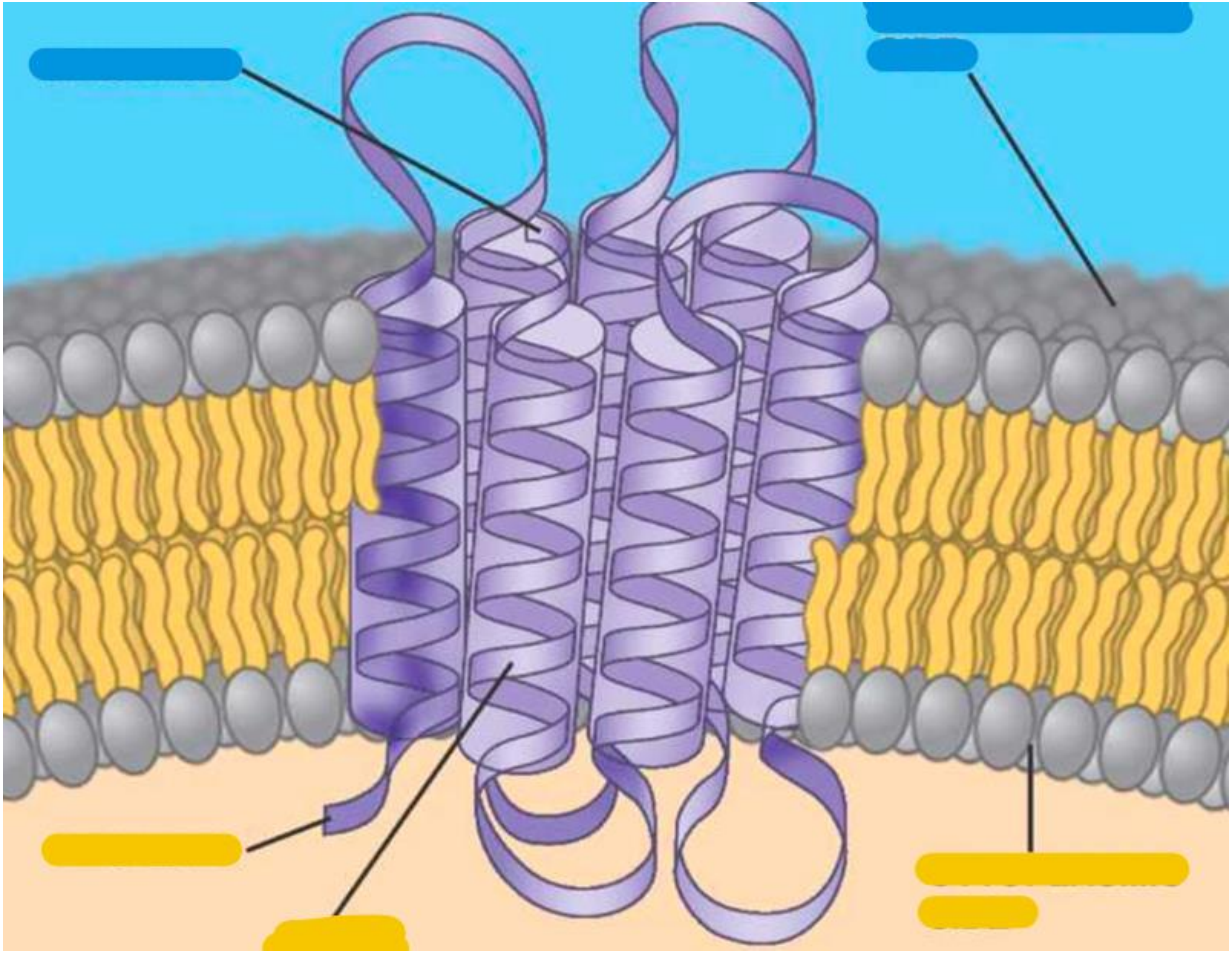
- Lipids in the bilayer are in constant motion
- Proteins are also able to move laterally within the membrane, though some are anchored to internal structural elements
- Anchored proteins have restricted mobility

Unwin and Henderson: Most Membrane Proteins Contain Transmembrane Segments

- Most integral membrane proteins have one or more hydrophobic segments that span the lipid bilayer

- These *transmembrane segments anchor* the protein to the membrane

- *Bacteriorhodopsin* was the first membrane protein shown to possess this structural feature



Recent Findings Further Refine Our Understanding of Membrane Structure

- Membranes are
  - **not homogenous freely mixing**
  - ordered through dynamic microdomains called **lipid rafts**
- Most cellular processes that involve membranes depend on structural complexes of specific lipids and proteins

Membrane Lipids: The "Fluid" Part of the Model

- Membrane lipids are important components of the "fluid" part of the fluid mosaic model
- Membranes contain several types of lipids
- The main classes of ***membrane lipids*** are ***phospholipids***, ***glycolipids***, and ***sterols***

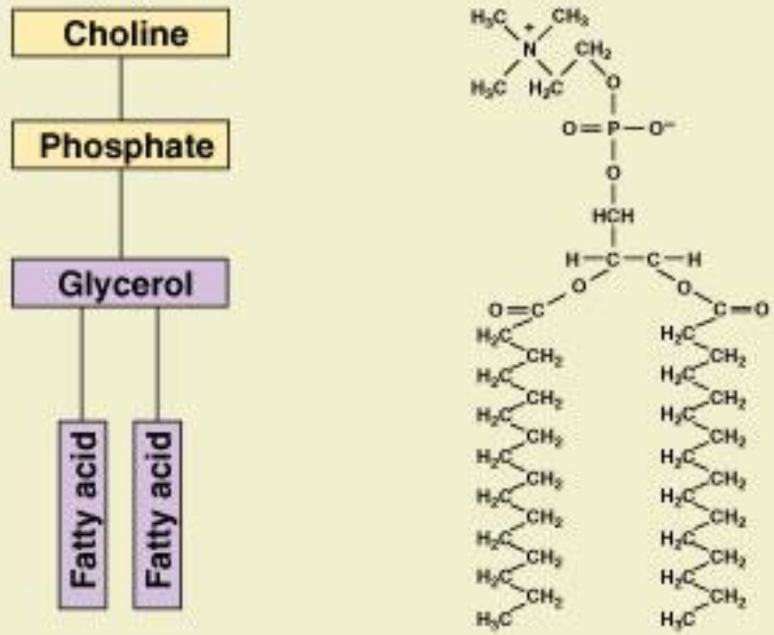
Phospholipids

- **Phospholipids** are the most abundant lipids in membranes
- They include the glycerol-based **phosphoglycerides** and the sphingosine-based **sphingolipids**
- The kinds and relative proportions of phospholipids vary greatly among types of membranes

Schematic diagram | Chemical structure

(a) PHOSPHOLIPIDS

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



Sphingomyelin (a sphingolipid)

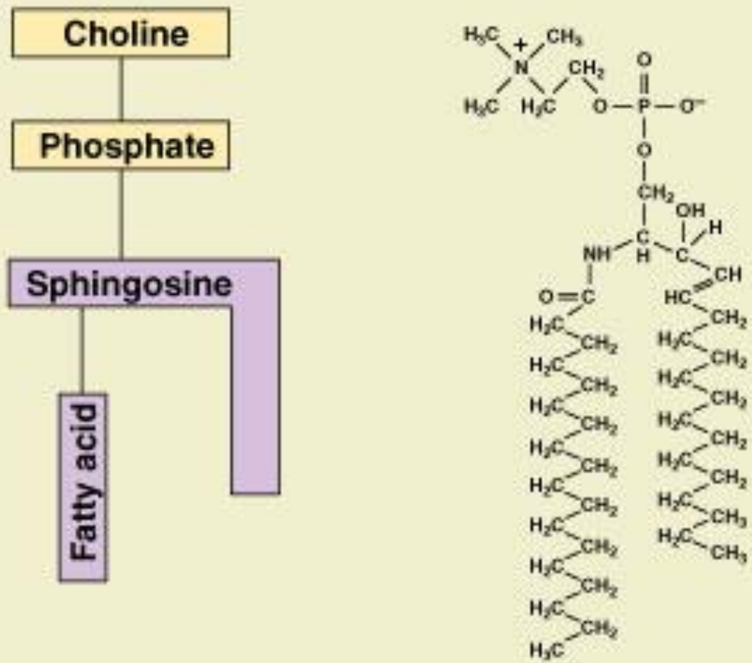


Figure 7-6A

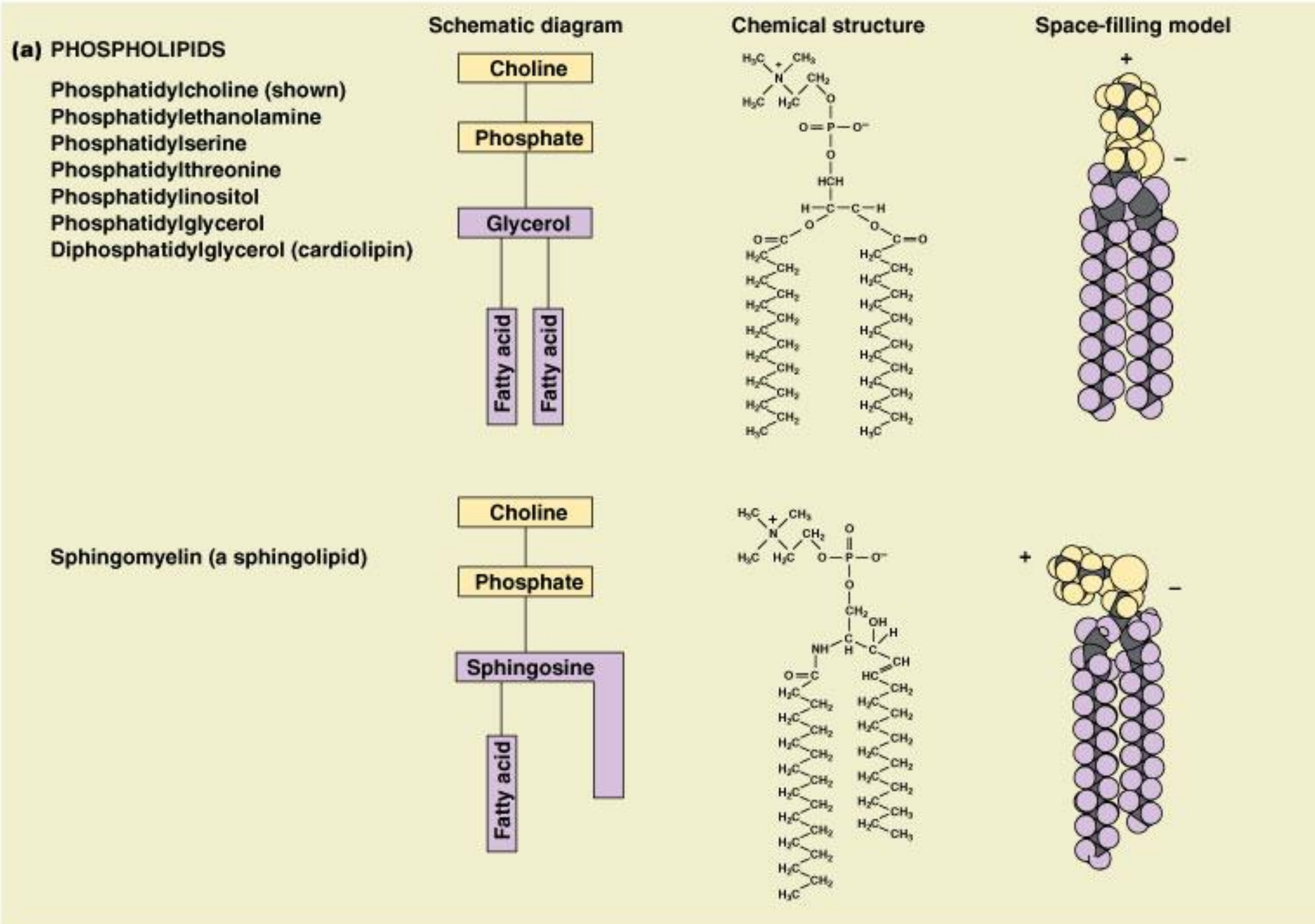


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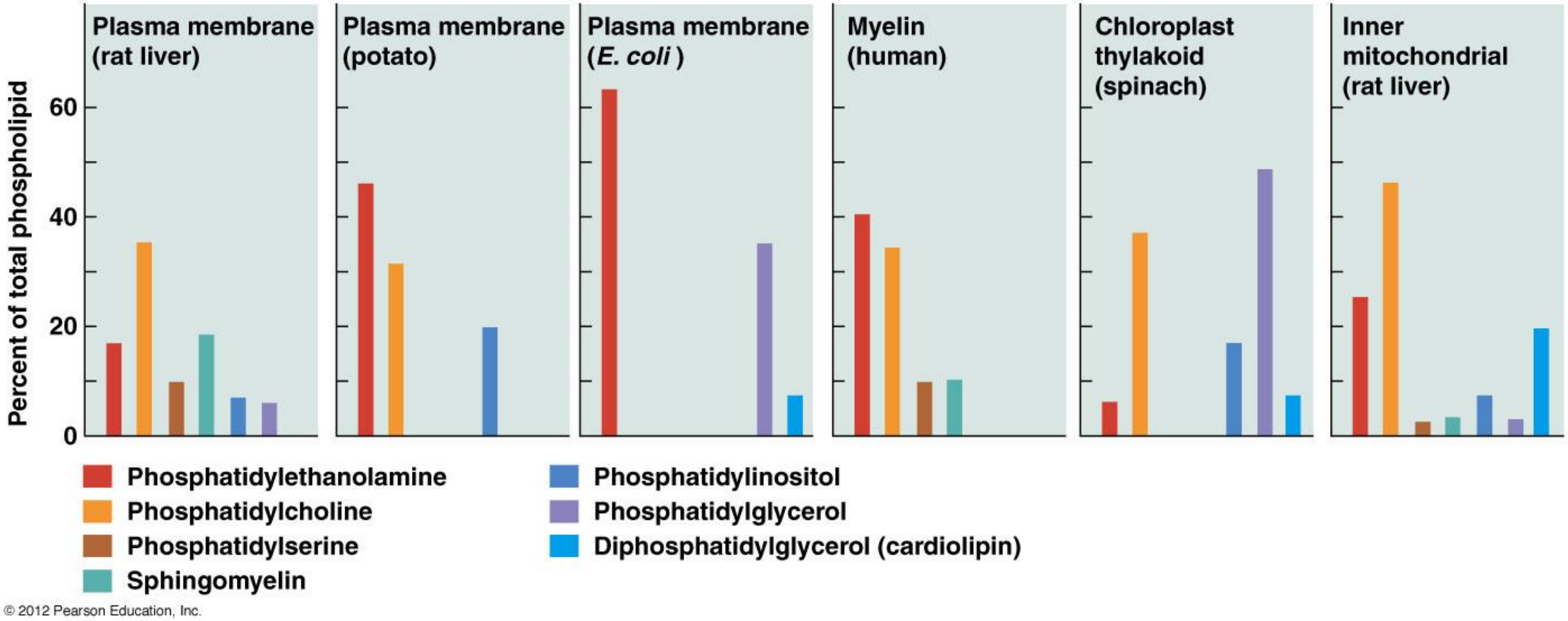
Figure 7-7



Percent of total phospholipid
0
20
40
60
Plasma membrane (rat liver)
Plasma membrane (potato)
Plasma membrane (*E. coli*)
Myelin (human)
Chloroplast thylakoid (spinach)
Inner mitochondrial (rat liver)
Phosphatidylethanolamine
Phosphatidylcholine
Phosphatidylserine
Sphingomyelin
Phosphatidylinositol
Phosphatidylglycerol
Diphosphatidylglycerol (cardiolipin)
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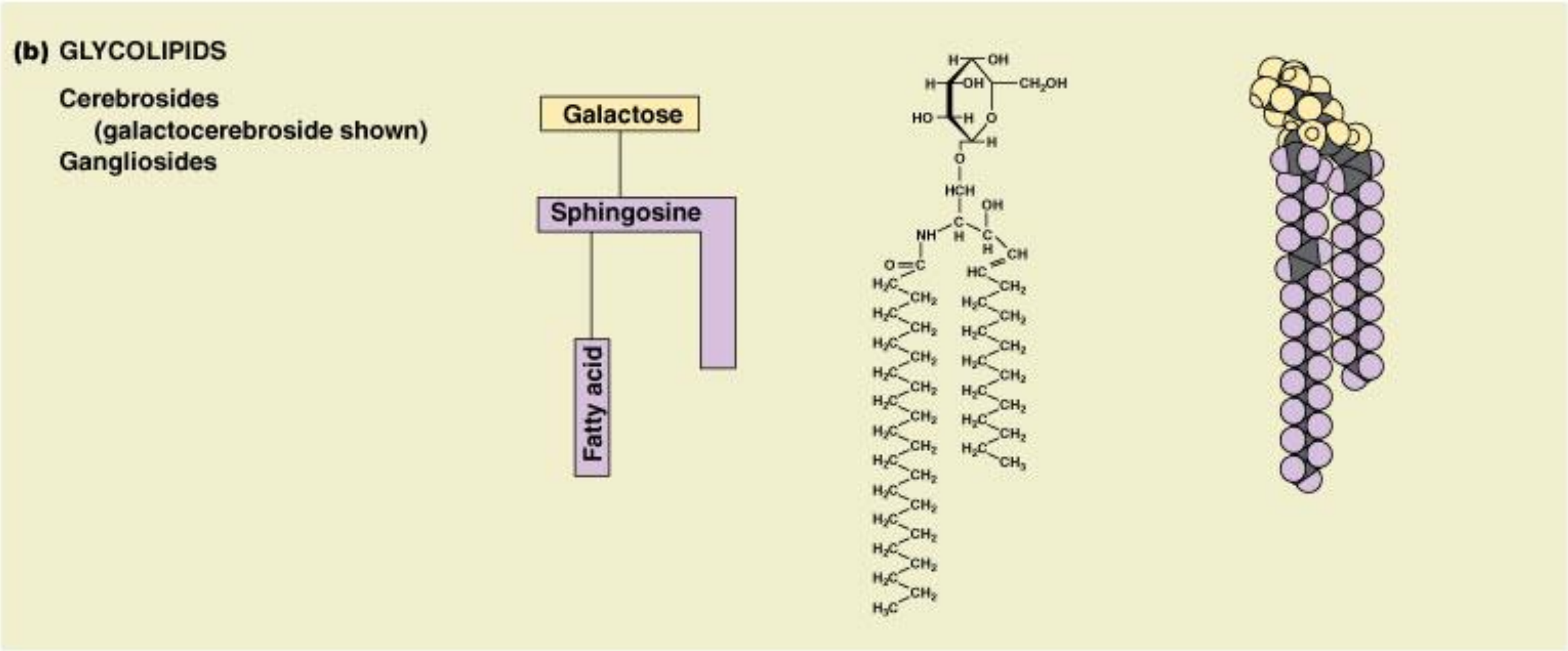
Glycolipids

- **Glycolipids** are formed by the addition of carbohydrates to lipids
- Some are glycerol-based and some are sphingosine-based; the *glycosphingolipids*
- The most common glycosphingolipids are **cerebrosides** and **gangliosides**

Figure 7-6B



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Cerebrosides and gangliosides

- Cerebrosides are *neutral glycolipids;* each molecule has an uncharged sugar as its head group
- A ganglioside has an oligosaccharide head group with *one or more negatively charged* sialic acid residues
- Cerebrosides and gangliosides are especially prominent in brain and nerve cells

Sterols

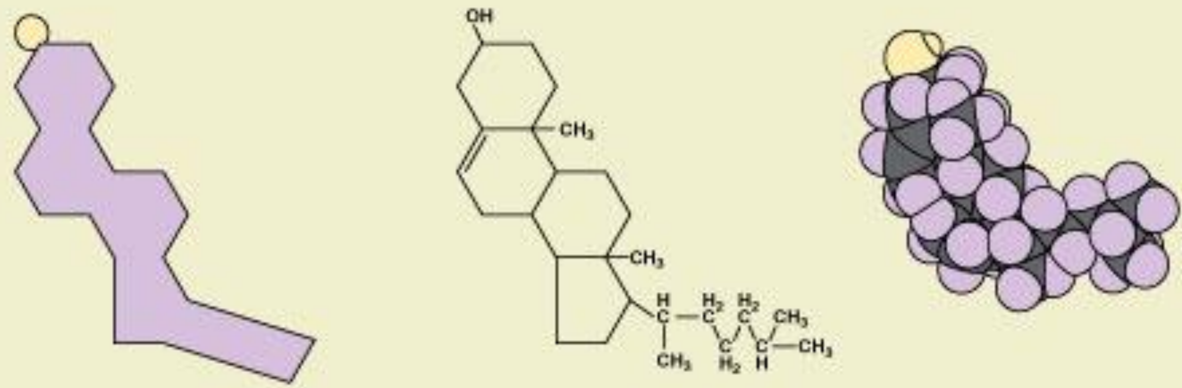
- The membranes of most eukaryotes contain significant amounts of **sterols**
- The main sterol in animal cell membranes is **cholesterol,** which is needed to stabilize and maintain membranes
- Plant cell membranes contain small amounts of **phytosterols**, whereas fungal cell membranes contain ergosterol, similar to cholesterol

Figure 7-6C

c) STEROLS

- Cholesterol (shown)
- Campesterol
- Sitosterol
- Stigmasterol
- Ergosterol
- Hopanoids

Campesterol, Sitosterol, Stigmasterol } Phytosterols

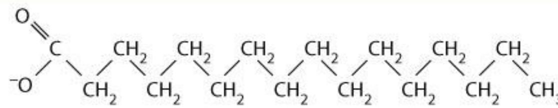
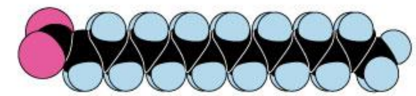
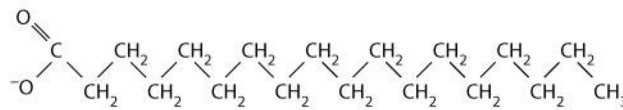
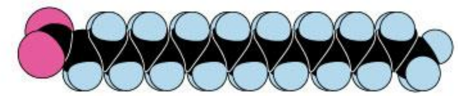
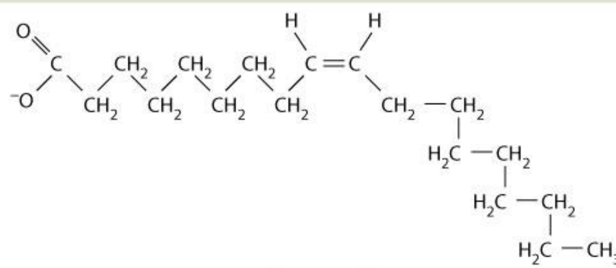
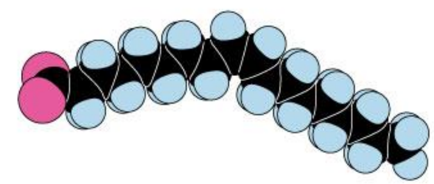
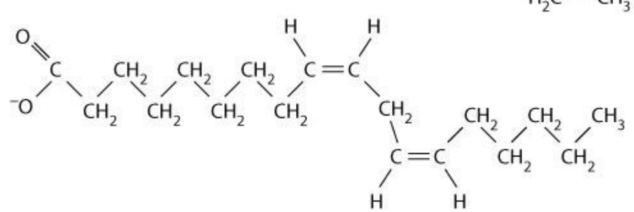
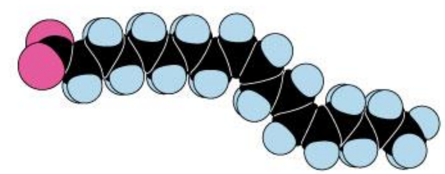


Fatty Acids Are Essential to Membrane Structure and Function

- *Fatty acids* are components of all membrane lipids except the sterols
- Their long hydrocarbon tails provide a barrier to diffusion of polar solutes
- The sizes of membrane fatty acids range between 12–20 carbons long, which is optimal for bilayer formation and dictates the usual thickness of membranes (6–8 nm)

Table 7-2

Table 7-2 **Structures of Some Common Fatty Acids Found in Lipid Bilayers**

Name of Fatty Acid	Number of Carbon Atoms	Number of Double Bonds	Structural Formula	Space-Filling Model
Saturated				
Palmitate	16	0		
Stearate	18	0		
Unsaturated				
Oleate	18	1		
Linoleate	18	2		

Fatty acids vary in degree of saturation

- Fatty acids vary considerably in the presence and number of double bonds
- *Palmitate* (16C) and *stearate* (18C) are common saturated fatty acids
- *Oleate* (one double bond) and *linoleate* (two double bonds), are both 18C unsaturated fatty acids

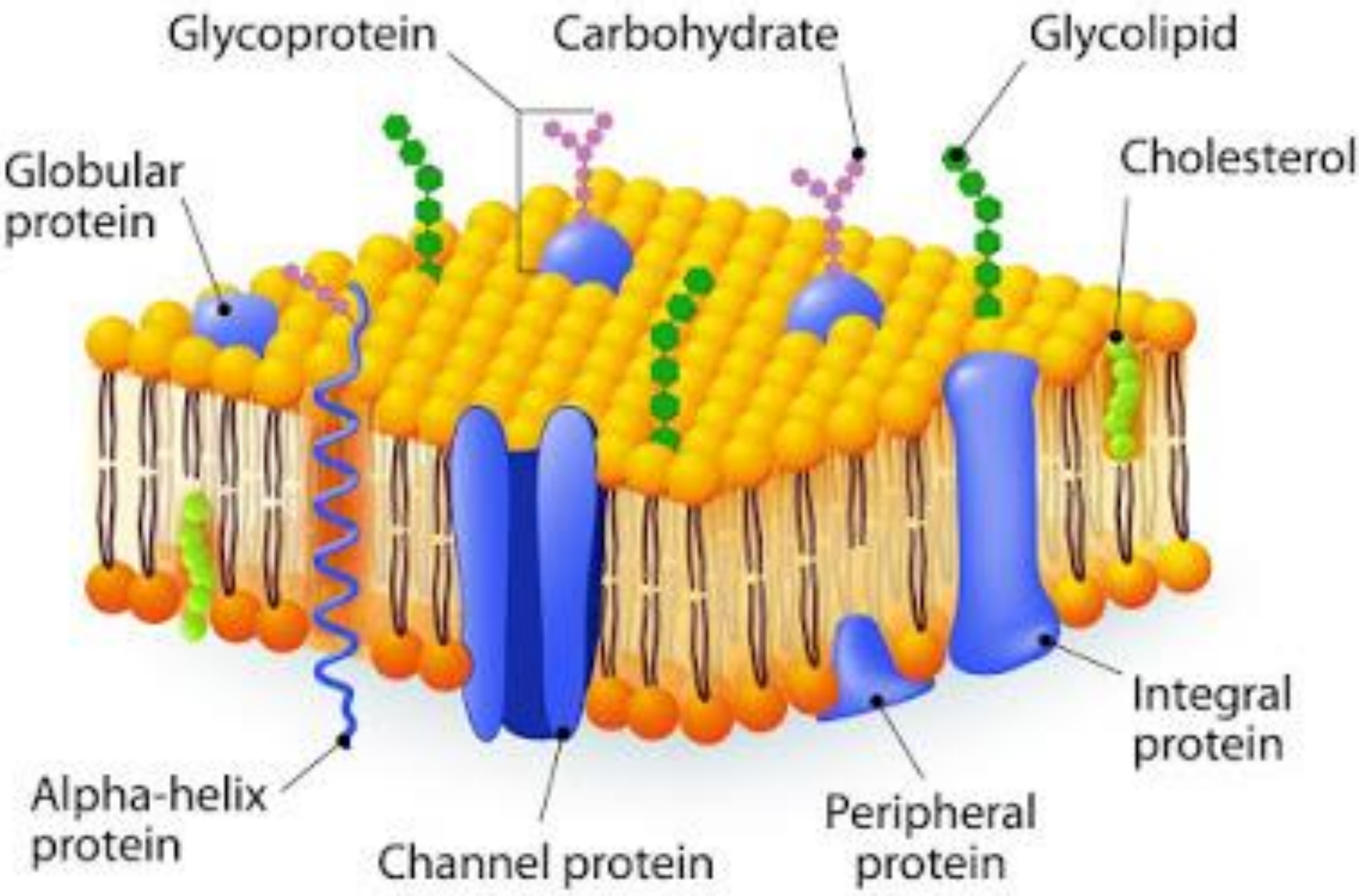
Membrane Asymmetry: Most Lipids Are Distributed Unequally Between the Two Monolayers

- Membrane asymmetry is the difference between the monolayers regarding the

1. kind of lipids present
2. the degree of saturation of fatty acids in the phospholipids

- Most of the glycolipids in the plasma membrane of animal cells are in the outer layer

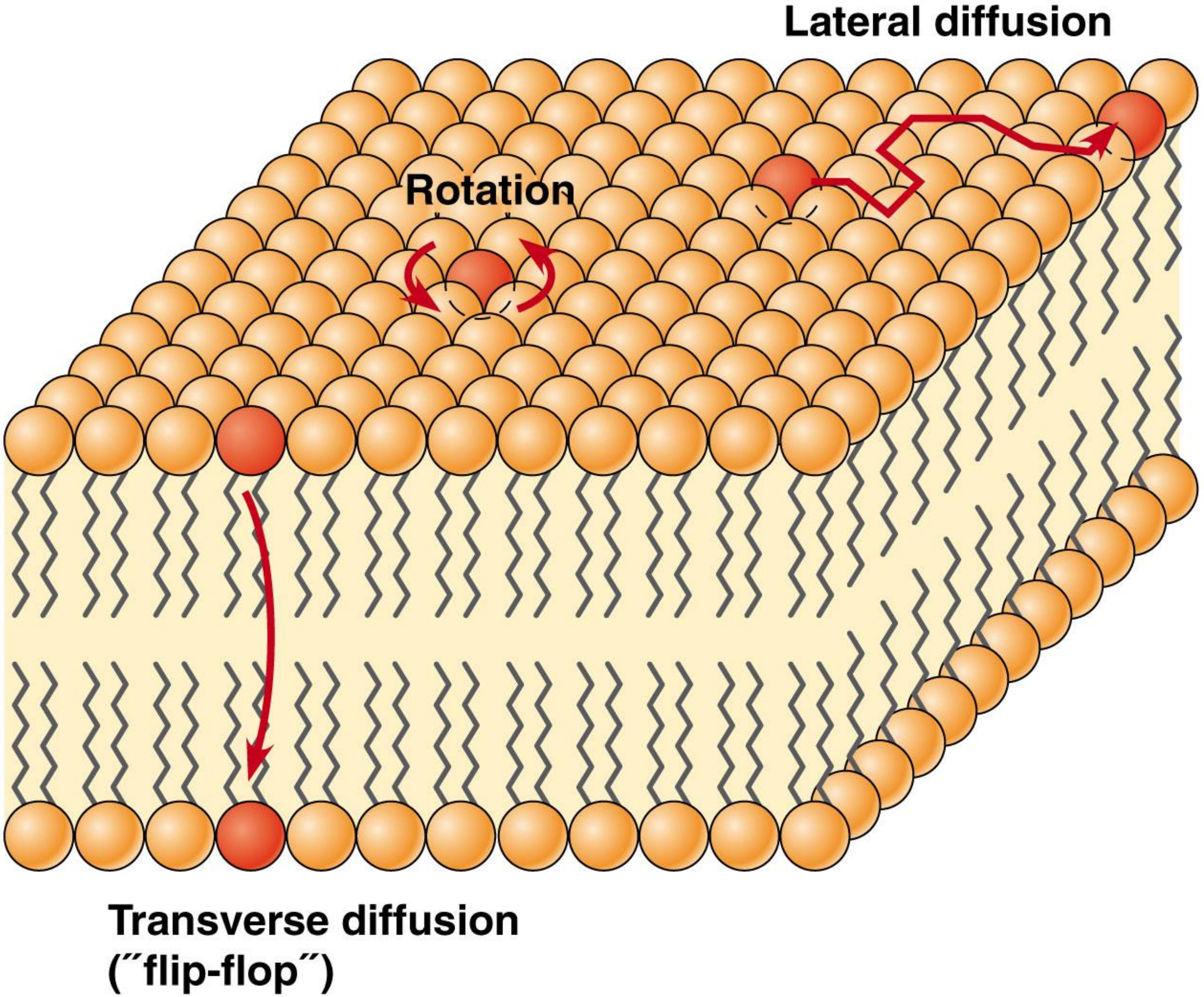
CELL MEMBRANE



Membrane asymmetry tends to be maintained

- Once established, membrane asymmetry does not change much
- The movement of lipids from one monolayer to another requires their hydrophilic heads to move all the way through the hydrophobic interior of the bilayer
- This **transverse diffusion** (or “flip-flop”) is relatively rare

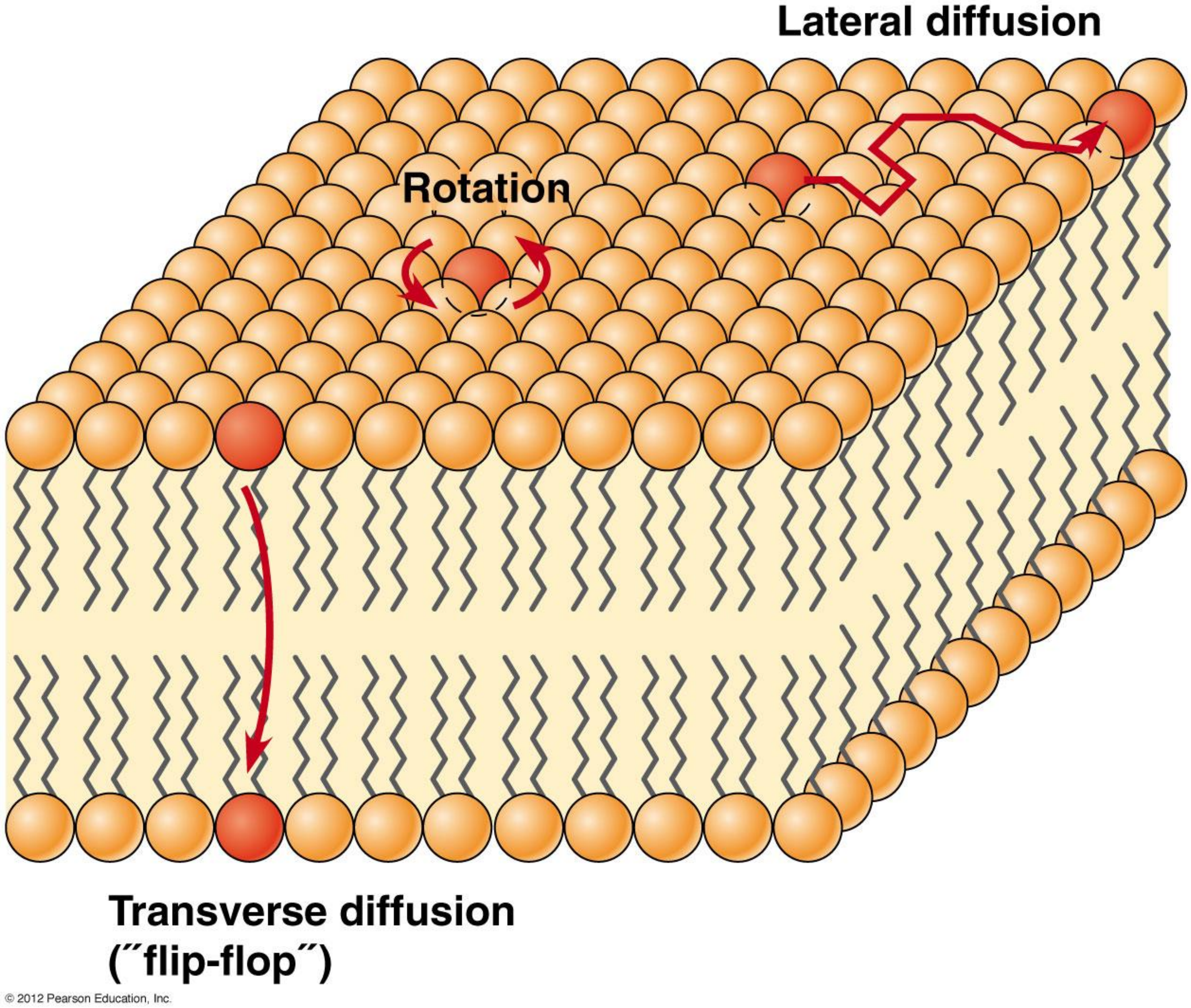
Figure 7-10


Lateral diffusion
Rotation
Transverse diffusion
("flip-flop")

Lipids move freely within their monolayer

- Lipids are mobile within their monolayer
- **Rotation** of phospholipids about their axes can occur
- Phospholipids can also move within the monolayer, via **lateral diffusion**
- Both types of movement are rapid and random

Figure 7-10


Lateral diffusion
Rotation
Transverse diffusion
("flip-flop")
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Transverse diffusion does occur

- Though rare, phospholipid flip-flop does occur in natural membranes

- Some membranes, in particular the smooth ER membrane, have proteins that catalyze the flip-flop of membrane lipids

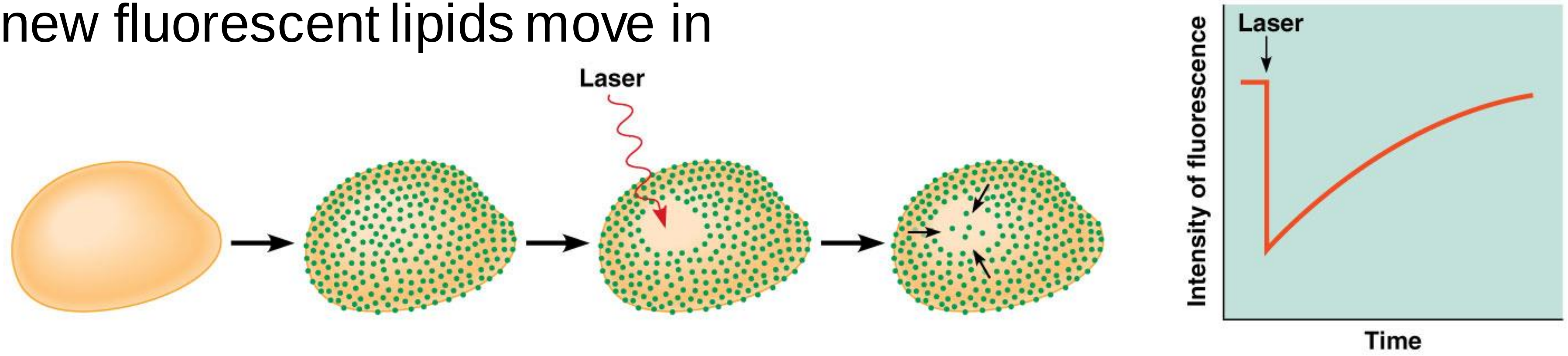
- These proteins are called **phospholipid translocators** or **flippases**

The Lipid Bilayer Is Fluid

- The lipid bilayer behaves as a fluid that permits the movement of both lipids and proteins
- Lipids can move as much as several μm per second within the monolayer
- Lateral diffusion can be demonstrated using **<u>f</u>luorescence <u>r</u>ecovery <u>a</u>fter <u>p</u>hotobleaching** (FRAP)

Measuring lipid mobility with FRAP

- Investigators label lipid molecules in a membrane with a fluorescence dye
- A laser beam is used to bleach the dye in a small area, creating a dark spot on the membrane
- The membrane is observed afterward to determine how long it takes for the dark spot to disappear, a measure of how quickly new fluorescent lipids move in



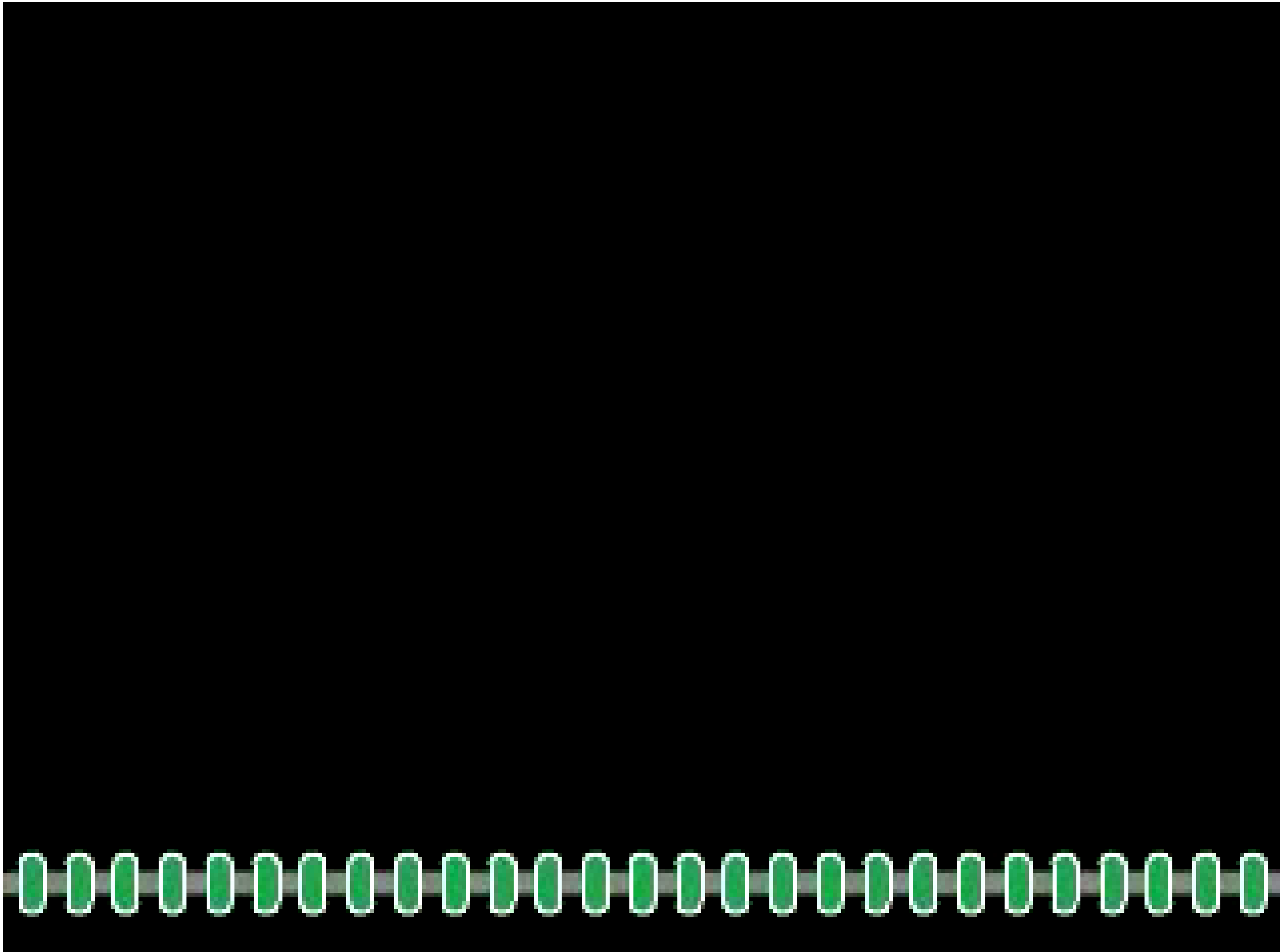
Unlabeled cell surface

Cell surface molecules labeled with fluorescent dye

Laser beam bleaches an area of the cell surface

Fluorescent-labeled molecules diffuse into bleached area

Rate of diffusion of fluorescence into bleached area measured over time



Membranes Function Properly Only in the Fluid State

- Membrane fluidity changes with temperature, decreasing as temperature falls and vice versa

- Every lipid bilayer has a characteristic **transition temperature $\boldsymbol{T_m}$,** the **temperature at which it becomes fluid**

This change of state is called a **phase transition**, in this case from solid to liquid

Below the T_m, any functions that rely on membrane fluidity will be disrupted

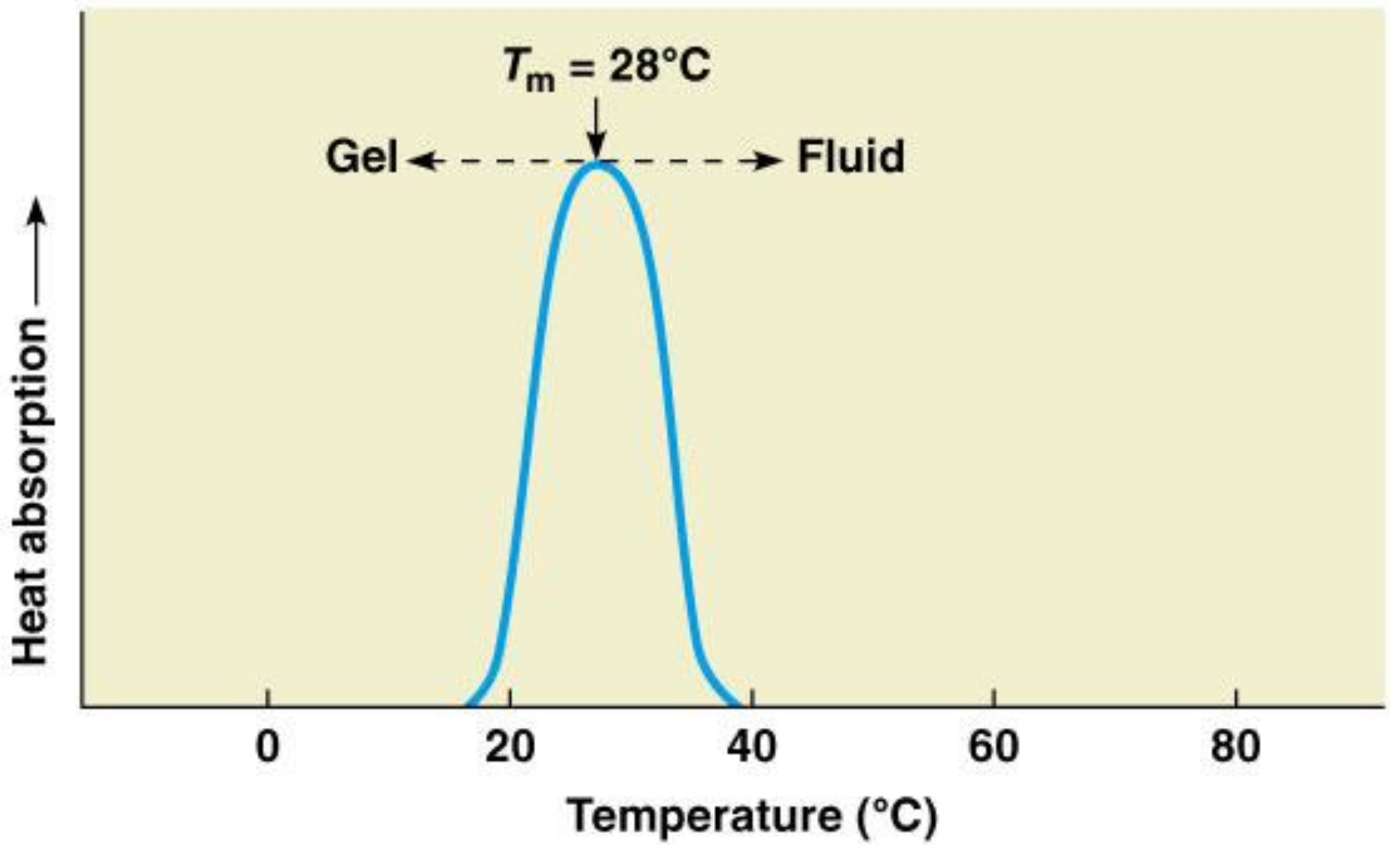
$< \quad \mathbf{Tm} \quad <$

Measuring the transition temperature of a membrane

- The transition temperature can be measured by **differential scanning calorimetry**
- The membrane is placed inside a *calorimeter* and the uptake of heat is measured as temperature is increased
- The T_m is the point of maximum heat absorption as the membrane changes from the gel to the fluid state

Figure 7-12A

(a) Normal membrane. When the temperature of a typical membrane preparation is increased slowly in a calorimeter chamber, a peak of heat absorption marks the gel-to-fluid transition temperature, T_m.



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Effects of Fatty Acid Composition on Membrane Fluidity

- Fluidity of a membrane depends mainly on the fatty acids that it contains

1. The length of fatty acid chains
2. The degree of saturation

- Long-chain and saturated fatty have higher T_m, whereas short-chain and unsaturated fatty acids have lower T_ms

Figure 7-12B

(b) Membranes enriched in unsaturated or saturated fatty acids. Membranes from cells grown in media enriched in the unsaturated fatty acid oleate (left) are more fluid than normal membranes (lower T_m). Membranes from cells grown in media enriched in the saturated fatty acid stearate (right) are less fluid than normal membranes (higher T_m).

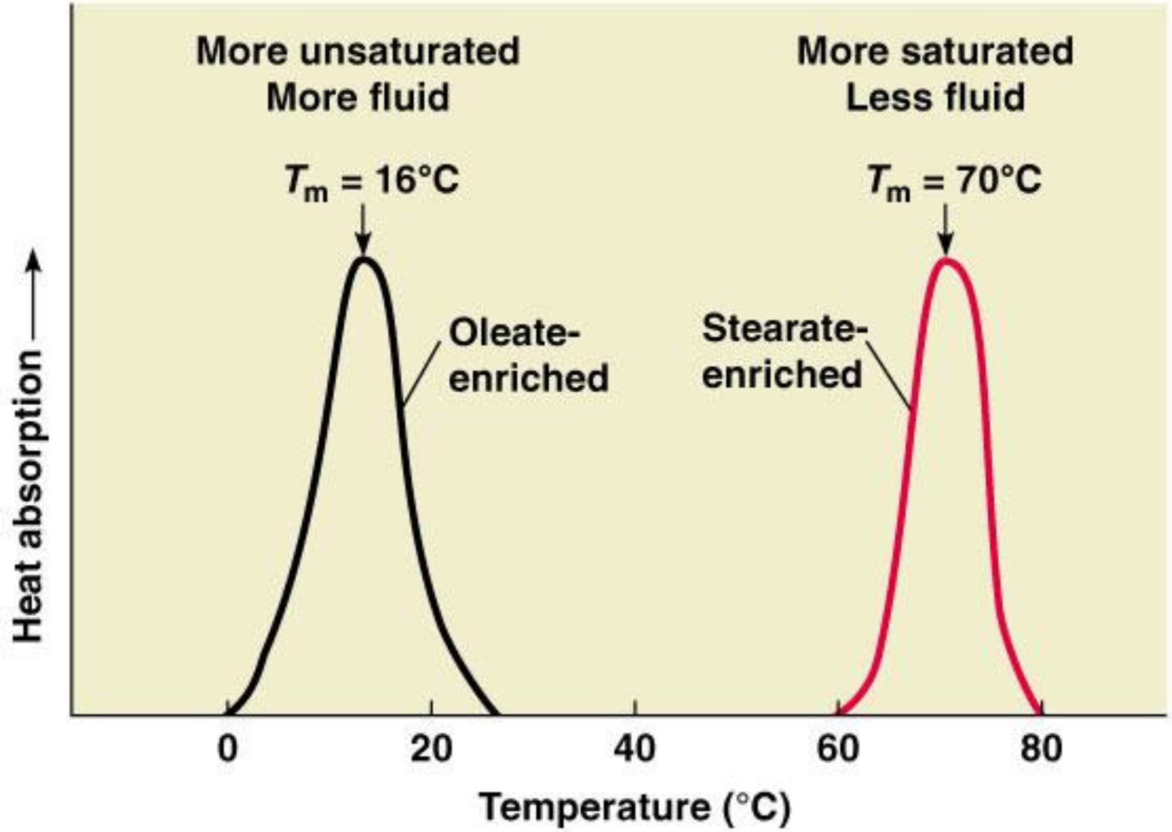

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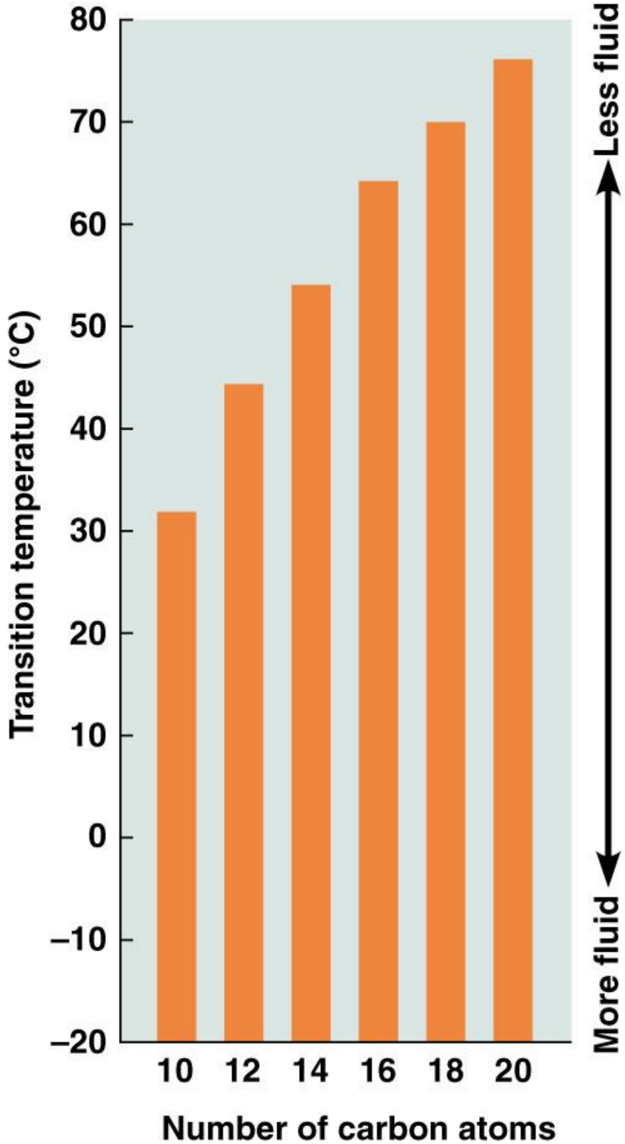
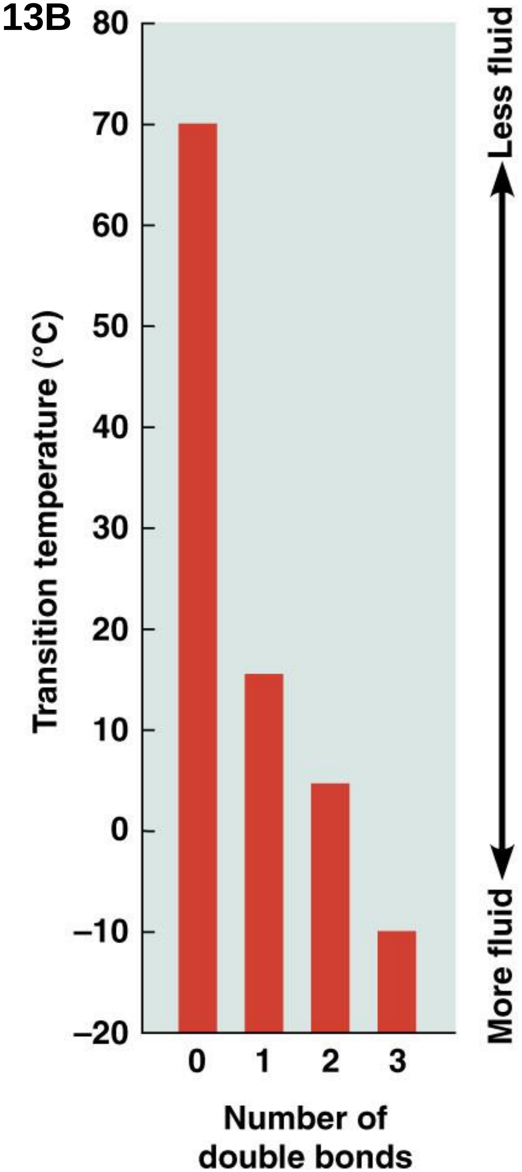
Figure 7-13A



(a) Effect of chain length on the melting point

Figure 7-13B

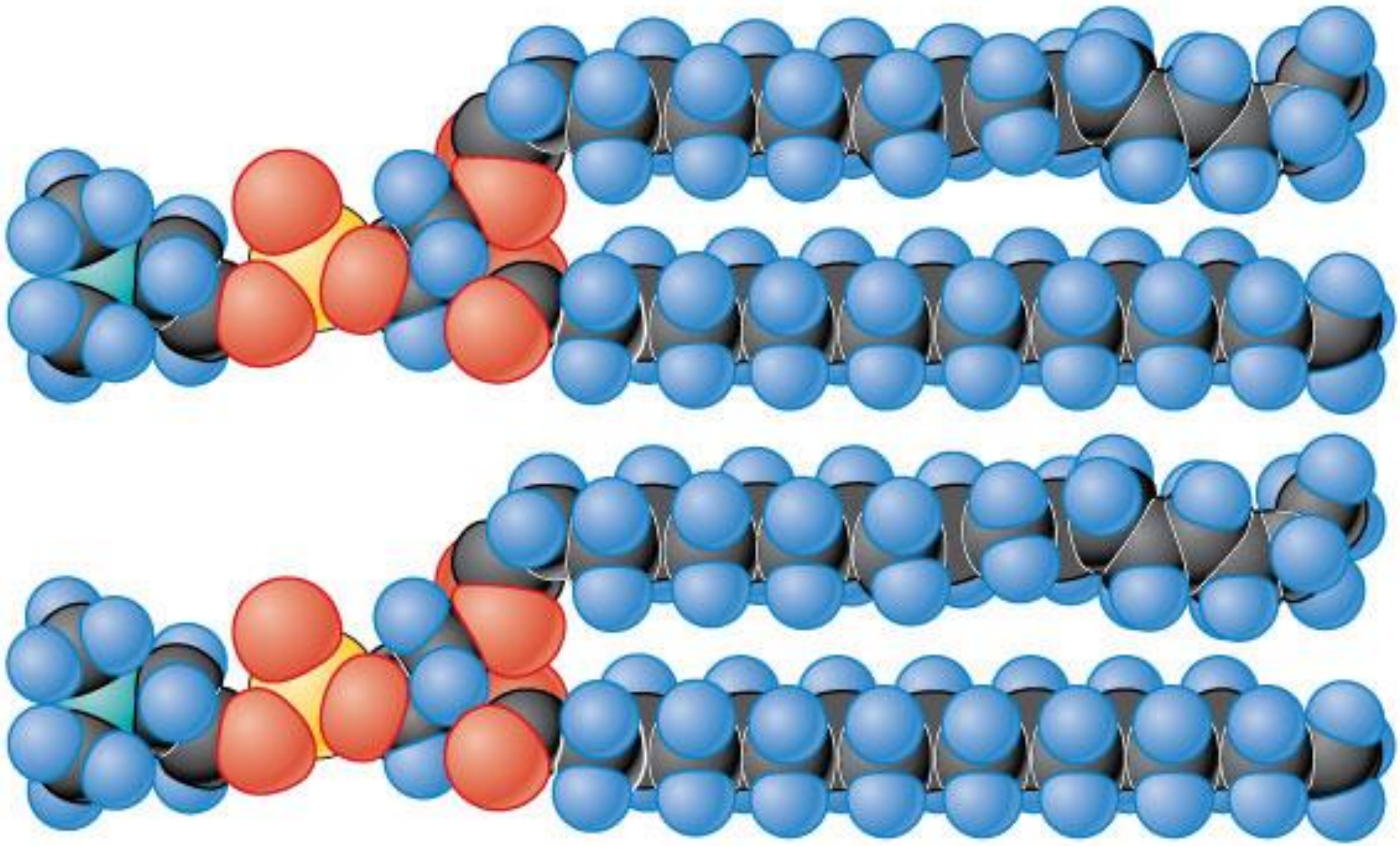


(b) Effect of unsaturation on the melting point

Fatty acid saturation and membrane fluidity

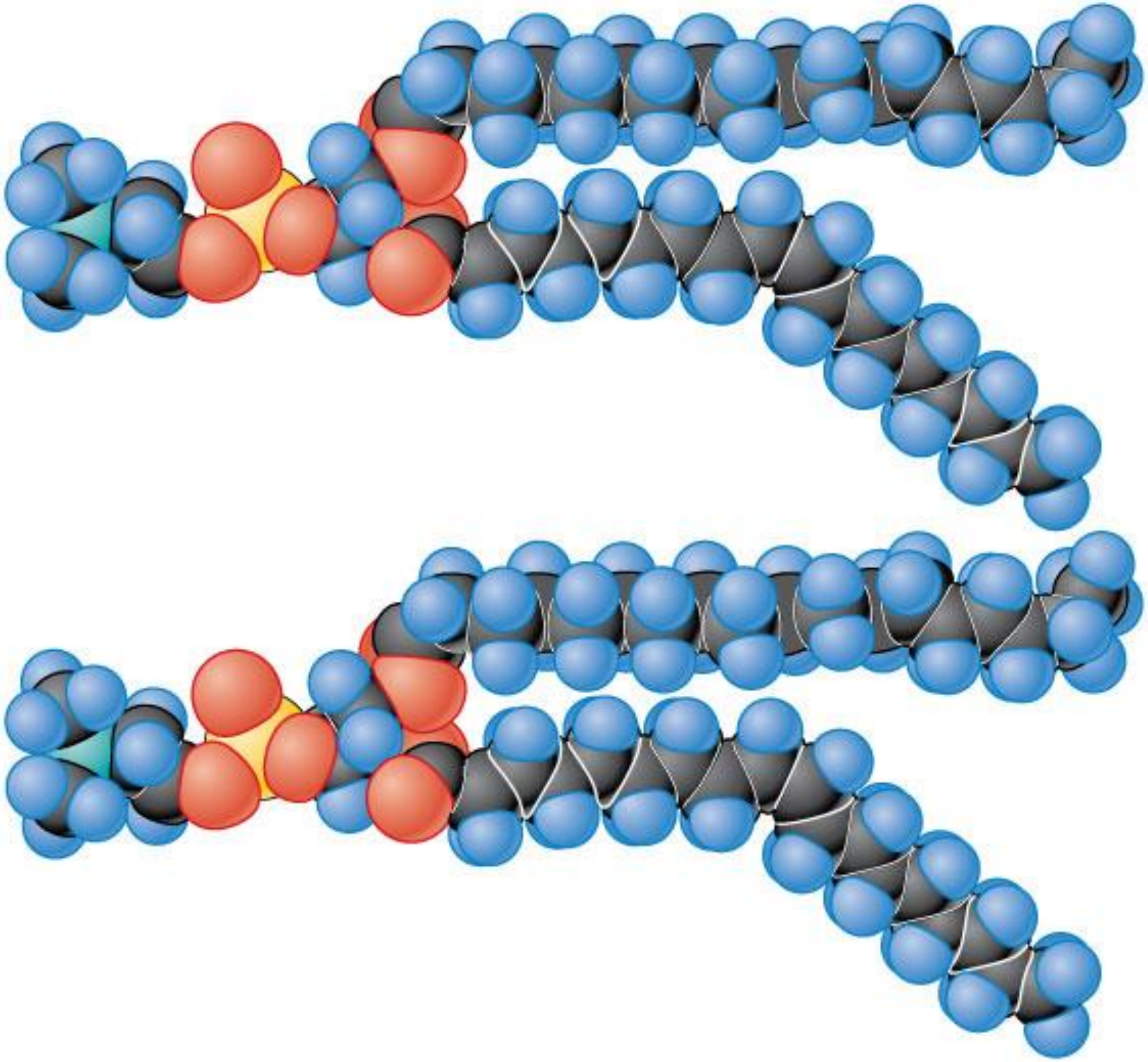
- Saturated fatty acids pack together well in the membrane
- Fatty acids with one or more double bonds have bends in the chains that prevent them from packing together neatly
- Thus unsaturated fatty acids are more fluid than saturated fatty acids, and have a lower T_m

Figure 7-14A



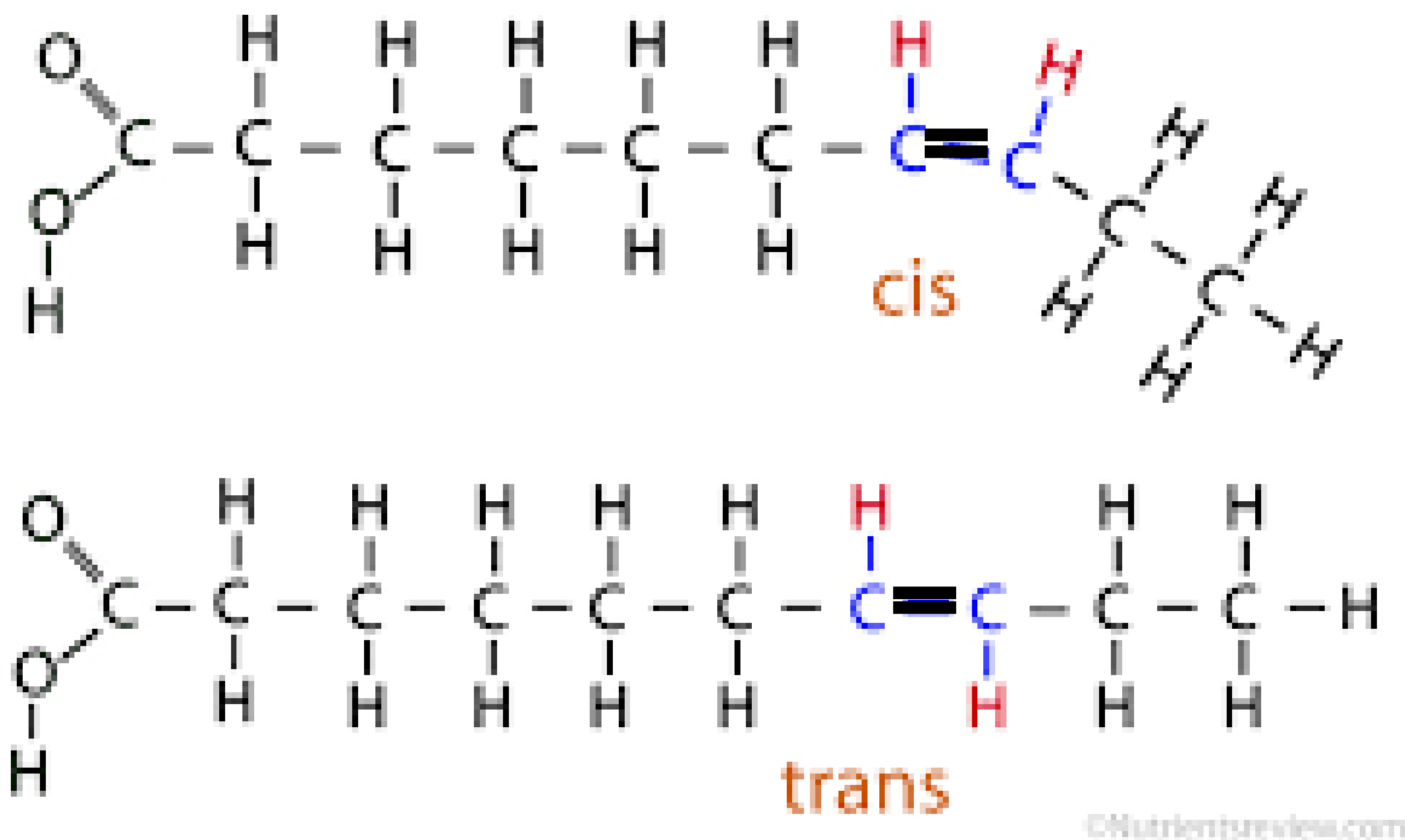
(a) Lipids with saturated fatty acids pack together well in the membrane

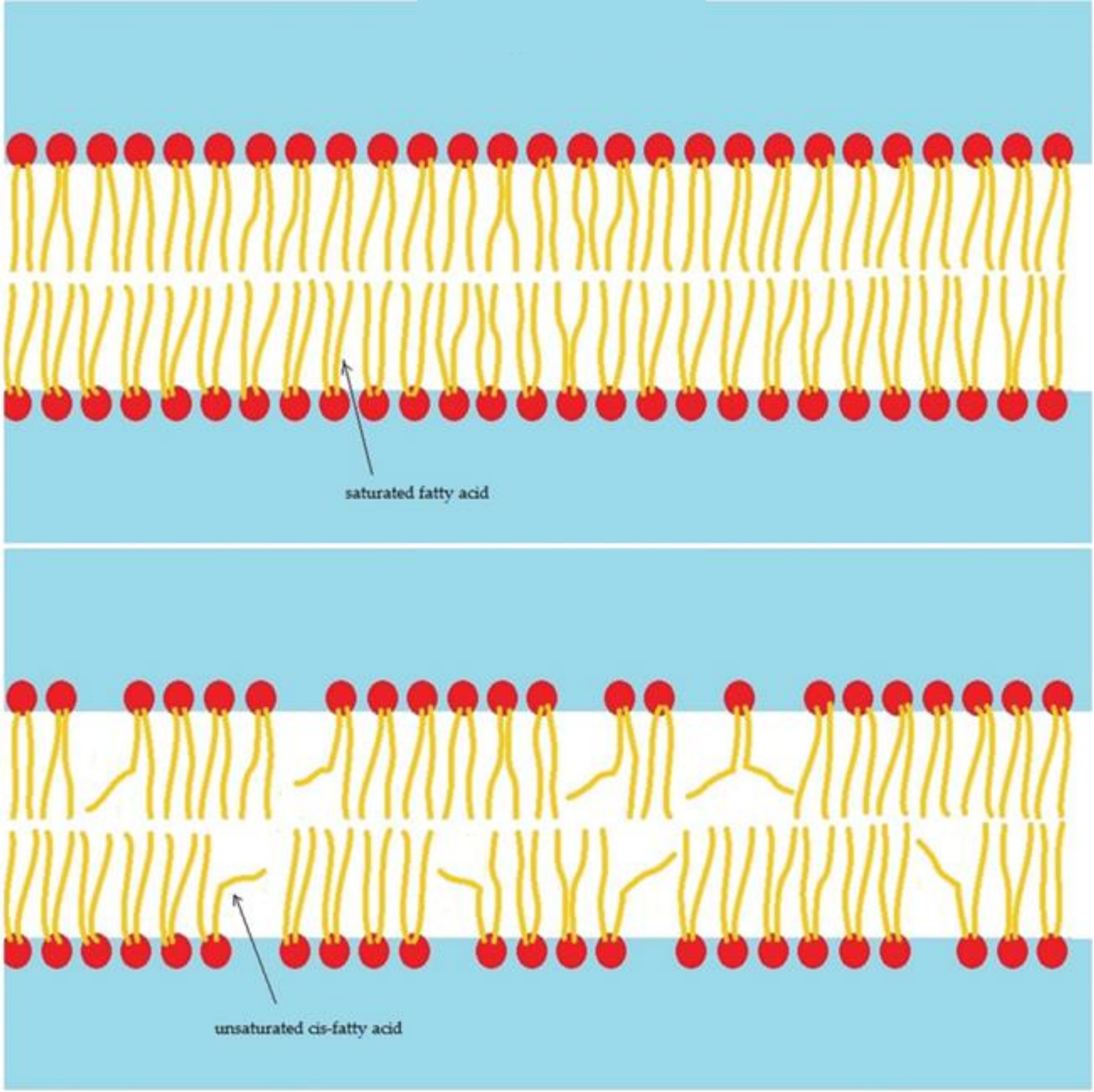
Figure 7.14B



(b) Lipids with a mixture of saturated and unsaturated fatty acids do not pack together well in the membrane

Cis- and Trans-Fatty Acids



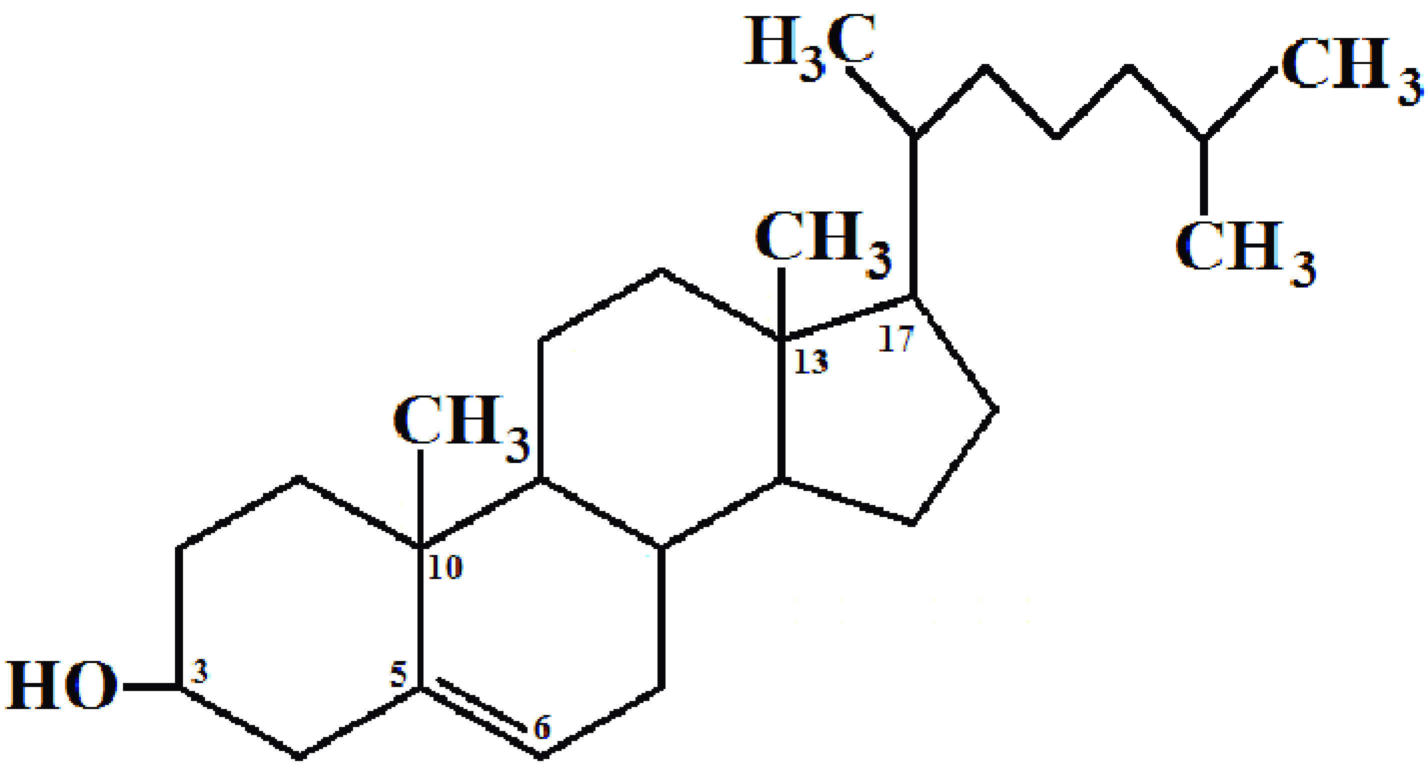

saturated fatty acid
unsaturated cis-fatty acid

Membrane composition

- Most plasma membrane fatty acids vary in chain length and degree of saturation
- This helps to ensure that membranes are **fluid at physiological temperatures**
- Most unsaturated fatty acids have *cis* double bonds, unlike the commercially produced *trans* fats, which pack together like saturated fats do

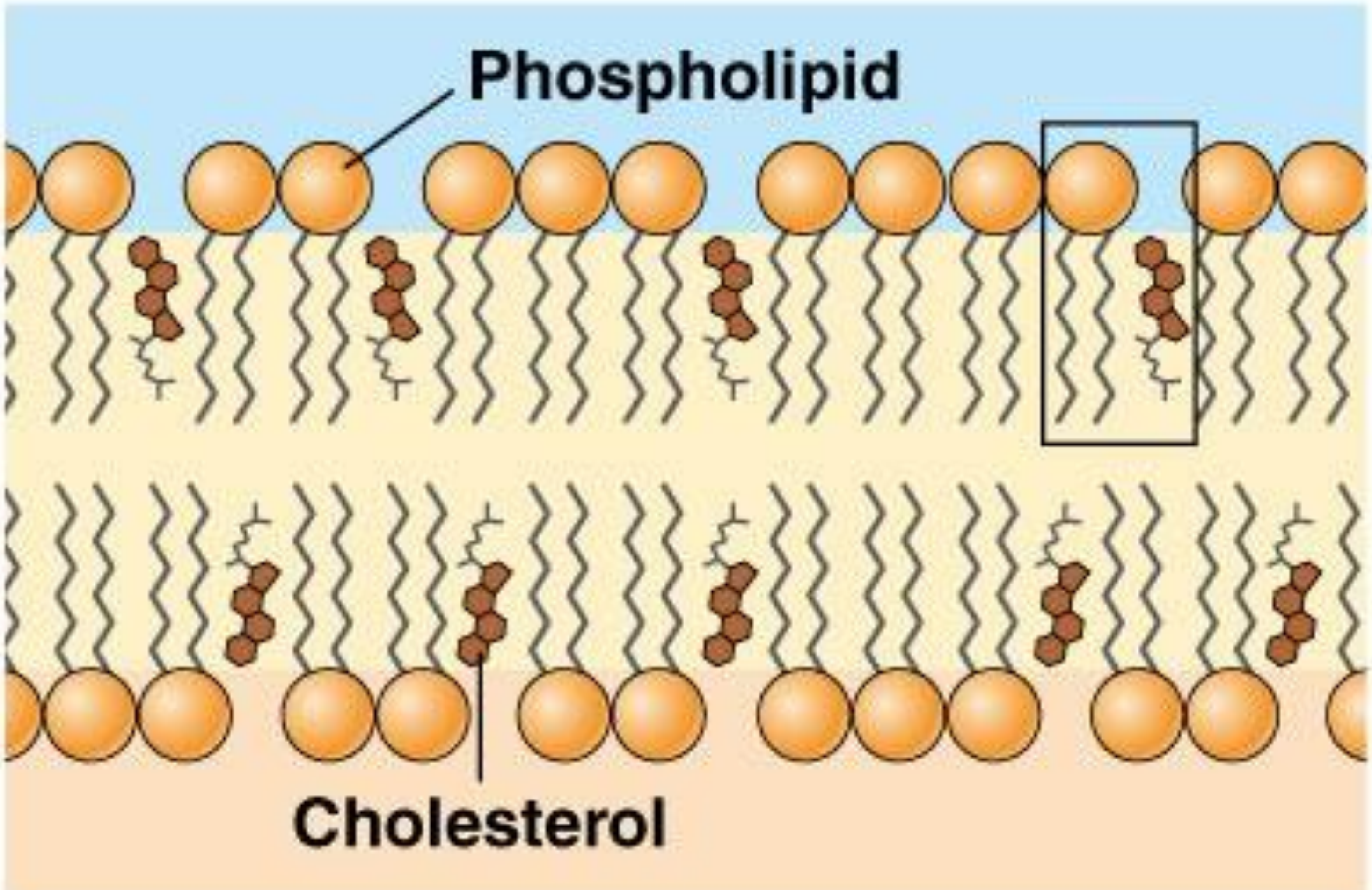
Effects of Sterols on Membrane Fluidity

- Membrane fluidity is influenced by sterols
- The intercalation of rigid cholesterol molecules into a membrane *decreases its fluidity and increases the T_m*
- However, cholesterol also prevents hydrocarbon chains of phospholipids from packing together tightly and so *reduces the tendency of membranes to gel upon cooling*



Cholesterol

Figure 7-15A

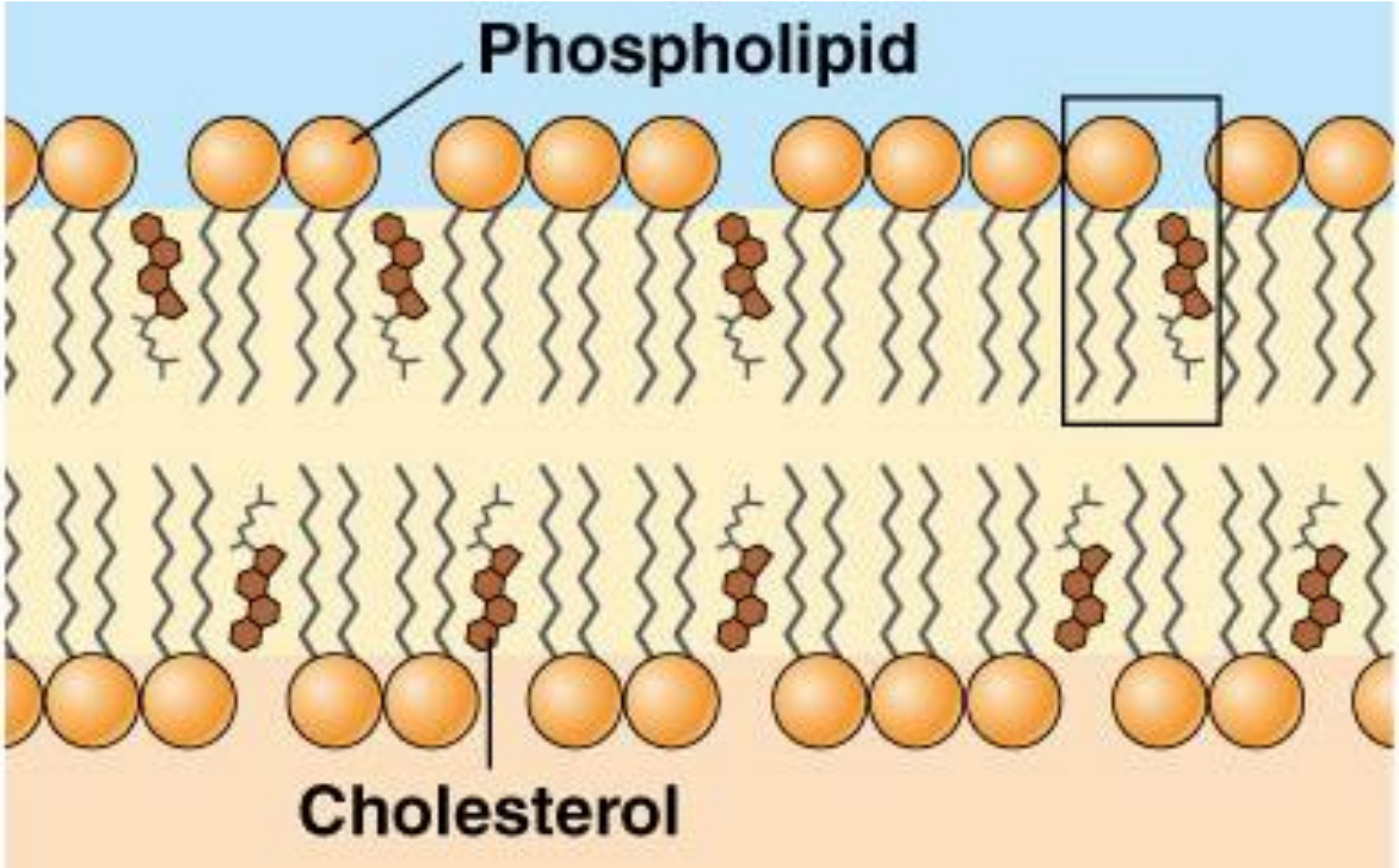


(a) Cholesterol in plasma membrane

Cholesterol is a fluidity buffer

T > T_m Cholesterol **decreases** membrane fluidity

T < T_m Cholesterol **increases** membrane fluidity



Other effects of sterols on membranes

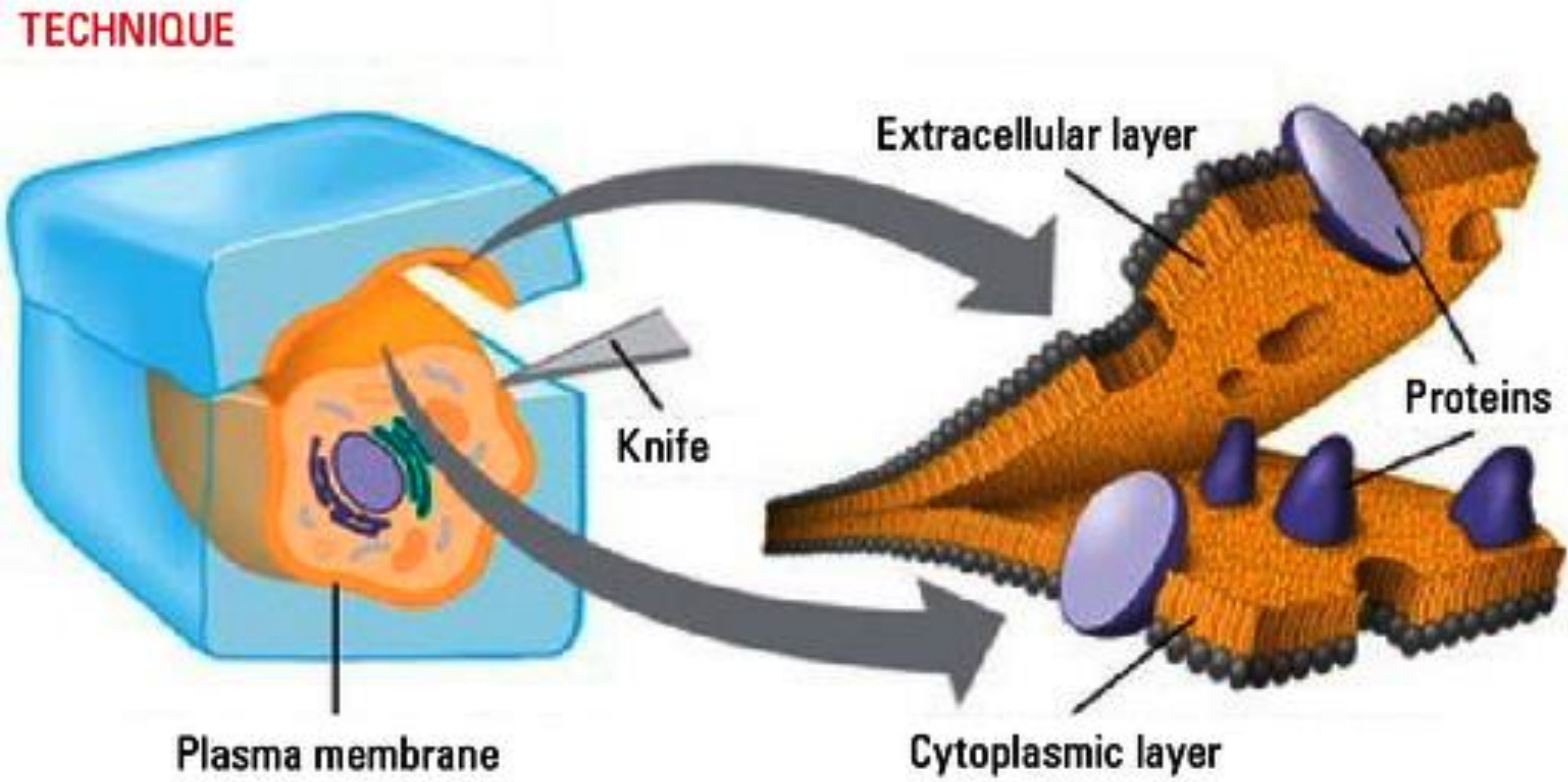
- Sterols decrease the permeability of membranes to ions and small polar molecules
- This is likely because they fill spaces between the hydrocarbon chains of phospholipids
- This effectively blocks the routes that ions and small molecules would take through the membrane

Membrane Proteins: the "Mosaic" Part of the Model

- The mosaic part of the fluid mosaic model includes lipid rafts and other lipid domains

- However, it is membrane proteins that are the main components

The membrane consists of a mosaic of proteins: evidence from freeze facture microscopy



Membranes Contain Integral, Peripheral and Lipid-Anchored Proteins

- Membrane proteins have different hydrophobicites and so occupy different positions in or on membranes
- This, in turn, determines how easily such proteins can be extracted from membranes
- Membrane proteins fall into three categories: *integral, peripheral,* and *lipid-anchored*

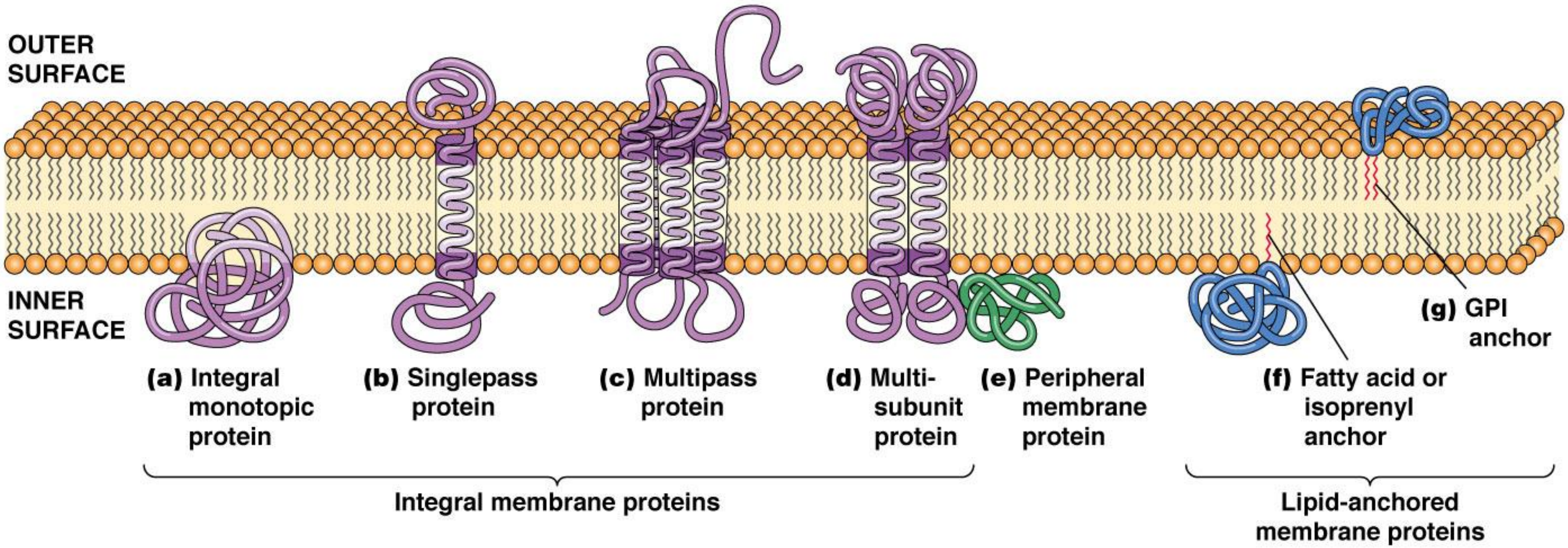
Integral Membrane Proteins

- Most membrane proteins possess one or more hydrophobic regions with an affinity for the interior of the lipid bilayer
- These are **integral membrane proteins**, with hydrophobic regions embedded in the interior membrane bilayer
- They are difficult to remove from membranes by standard isolation procedures

Integral Membrane Proteins

- Some integral membrane proteins, called **integral monotropic proteins**, are embedded in just one side of the bilayer
- However, most are **transmembrane proteins** that span the membrane and protrude on both sides (anchored to the lipid bilayer by one or more hydrophobic **transmembrane segments**)
- Transmembrane proteins cross either once (*singlepass proteins*) or several times (*multipass proteins*)

Figure 7-19

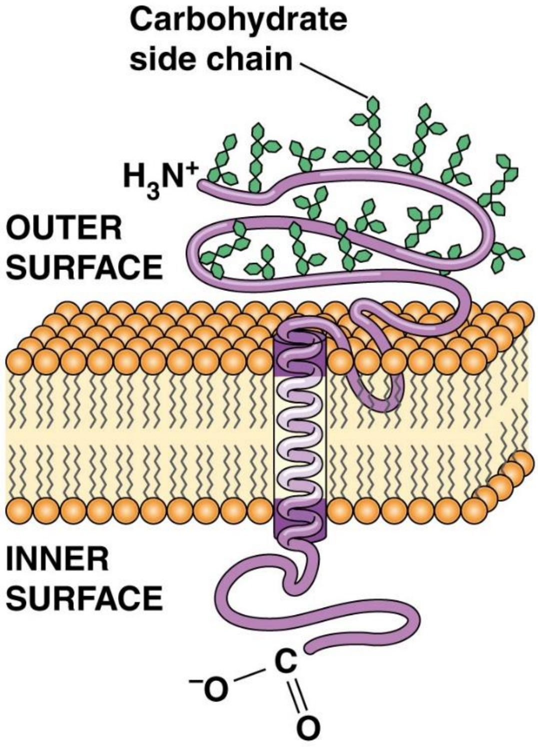


OUTER SURFACE
INNER SURFACE
(a) Integral monotopic protein
(b) Singlepass protein
(c) Multipass protein
(d) Multi-subunit protein
(e) Peripheral membrane protein
(f) Fatty acid or isoprenyl anchor
(g) GPI anchor
Integral membrane proteins
Lipid-anchored membrane proteins

Singlepass membrane proteins

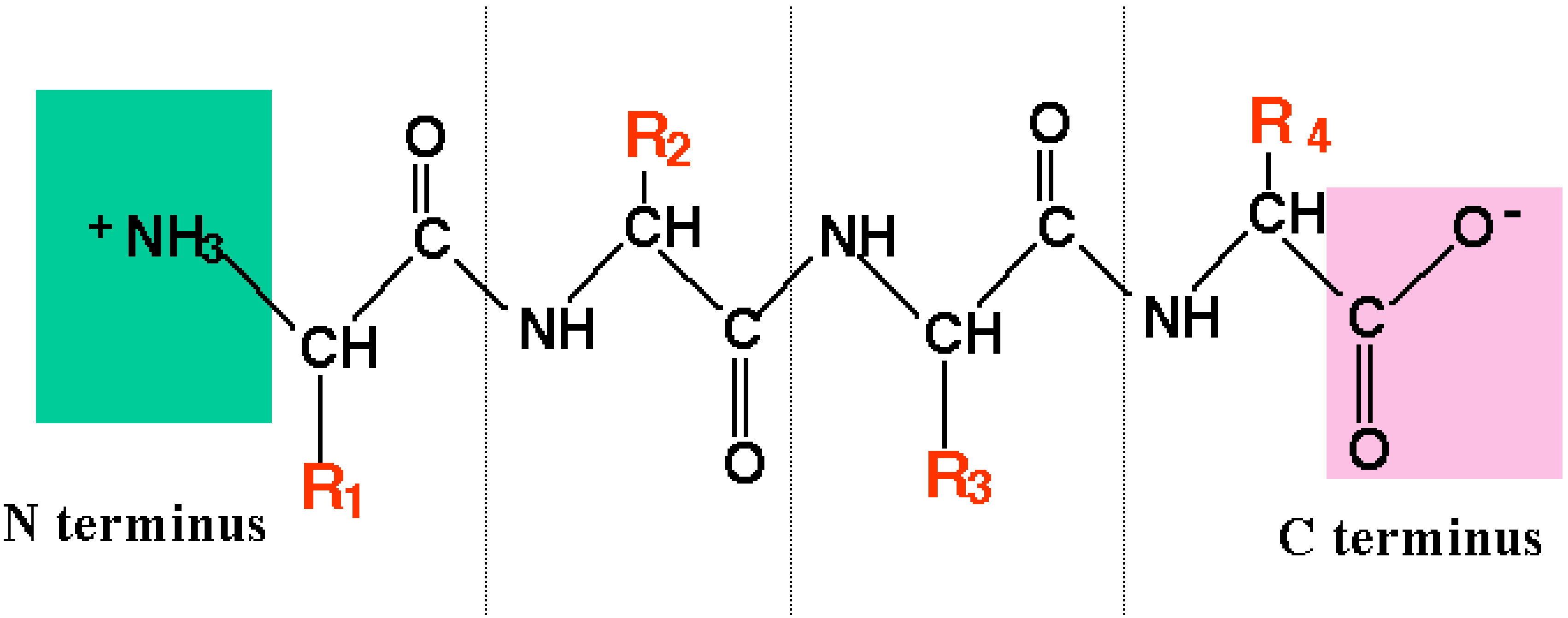
- Singlepass membrane proteins have the
  C-terminus extending from one surface of the membrane and the N-terminus from the other

- For example, *glycophorin* is a singlepass protein on the erythrocyte plasma membrane, that is oriented so the C-terminus is on the inner surface and the N-terminus is on the outer



(a) Glycophorin

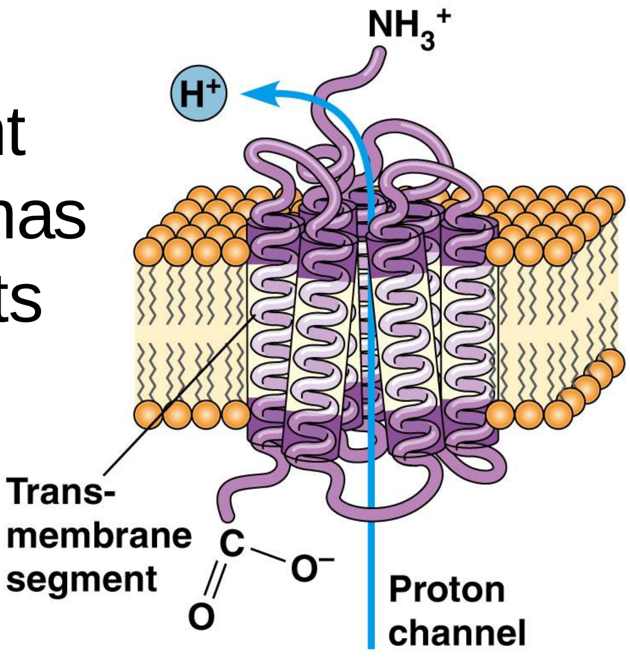
Peptide = chain of amino acids



polypeptide chain

Multipass membrane proteins

- Multipass membrane proteins have 2-20 (or more) transmembrane segments
  - For example, *band 3 protein* (*anion exchange protein*) is present in the erythrocyte membrane and has at least 6 transmembrane segments
  - For example, bacteriorhodopsin has 7 transmembrane segments positioned to form a channel



(b) Bacteriorhodopsin

Peripheral Membrane Proteins

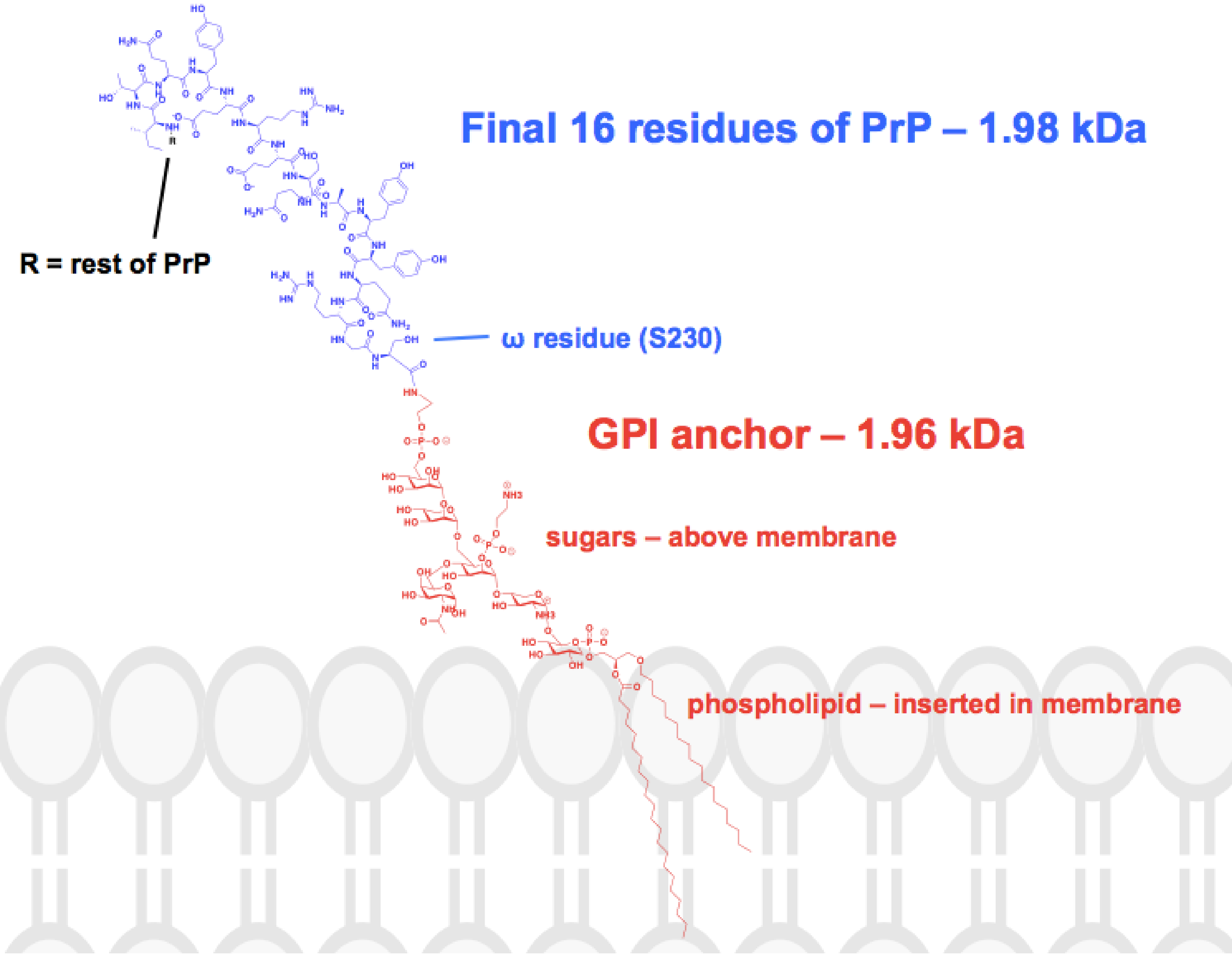
- Membrane proteins that lack **discrete** hydrophobic regions do not penetrate the lipid bilayer
- These **peripheral membrane** proteins are bound to membrane surfaces through weak electrostatic forces and hydrogen bonds
- Some hydrophobic residues play a role in anchoring them to the membrane surface

Peripheral membrane proteins (continued)

- Peripheral membrane proteins are easily separated from membranes by changing pH or ionic strength
- The main peripheral membrane proteins of erythrocytes are *spectrin*, *ankyrin*, and *band 4.1*
- These are on the inner surface of the plasma membrane

Lipid-Anchored Membrane Proteins

- The polypeptide chains of **lipid-anchored membrane proteins** are located on the surfaces of membranes
- They are *covalently bound* to lipid molecules embedded in the bilayer
- Proteins bound to the inner surface or the external surface of the plasma membrane are linked to fatty acids or GPI
- **GPI-anchored membrane proteins** are covalently linked to *glycosylphosphatidylinositol*


Final 16 residues of PrP – 1.98 kDa
R = rest of PrP
ω residue (S230)
GPI anchor – 1.96 kDa
sugars – above membrane
phospholipid – inserted in membrane

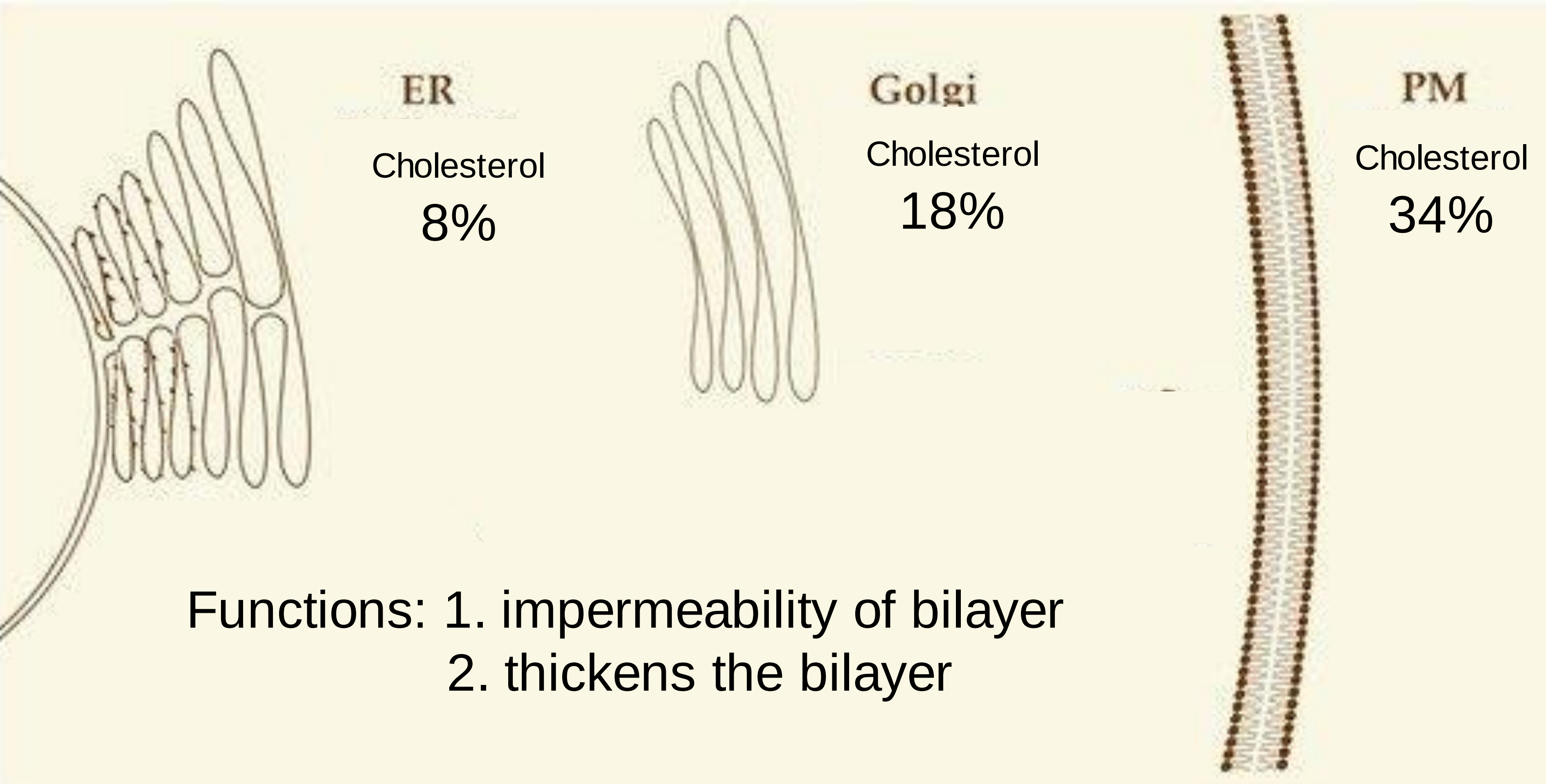
Cellular lipidome vs membrane proteomics

- Proteins received most of the scientific attention
- 1/3 genome encodes for membrane proteins embedded in the bilayer

- Cellular lipidomes may contain up to 7000 distinct lipid species **WHY??**

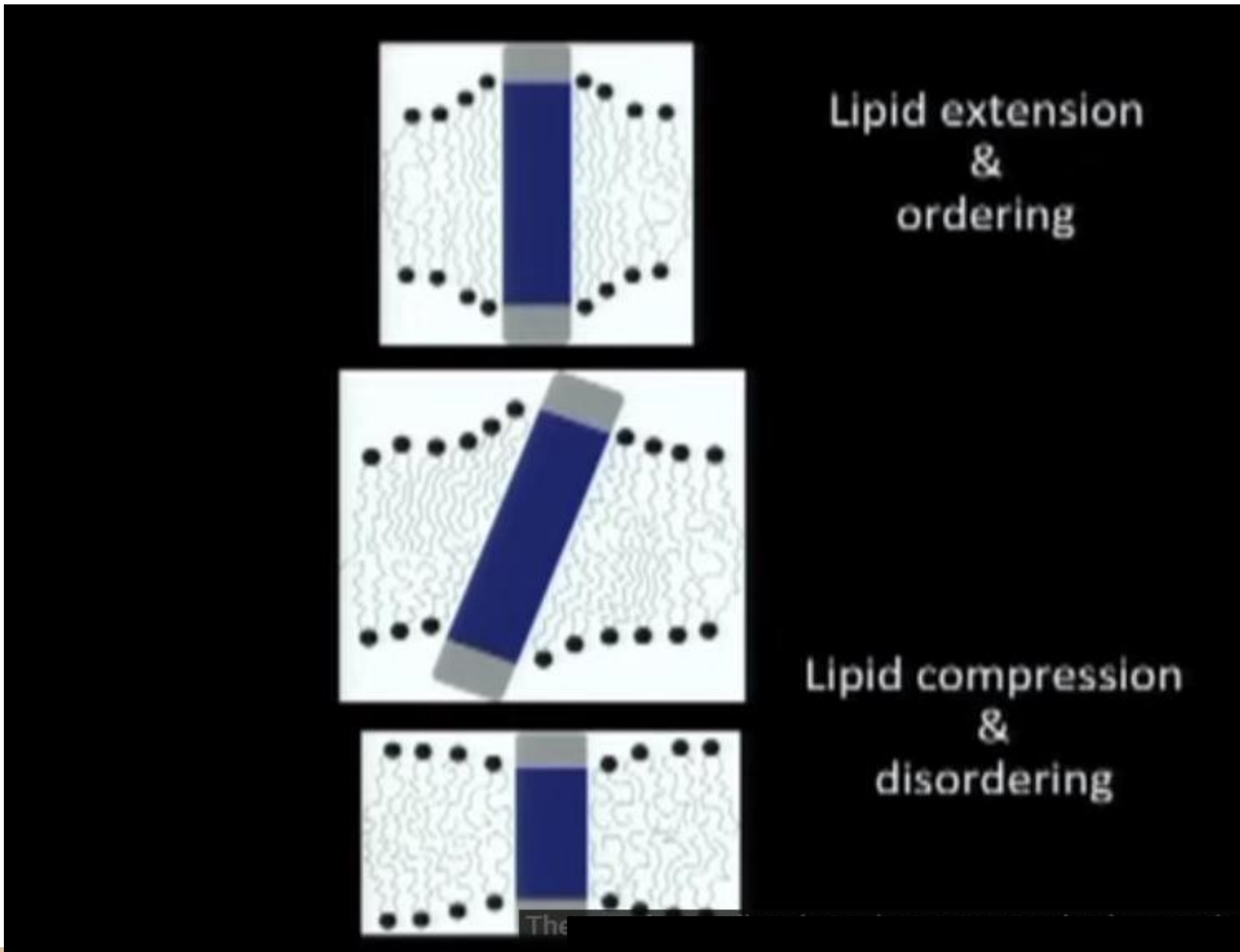
1. Cellular architecture
2. Lipid signaling
3. Regulating membrane proteins
4. Membrane trafficking
5. Part of SUBCOMPARTMENTALIZATION in cells

Lipids are distributed heterogeneously in several ranges: subcellular organelles show lateral differences

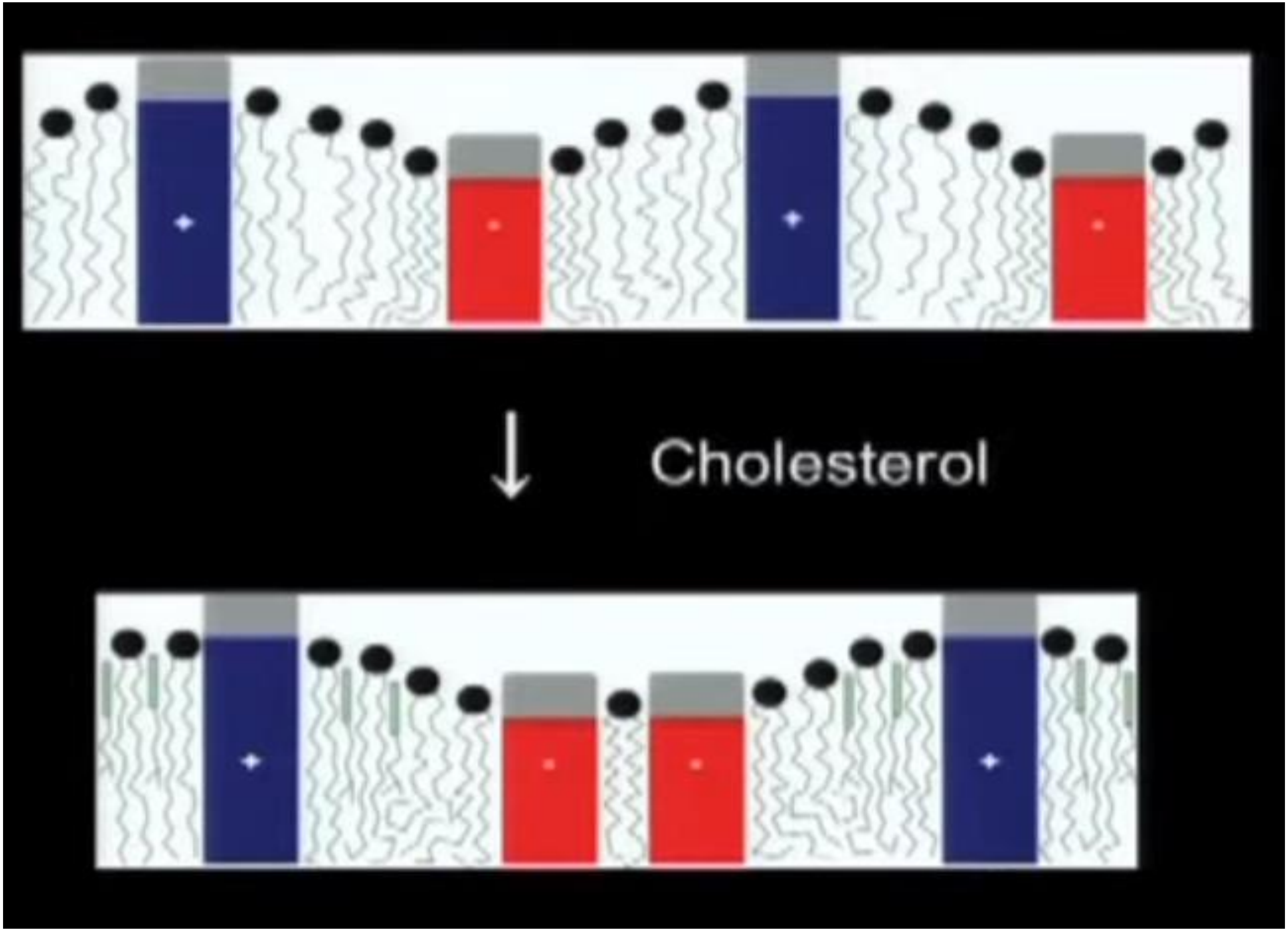


Functions: 1. impermeability of bilayer
2. thickens the bilayer

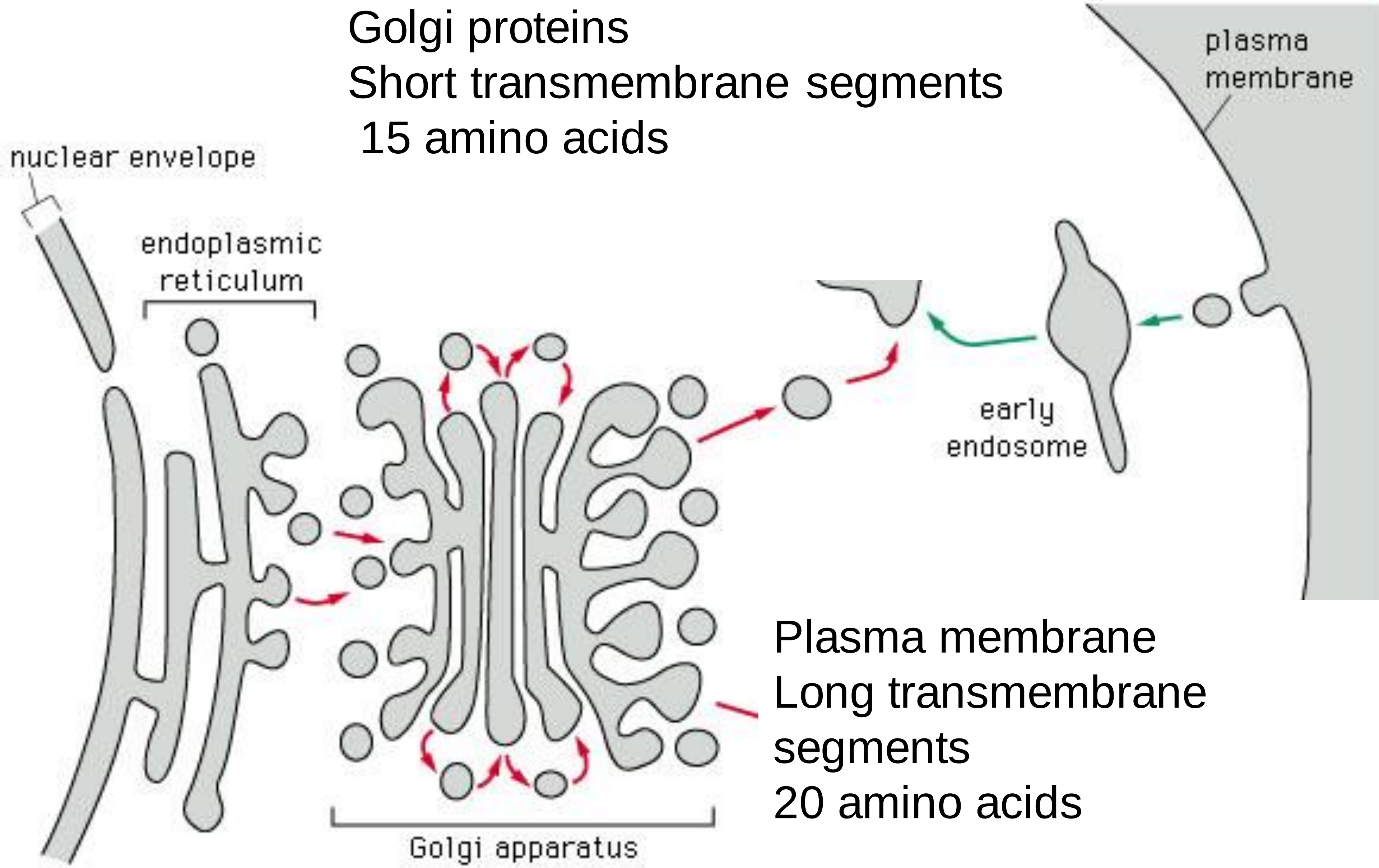
Matching the thickness of the bilayer with hydrophobic transmembrane domains in proteins



Cholesterol induces protein sorting

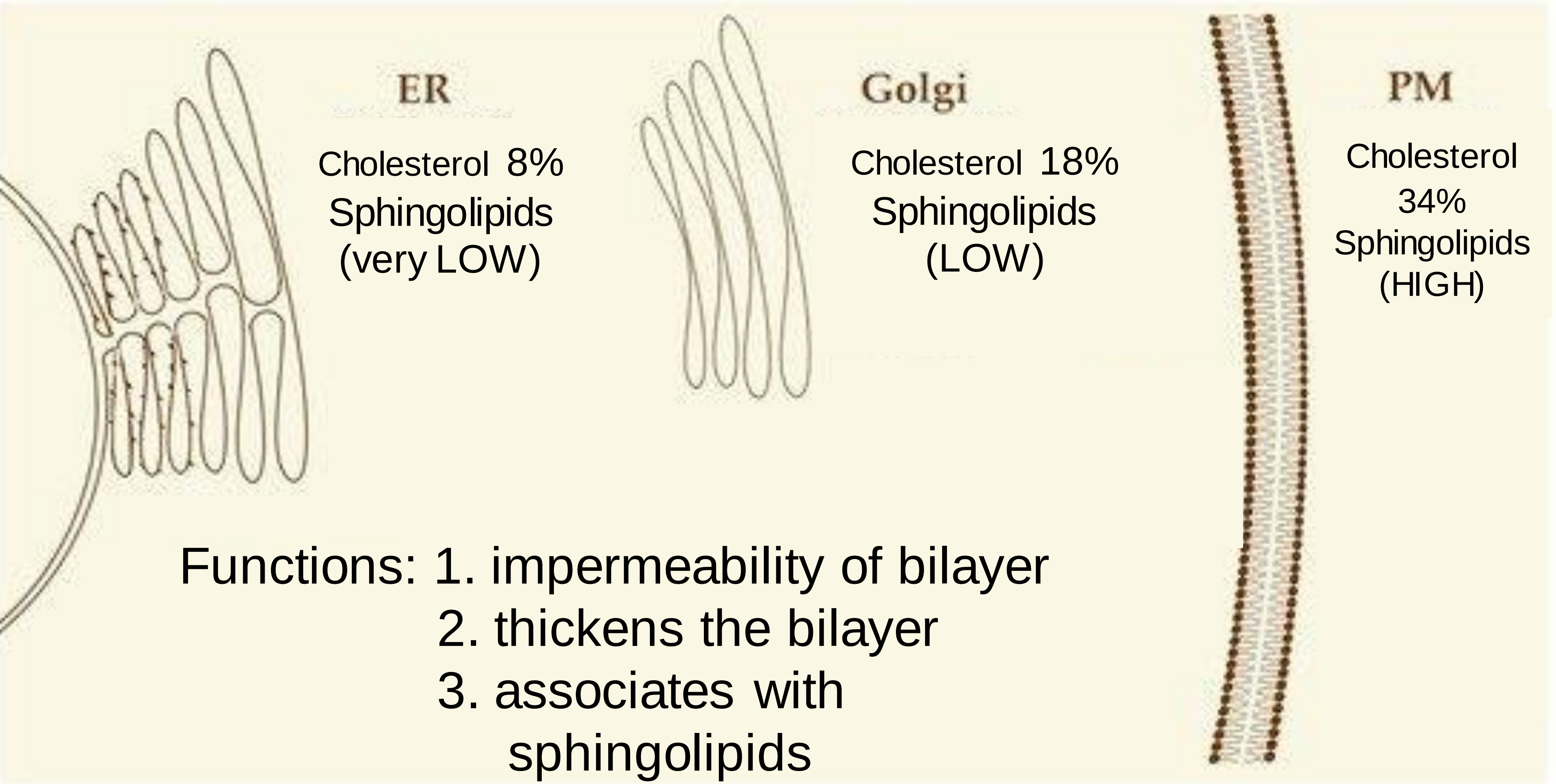


Golgi proteins
Short transmembrane segments
15 amino acids



Plasma membrane
Long transmembrane segments
20 amino acids

This is NOT enough!!!

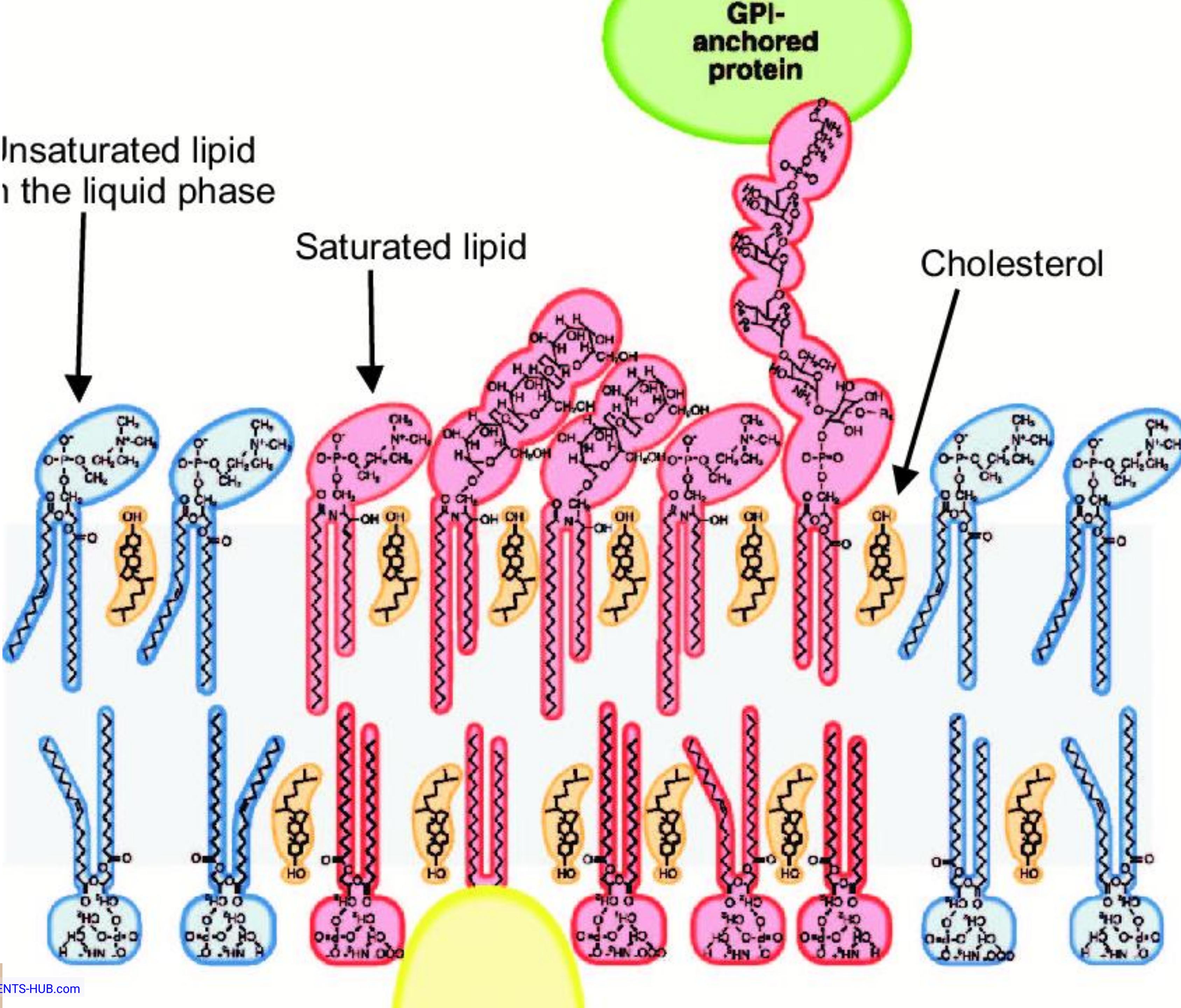


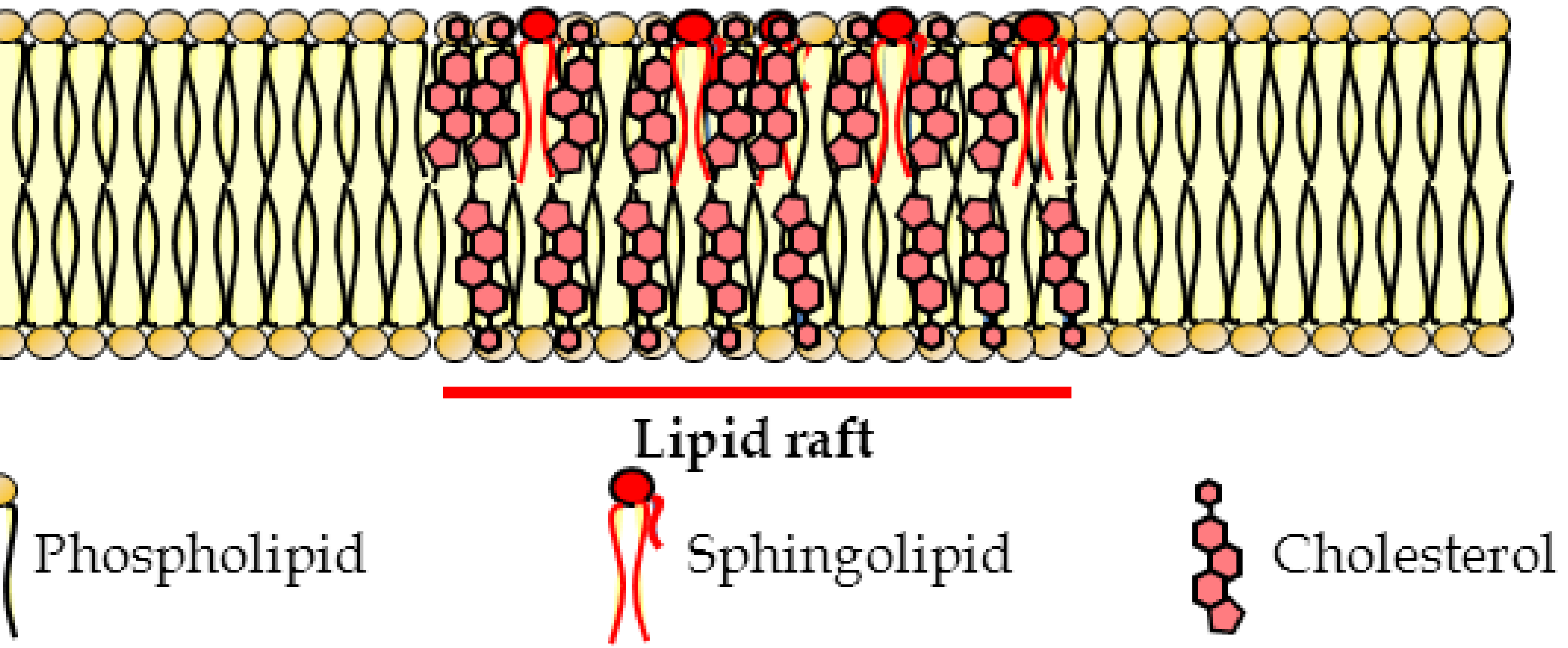
Cholesterol 8%
Sphingolipids
(very LOW)

Cholesterol 18%
Sphingolipids
(LOW)

Cholesterol
34%
Sphingolipids
(HIGH)

Functions: 1. impermeability of bilayer
2. thickens the bilayer
3. associates with sphingolipids


GPI-
anchored
protein
Insaturated lipid
ı the liquid phase
Saturated lipid
Cholesterol


Lipid raft
Phospholipid
Sphingolipid
Cholesterol

Sub-compartmentalization