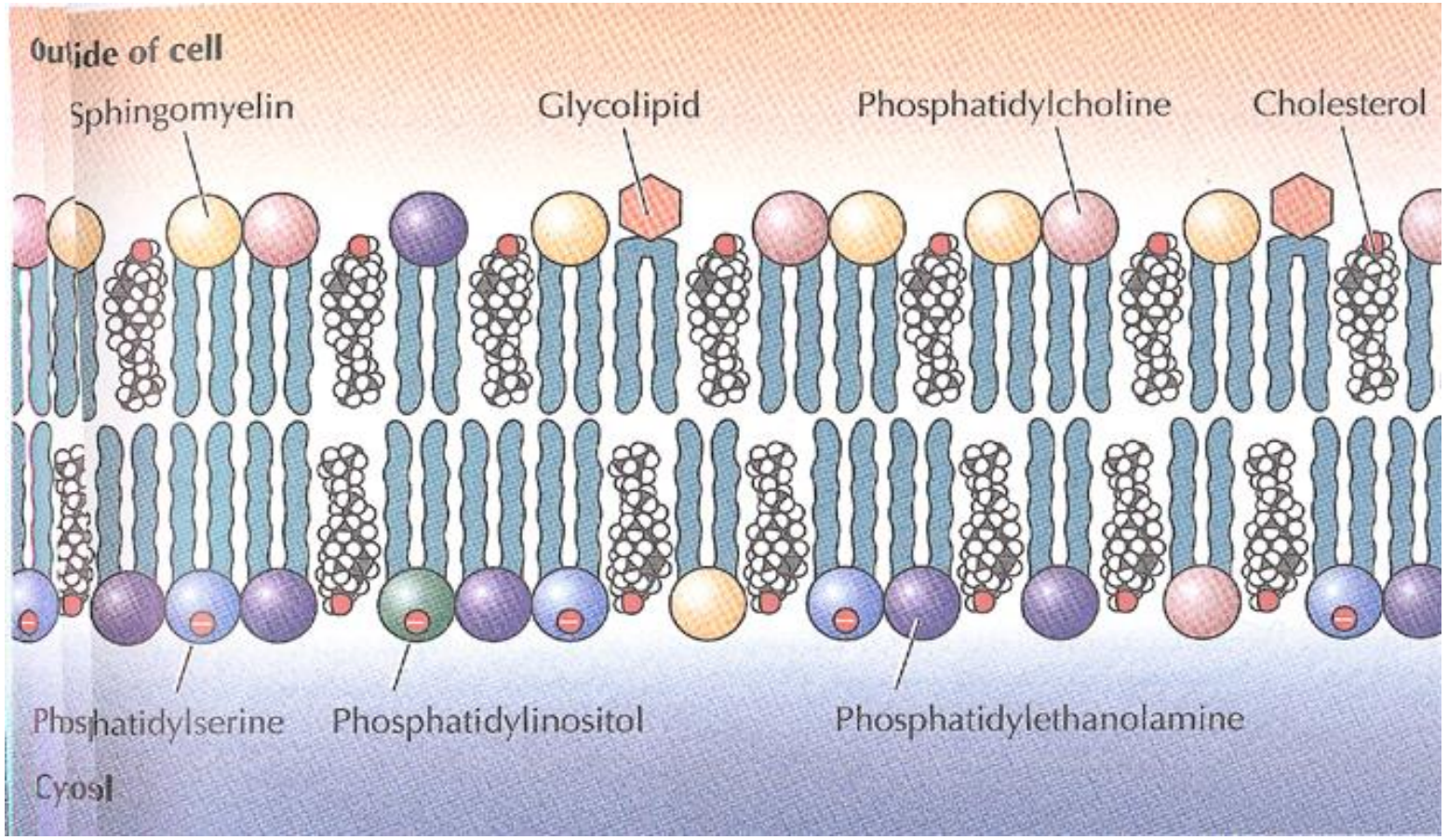
The lipid bilayer



Lecture 2: Membranes and their Lipids

Chapter 10, 565-576

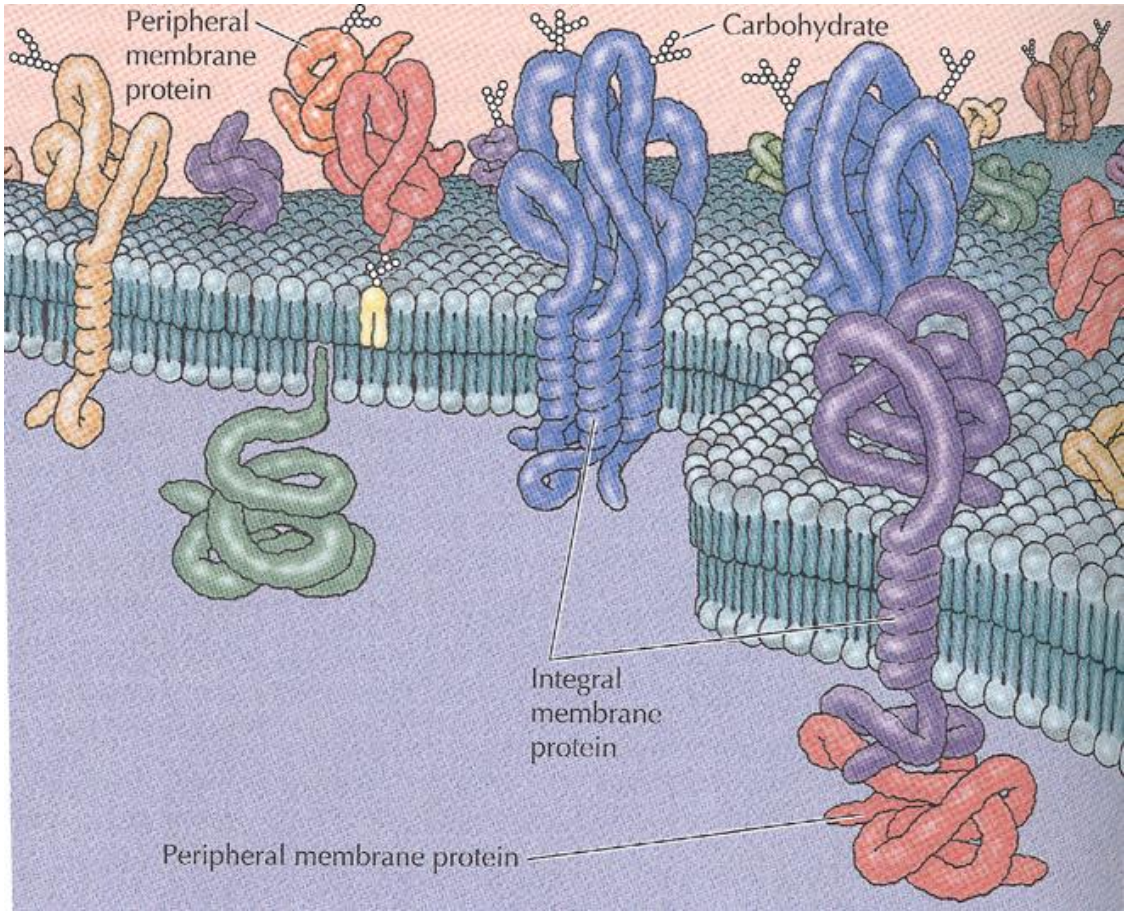
- Compartmentalization
  - Inside / Outside
  - Membrane asymmetry
- Different types of lipids
  - Membrane composition
- The molecular basis of membrane fluidity
  - Unsaturated fatty acids / Cholesterol
- Cholesterol and microdomains ('lipid rafts')
- Permeability properties of lipid bilayers
- Lipids in signaling

General properties

Membranes constitute thin, strong, non-elastic, self-sealing, flexible, deformable, hydrophobic barriers between aqueous compartments.

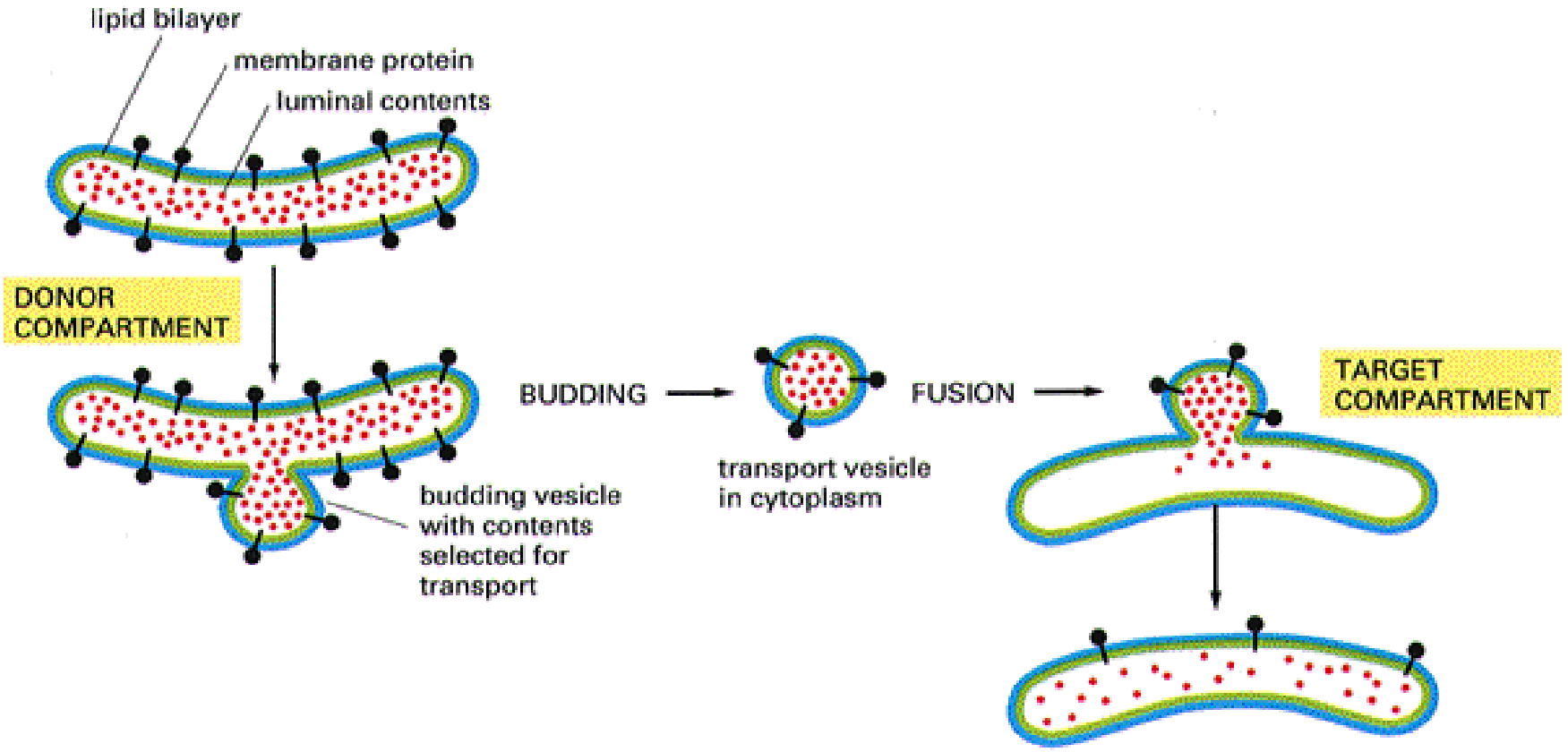
- Extremely thin (the bilayer is 4-5 nm thick, a biological membrane up to 7 nm with proteins included)
- Impermeable to most polar solutes, therefore insulating
- Extremely stable thermodynamically
- Virtually non elastic (allows only about 4% stretching before tearing)
- No edges, only closed structures

Membrane proteins are always oriented in respect to outside and inside. Their ‘topology’ is thus defined. They cannot rotate.



Vesicular transport:

Coupled membrane fission and fusion

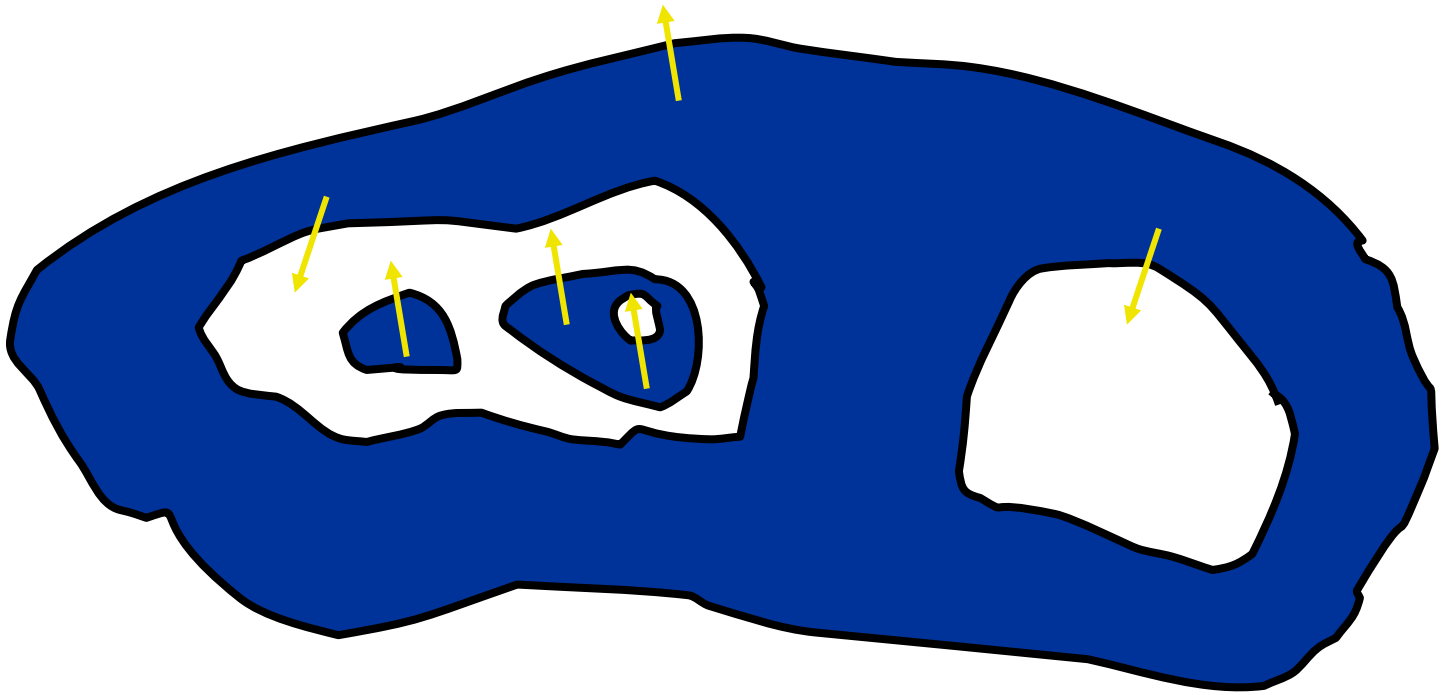


Two principal compartments in the living world:

1) cytosol and topologically equivalent volumes (blue)
2) the extra cellular space (the primordial ocean) and topologically equivalent volumes (white)

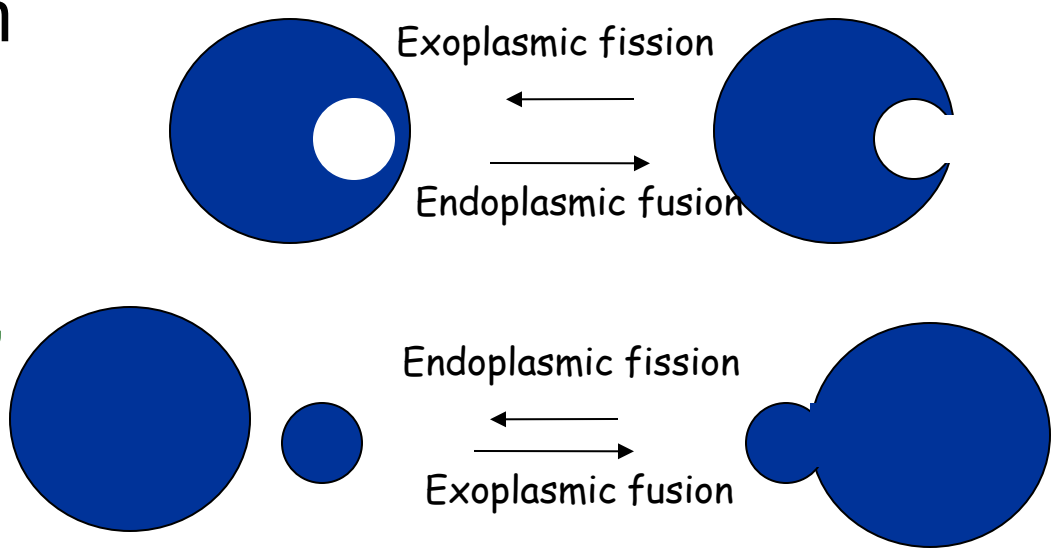
Forever separated!

Every membrane separates white from blue.

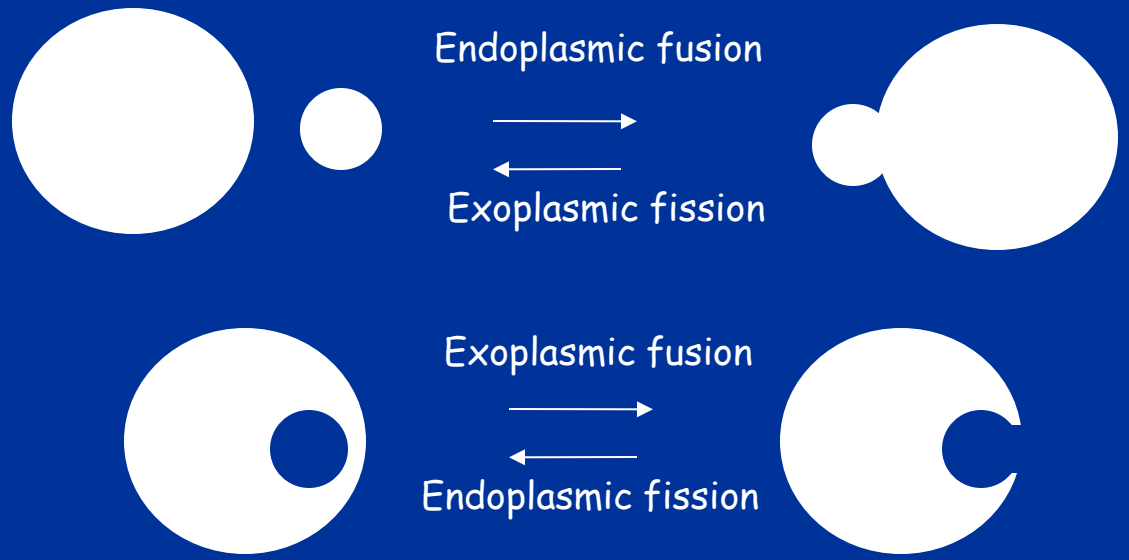


Exoplasmic and endoplasmic fusion and fission

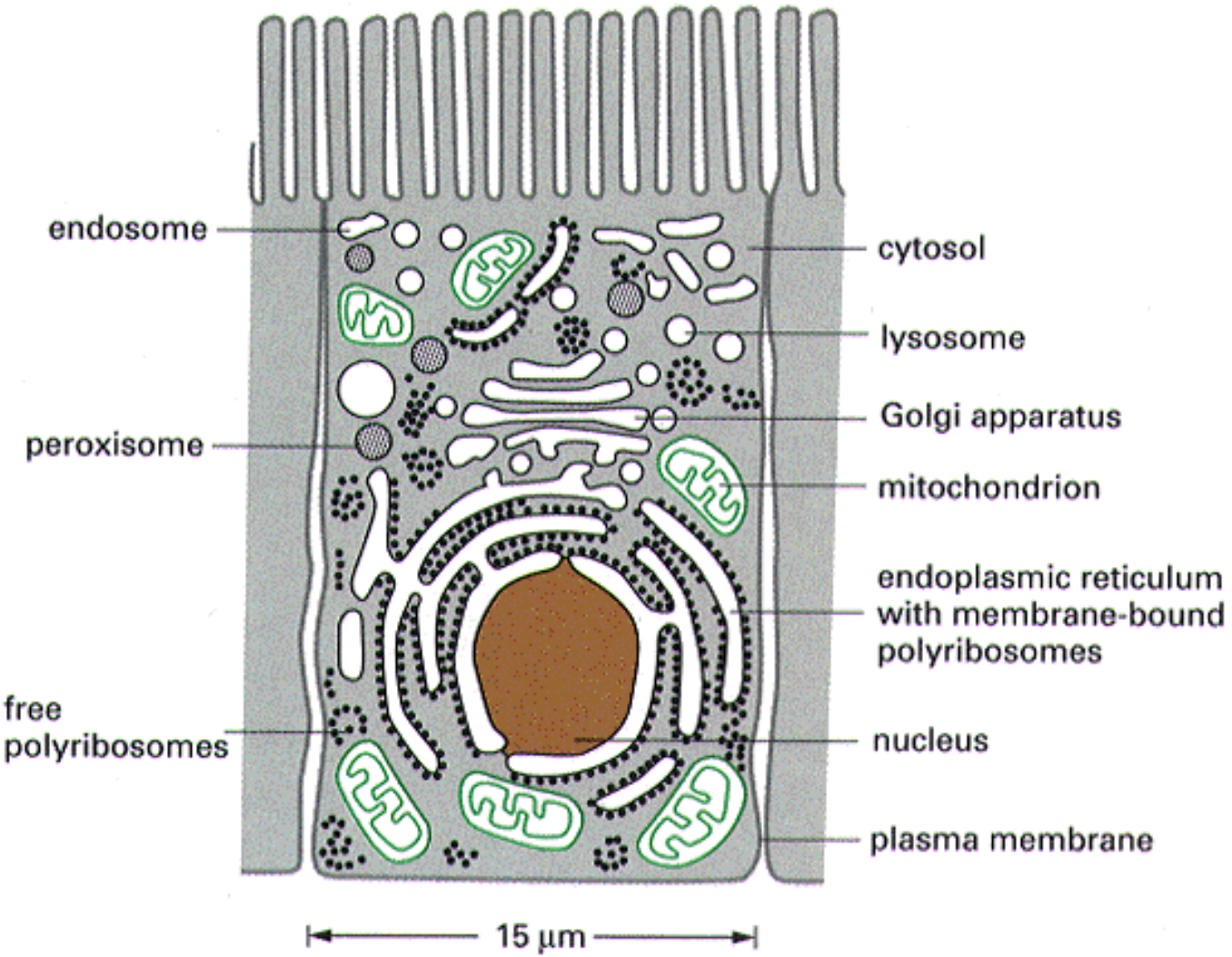
Endoplasmic when the surface(s) that come together face the cytosol, and exoplasmic when they face the extra cytosolic volume



CYTOSOL



Organelles and compartments in animal cells (eukariotic)



Membranes contain two general types of lipids

- Lipids that spontaneously form a bilayer when mixed with water.

  Phospholipids and some glycolipids

- Lipids that alone cannot form a bilayer, but can be dissolved into - and thus accommodated in - a bilayer

  Cholesterol, gangliosides, lysophosholipids, tri- and di-glycerides, isoprenoids, lysophospholipids, long chain fatty acids.

Bilayer forming lipids are ‘amphiphilic’, they have two long alkyl chains, and take up a roughly cylindrical space:

A prime example is phosphatidylcholine (PC), also

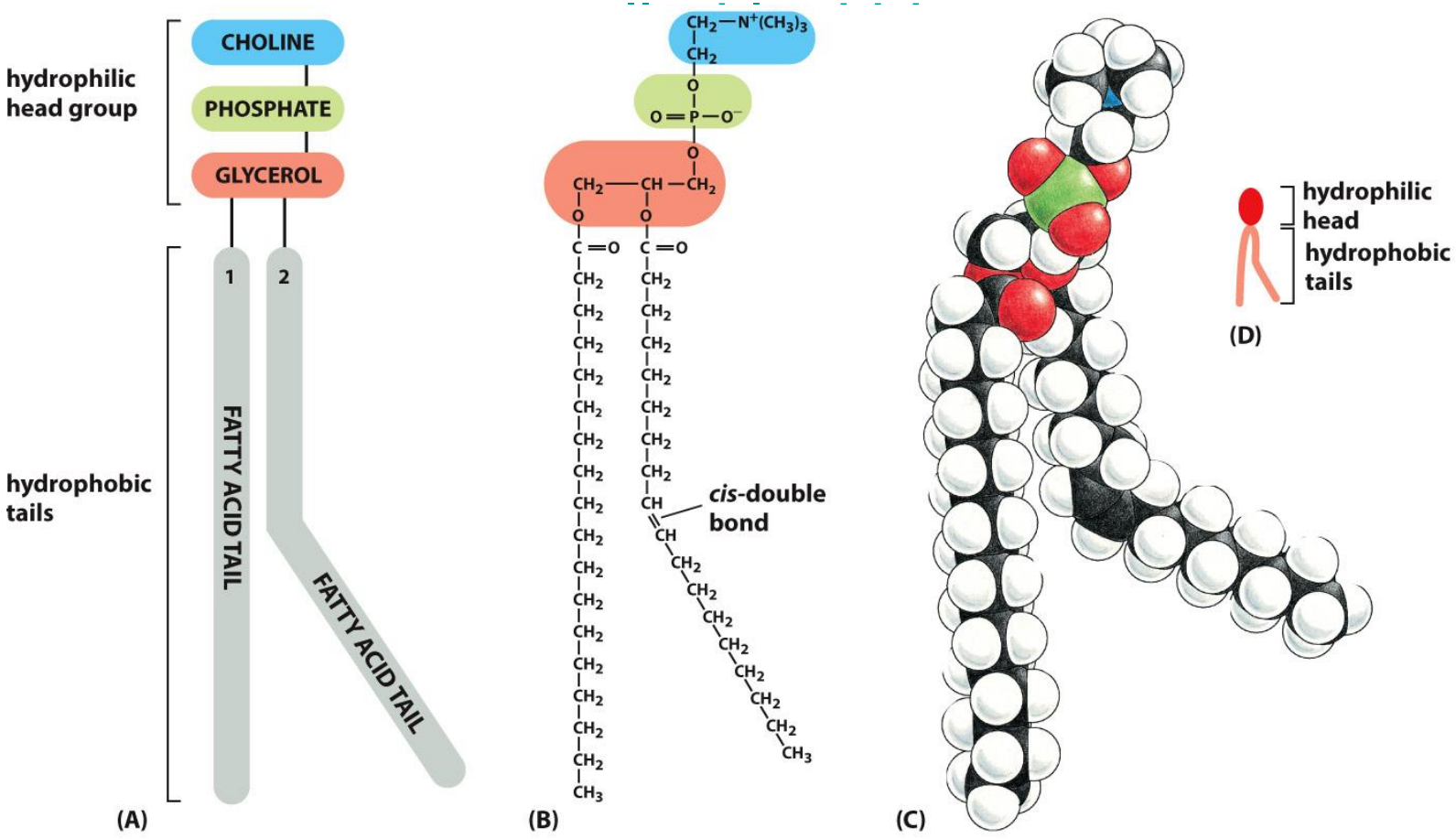


Figure 10-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

Classes of Phospho-Lipids

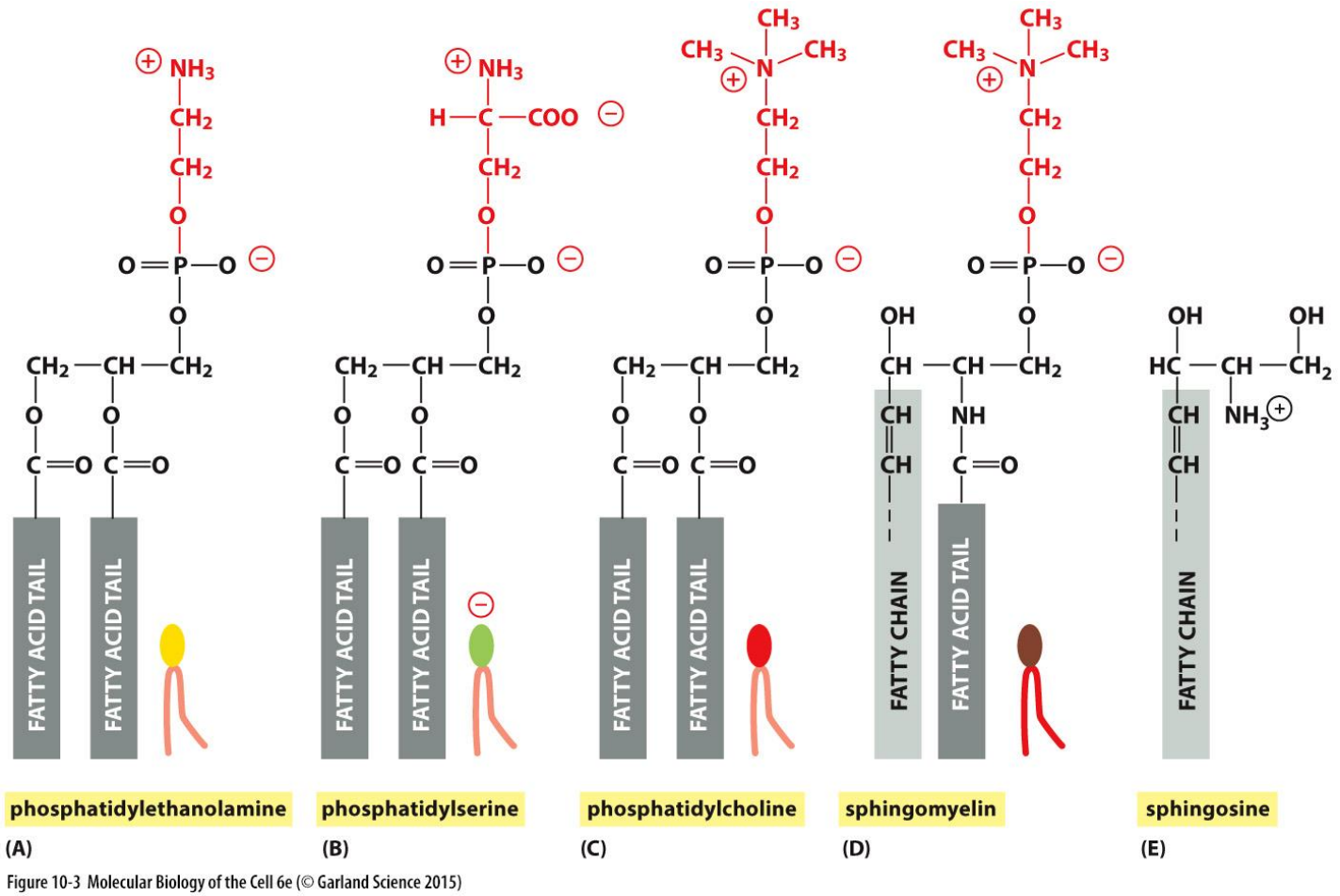


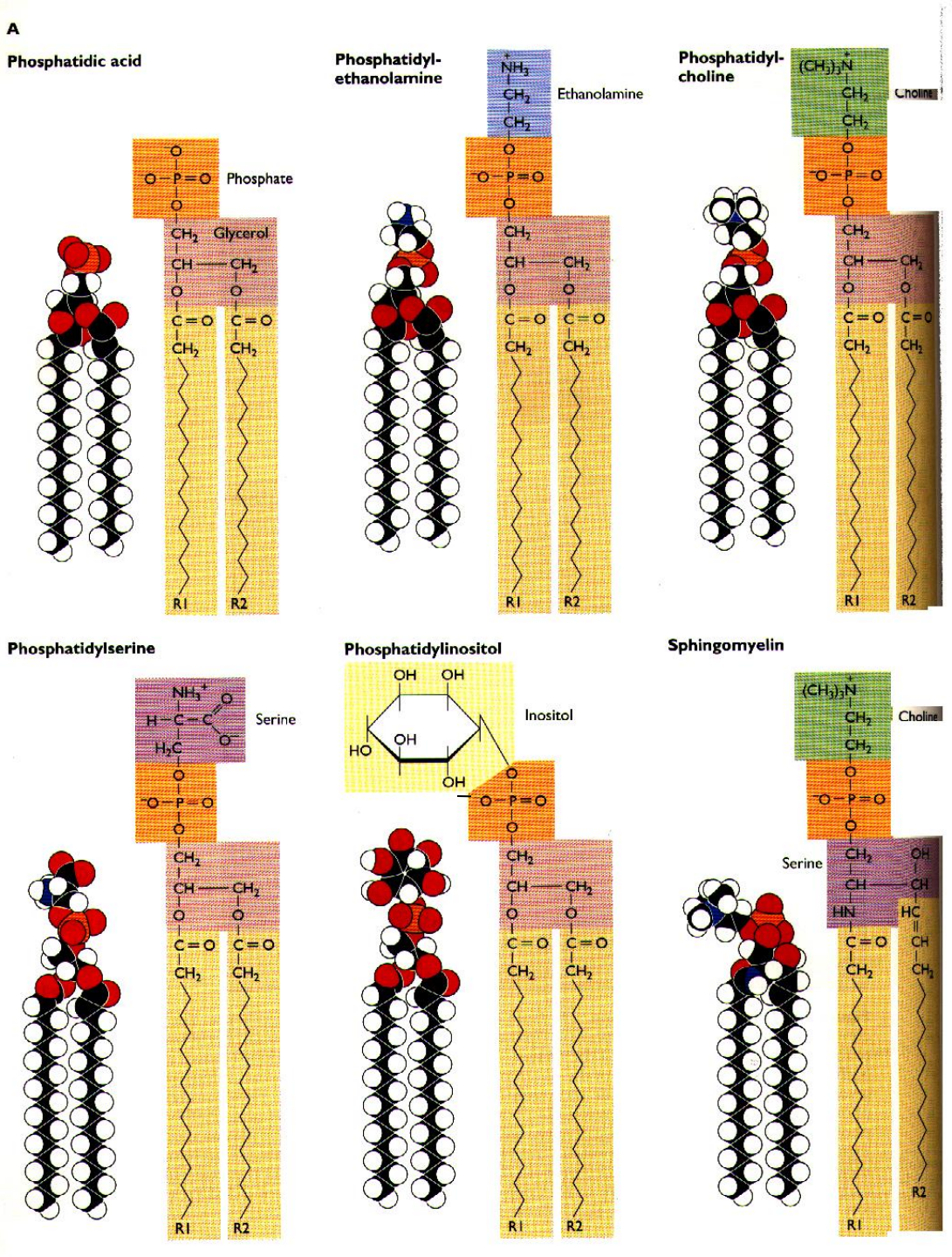
Figure 10-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

Note that PE ,PS and PC are glycerophospholipids
SPH is a sphingolipid (instead of glycerol and one of the fatty acids it contains a sphingosine).

Two more phospholipids that you need to know

Phosphatidic acid (PA)
Phosphatidylinositol(PI)

Which phospholipids are negatively charged?
Which have amino groups?
Which are not glycerolipids?



Many different fatty acids are associated with these lipids

Some Naturally Occurring Fatty Acids

Symbol	Structure	Systematic name	Common name	m.p. (°C)
	A. Saturated fatty acids			
12 : 0	$CH_3(CH_2)_{10}COOH$	*n*-Dodecanoic	Lauric	44.2
14 : 0	$CH_3(CH_2)_{12}COOH$	*n*-Tetradecanoic	Myristic	53.9
16 : 0	$CH_3(CH_2)_{14}COOH$	*n*-Hexadecanoic	Palmitic	63.1
18 : 0	$CH_3(CH_2)_{16}COOH$	*n*-Octadecanoic	Stearic	69.6
20 : 0	$CH_3(CH_2)_{18}COOH$	*n*-Eicosanoic	Arachidic	76.5
24 : 0	$CH_3(CH_2)_{22}COOH$	*n*-Tetracosanoic	Lignoceric	86.0
	B. Unsaturated fatty acids			
16 : 1	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$		Palmitoleic	−0.5
18 : 1	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$		Oleic	13.4
18 : 2	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$		Linoleic	−5
18 : 3	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$		Linolenic	−11
20 : 4	$CH_3(CH_2)_4(CH{=}CHCH_2)_3CH{=}CH(CH_2)_3COOH$		Arachidonic	−49.5

Note the nomenclature: 18:2 means 18 carbons, 2 double bonds

Fatty acid composition determined by:

- Organism
- Ambient temperature
- Cell type
- Membrane organelle
- Lipid class

The fatty acid in R1 position of glycerol often saturated, in R2 position

usually unsaturated

Fatty Acids Found in Egg Phosphatidylcholine and Human Erythrocyte Phosphatidylethanolamine

A. Fatty Acid Composition of Phospholipids[a]

Fatty acid	Mol % in egg PC	Mol % in erythrocyte PE
16 : 0	33	19
16 : 1	2	—
18 : 0	15	13
18 : 1	32	22
18 : 2	17.8	7
20 : 4	4.3	19
22 : 4	—	5
22 : 6	1.7	4

B. Fatty Acid Combination Found in Egg PC[b]

	R_1	R_2	Mol %
	16 : 0	18 : 1	45
	16 : 0	18 : 2	31
→	18 : 0	18 : 2	12
	18 : 0	18 : 1	10
	18 : 0	20 : 4	8

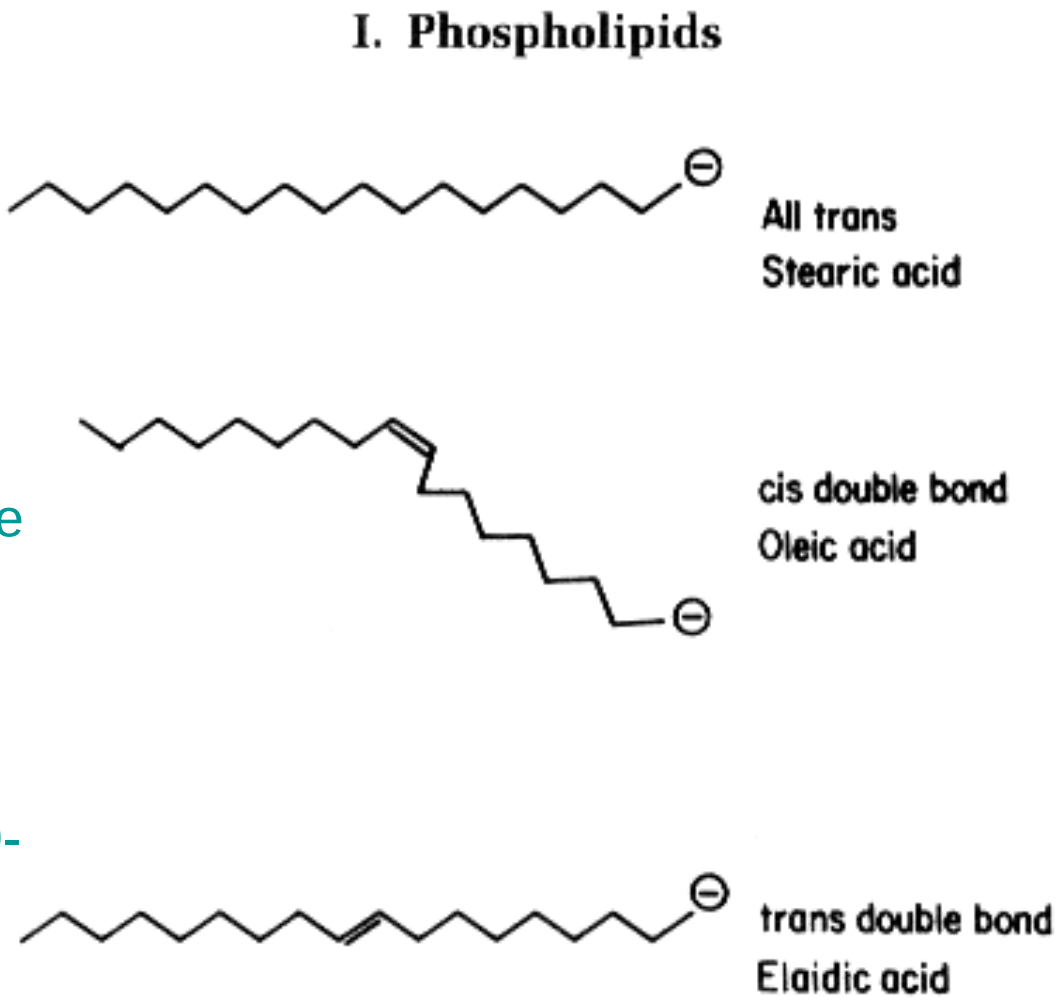
Cis-double bonds introduce a kink in the FA chain.

A permanent feature commonly found in biological membrane lipids.

Tends to perturb membrane more than the trans form.

Makes membranes more fluid!

When one double bond, the position is C9-C10, when two C9-C10 and C12-C13.



Glycosphingolipids and gangliosides are often present in the plasma membrane

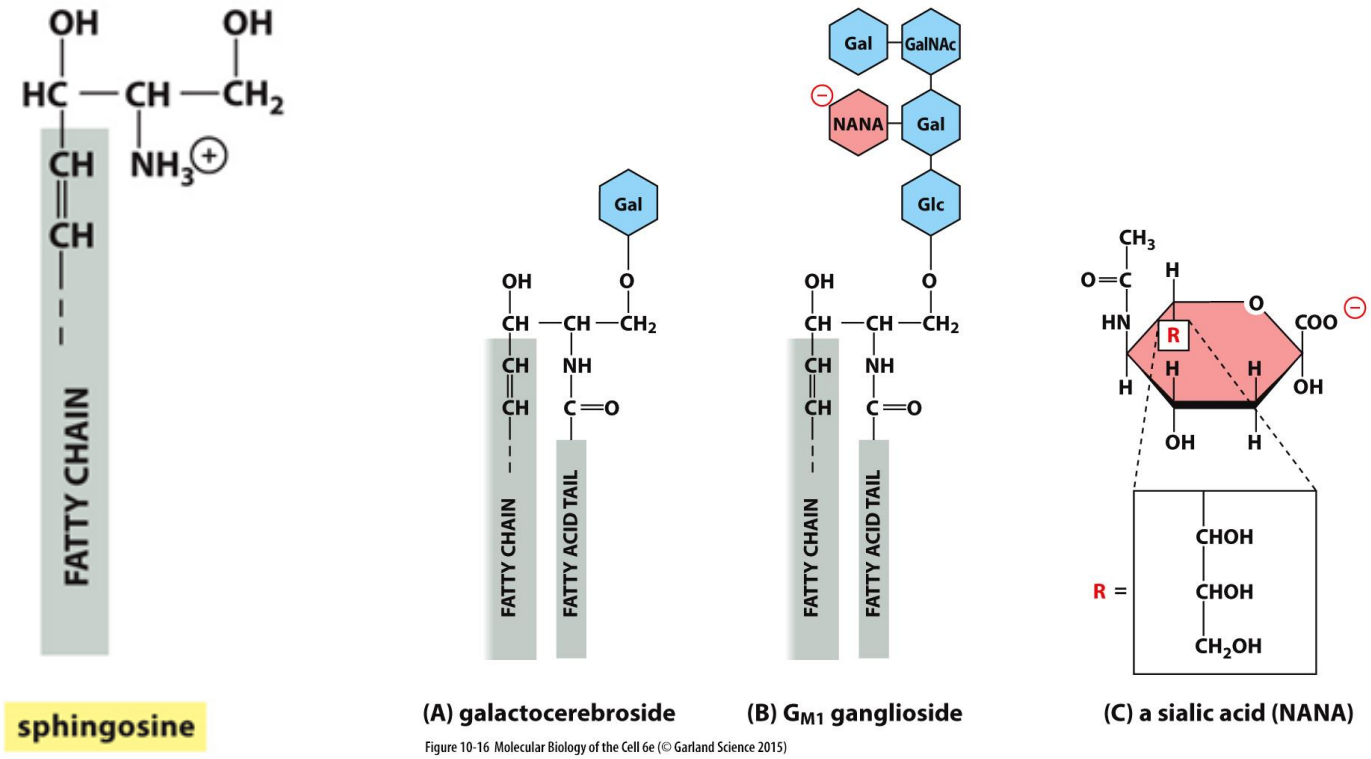
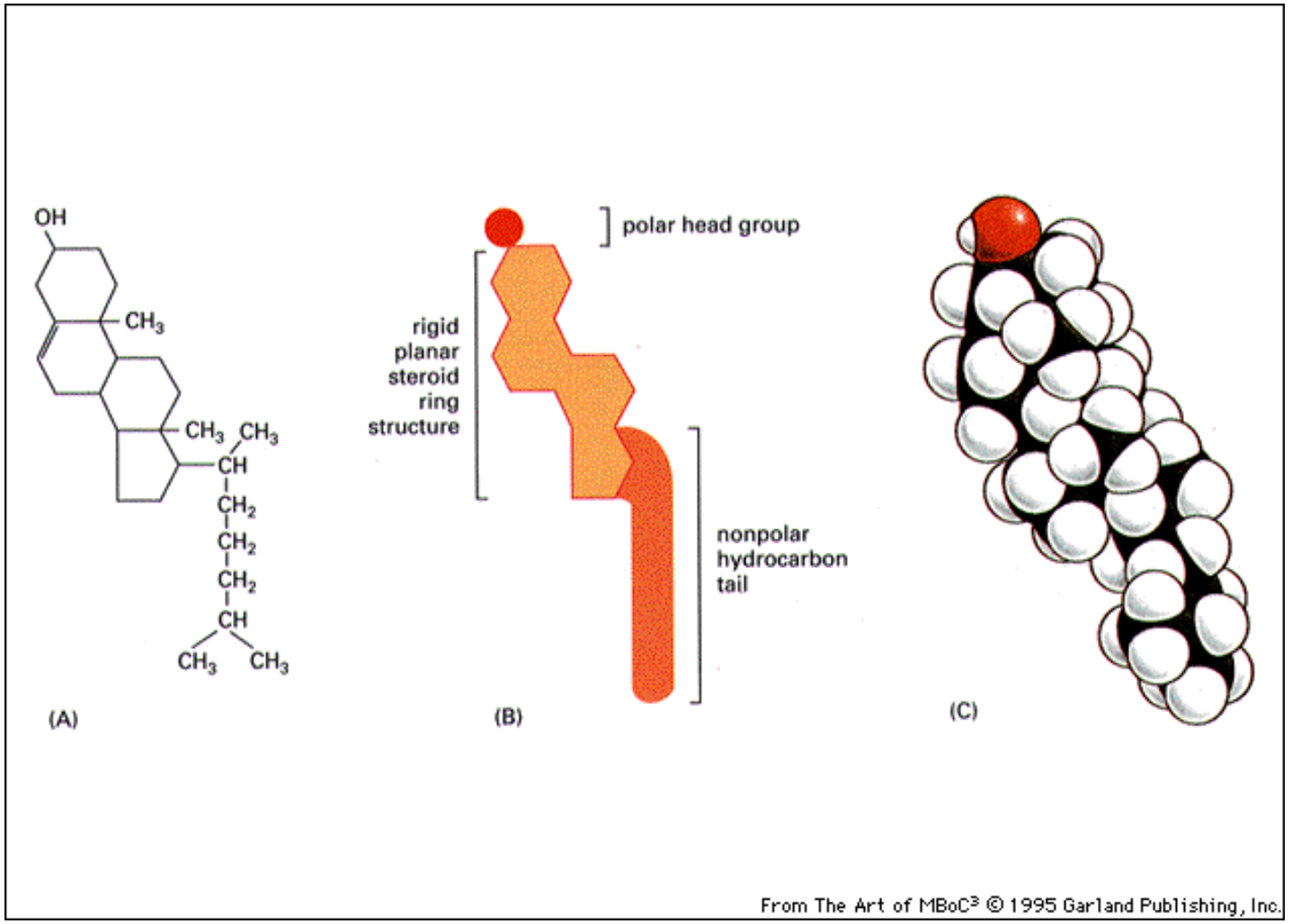


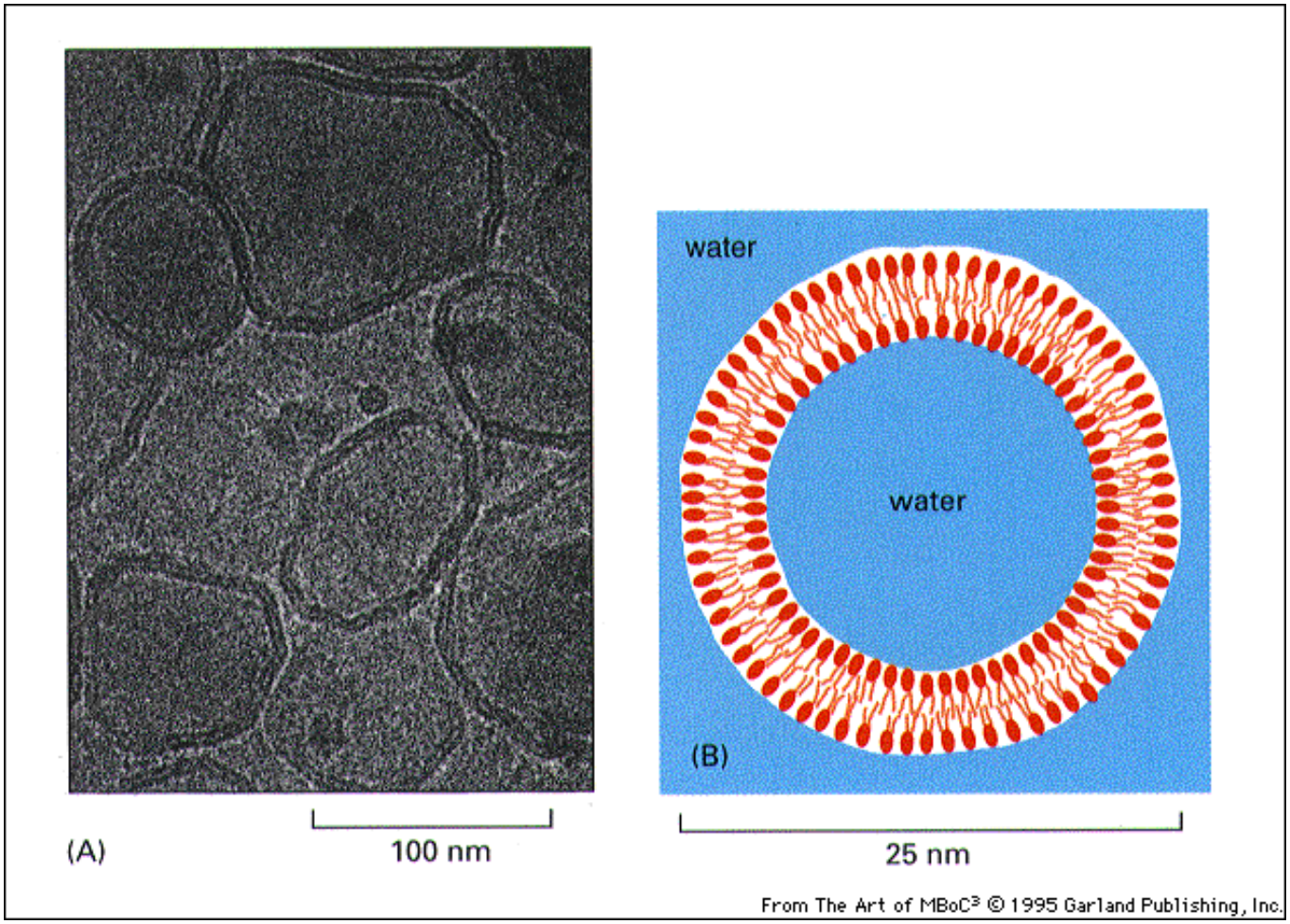
Figure 10-16 Molecular Biology of the Cell 6e (© Garland Science 2015)

- Sphingosine as starting point
- Not a phospholipid
- Mainly on the outside (sugar addition in the Golgi)
- Gangliosides: glycosphingo-lipids that contain one or more sialic acids (in humans N-acetyl neuraminic acid =NANA)

Cholesterol: Important component of animal cell plasma membrane



Bilayer membranes in the form of ‘liposomes’ form spontaneously when lipids are suspended in water



Liposome formation

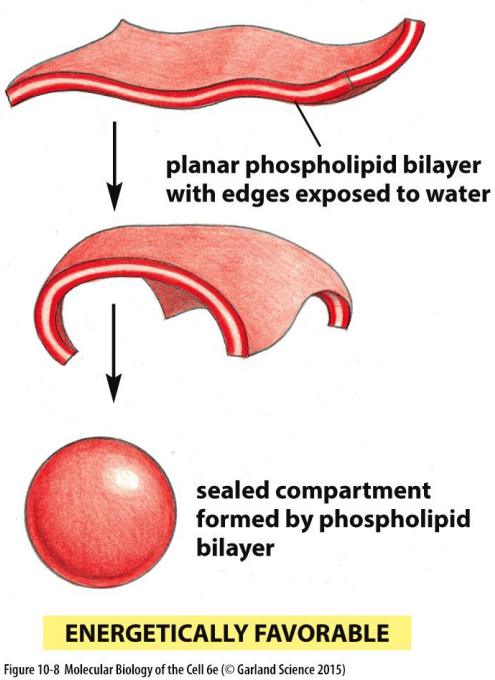
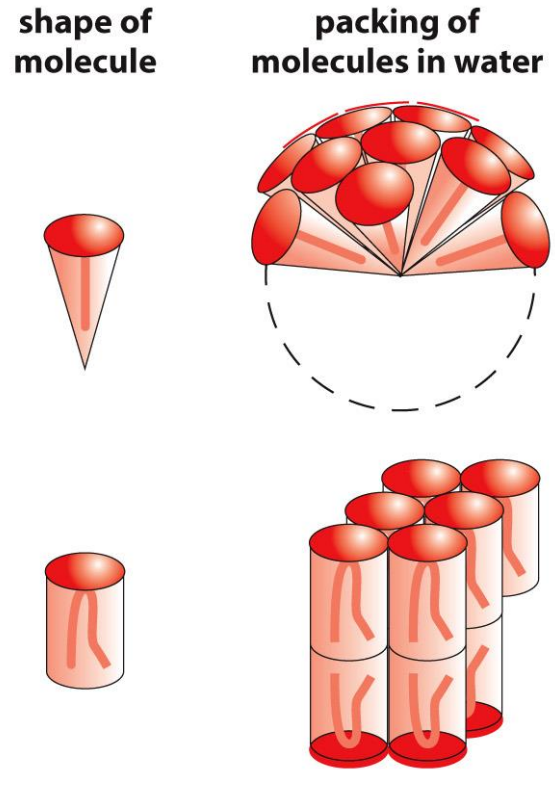


Figure 10-8 Molecular Biology of the Cell 6e (© Garland Science 2015)



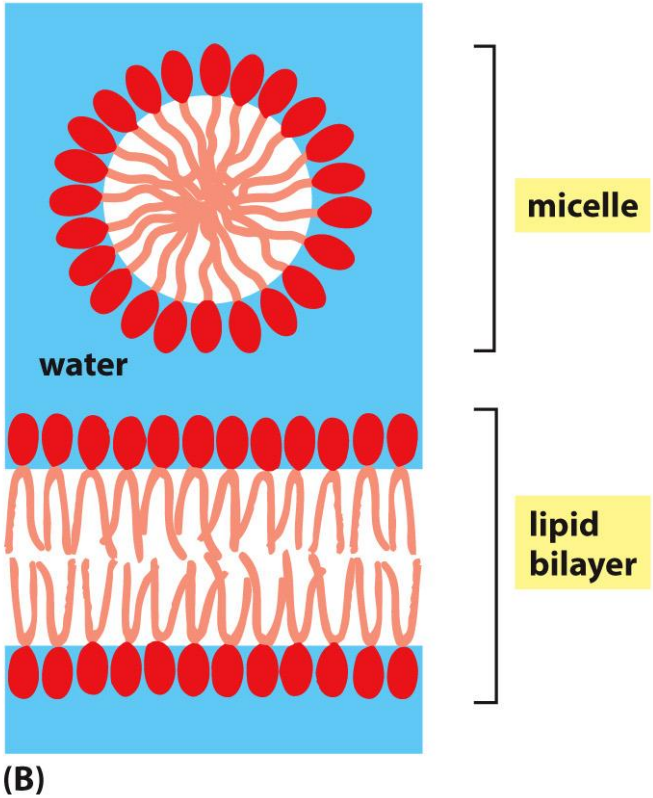
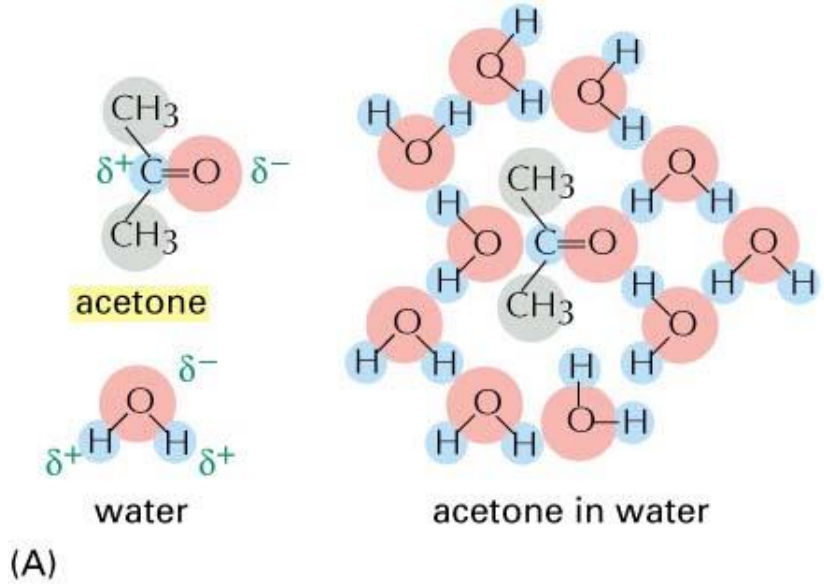


Figure 10-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

The hydrophobic effect: How polar and apolar molecules interact with water



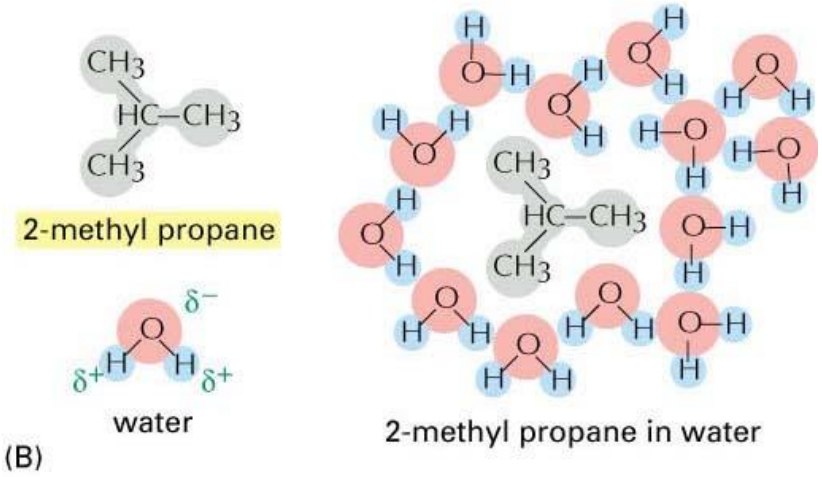


Figure 10–3. Molecular Biology of the Cell, 4th Edition.

The carbonyl group of acetone can form hydrogen bonds with water. This means that it will not disrupt water structure very much.

2-methyl propane has no groups that can participate in hydrogen bonds with water. A hydrogen-bonded 'cage' of water molecules therefore has to form around it. This limits the degrees of freedom of the system, and is energetically unfavourable.

Some additional facts about the hydrophobic effect

- Unsaturated alkyl chains are less hydrophobic than the corresponding saturated chains
- A methyl group as a branch adds less hydrophobicity than an additional methyl group at the end of the chain
- Two separate chains are worth more in energy than two connected as in phospholipids

The rule is that the larger the surface area of the clathrate structure the stronger the hydrophobic effect

A fluid membrane: Mobility in a lipid bilayer

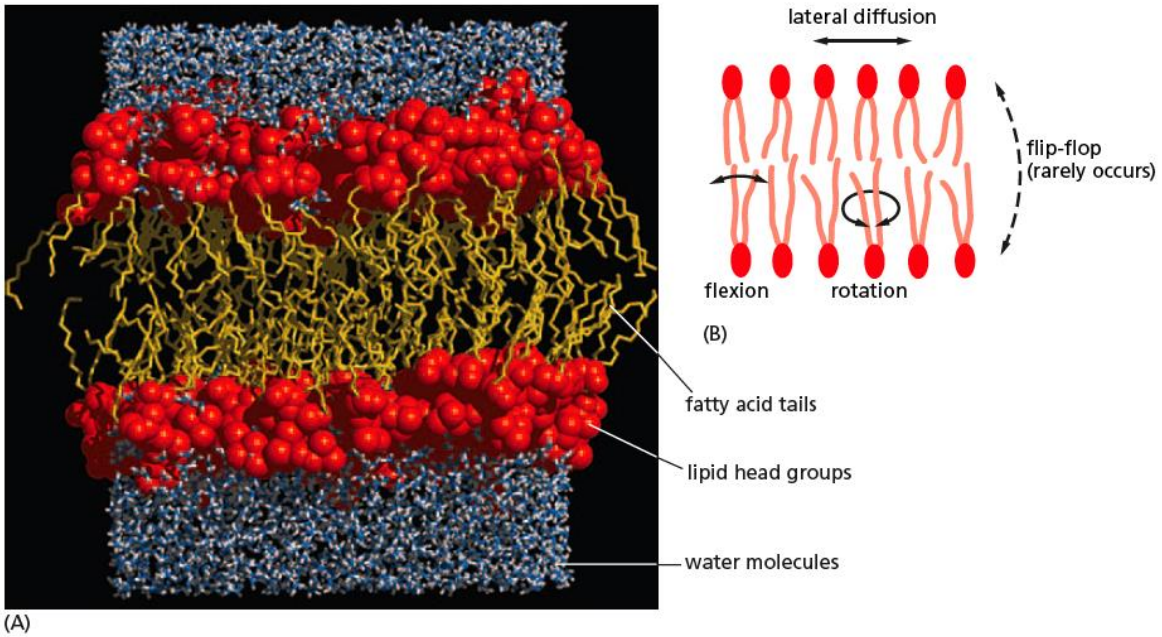
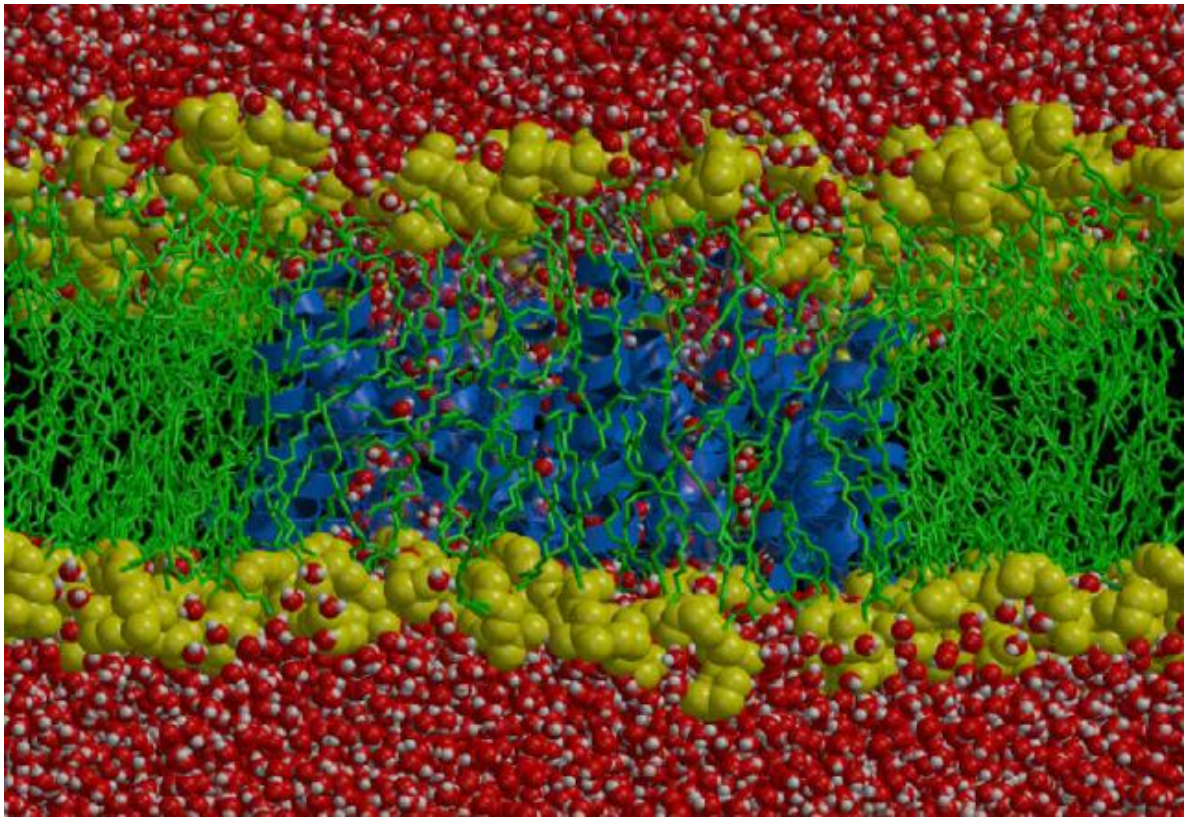


Figure 10–11 The mobility of phospholipid molecules in an artificial lipid bilayer. <CACA> Star

Lateral diffusion: average distance 2 μm per sec (i.e. length of a bacterial cell)
Rotational: Frequency 10^8 per sec
Flip-Flop: once in about three days

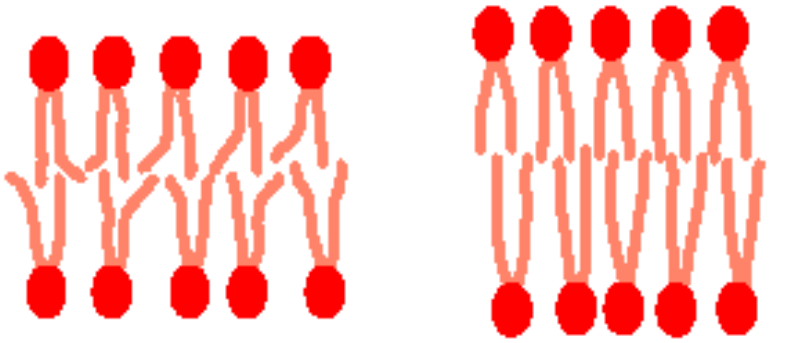
Simulation fragment of 200 ps of aquaporin-1(a water channel)



(B.L. de Groot and H. Grubmüller: Science 294, 2353-2357 (2001)

www.mpibpc.mpg.de/groups/de_groot/gallery.html

Influence of cis-double bonds: they make membranes more fluid and thinner

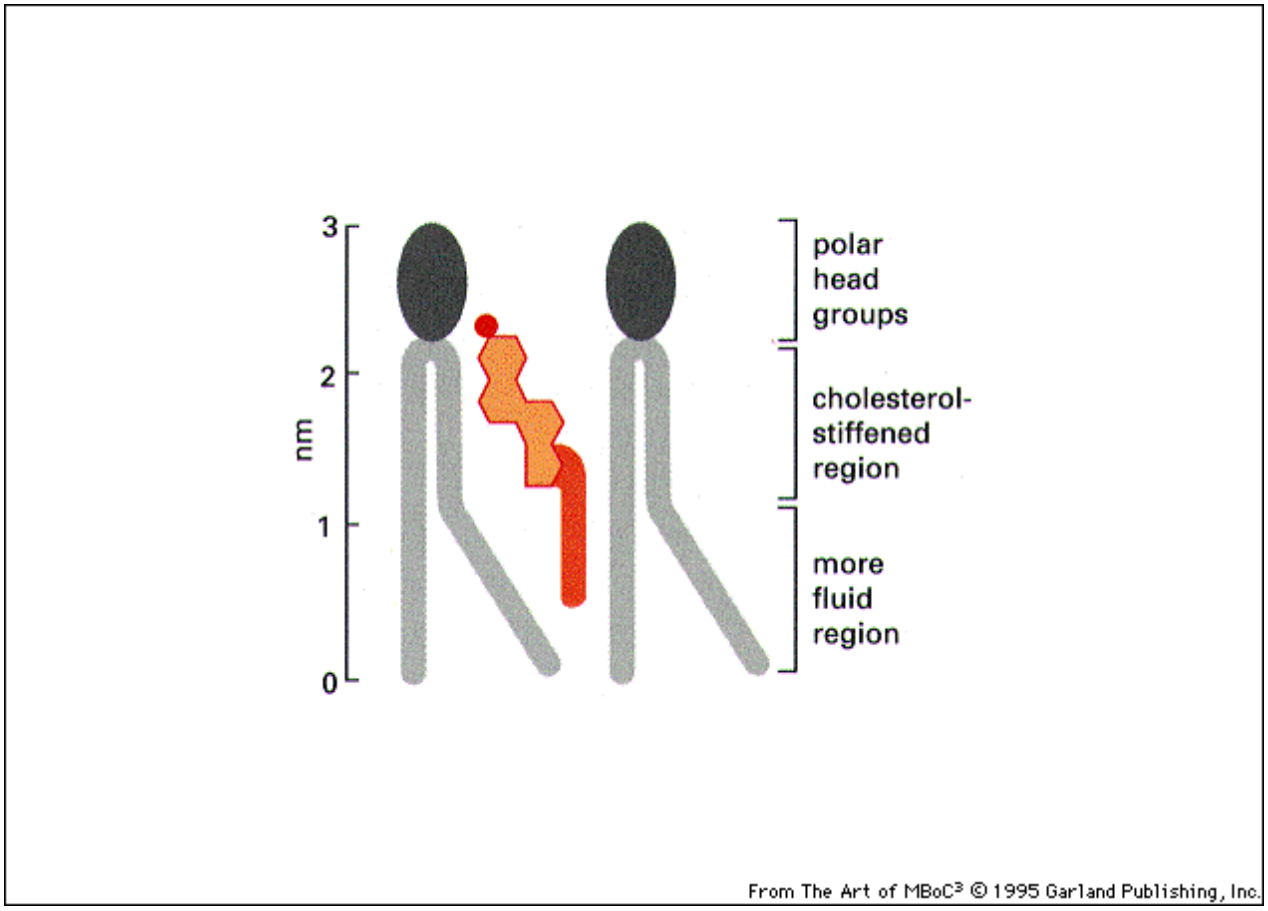


unsaturated hydrocarbon chains with *cis*-double bonds

saturated hydrocarbon chains

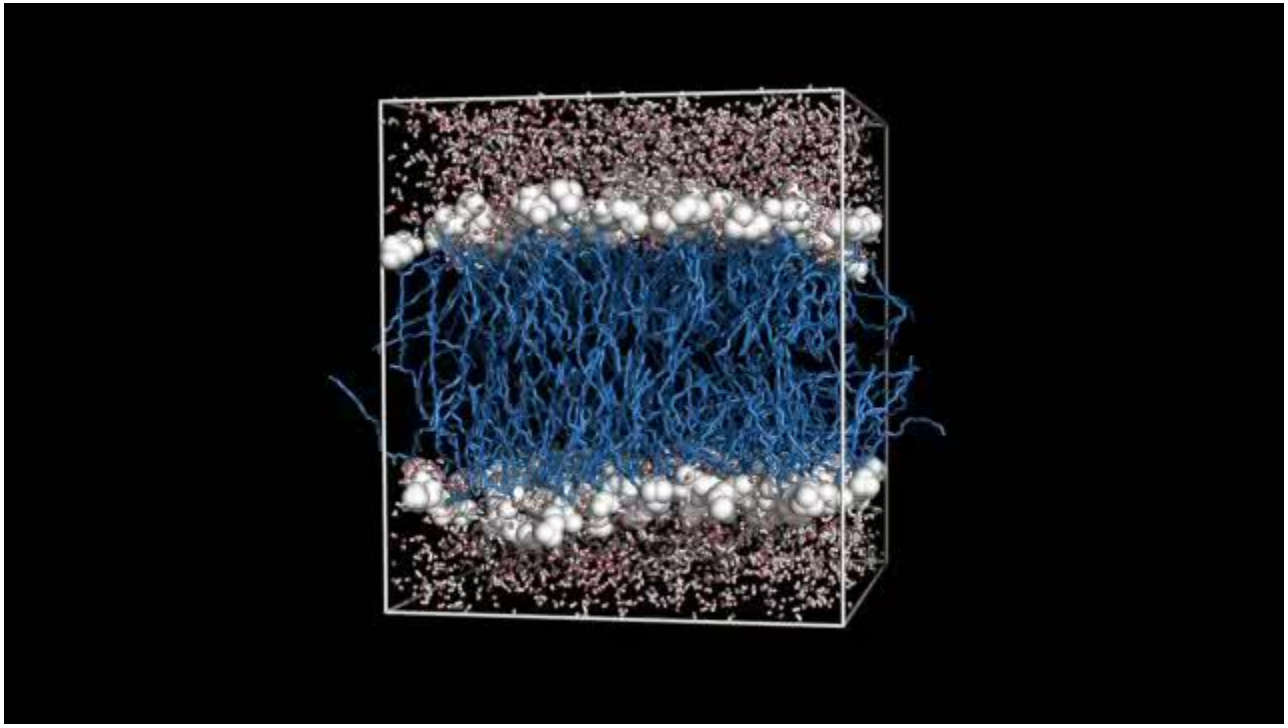
Figure 10–7/10–9 The influence of *cis*-double bonds in hydrocarbon chains. The double bonds make it more difficult to pack the chains together, thereby making the lipid bilayer more difficult to freeze. Because the fatty acid chains of unsaturated lipids are more spread apart, lipid bilayers containing them are thinner than bilayers formed exclusively from saturated lipids.

Cholesterol stiffens the bilayer but keeps it fluid



Movie

Cholesterol stiffens the bilayer but keeps it fluid



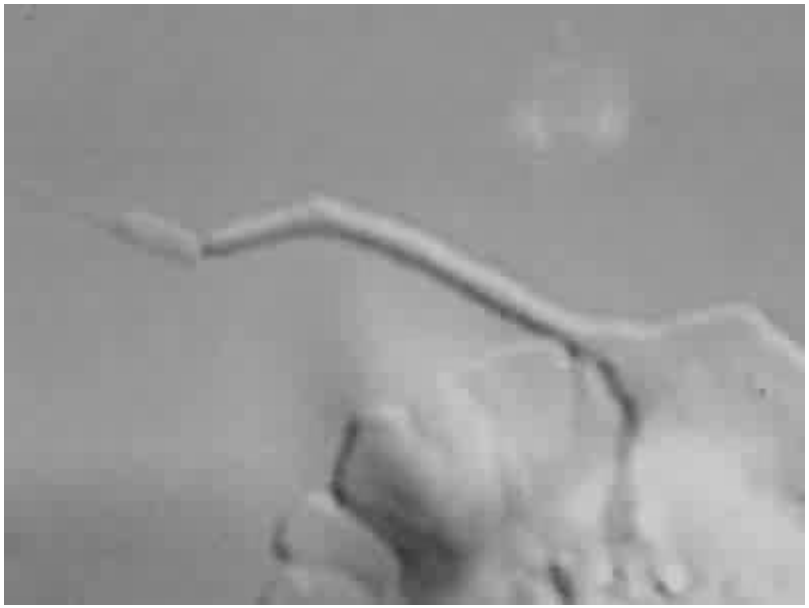
Movie 10.3_Cholesterol

- Biological membranes must be fluid to be functional
- Lipid composition is, in fact, adjusted to fine-tune the degree of fluidity in response for example to temperature changes

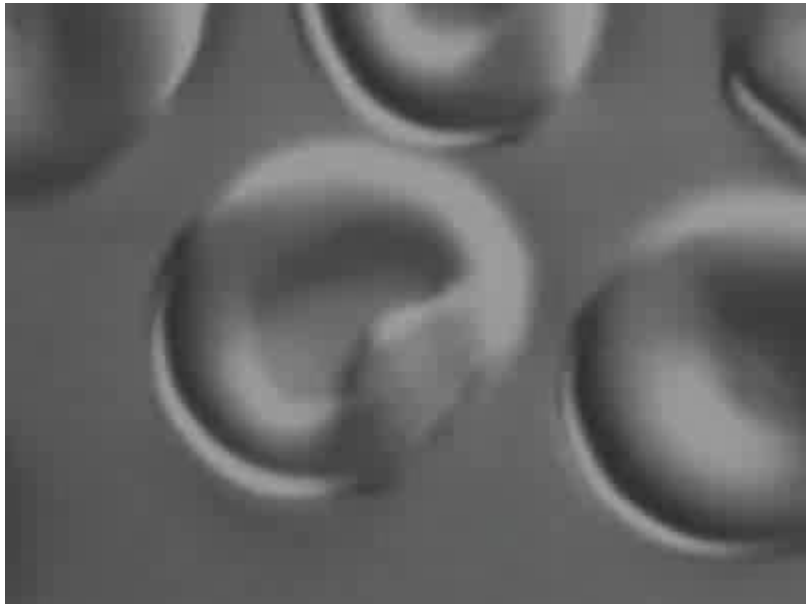
Why is fluidity important?

- Most membrane functions depend on the mobility of membrane components
- Many membrane-bound enzymes only work in fluid environment
- Fusion and fission requires fluidity
- Lateral movement needed for transport, etc.

Movie

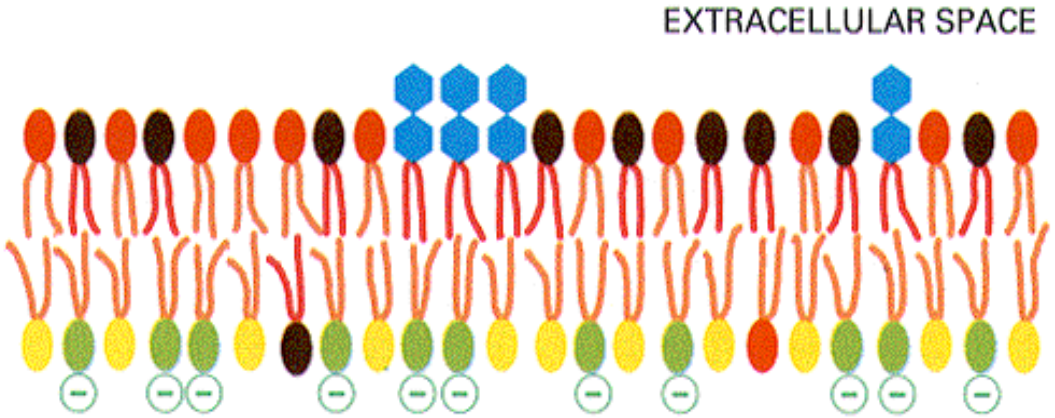


Movie 10.2_Fluidity_of_the_Lipid_Bilayer



10.7_Membrane_Effects_in_a_Red_Blood_Cell

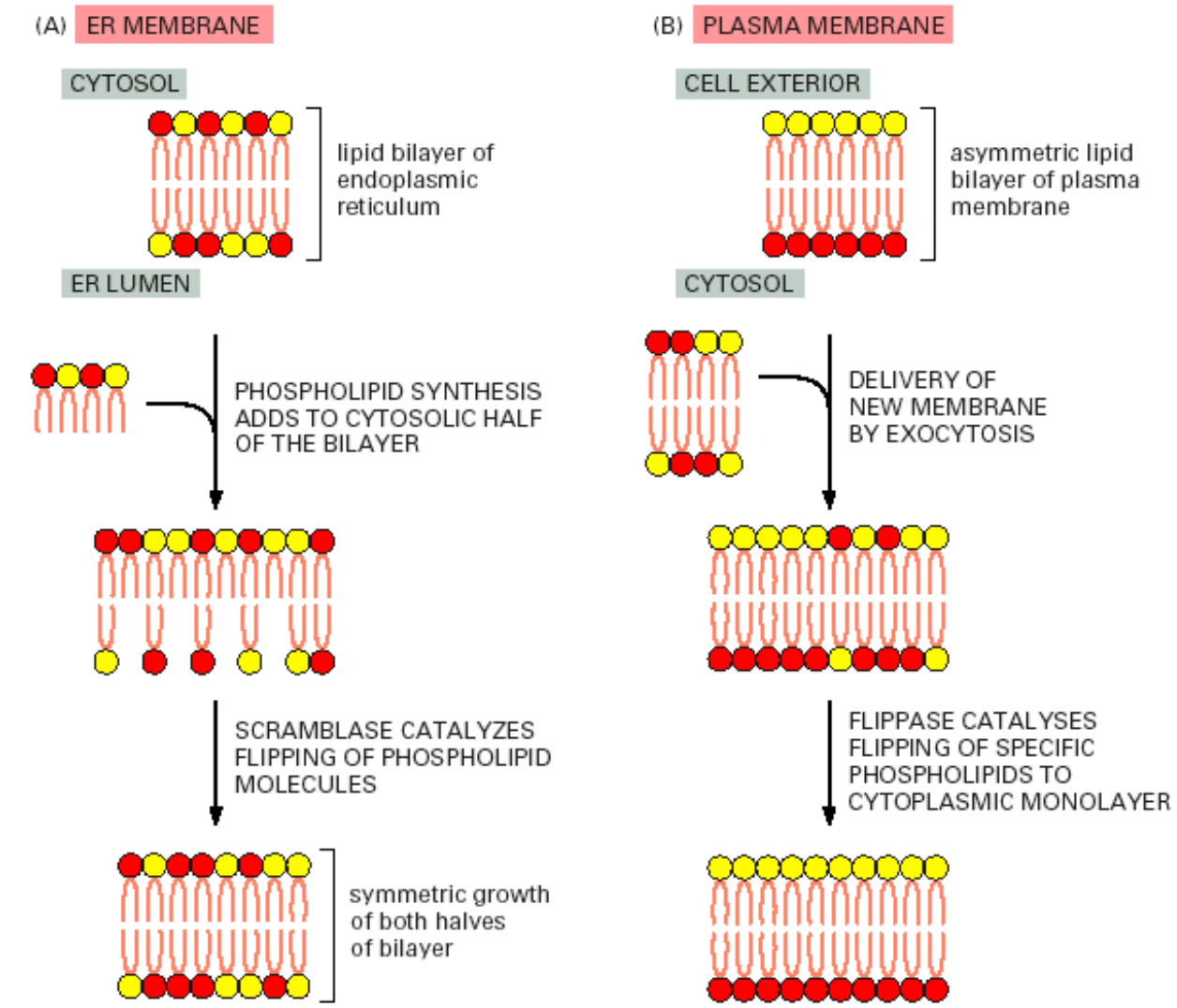
Lipid asymmetry in the plasma membrane



Asymmetry in PM

- Phospholipids with choline head groups are enriched in extra-cytosolic leaflet (PC,SPH)
- Phospholipids with terminal amino group enriched in head group in cytosolic leaflet(PE, PS and also PI)
- Glycolipids in the outer leaflet
- Cholesterol in both
- Charge difference: majority of negative phospholipids (PS, PI) face inside
- Asymmetry is generated by 'flippases' and 'scramblases', -> enzymes in ER and PM that flip specific P-lipids across

Scrambling and flipping



Why are membrane lipids so heterogenous?

- To secure fluidity of membrane at ambient temperature
- To allow formation of lipid micro-domains
- Some lipids bind to specific proteins
- Some serve as second messengers in signal transduction
- To give identity to cells

Liquid ordered and liquid disordered phases can occur together

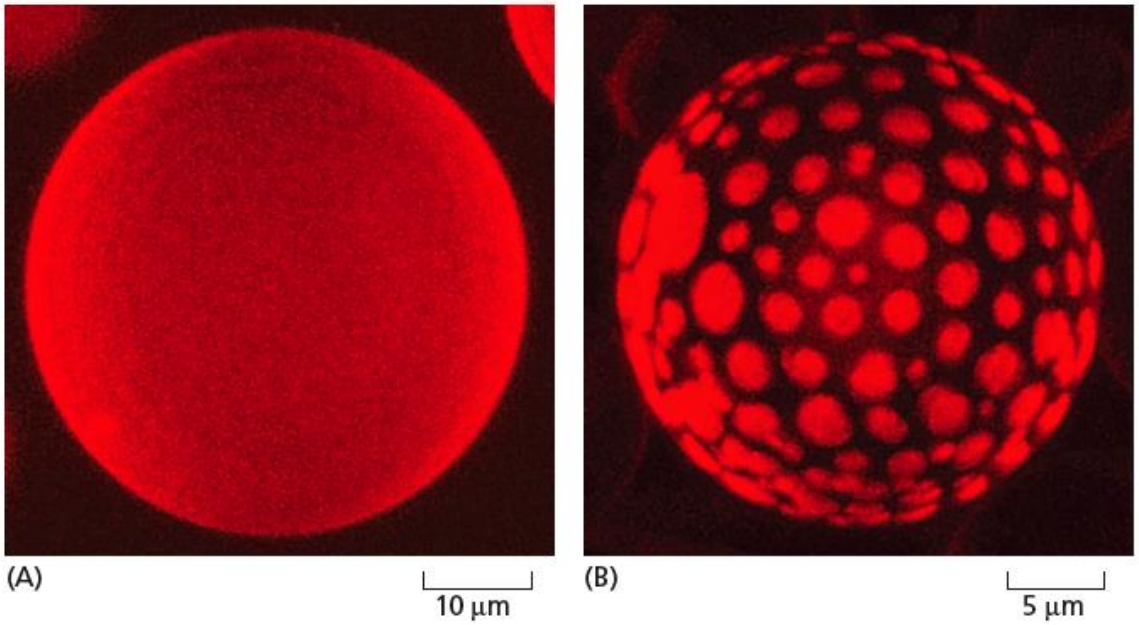
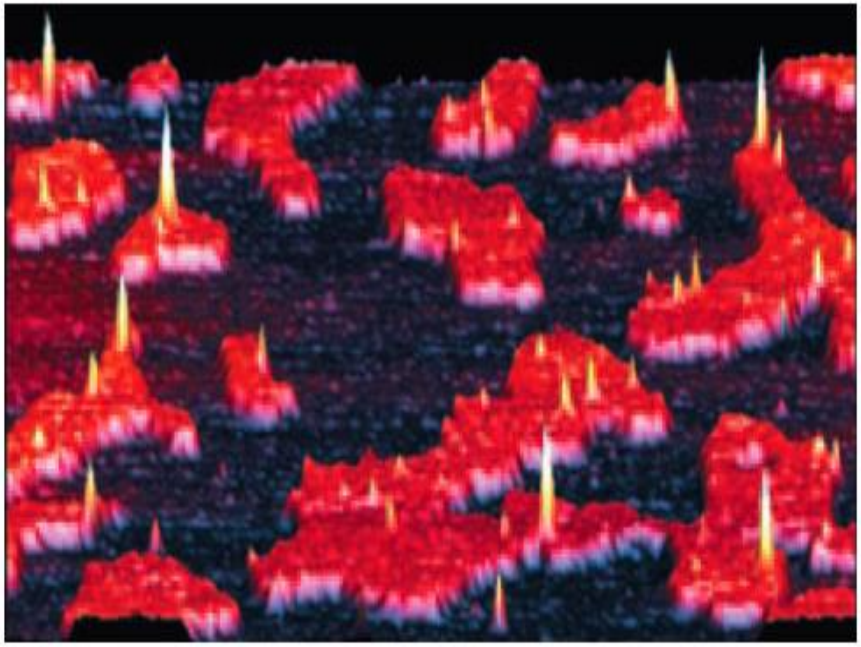


Figure 10–13 Lateral phase separation in artifical lipid bilayers. (A) Giant liposomes produced from a 1:1 mixture of phosphatidylcholine and spingomyelin form uniform bilayers, whereas (B) liposomes produced from a 1:1:1 mixture of phosphatidylcholine, spingomyelin, and cholesterol form bilayers with two immiscible phases. The liposomes are stained with trace concentrations of a fluorescent dye that preferentially partitions into one of the phases. The average size of the domains formed in these giant artificial liposomes is much larger than that expected in biological membranes, where rafts may be as small as a few nanometers in diameter. (A, from



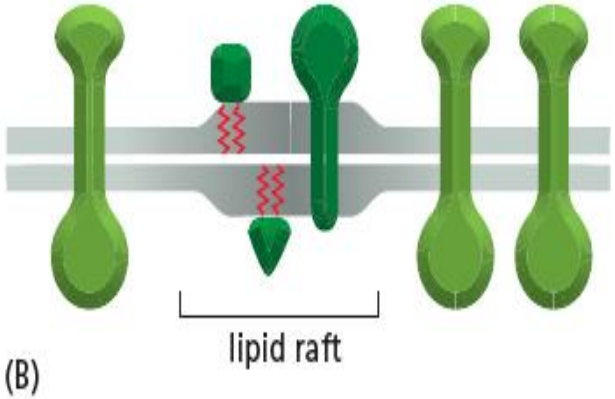
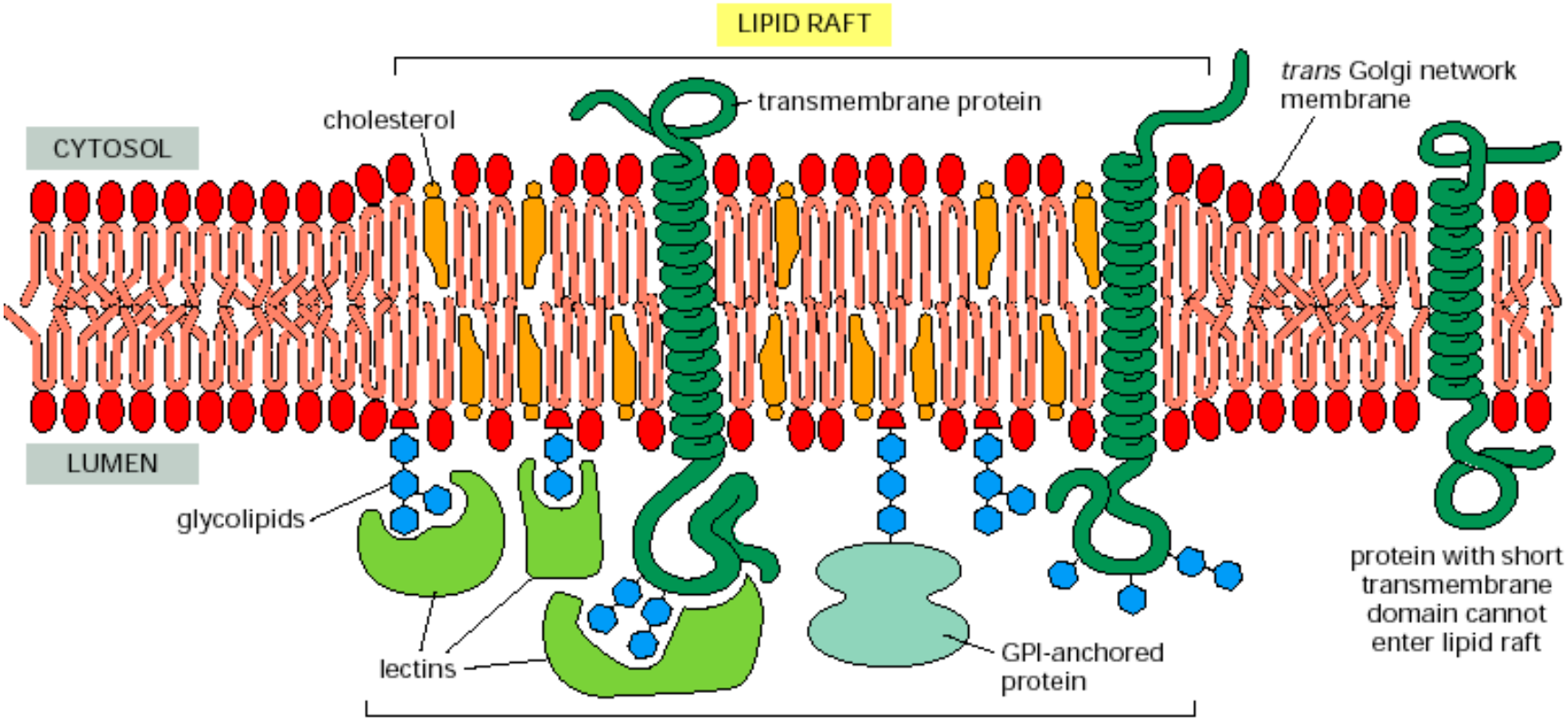


Figure 10–14 The effects of lipid rafts in artificial lipid bilayers. (A) The surface contours of a synthetic bilayer containing lipid rafts, analyzed by atomic force microscopy. Note that the raft areas, shown in *orange*, are thicker than the rest of the bilayer; as in Figure 10–13, the rafts primarily contain sphingomyelin and cholesterol. The sharp, yellow spikes are incorporated protein molecules, which are attached to the bilayer by a glycosylphosphatidyl-inositol (GPI) anchor (illustrated in Figure 10–19, example 6), and preferentially partition into the raft domains. (B) Because of both their increased thickness and lipid composition, rafts are thought to concentrate specific membrane proteins *(dark green)*. (A, from D.E. Saslowsky et al., *J. Biol.*

Microdomains:Lipid rafts



About lipid rafts

- Present in PM of eukariotic cells, often induced by clustering of membrane components
- Also in the Golgi and endosomes
- Enriched in cholesterol and sphingolipids
- Sphingolipids have alkyl chains that are more saturated and longer: raft domains are therefore less fluid and thicker
- Rafts form a separate phase in membrane
- The are enriched in GPI-anchored proteins, signaling proteins, and proteins with double FA-acyl groups

More about lipid rafts

- Insoluble in certain detergents (Triton X100)
- Present in PM specializations called caveolae (small flask shaped indentations)
- Serve as 'platforms' for special functions in the membrane

- Formation of rafts may be why we need cholesterol!

Phosphoinositides as Signaling Molecules

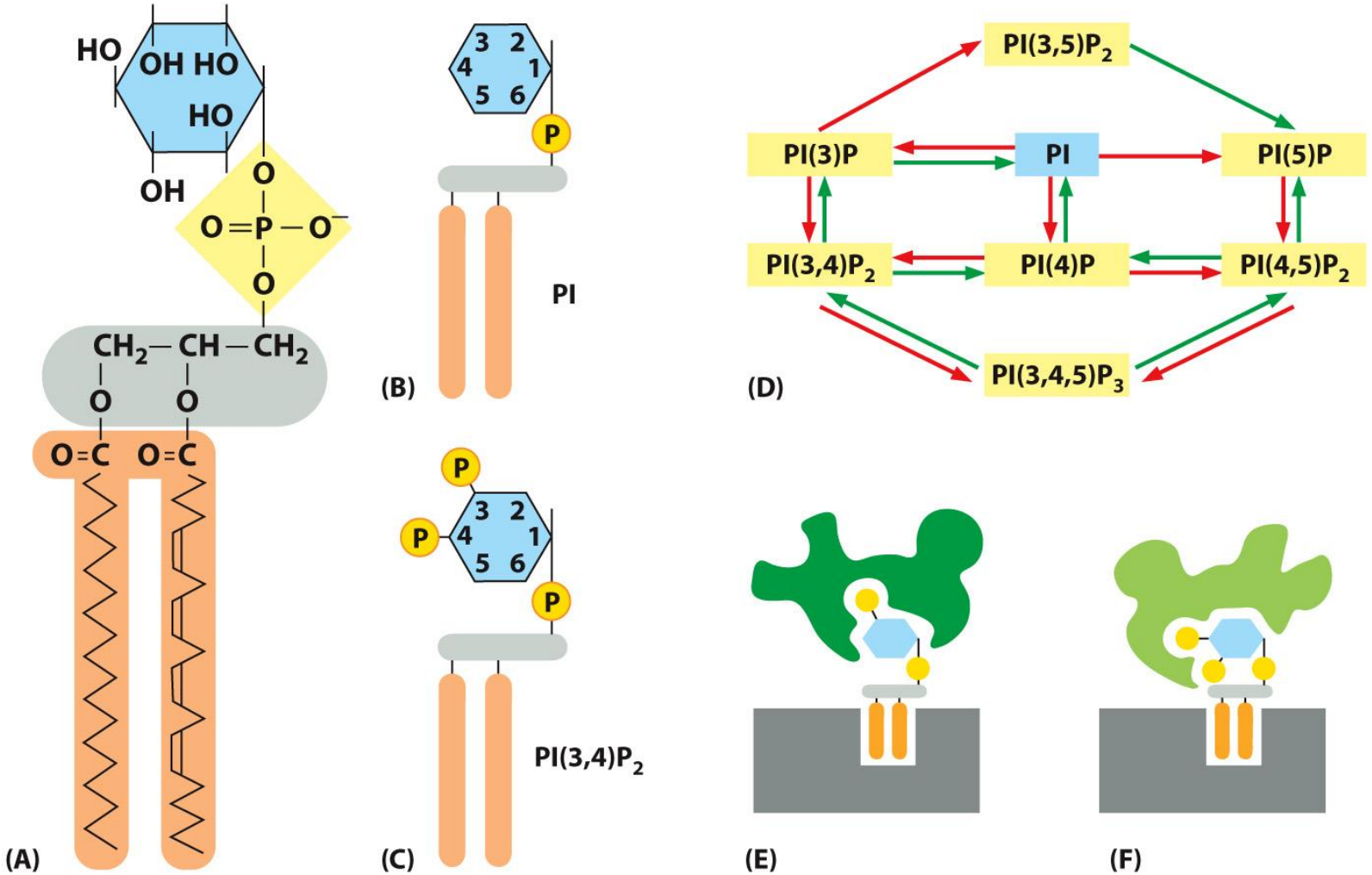


Figure 13-10 Molecular Biology of the Cell 6e (© Garland Science 2015)

How PI and phosphoinositides serve as second messengers during signal transduction

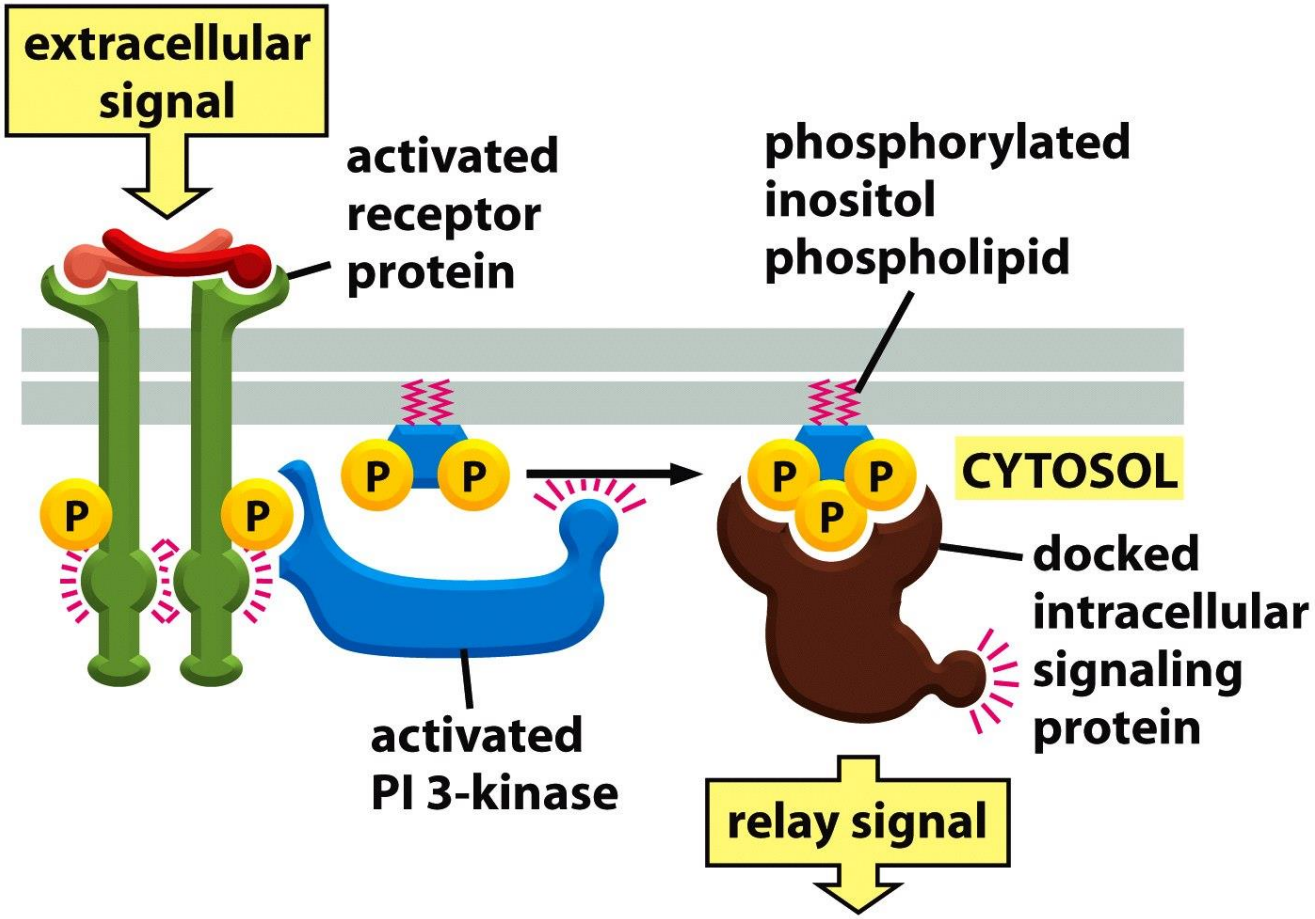


Figure 10-17a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Signal activated phospholipases generate messengers

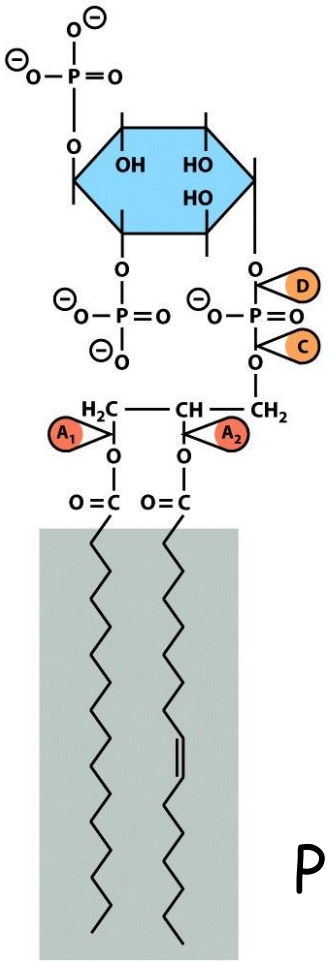


Figure 10-17c Molecular Biology of the Cell 5/e (© Garland Science 2008)

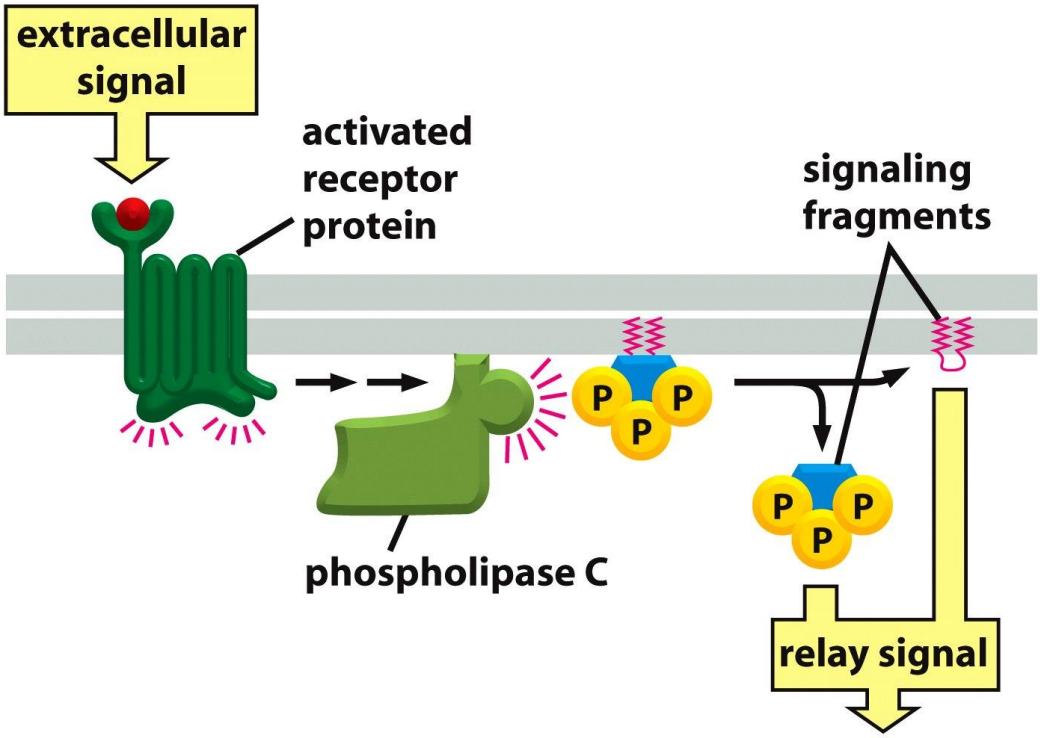


Figure 10-17b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Phospholipases can hydrolyse different bonds

Different strategies to anchor proteins in a membrane

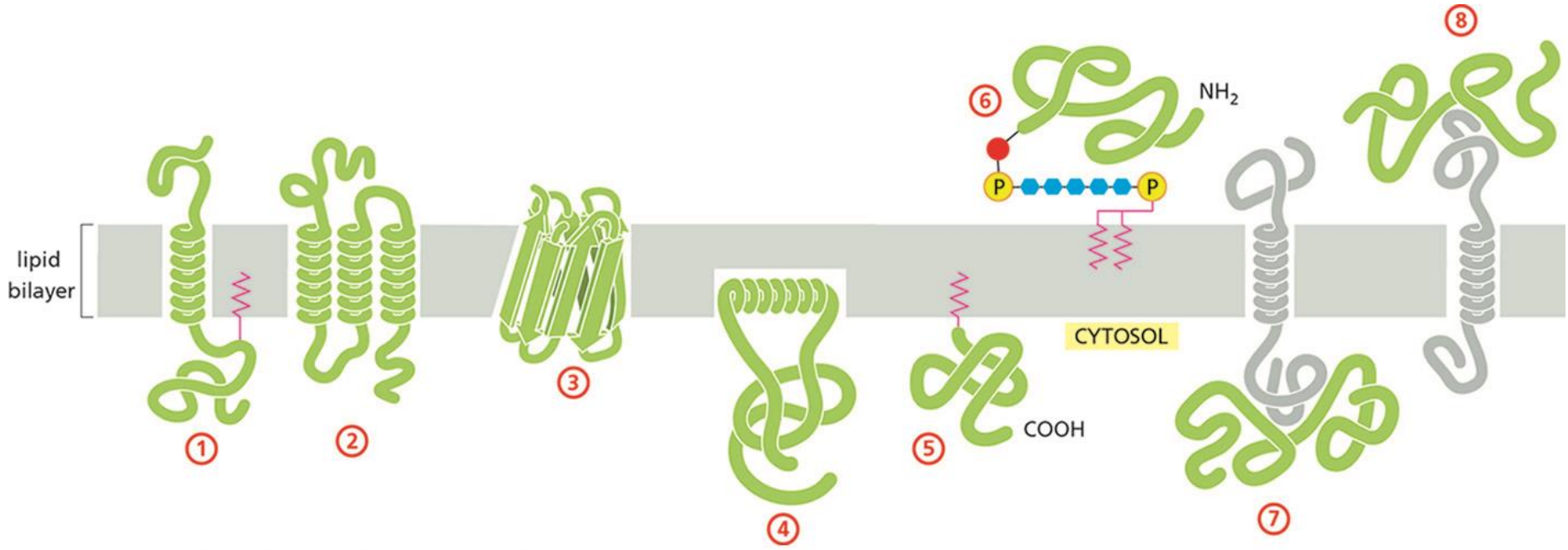


Figure 10-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

Lipid anchors

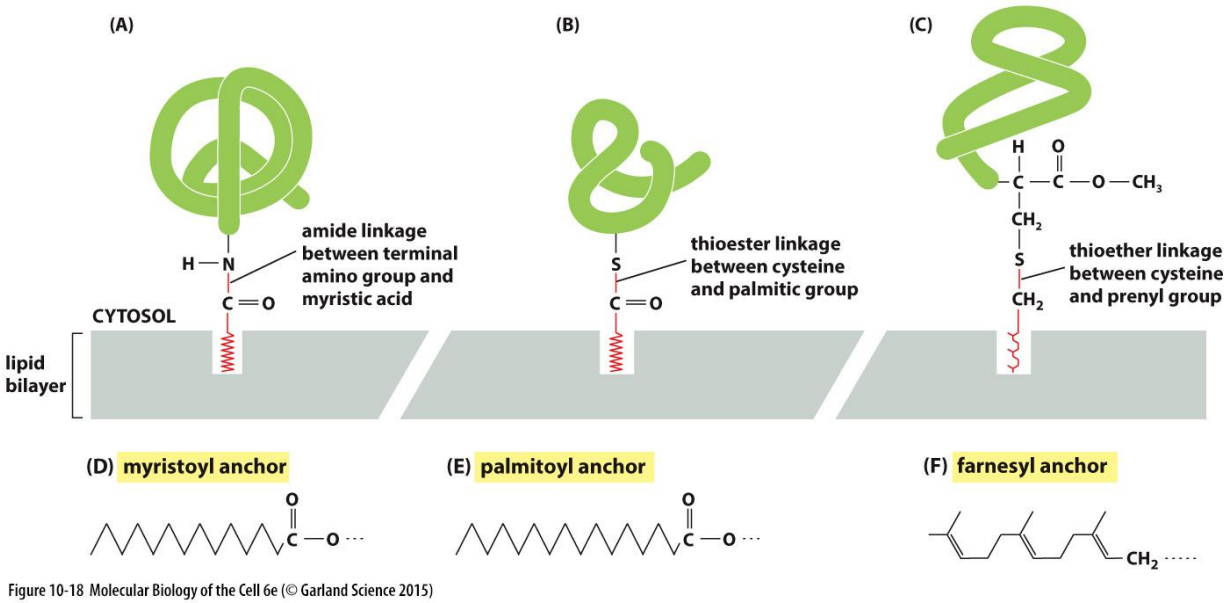
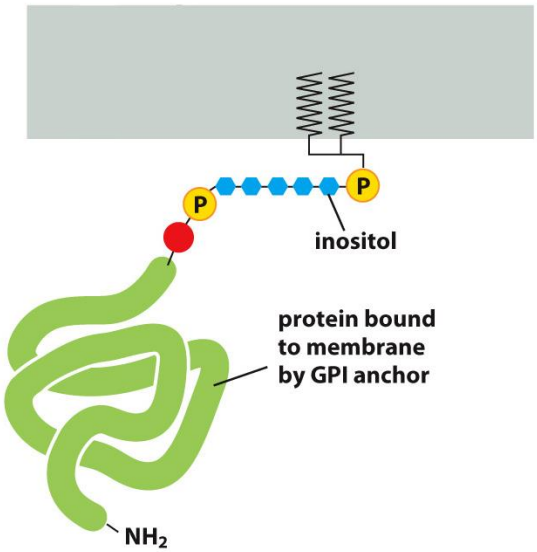


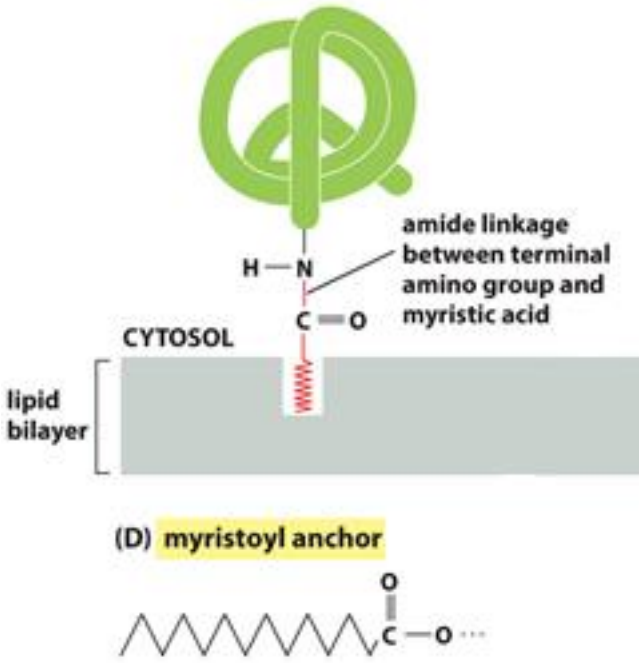
Figure 10-18 Molecular Biology of the Cell 6e (© Garland Science 2015)



- Lipid anchor can be used to link proteins to membranes
- Not very strong binding
- often used to shuttle proteins between cytoplasm and membran

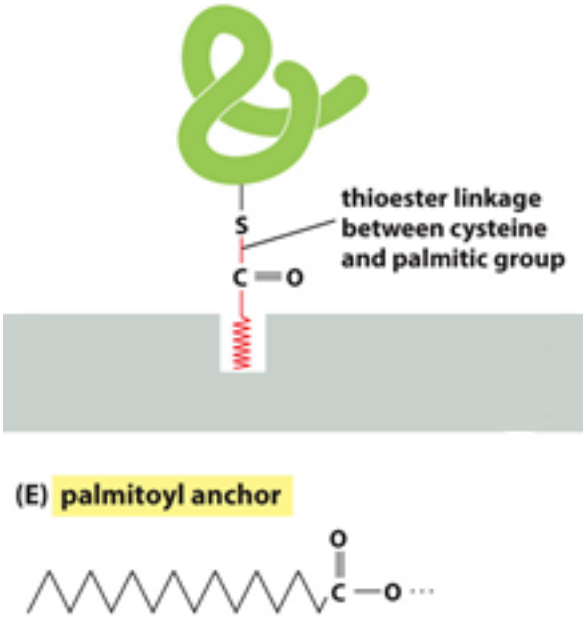
Myristoylation

- Cytoplasmic side
- C14 fatty acid, amide linkage
- Linked to N-terminal glycine
- Protease cleavage necessary
  - e.g. caspase 3 => apoptosis
- other targets:
  - G-proteins, c-src
- often in combination with other lipids
- Shuttle between cytoplasm and plasma membrane



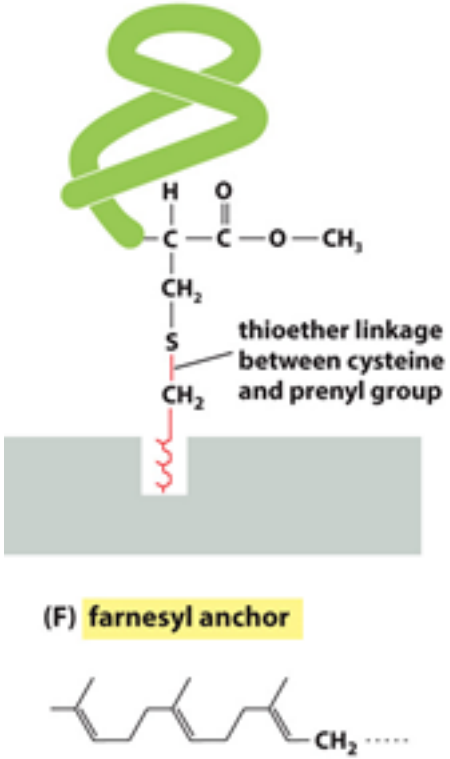
Palmitoylation

- cytoplasmic side
- C16 fatty acid, thioester linkage
- linked to internal cysteine (Ser, Thr)
- targets
  - β2 adrenergic receptor, Galpha
- reversible
- often in combination with other lipids
- shuttle between cytoplasm and plasma membrane



Prenylation

- Prenylation = Farnesylation (C15) + Geranylgeranylation (C20)
- thioether linkage
- cytoplasmic side
- linked to cysteine near C-terminus
- often two moieties
- tagged proteins are no longer soluble => binding proteins
- CAAX recognition motif (exceptions possible)
- targets
  - Rab GTPases, RAS



GPI anchor

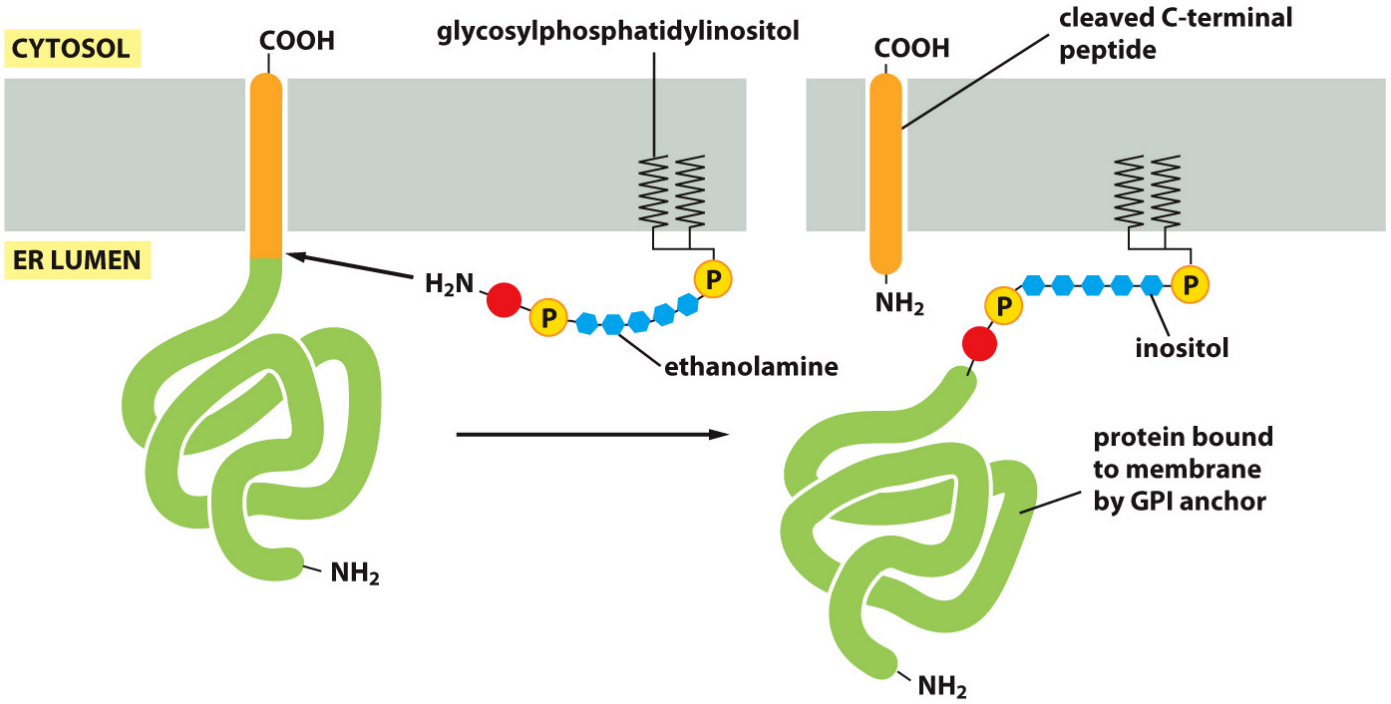
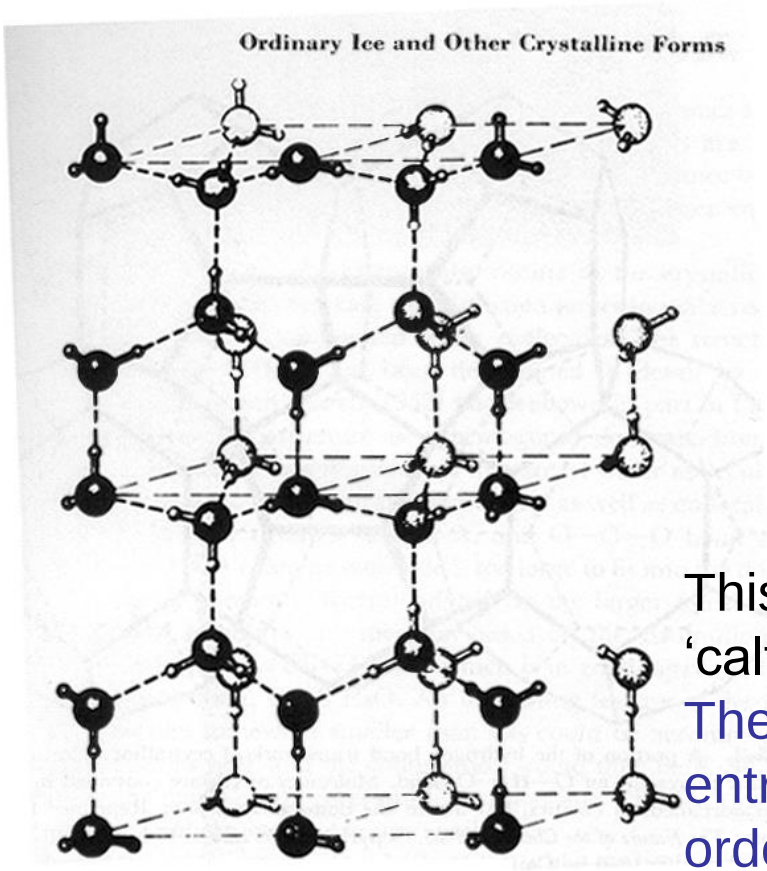
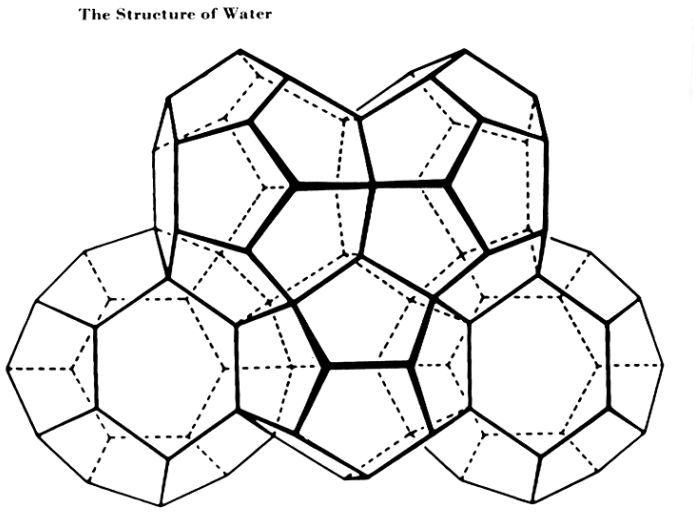


Figure 12-52 Molecular Biology of the Cell 6e (© Garland Science 2015)

- extracellular side
- guides proteins to lipid raft
- active release from plasma membrane is possible (PLC)
- examples
  - Acetylcholine esterase, membrane proteins of Trypanosoma

The strength of the hydrophobic effect depends on the surface area of the cavity needed to fit the apolar moiety.





This structure is called ‘calthrate’.

The hydrophobic effect is primarily due to the entropic effect due to its ice-like, highly ordered structure