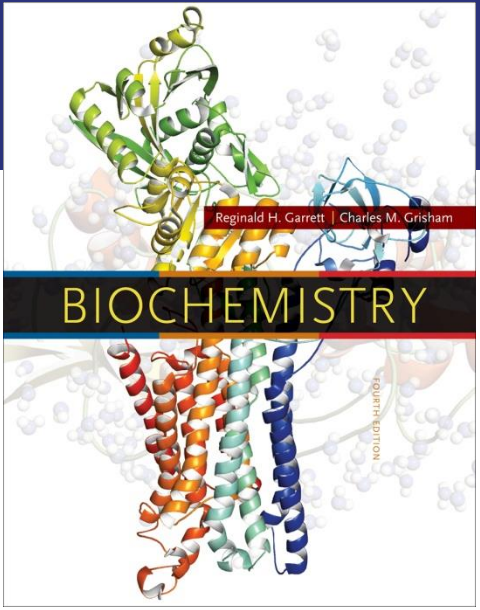


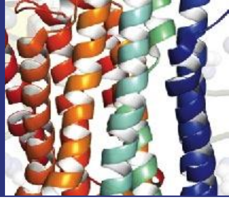
BROOKS/COLE
CENGAGE Learning

Reginald H. Garrett
Charles M. Grisham

Chapter 9
Membranes and Membrane Transport

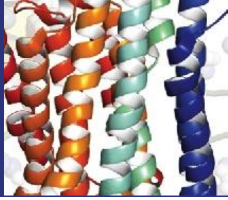
What are the properties and characteristics of biological membranes that account for their broad influence on cellular processes and transport?

Outline



- What are the chemical and physical properties of membranes?
- What are the structure and chemistry of membrane proteins?
- How are biological membranes organized?
- What are the dynamic processes that modulate membrane function?
- How does transport occur across biological membranes?
- What is passive diffusion?
- How does facilitated diffusion occur?
- How does energy input drive active transport processes?
- How are certain transport processes driven by light energy?
- How is secondary active transport driven by ion gradients?

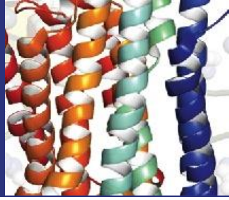
Membranes are Key Structural and Functional Elements of Cells



Some of the many functions of membranes:

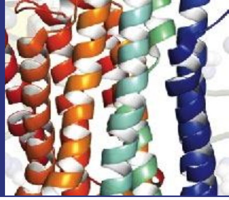
- Barrier to toxic molecules
- Transport and accumulation of nutrients
- Energy transduction
- Facilitation of cell motion
- Reproduction
- Signal transduction
- Cell-cell interactions

9.1 What Are the Chemical and Physical Properties of Membranes?



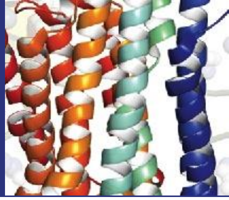
- Lipids self-associate to form membranes because:
  - Water prefers polar interactions and prefers to self-associate with H bonds
  - The hydrophobic effect promotes self-association of lipids in water to maximize entropy
- These forces drive amphiphilic lipids to form membranes
- These forces also facilitate the association of proteins with membranes

The Composition of Membranes Suits Their Function



- Biological membranes may contain as much as 75% to 80% protein or as little as 15-20% protein
- Membranes that carry out many enzyme-catalyzed reactions and transport activities are richer in protein
- Membranes that carry out fewer such functions (such as myelin sheaths) are richer in lipid
- Cells adjust the lipid composition of membranes to suit functional needs

The Composition of Membranes Suits Their Function



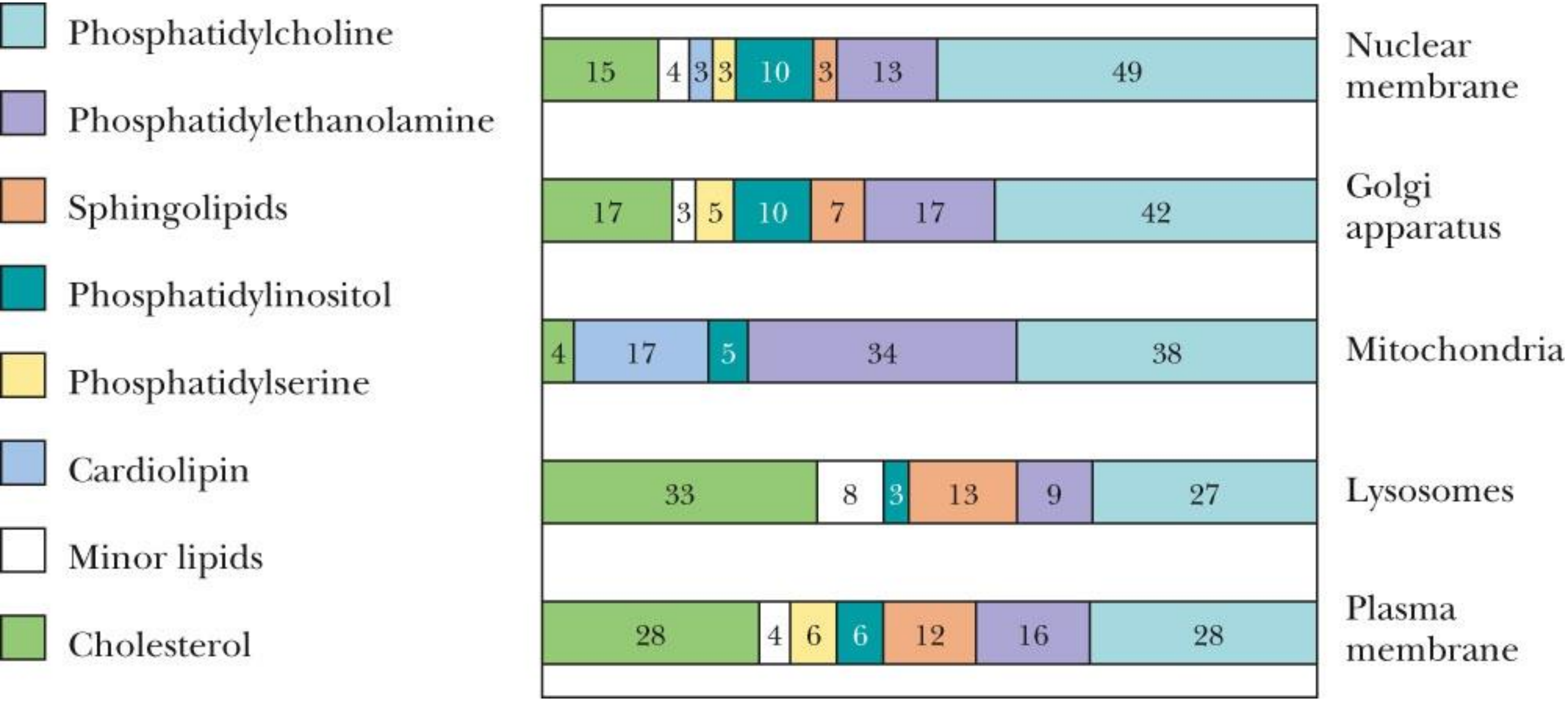
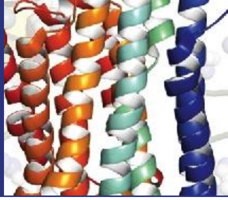


Figure 9.2 The lipid composition of rat liver cell membranes, in weight percent.

9.1 What Are the Chemical and Physical Properties of Membranes?



- Lipids form ordered structures spontaneously in water
- Very few lipids exists as monomers
- Monolayers on the surface of water arrange lipid tails in the air
- Micelles bury the nonpolar tails in the center of a spherical structure
- Micelles reverse in nonpolar solvents
- The amphipathic molecules that form micelles are each characterized by a **critical micelle concentration (CMC)**

9.1 What Are the Chemical and Physical Properties of Membranes?

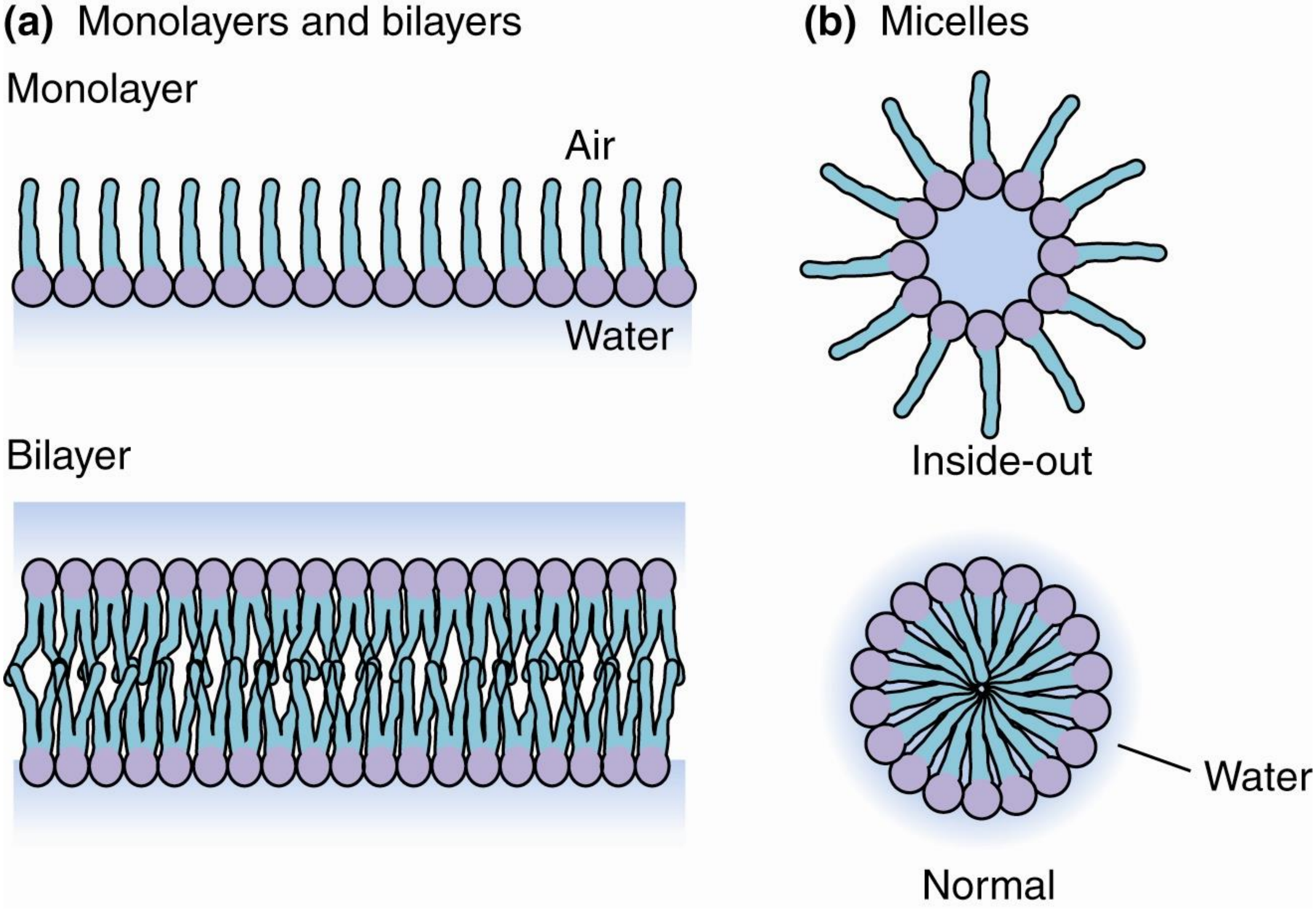


Figure 9.3 Spontaneously formed lipid structures.

9.1 What Are the Chemical and Physical Properties of Membranes?

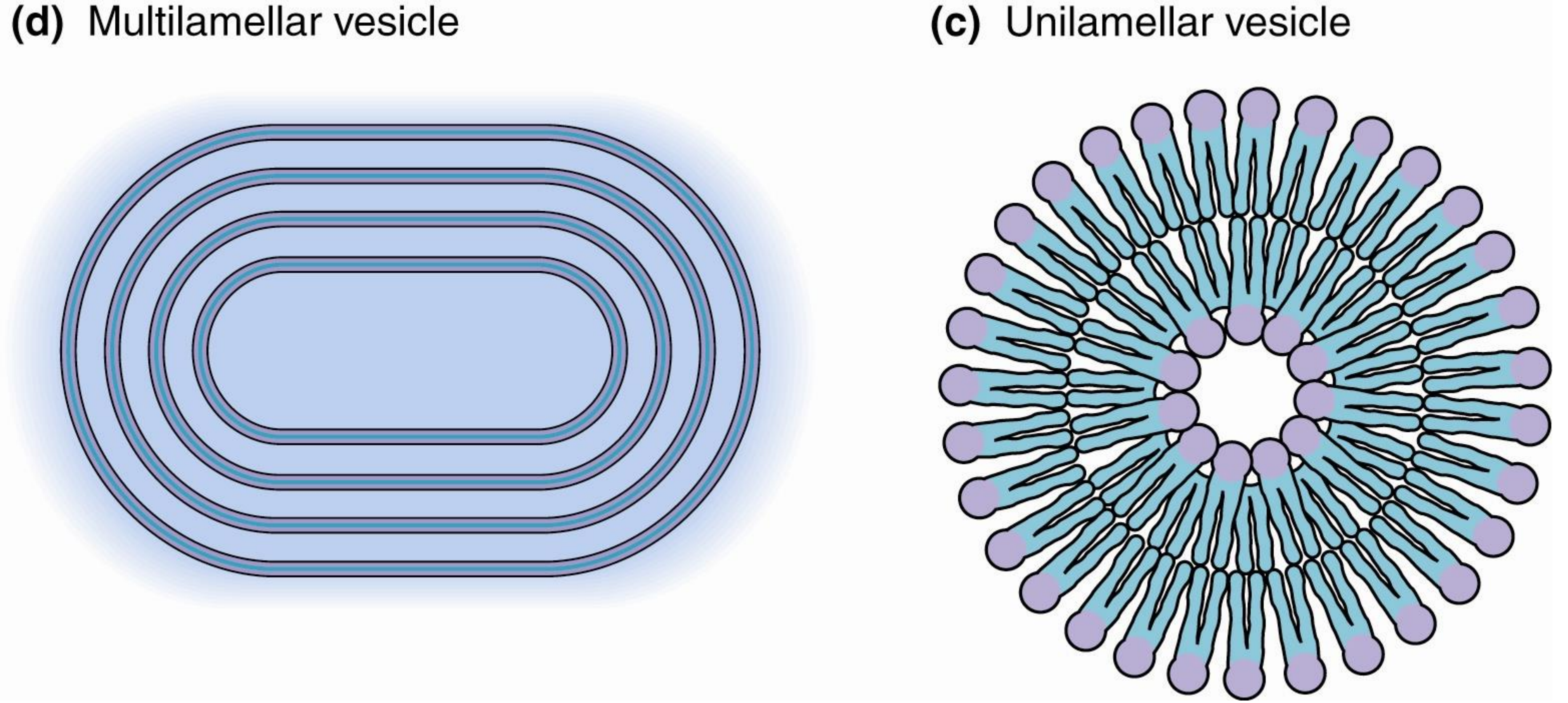
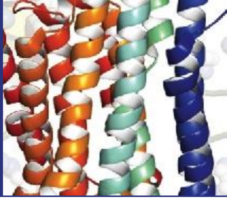


Figure 9.3 Spontaneously formed lipid structures. Unilamellar vesicles (aka liposomes) are highly stable structures.

The Critical Micelle Concentrations of Detergents Vary Widely



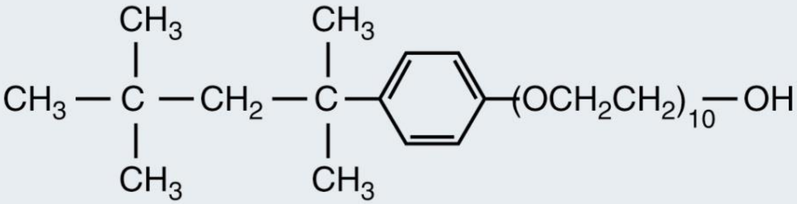
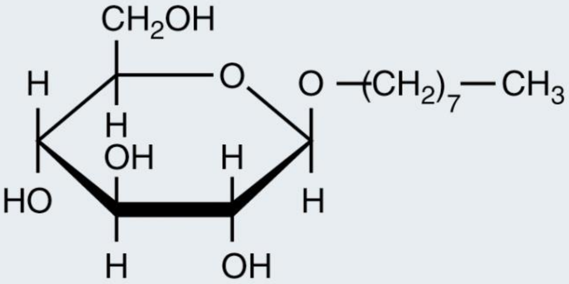
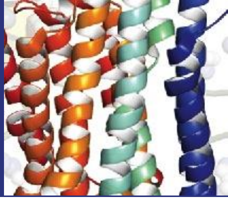
Structure	M_r	CMC	Micelle M_r
Triton X-100 	625	0.24 m*M*	90–95,000
Octyl glucoside 	292	25 m*M*	
$C_{12}E_8$ (Dodecyl octaoxyethylene ether) $C_{12}H_{25}-(OCH_2CH_2)_8-OH$	538	0.071 m*M*	

Figure 9.4 The structures of some common detergents and their physical properties.

The Fluid Mosaic Model Describes Membrane Dynamics

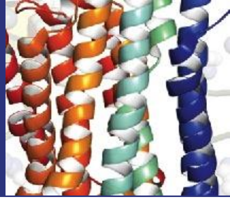


S. J. Singer and G. L. Nicolson

1972

- The phospholipid bilayer is a *fluid matrix*
- The bilayer is a two-dimensional solvent
- Lipids and proteins can undergo rotational and lateral movement
- Two classes of proteins:
  - peripheral proteins (extrinsic proteins)
  - integral proteins (intrinsic proteins)

The Fluid Mosaic Model Describes Membrane Dynamics



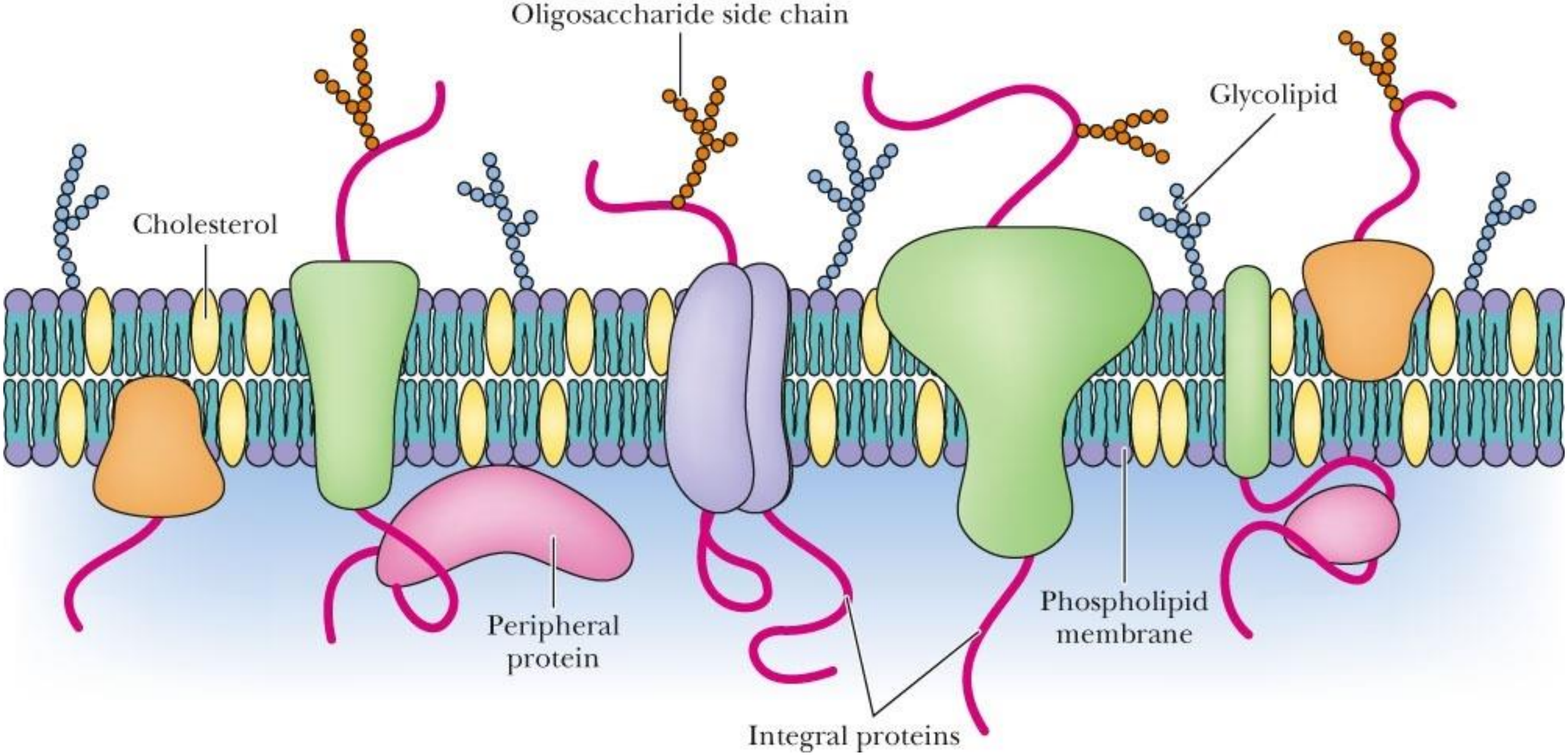
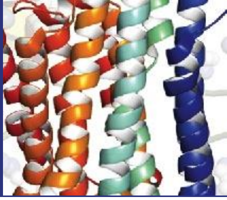


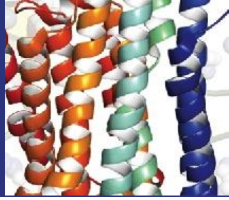
Figure 9.5 The fluid mosaic model of membrane structure proposed by S.J. Singer and G.L. Nicolson. The lipids and proteins are mobile; they can diffuse laterally in the membrane plane. Transverse motion is much slower.

There is Motion in the Bilayer



- Lipid chains can bend, tilt, rotate
- The portions of the lipid chain near the membrane surface lie most nearly perpendicular to the membrane plane
- Lipid chain ordering decreases (and motion increases) toward the end of the chain (toward the middle of the bilayer)
- Lipids and proteins can migrate ("diffuse") in the bilayer (more about this in Section 9.4)

Peripheral Membrane Proteins Associate Loosely with the Membrane



- Peripheral proteins are not strongly bound to the membrane
  - They may form ionic interactions and H bonds with polar lipid headgroups or with other proteins
  - Or they may interact with the nonpolar membrane core by inserting a hydrophobic loop or an amphiphilic α-helix
- They can be dissociated with mild detergent treatment or with high salt concentrations

Peripheral Membrane Proteins Associate Loosely with the Membrane

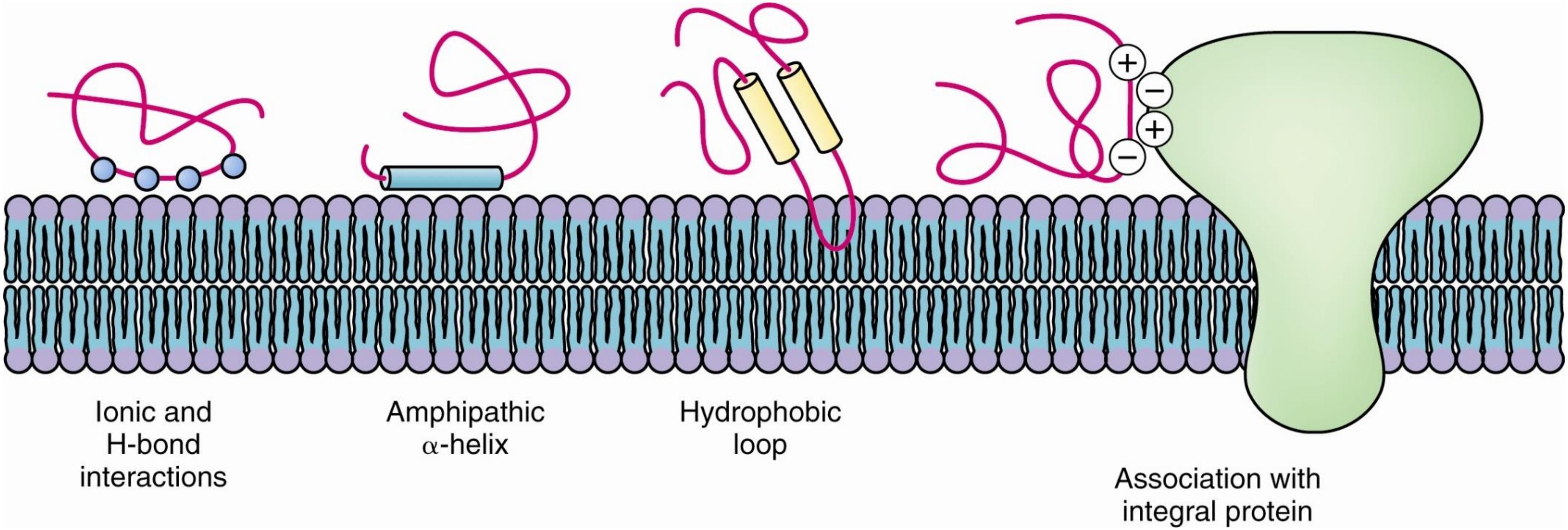
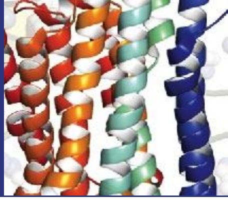


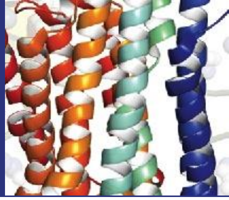
Figure 9.8 Four possible modes for the binding of peripheral membrane proteins.

Glyophorin is an Integral Protein With a Single Transmembrane Segment



- The transmembrane segment of glycophorin has globular domains on either end
- Transmembrane segment is α-helical and consists of 19 hydrophobic amino acids
- Extracellular portion contains oligosaccharides and these constitute the ABO and MN blood group determinants
- Mark Bretscher showed that glycoprotein was a transmembrane protein

Glyophorin is an Integral Protein With a Single Transmembrane Segment



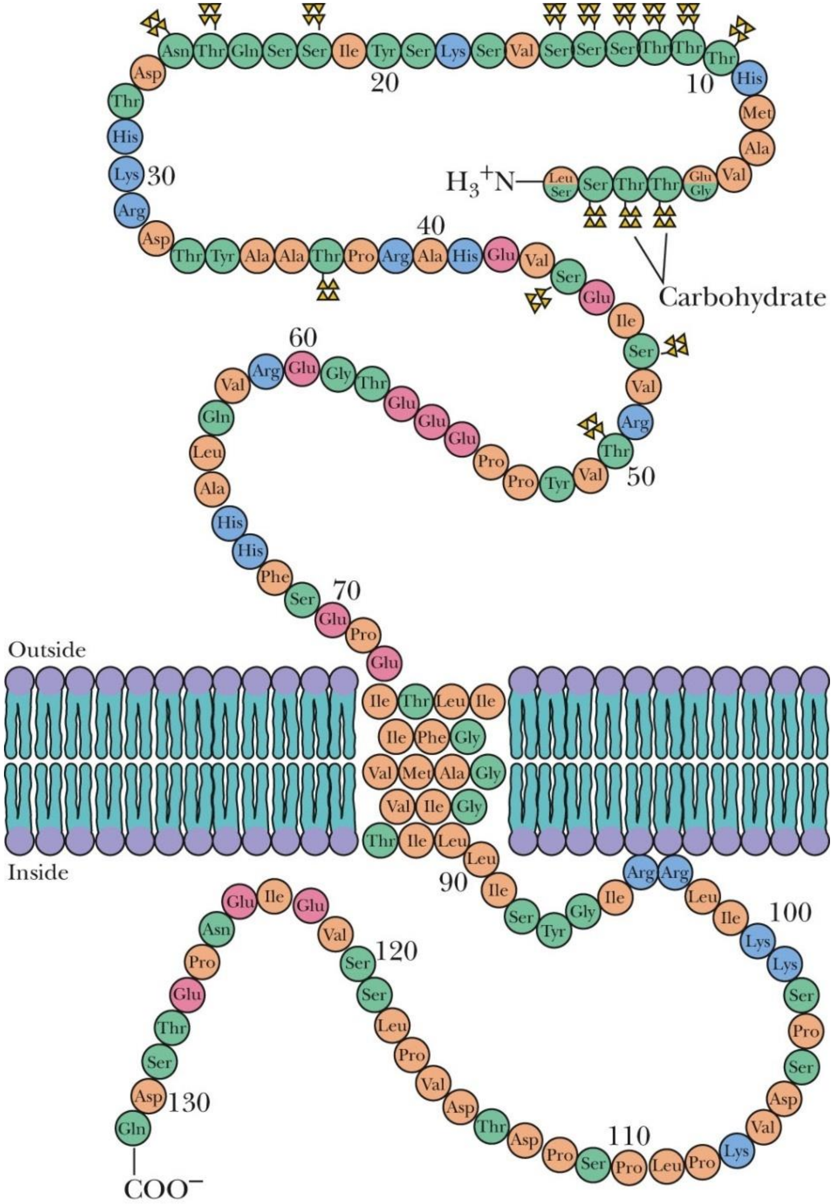
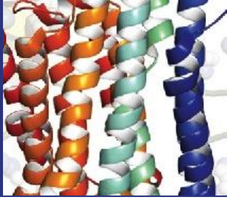


Figure 9.10 Glycophorin A spans the membrane of the human erythrocyte via a single –helical transmembrane segment. The C-terminus of the peptide faces the cytosol of the erythrocyte; the N-terminus is extracellular. Points of attachment of carbohydrate groups are indicated by triangles.

Two More Integral Membrane Proteins With a Single Transmembrane Segment



Major histocompatibility anigen HLA-A2

Monoamine oxidase

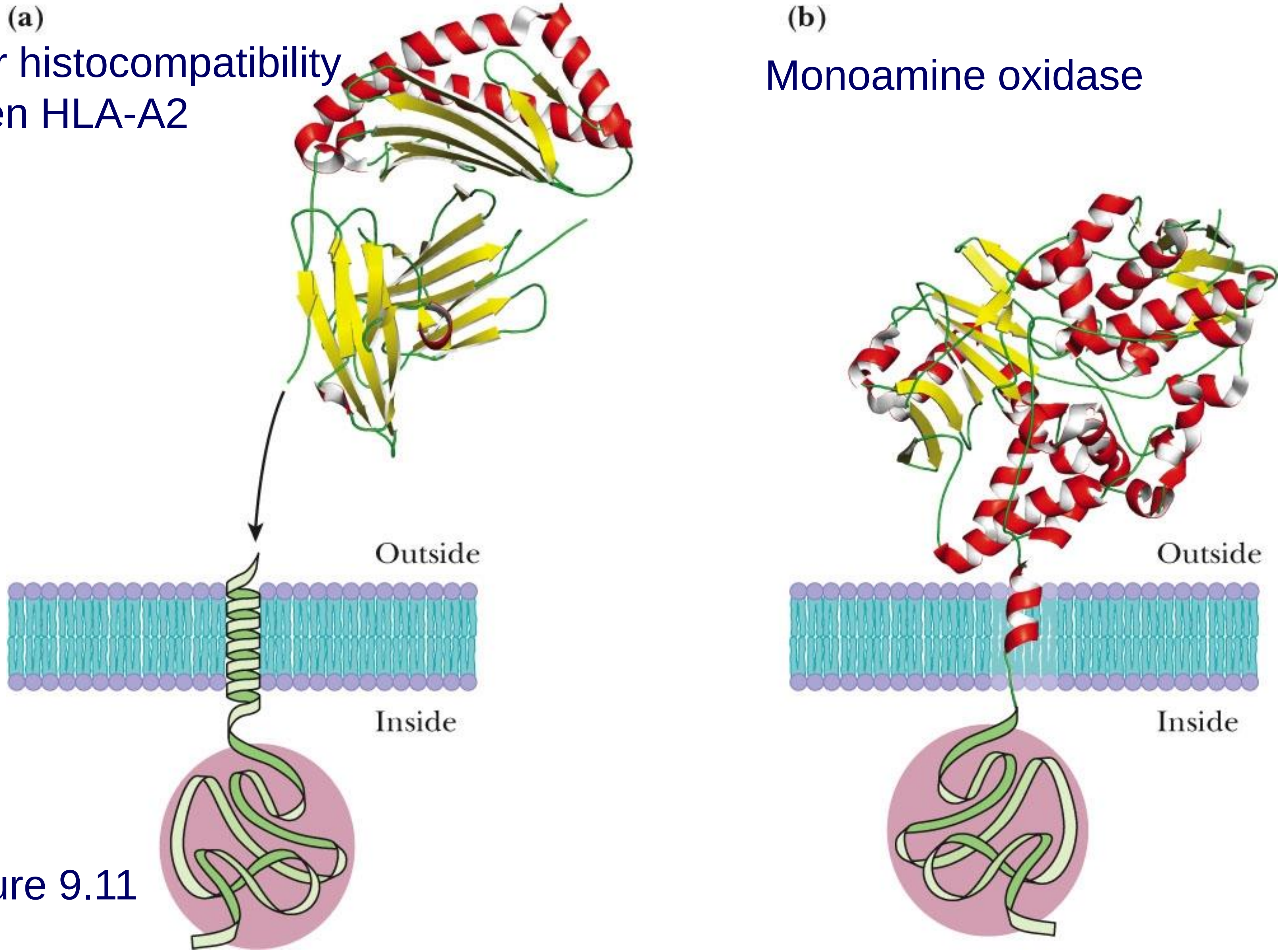
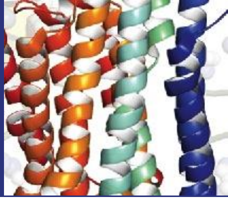


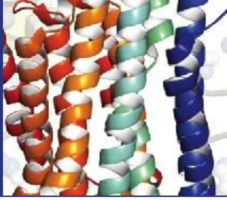
Figure 9.11

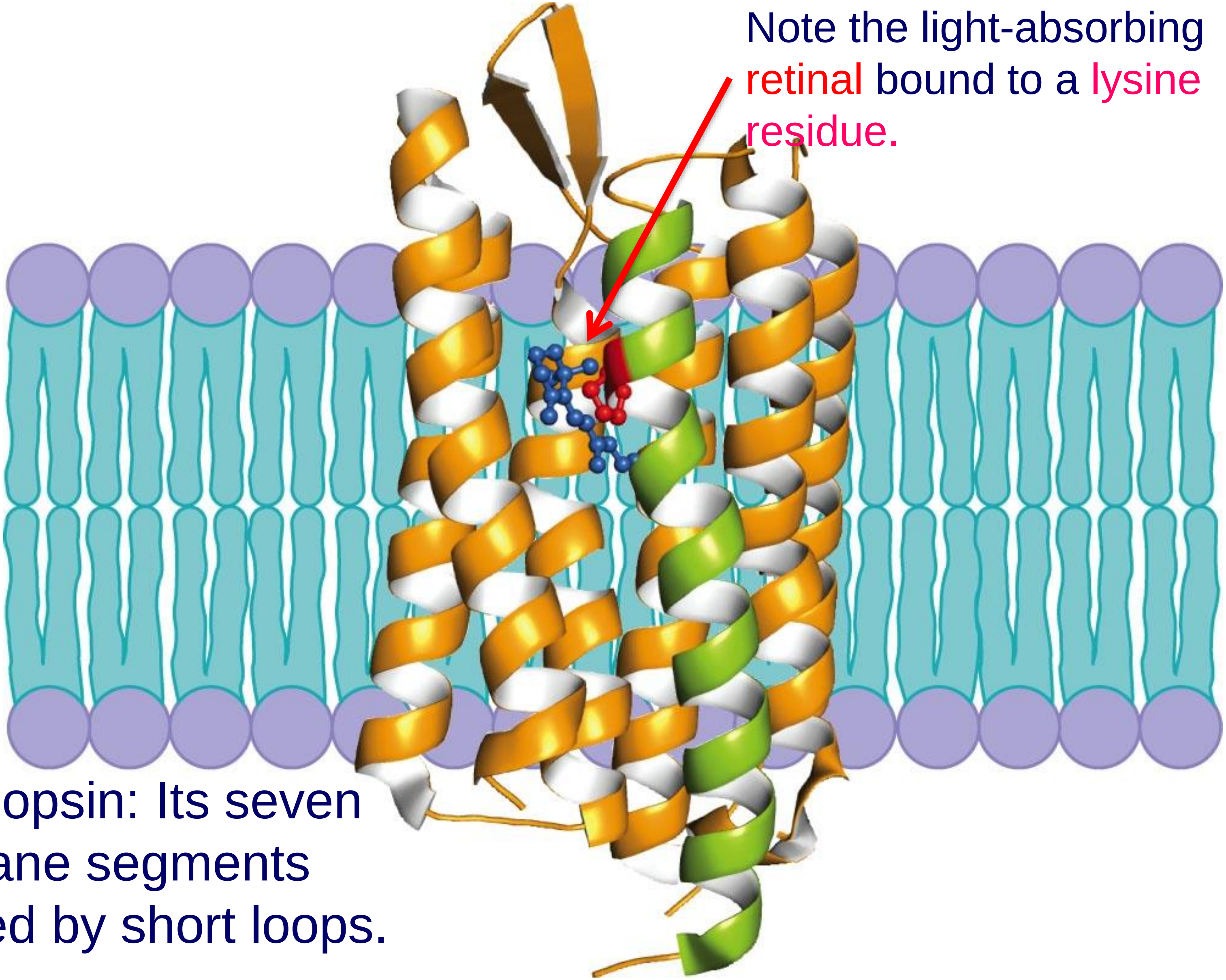
Bacteriorhodopsin is a Paradigm for Membrane Proteins with 7 Helical Segments



- Found in purple patches of *Halobacterium halobium*
- Consists of 7 transmembrane helical segments with short loops that interconnect the helices
- Note the symmetry of packing in bacteriorhodopsin (bR) (see Figure 9.13)
- bR is a light-driven proton pump!

Bacteriorhodopsin is a Paradigm for Membrane Proteins with 7 Helical Segments

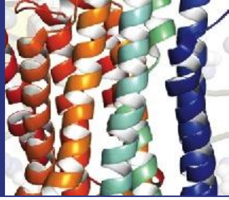




Note the light-absorbing retinal bound to a lysine residue.

Figure 9.13 Bacteriorhodopsin: Its seven transmembrane segments are connected by short loops.

Membrane Protein Topology Can Be Revealed by Hydropathy Plots



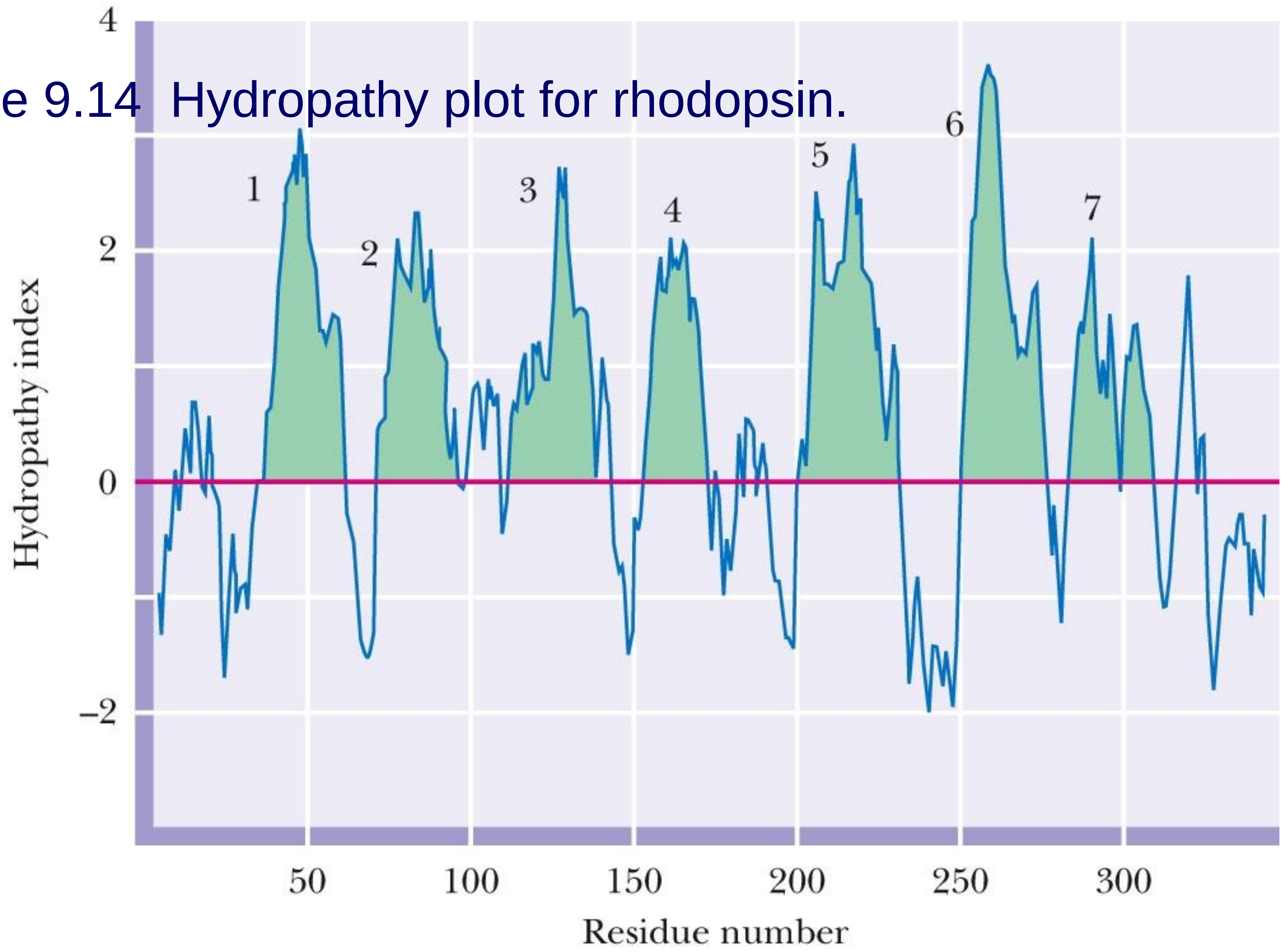


Figure 9.14 Hydropathy plot for rhodopsin.

Snorkeling and antisnorkeling behavior in membrane proteins

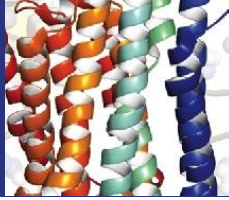
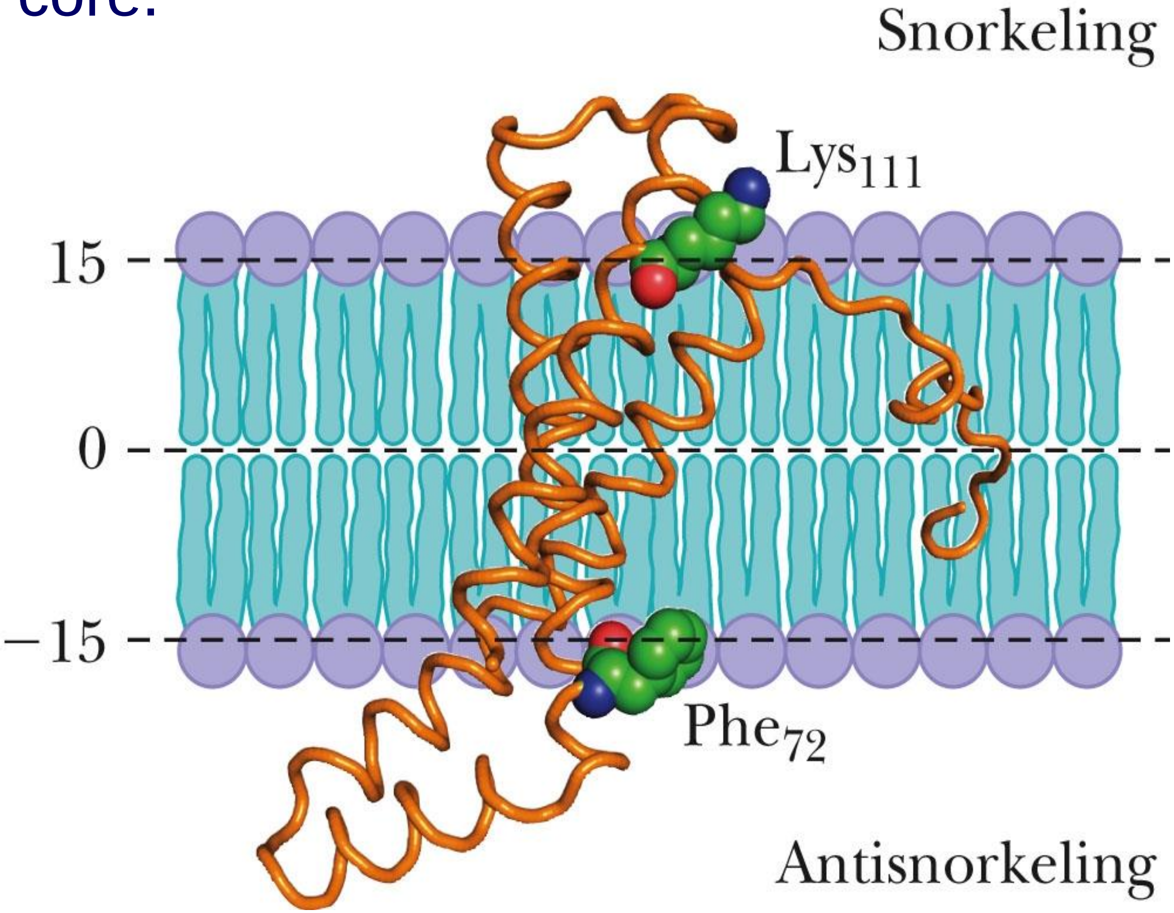
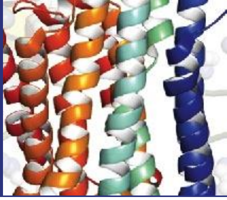


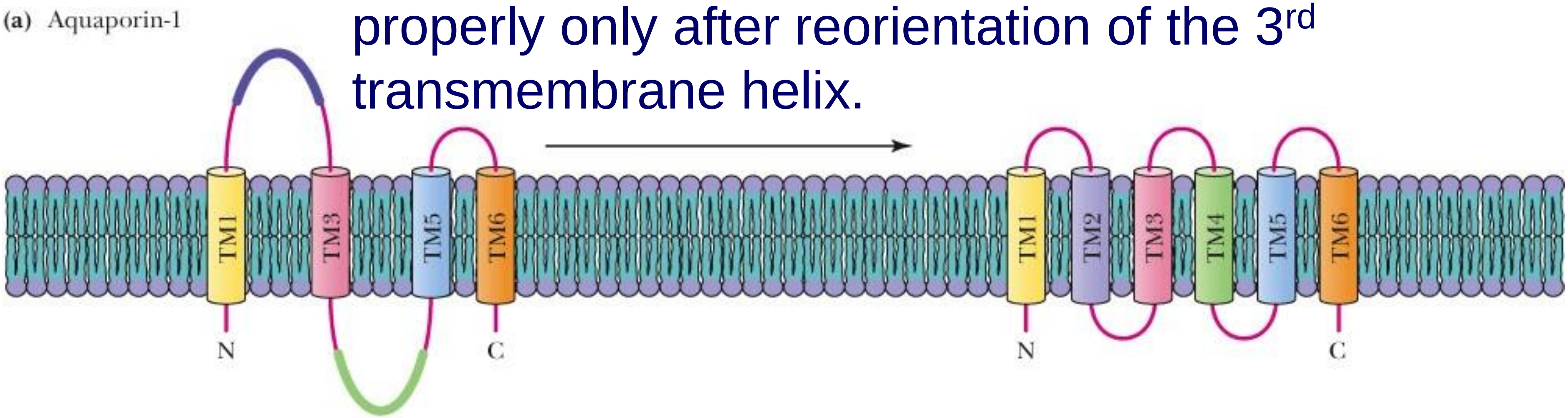
Figure 9.16 Lys^{111} snorkels away from the membrane core. Phe^{72} antisnorkels toward the membrane core.



Some Membrane Proteins are Known to Change Their Membrane Orientation



The 2nd and 4th helices of aquaporin-1 insert properly only after reorientation of the 3rd transmembrane helix.



The N-terminal "pre-S" domain of the hepatitis B envelope protein translocates in a slow process.

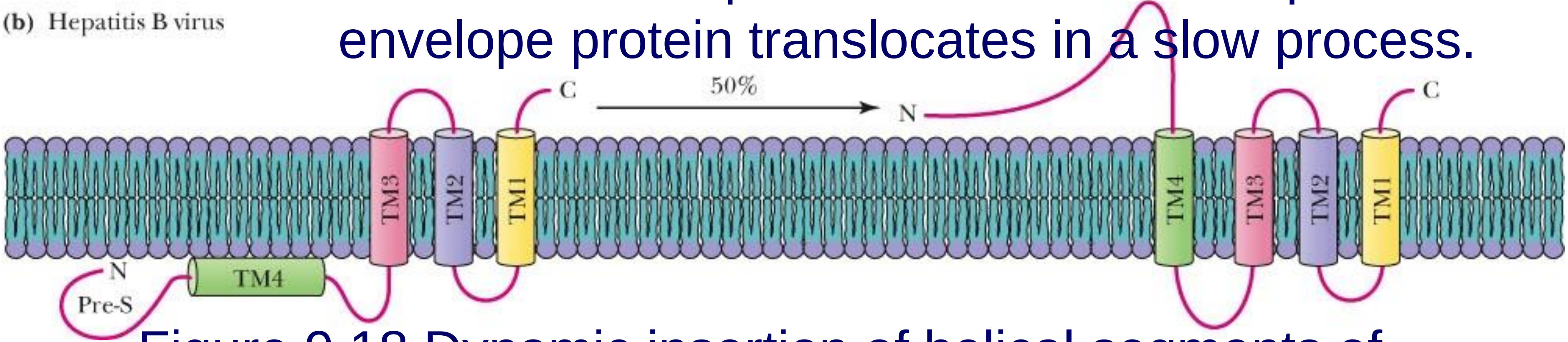
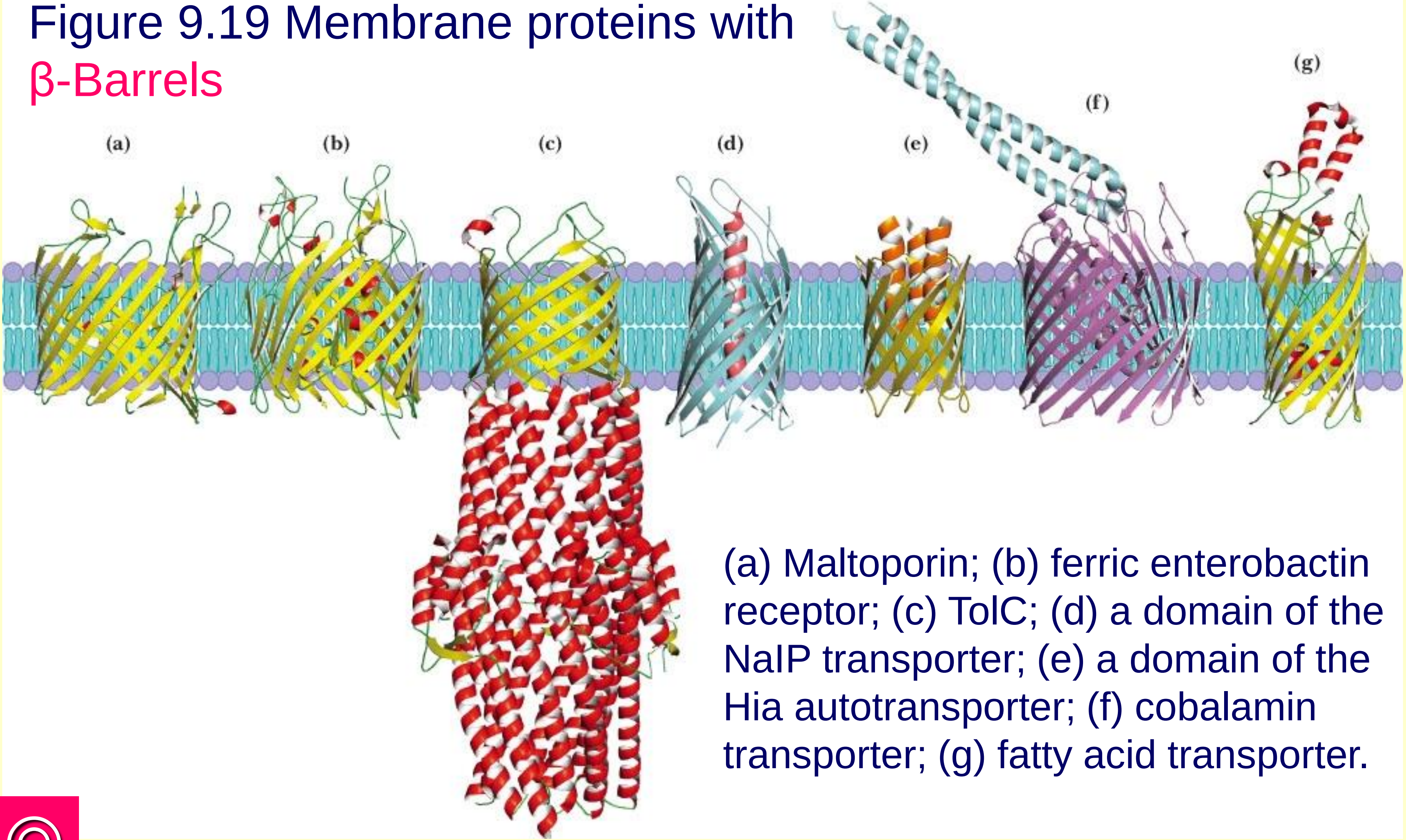


Figure 9.18 Dynamic insertion of helical segments of membrane proteins.

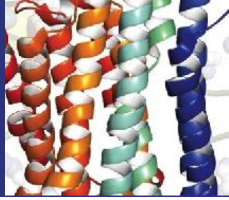
Some Proteins use β–Strands and β–Barrels to Span the Membrane

Figure 9.19 Membrane proteins with β-Barrels



(a) Maltoporin; (b) ferric enterobactin receptor; (c) TolC; (d) a domain of the NalP transporter; (e) a domain of the Hia autotransporter; (f) cobalamin transporter; (g) fatty acid transporter.

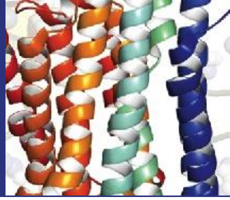
Porin Proteins Span Their Membranes with Large β-Barrels



Porins are found both in Gram-negative bacteria and in the mitochondrial outer membrane

- Porins are pore-forming proteins - 30-50 kD
- General or specific - exclusion limits 600-6000
- Most arrange in membrane as trimers
- High homology between various porins
- Porin from *Rhodobacter capsulatus* has 16-stranded beta barrel that traverses the membrane to form the pore (with an eyelet!)

Porin Proteins Span Their Membranes with Large β-Barrels



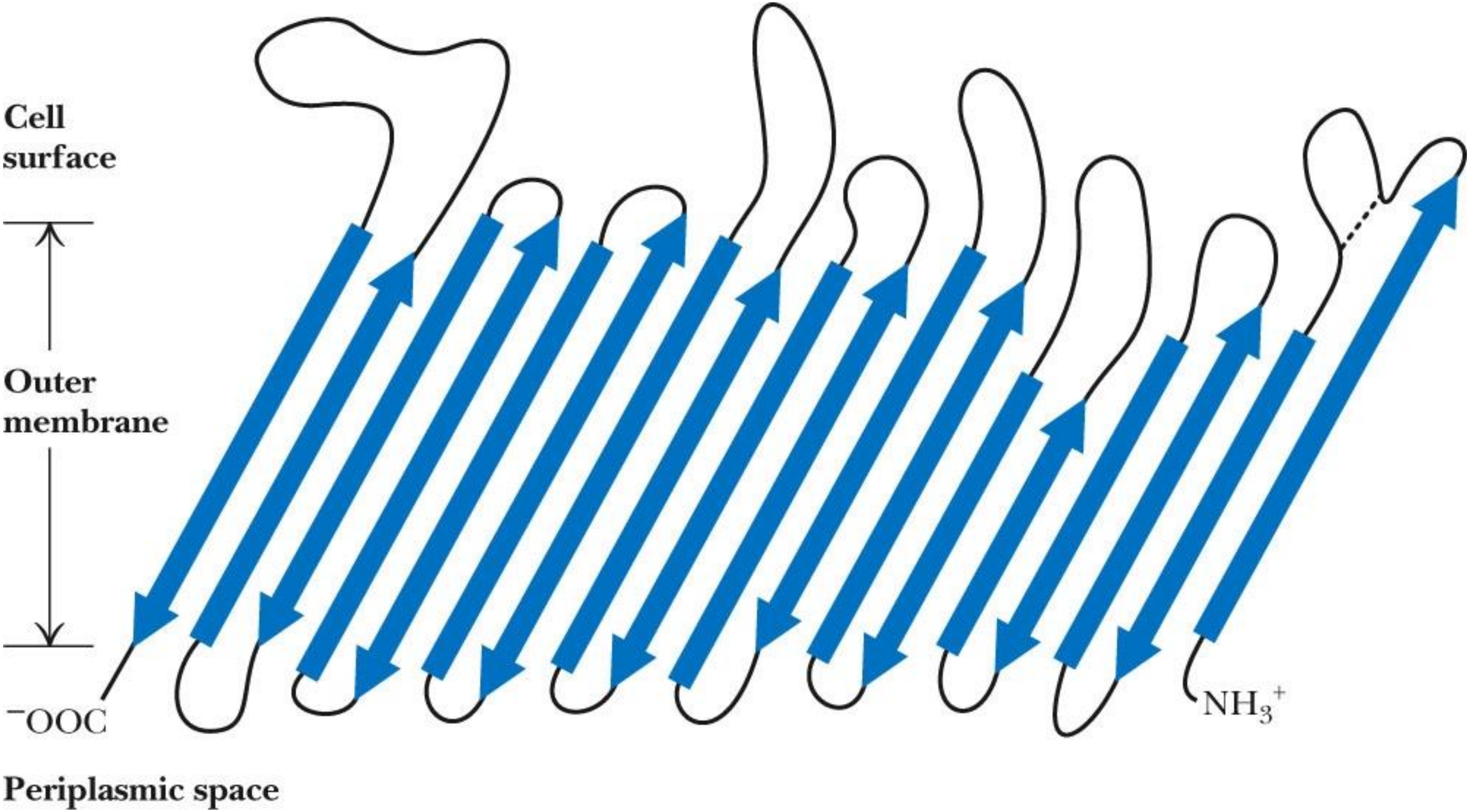


Figure 9.20 The arrangement of the peptide chain in maltoporin from *E.coli*.

α-Hemolysin – a β-Barrel Constructed From Multiple Subunits

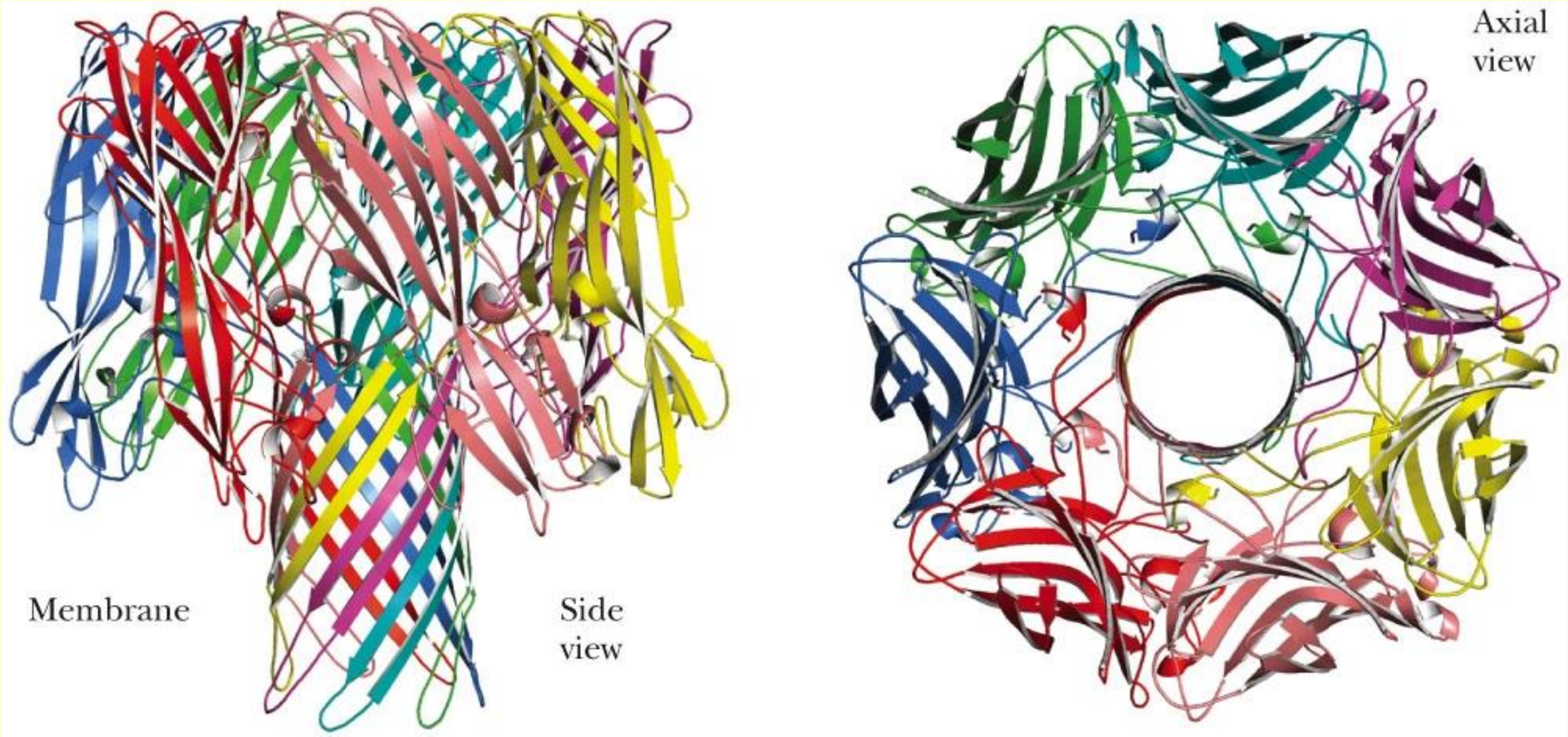
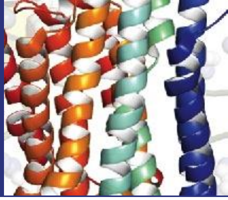


Figure 9.21 The heptameric channel formed by *S. aureus* α-hemolysin. Each of the seven subunits contributes a β–sheet hairpin to the transmembrane channel.

Transmembrane Barrels Can also Be Formed with α-Helices



- Bacteria, such as *E. coli*, produce extracellular polysaccharides, some of which form a discrete structural layer – the capsule, which shields the cell
- Components of this capsule are synthesized inside the cell and then transported outward through an octameric outer membrane protein called **Wza**
- Wza forms a novel octameric α-helical barrel structure across the outer membrane
- The eight transmembrane helices of the Wza barrel form an amphiphilic pore across the membrane

Transmembrane Barrels Can also Be Formed with α-Helices

Each Wza helix has a nonpolar outer surface facing the bilayer and a hydrophilic inner surface that faces the water-filled pore

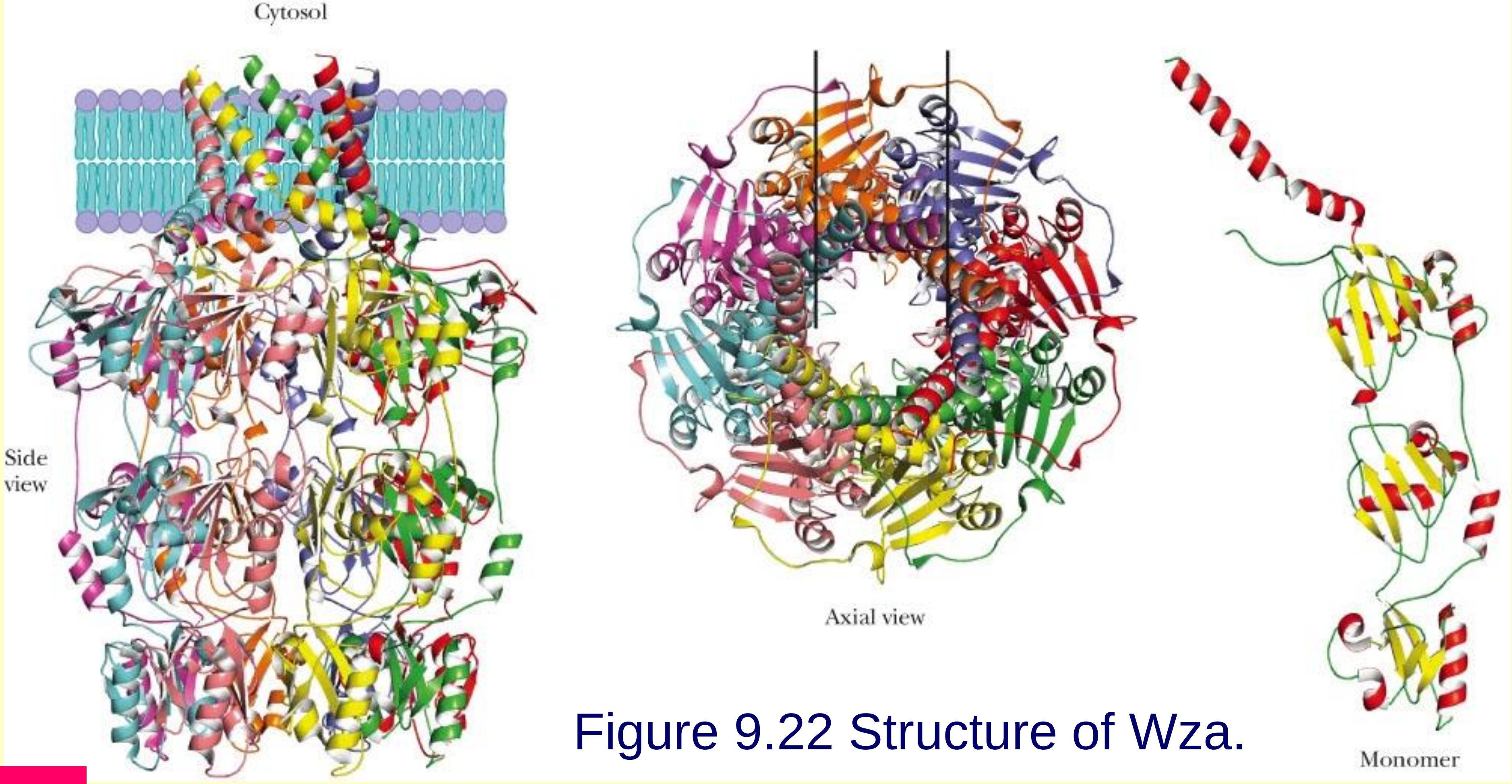
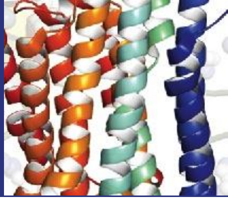


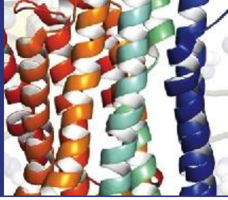
Figure 9.22 Structure of Wza.

Lipid-Anchored Membrane Proteins Are Switching Devices



- Certain proteins are found covalently linked to lipids in the membrane
- Lipid anchors may be transient – lipid anchors can be reversibly bound to proteins
- Attachment to the lipid membrane via the lipid anchor can modulate the activity of the protein
- Four types of lipid-anchored proteins are known:
  - Amide-linked myristoyl anchors
  - Thioester-linked fatty acyl anchors
  - Thioether-linked prenyl anchors
  - Glycosyl phosphatidylinositol anchors

Amide-Linked Myristoyl Anchors



- The lipid anchor is always myristic acid
- It is always N-terminal
- It is always a Gly residue that links
- Examples: cAMP-dependent protein kinase, pp60$^{\text{src}}$ tyrosine kinase, calcineurin B, alpha subunits of G proteins, gag protein of HIV-1

Amide-Linked Myristoyl Anchors and Thioester-linked and Acyl Anchors

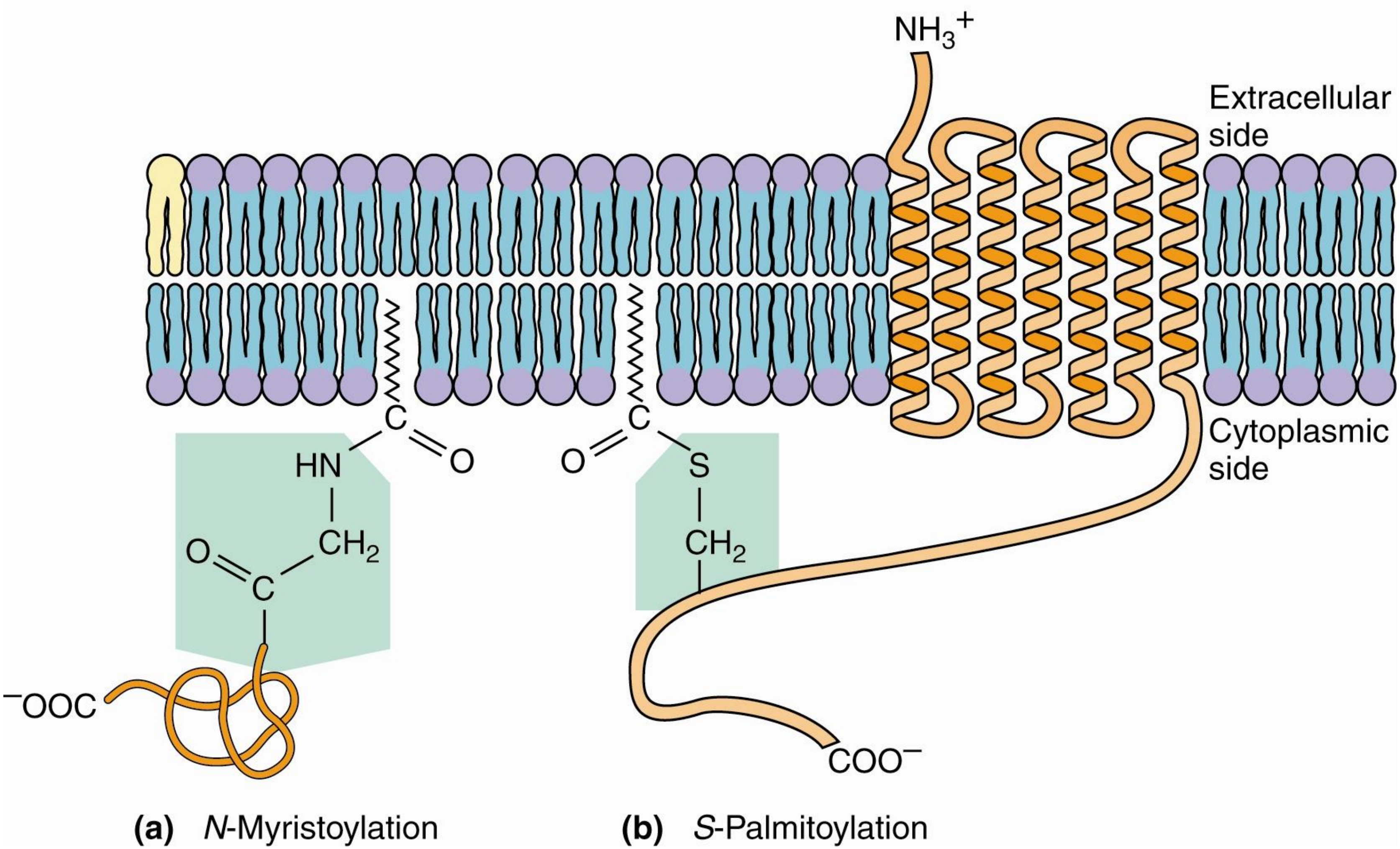
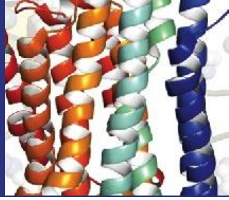


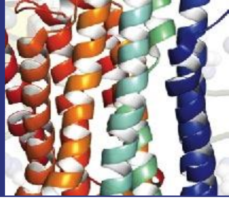
Figure 9.23 Certain proteins are anchored to biological membranes by myristyl and thioester-linked anchors.

Thioester-linked and Acyl Anchors



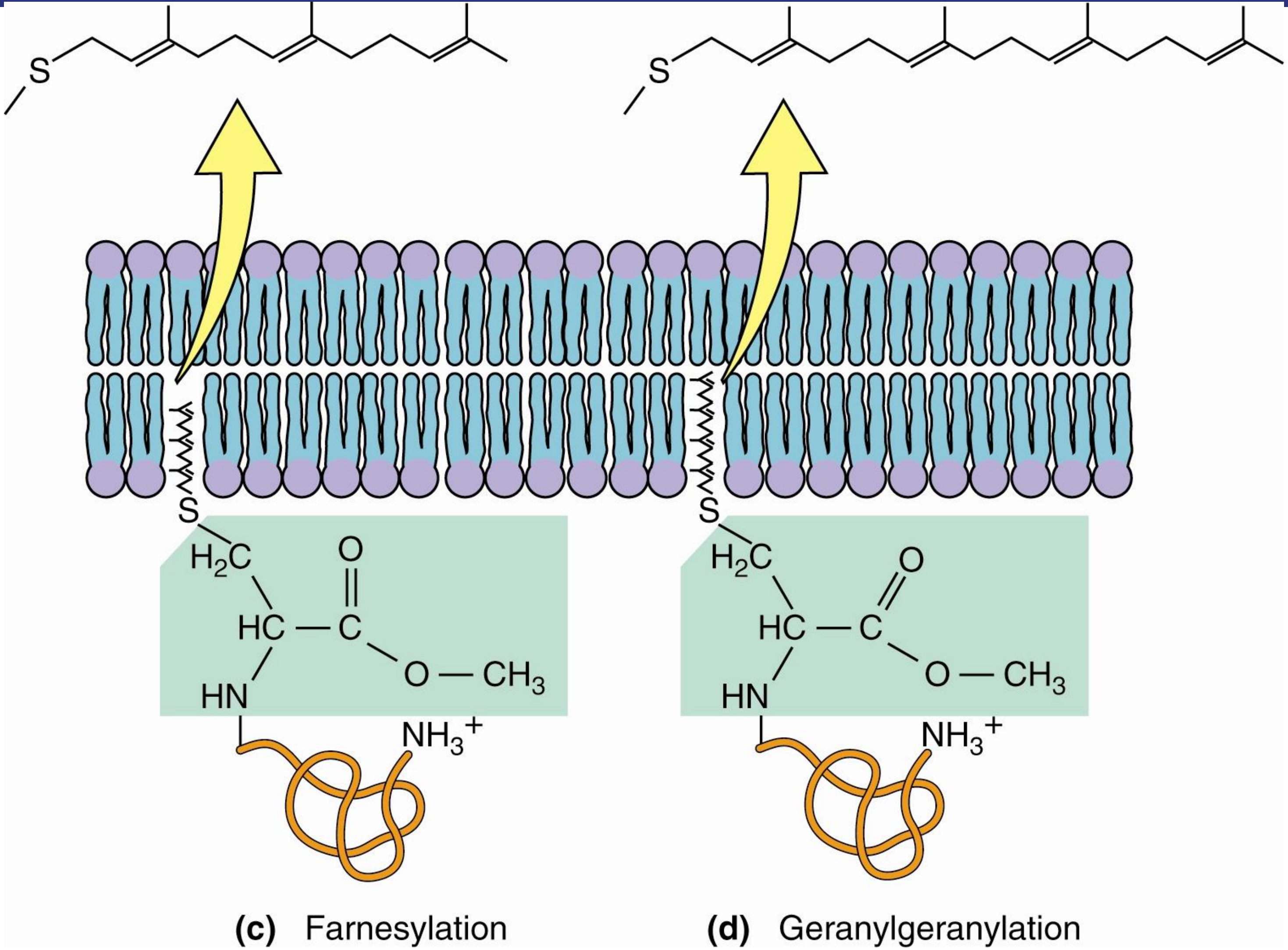
- A broader specificity for lipids - myristate, palmitate, stearate, oleate all found
- Broader specificity for amino acid links - Cys, Ser, Thr are all found
- Examples: G-protein-coupled receptors, surface glycoproteins of some viruses, transferrin receptor triggers and signals

Thioether-linked Prenyl Anchors



- Prenylation refers to linking of "isoprene"-based groups
- Always linked to Cys of CAAX (C=Cys, A=Aliphatic, X=any residue)
- Isoprene groups include farnesyl (15-carbon, three double bond) and geranylgeranyl (20-carbon, four double bond) groups
- Examples: yeast mating factors, $p21^{ras}$ and nuclear lamins

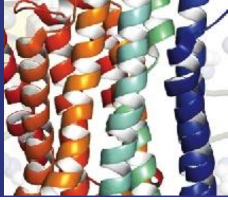
Thioether-linked Prenyl Anchors



(c) Farnesylation **(d)** Geranylgeranylation

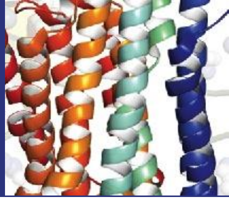
Figure 9.23 Certain proteins are anchored to membranes by prenyl anchors.

Prenylation Reactions as Possible Chemotherapy Targets

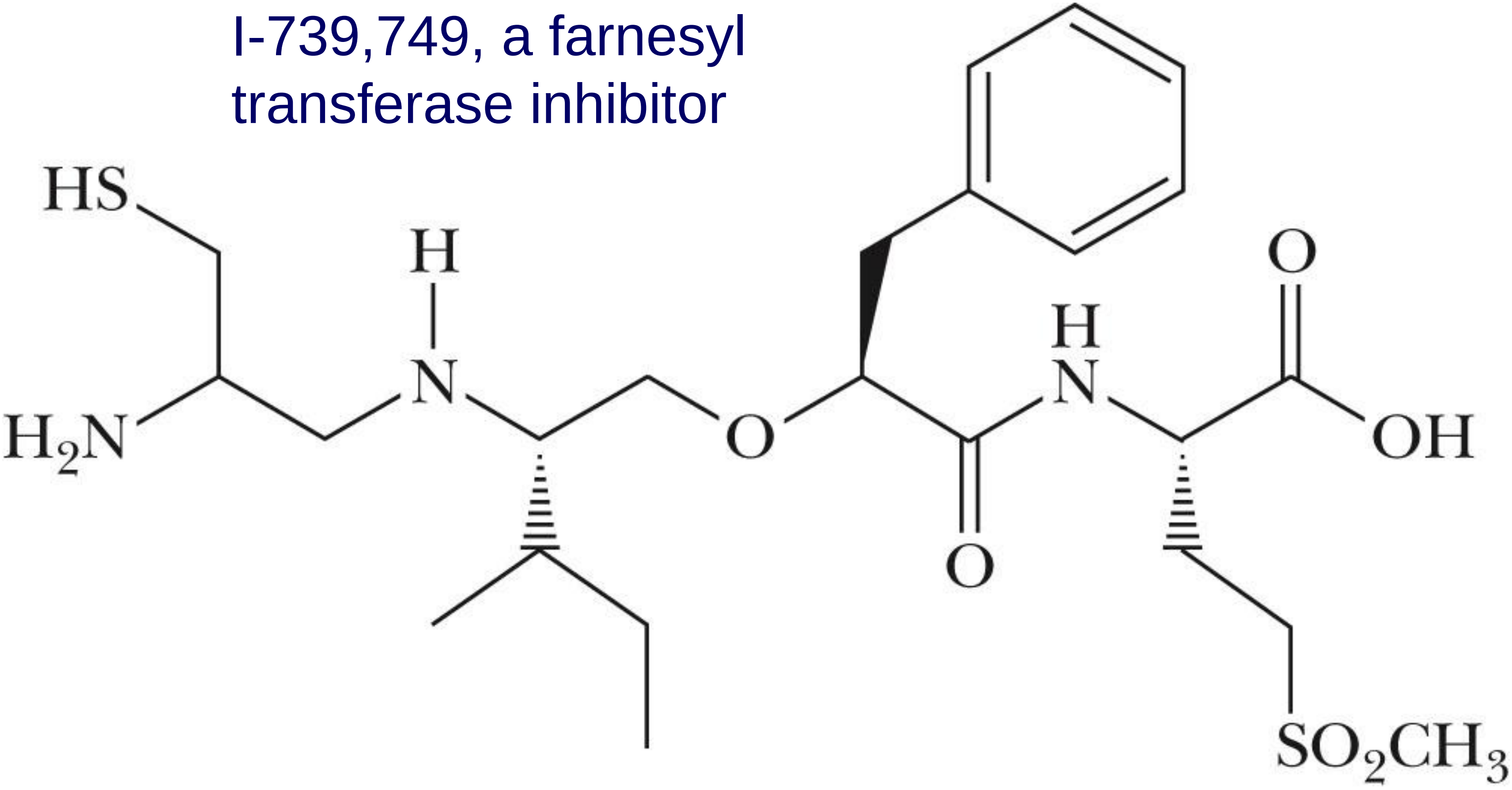


- Ras is a small GTP-binding protein involved in cell signaling
- The signaling activity of Ras depends on prenylation
- Thus the prenylation reaction is a target for chemotherapy strategies
- Farnesyl transferase inhibitors are potent suppressors of tumor growth
- However, the protease that cleaves the –AAX motif from Ras following prenylation may be a better target
- Inhibitors of CAAX proteases may be valuable chemotherapeutic agents than prenyl transferase inhibitors

Prenylation Reactions as Possible Chemotherapy Targets



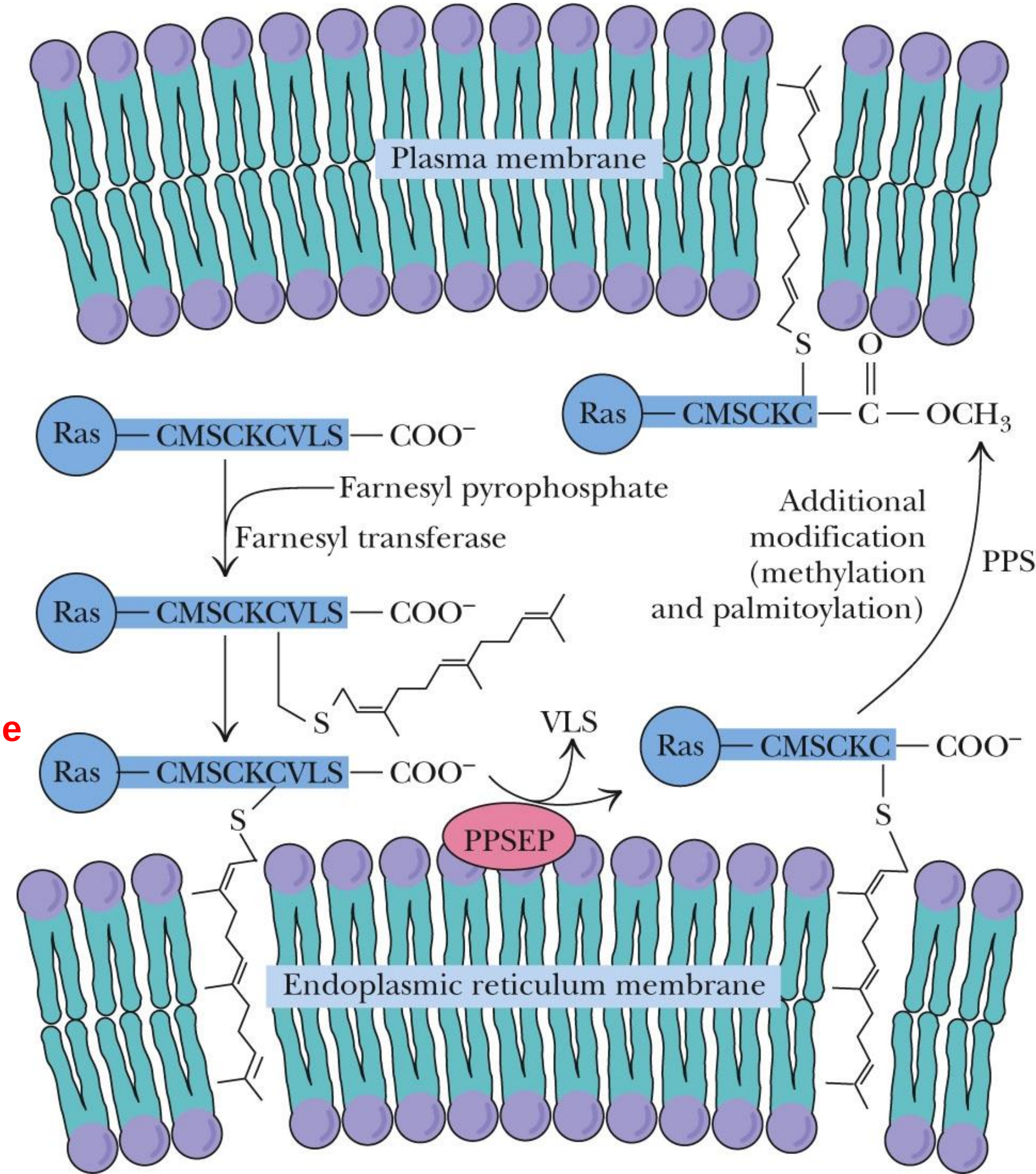
l-739,749, a farnesyl transferase inhibitor



2(*S*)-{(*S*)-[2(*R*)-amino-3-mercapto]propylamino-3(*S*)-methyl}pentyloxy-3-phenylpropionyl-methioninesulfone methyl ester

Prenylation Reactions as Possible Chemotherapy Targets

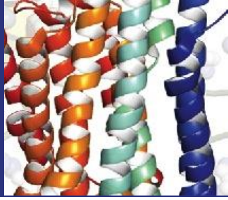
The farnesylation and subsequent processing of the Ras protein.



prenyl protein-specific endoprotease

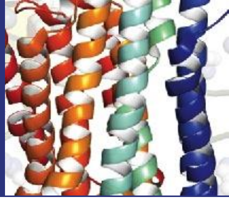
prenyl protein-specific methyltransferase

Glycosyl Phosphatidylinositol Anchors



- GPI anchors are more elaborate than others
- Always attached to a C-terminal residue
- Ethanolamine link to an oligosaccharide linked in turn to inositol of PI
- See Figure 9.20
- Examples: surface antigens, adhesion molecules, cell surface hydrolases

Glycosyl Phosphatidylinositol Anchors



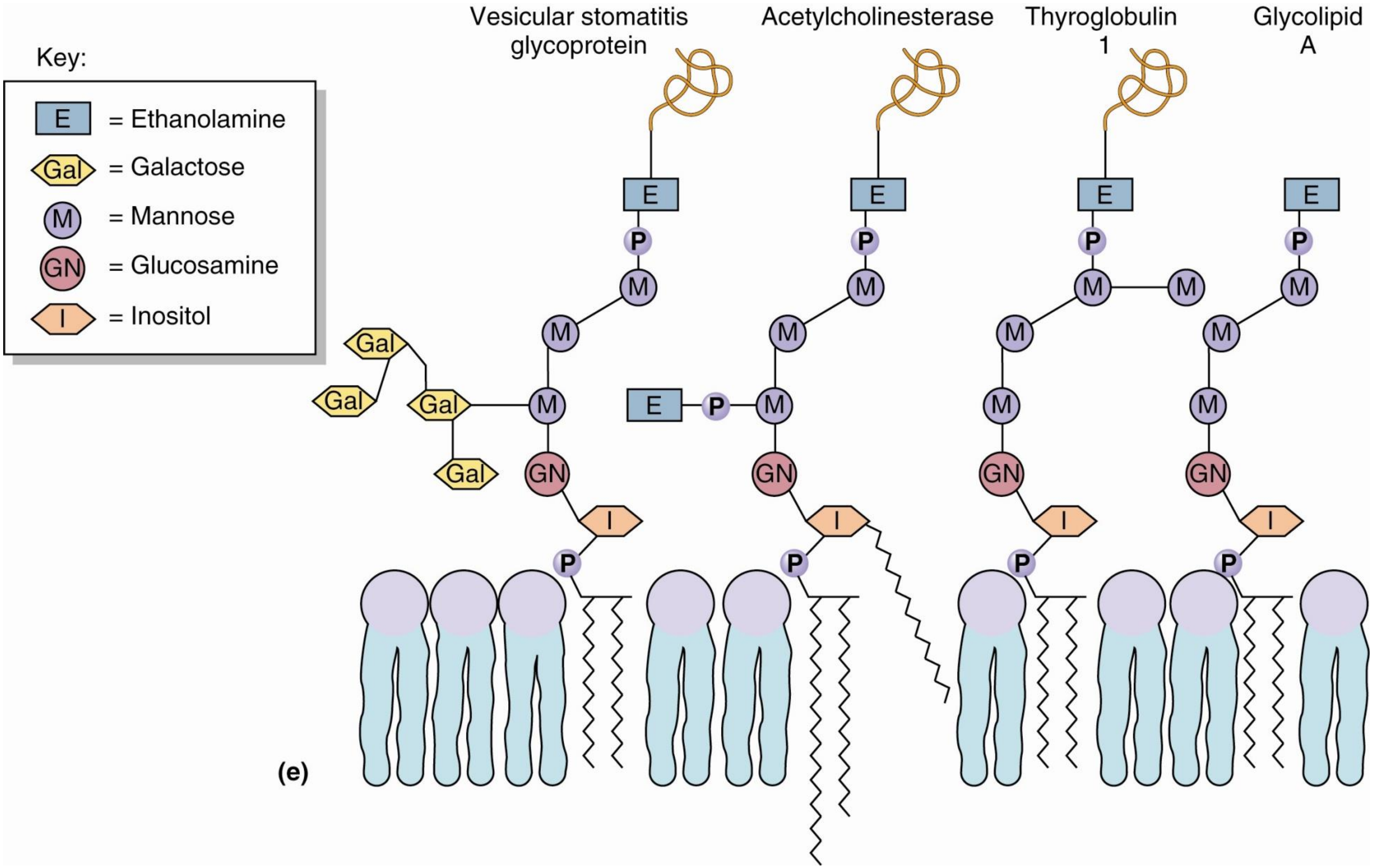
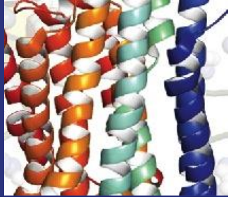


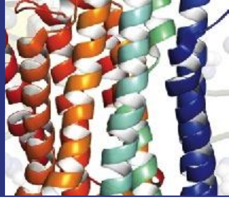
Figure 9.23 Certain proteins are anchored to membranes via glycosyl phosphatidylinositol anchors.

9.3 How Are Biological Membranes Organized?



- Membranes are asymmetric, heterogeneous structures
- The two monolayers of the bilayer have different lipid compositions and different protein complements
- The composition is also different across the plane of the membrane
- There are lipid clusters and lipid-protein aggregates
- Thus both the lipids and the proteins of the membrane exhibit lateral heterogeneity and transverse asymmetry

Membranes are Asymmetric Structures



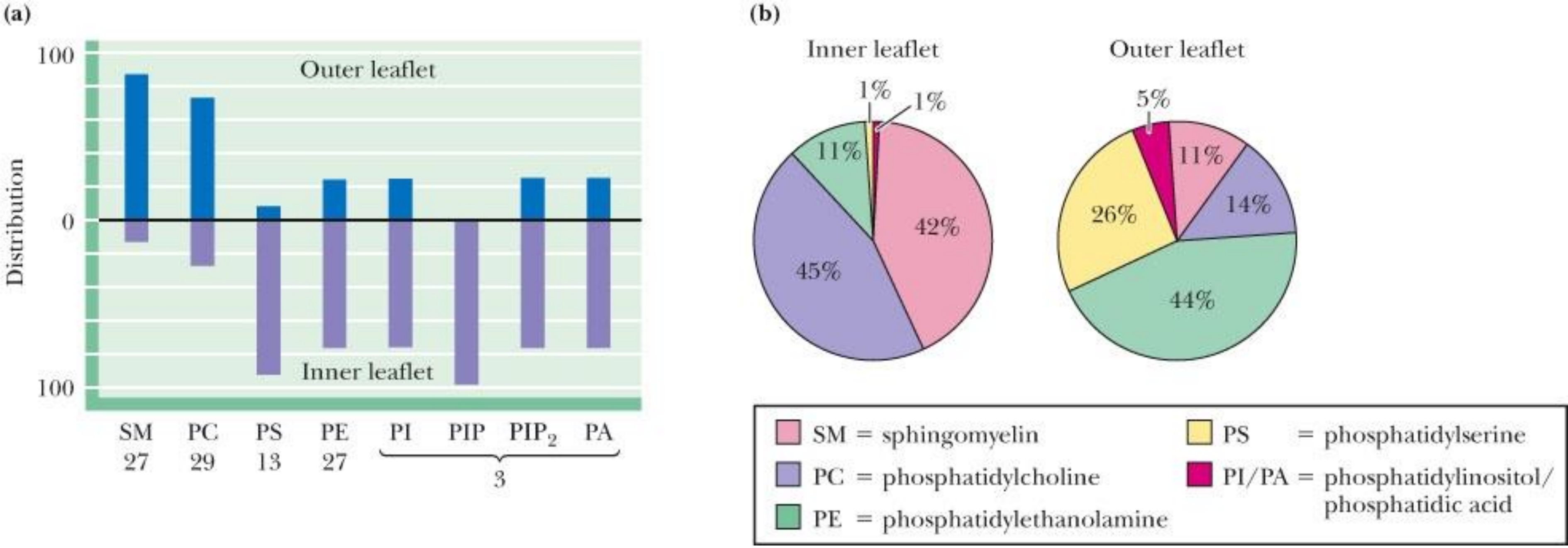
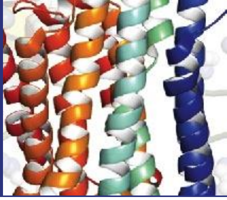


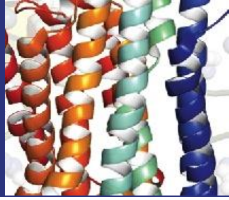
Figure 9.24 Phospholipids are distributed asymmetrically in most membranes, including the erythrocyte membrane, as shown here.

9.4 What Are the Dynamic Processes That Modulate Membrane Function?



- Lipids and proteins undergo a variety of movements in membranes
- These motions support a variety of cell functions
- These functions will be described throughout our text, especially in Chapters 9, 16, and 32
- The types and rates of lipid motions are described in Figure 9.25

Lipids and Proteins Undergo a Variety of Movements in Membranes



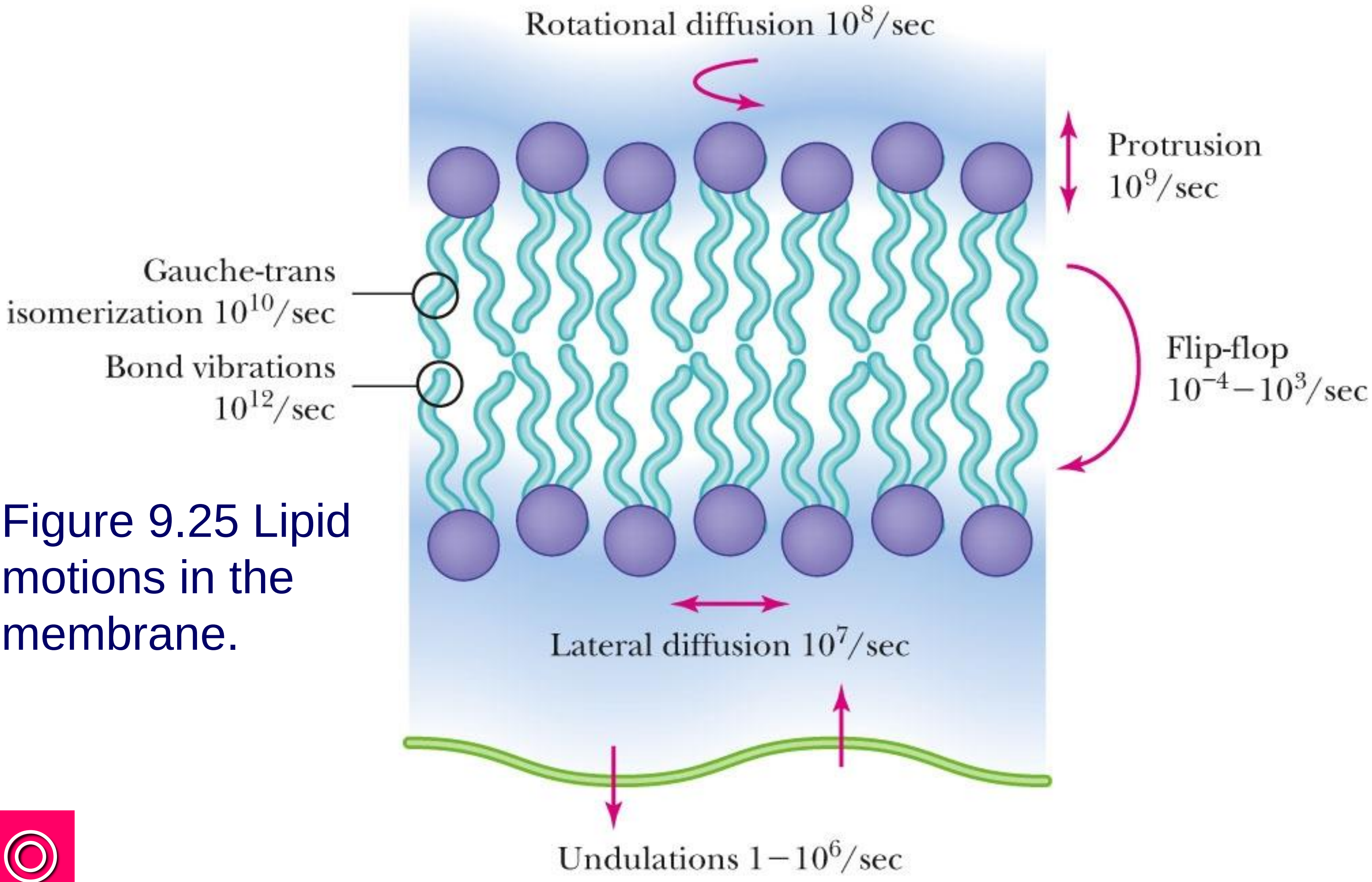
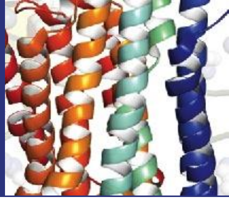


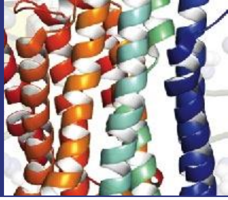
Figure 9.25 Lipid motions in the membrane.

Protein Motion in Membranes



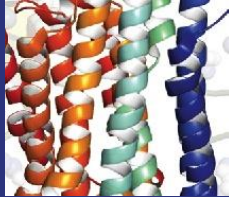
- A variety of protein motions in membranes supports their many functions
- Proteins move laterally (through the plane of the membrane) at a rate of a few microns per second
- Some integral membrane proteins move more slowly, at diffusion rates of 10 nm per sec – why?
- Slower protein motion is likely for proteins that associate and bind with each other, and also for proteins that are anchored to the **cytoskeleton** – a complex lattice structure that maintains cell shape

Flippases, Floppases, and Scramblases: Proteins That Redistribute Membrane Lipids



- Lipids can be moved from one monolayer to the other by **flippase** and **floppase** proteins
- Some flippases and floppases operate passively and do not require an energy source
- Others appear to operate actively and require the energy of hydrolysis of ATP
- Active (energy-requiring) flippases and floppases can generate membrane asymmetries

Flippases, Floppases, and Scramblases: Proteins That Redistribute Membrane Lipids



- ATP-dependent flippases move PS (and some PE) from the outer leaflet to the inner leaflet
- ATP-dependent floppases move amphiphilic lipids (including cholesterol, PC, and sphingomyelin) from the inner leaflet to the outer leaflet of the membrane
- Bidirectional **scramblases** (Ca2+-activated but ATP-independent) randomize lipids across the membrane and thus degrade membrane lipid asymmetry

Flippases, Floppases, and Scramblases: Proteins That Redistribute Membrane Lipids

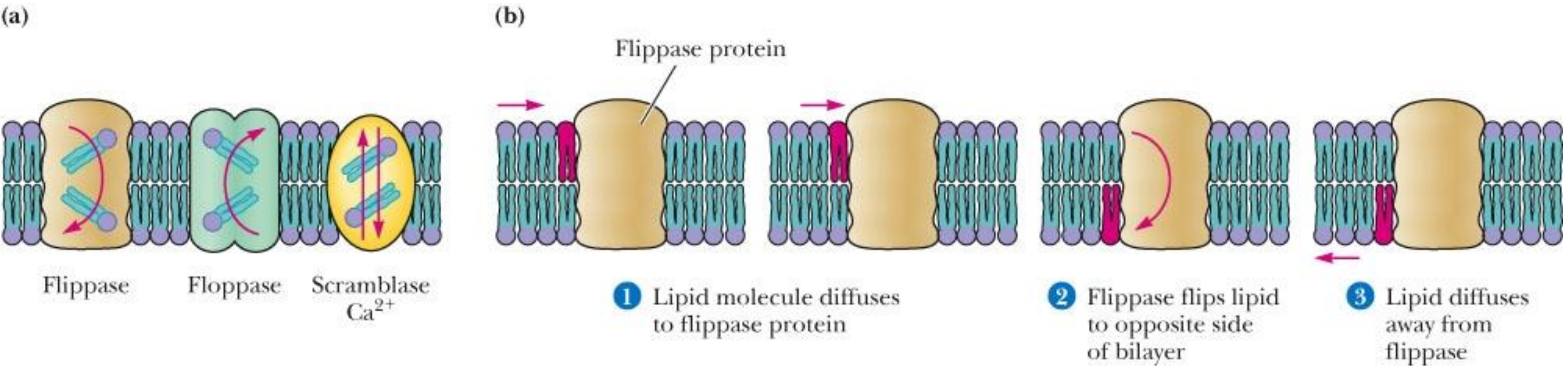
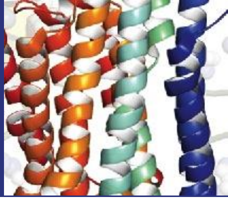


Figure 9.26 (a) Phospholipids can be flipped, flopped, or scrambled across a bilayer membrane by the action of flippase, floppase, and scramblase proteins. (b) When, by normal diffusion through the bilayer, the lipid encounters one of these proteins, it can be moved quickly to the other face of the bilayer.

Membranes Undergo Phase Transitions

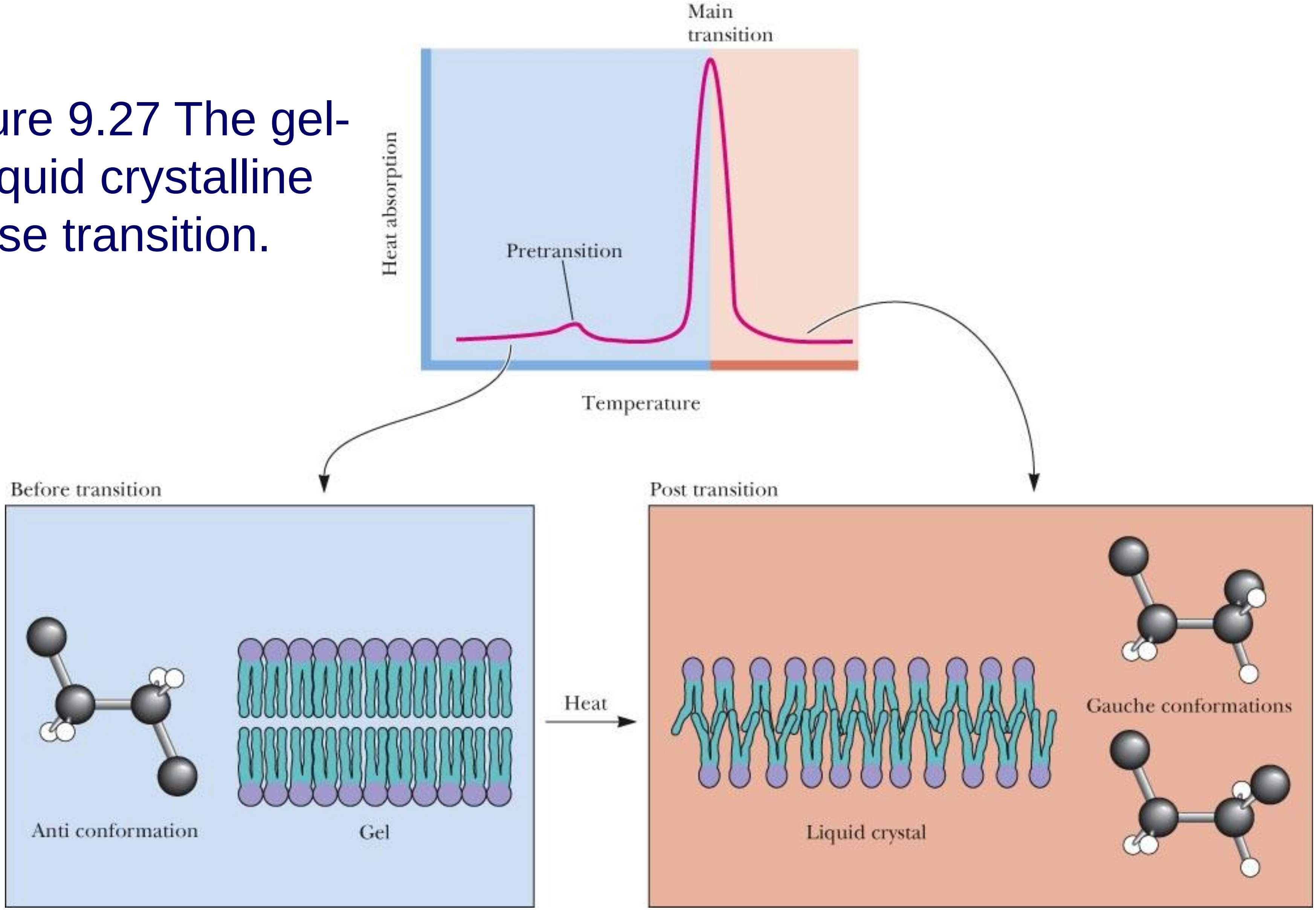


The "melting" of membrane lipids

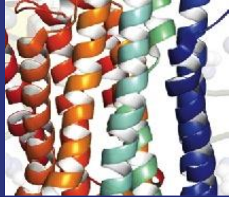
- The transition from the gel phase to the liquid crystalline phase is a true **phase transition**
- The temperature at which this occurs is the **transition temperature** or **melting temperature**
- The transition temperature (**T_m**) is characteristic of the lipids in the membrane
- Only pure lipid systems give sharp, well-defined transition temperatures

Membranes Undergo Phase Transitions

Figure 9.27 The gel-to-liquid crystalline phase transition.



Membranes Undergo Phase Transitions



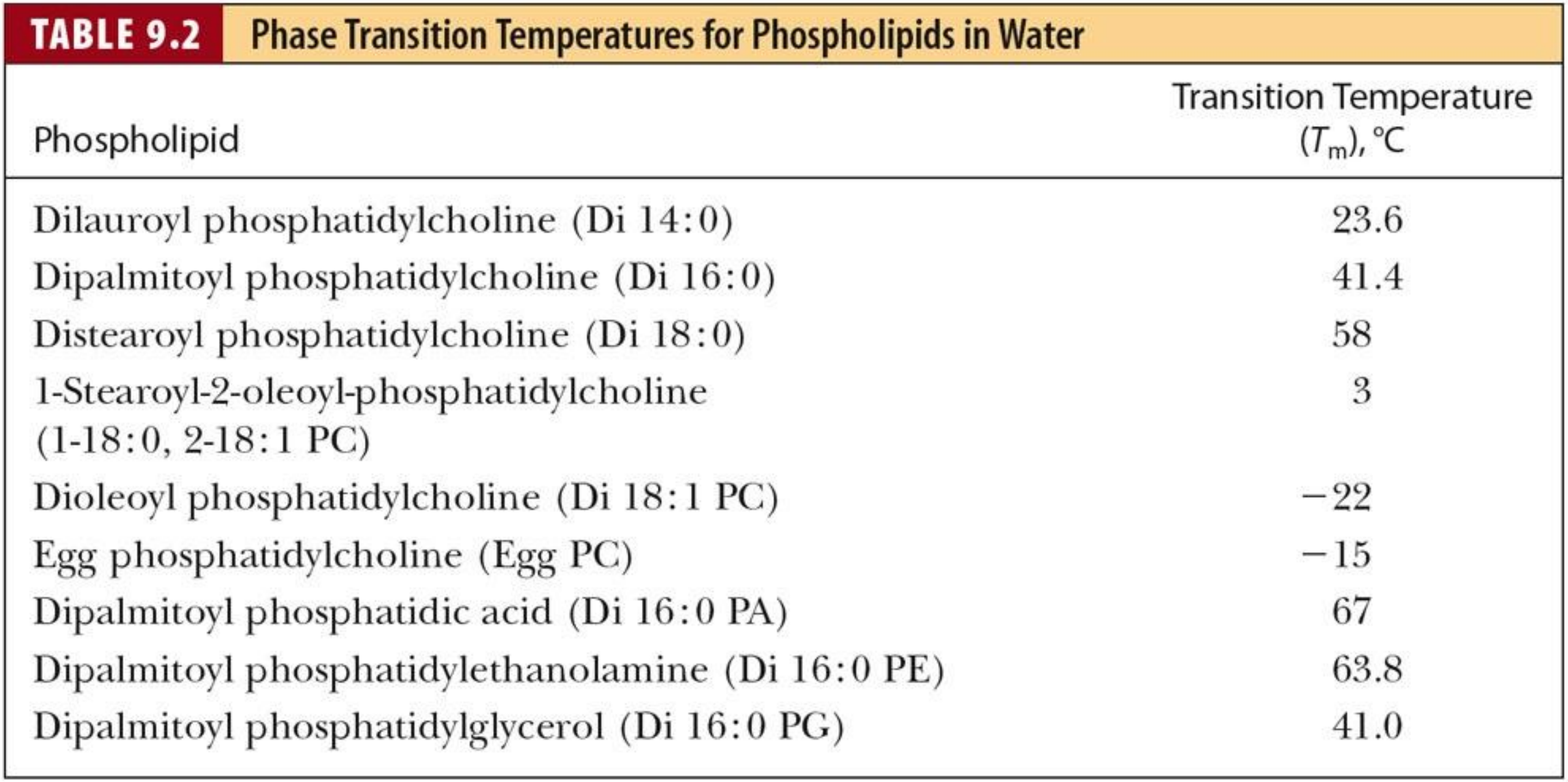
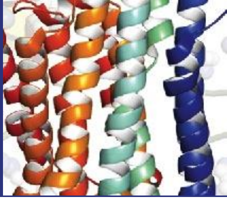


TABLE 9.2 Phase Transition Temperatures for Phospholipids in Water

Phospholipid	Transition Temperature (T_m), °C
Dilauroyl phosphatidylcholine (Di 14:0)	23.6
Dipalmitoyl phosphatidylcholine (Di 16:0)	41.4
Distearoyl phosphatidylcholine (Di 18:0)	58
1-Stearoyl-2-oleoyl-phosphatidylcholine (1-18:0, 2-18:1 PC)	3
Dioleoyl phosphatidylcholine (Di 18:1 PC)	−22
Egg phosphatidylcholine (Egg PC)	−15
Dipalmitoyl phosphatidic acid (Di 16:0 PA)	67
Dipalmitoyl phosphatidylethanolamine (Di 16:0 PE)	63.8
Dipalmitoyl phosphatidylglycerol (Di 16:0 PG)	41.0

Note the trend of increasing T_m as chain length increases.
Note also the effect of unsaturation on T_m.

The Evidence for Liquid Ordered Domains and Membrane Rafts



- In addition to the S_o and L_d states, model lipid bilayers can form a 3rd phase if the membrane contains sufficient cholesterol
- The **liquid-ordered state (L_o)** shows the high lipid ordering of the S_o state but the translational disorder of the L_d state
- Lipid diffusion in the L_o state is 2- to 3-fold slower than in the L_d phase
- Biological membranes are hypothesized to contain L_o phases – these **microdomains** are termed **lipid rafts**
- They contain large amounts of cholesterol, sphingolipids, and GPI-anchored proteins

The Evidence for Liquid Ordered Domains and Membrane Rafts

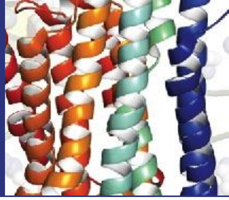
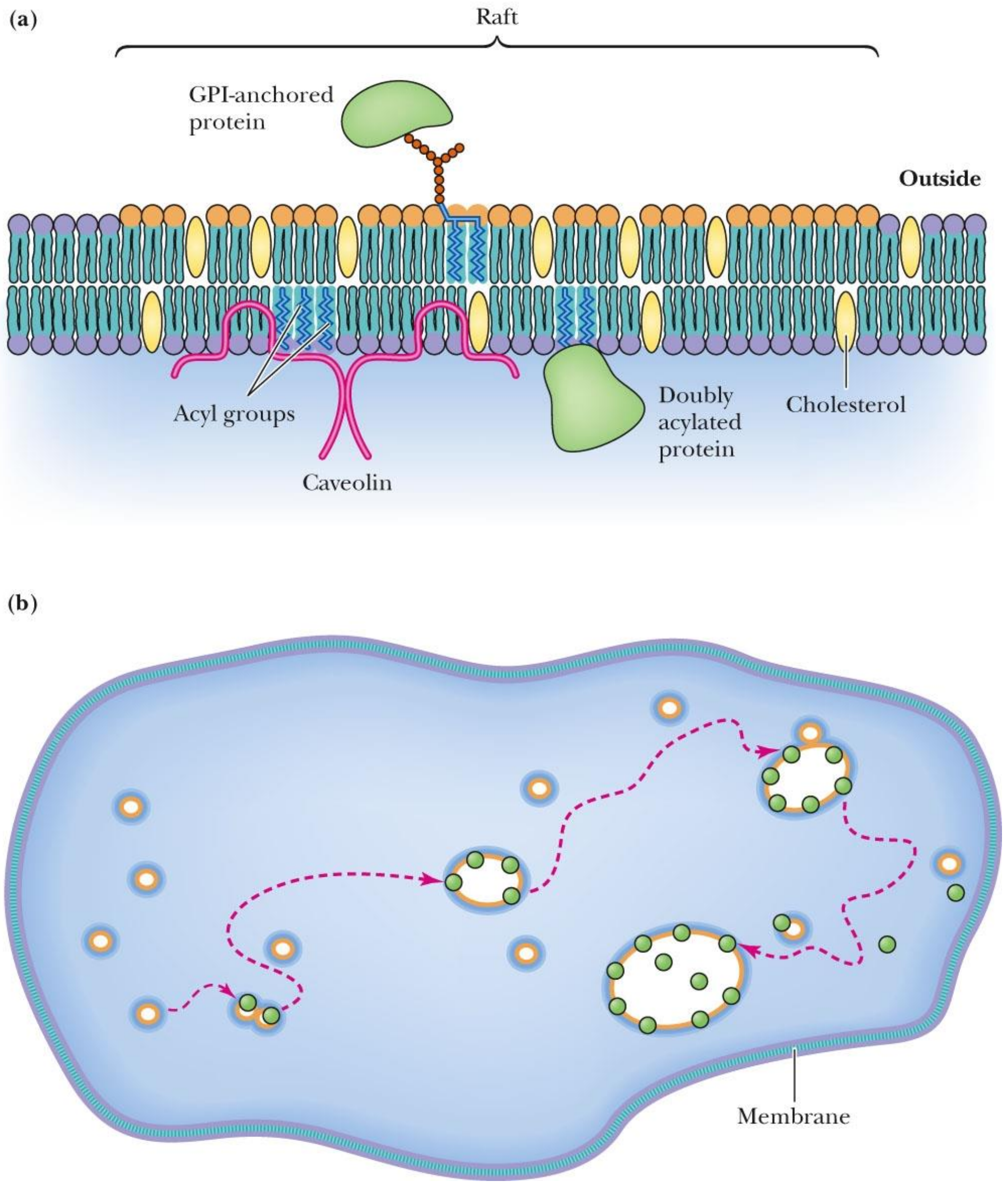
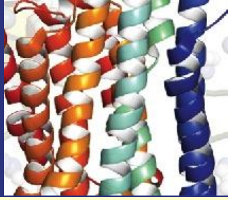


Figure 9.28 (a) Model for a membrane raft. (b) Rafts are postulated to grow by accumulation of cholesterol, sphingolipids, and GPI-anchored proteins.



Lipids and Proteins Direct Dynamic Membrane Remodeling and Curvature



- The complex shapes of cells and organelles, and the change of these shapes during movement and cell division, are all crafted by lipids and proteins
- Several ways to induce membrane curvature
- Lipids can influence or accommodate curvature
  - Due to novel lipid geometry
  - Due to imbalance in numbers of lipids in the inner and outer leaflets of the membrane
- Integral membrane proteins with conical shapes can induce curvature
- Scaffolding proteins can influence membrane shape in many ways

Lipids and Proteins Direct Dynamic Membrane Remodeling and Curvature

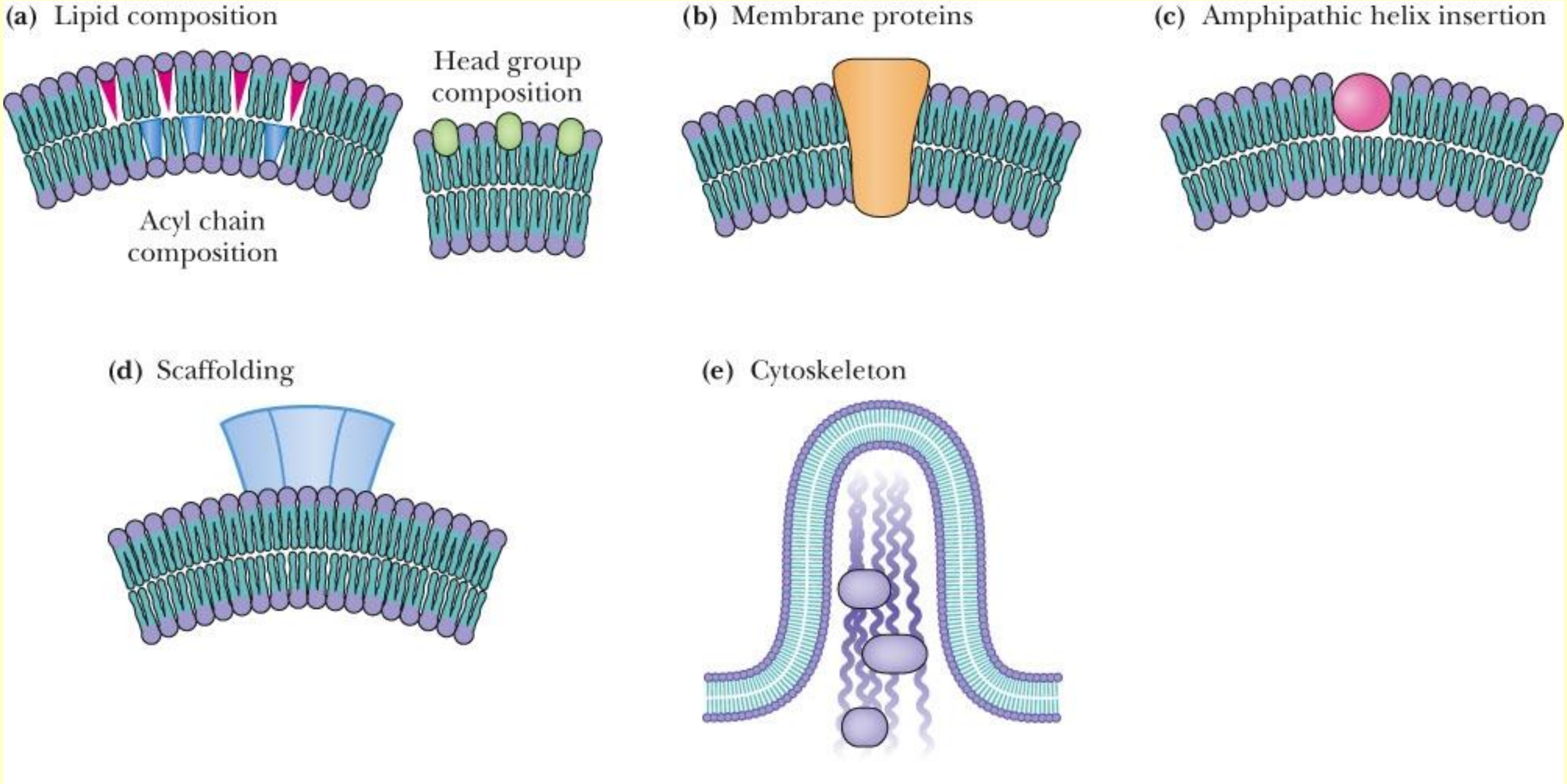


Figure 9.31 Membrane curvature can occur by several different mechanisms.

Lipids and Proteins Direct Dynamic Membrane Remodeling and Curvature

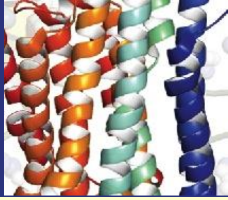
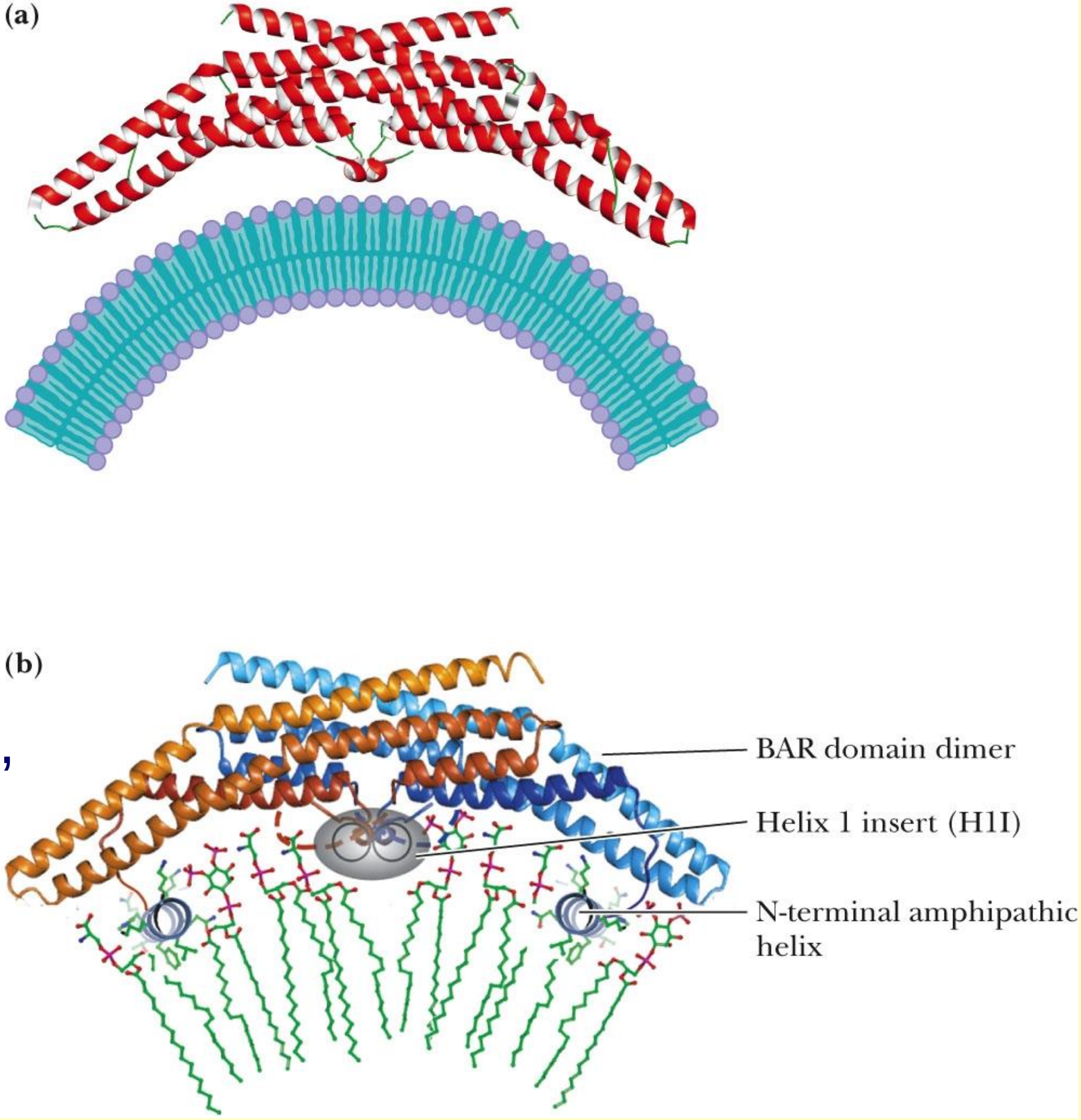


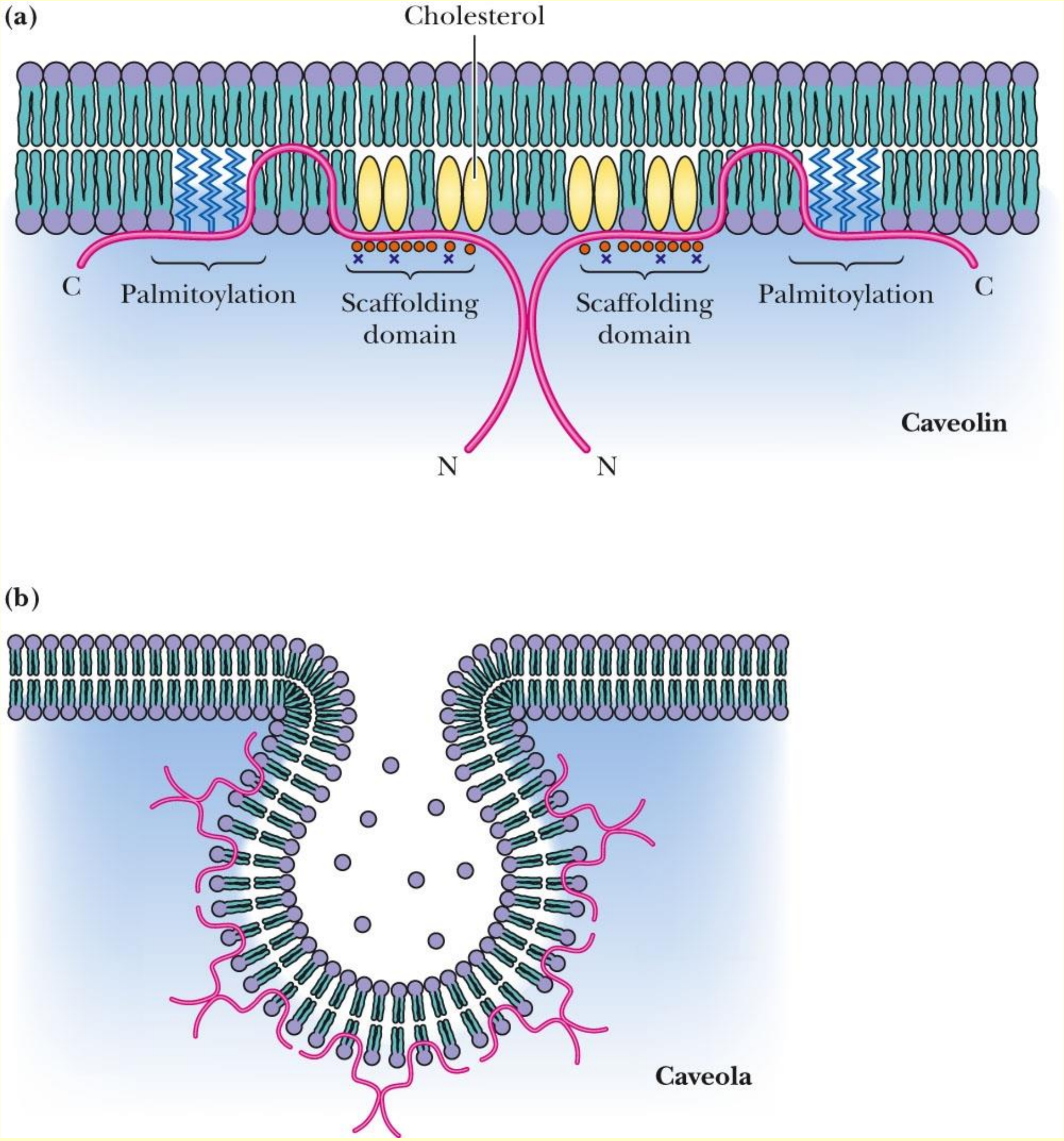
Figure 9.32 Model of BAR domain binding to membranes.

BAR domains are dimeric, banana-shaped structure that bind preferentially to and stabilize curved regions of the plasma membrane, thus forcing curvature on the membrane.



Caveolins and Caveolae Respond to Plasma Membrane Changes

Figure 9.33 Caveolin possesses a central hydrophobic segment flanked by three covalently bound fatty acyl anchors on the C-terminal side and a scaffolding domain on the N-terminal side.



Vesicle Formation and Fusion Are Essential Membrane Processes

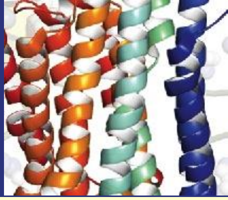
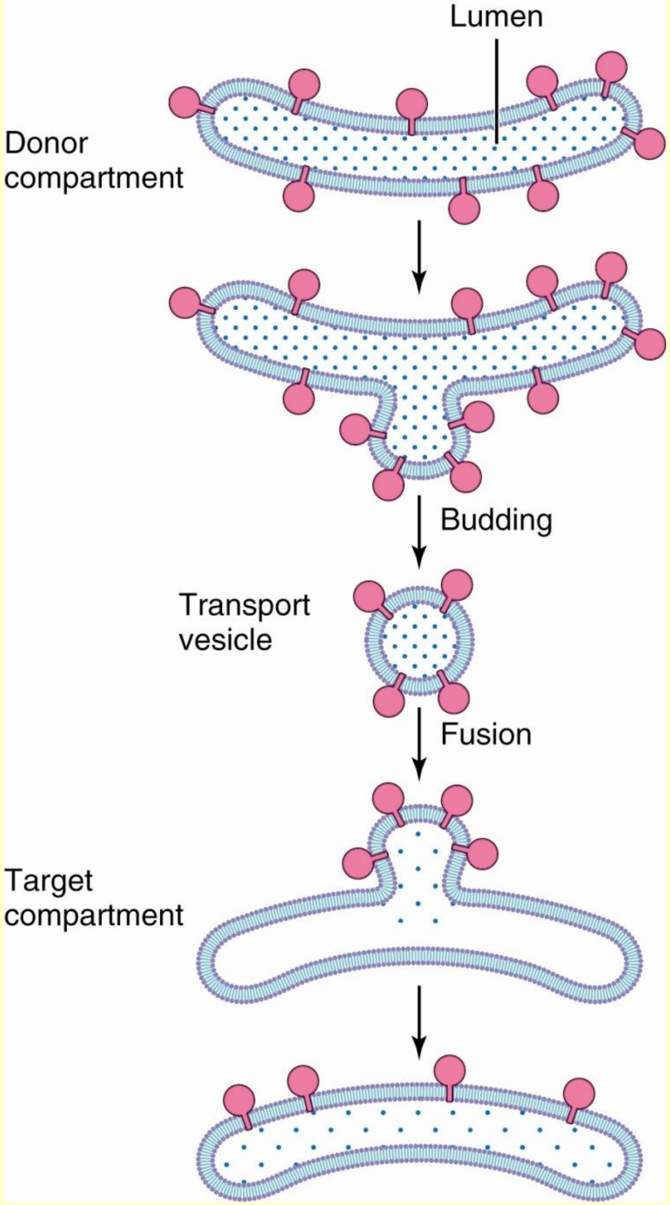


Figure 9.34 Vesicle-mediated transport in cells involves budding of vesicles from a donor membrane, followed by fusion of the vesicle membrane with the membrane of a target compartment, a process that transfers the contents of the donor compartment, as well as selected membrane proteins.

N-ethylmaleimide sensitive fusion protein

(Soluble NSF Attachment Protein) REceptor) SNARE



Vesicle Formation and Fusion Are Essential Membrane Processes

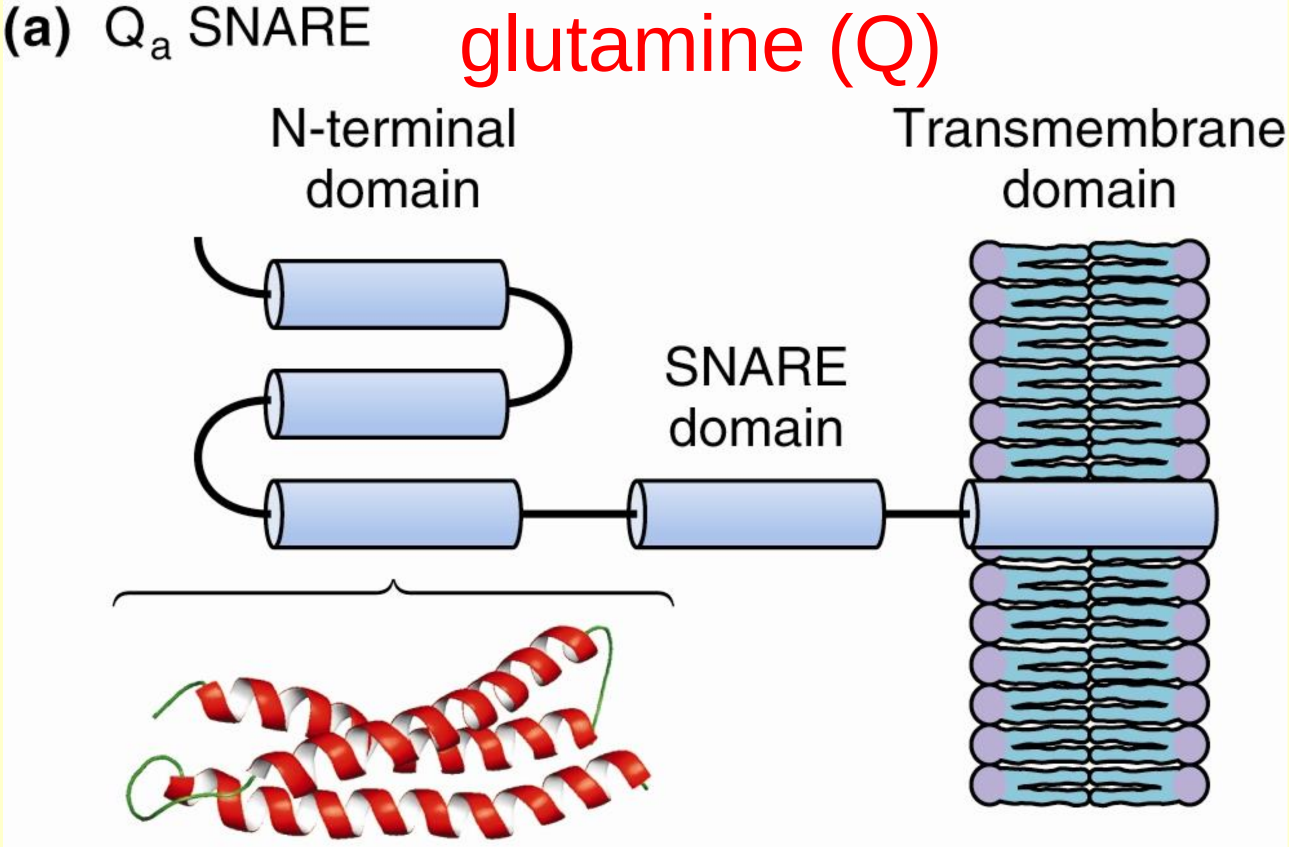


Figure 9.35 The domain structure of the SNARE protein families. A variety of N-terminal domains are found in Q_a SNARE proteins, including the three-helix bundle of syntaxin-1.

Vesicle Formation and Fusion Are Essential Membrane Processes

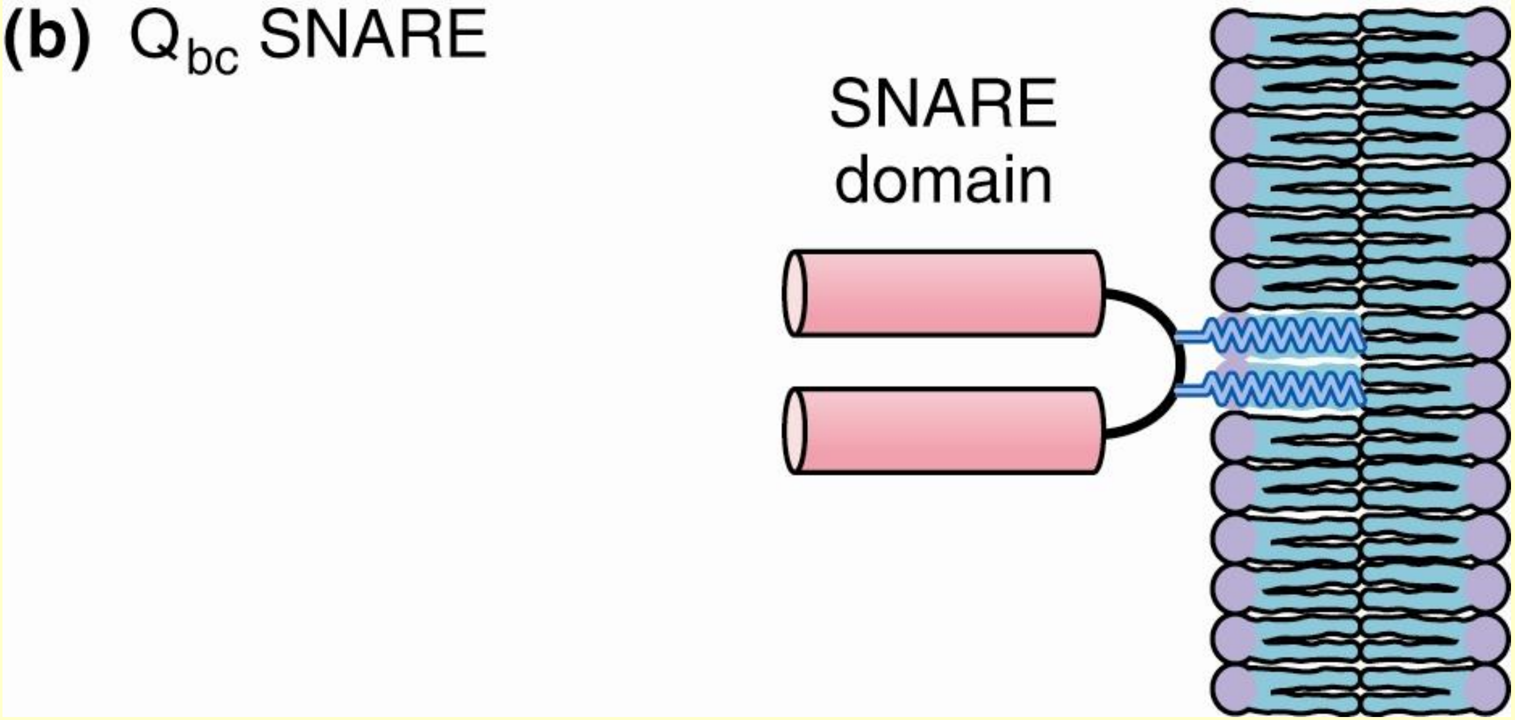
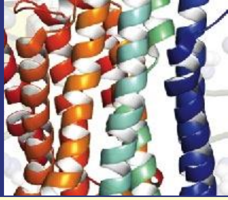


Figure 9.35 Q_{bc} SNAREs are anchored in the membrane by palmitic acid lipid anchors

Vesicle Formation and Fusion Are Essential Membrane Processes



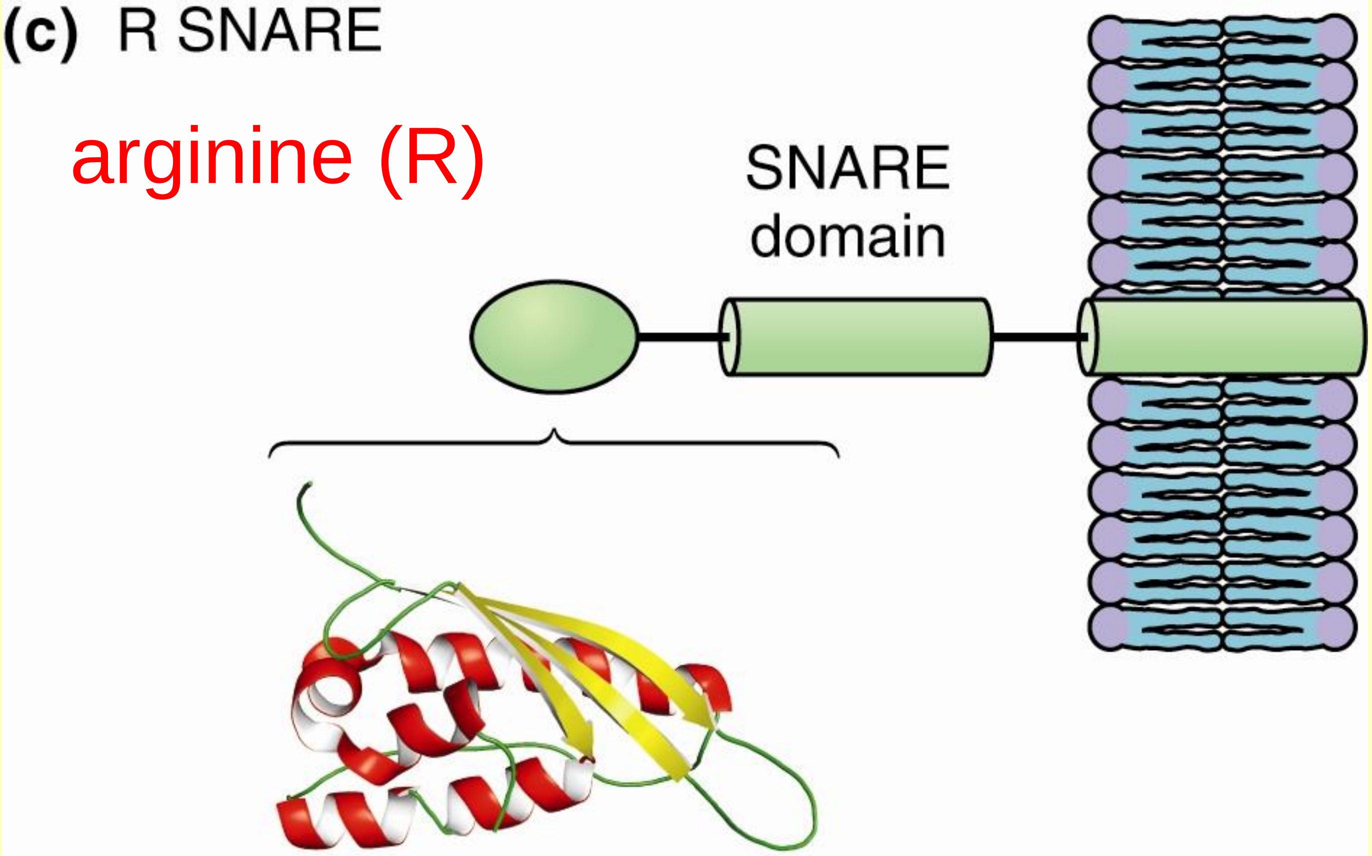
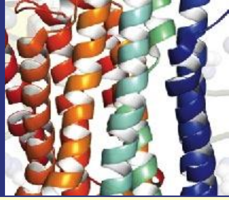


Figure 9.35 Many R SNAREs contain small globular N-terminal domains such as Vam7, a PX-homology domain.

The Fusion of Vesicles with the Plasma Membrane is Directed by SNAREs



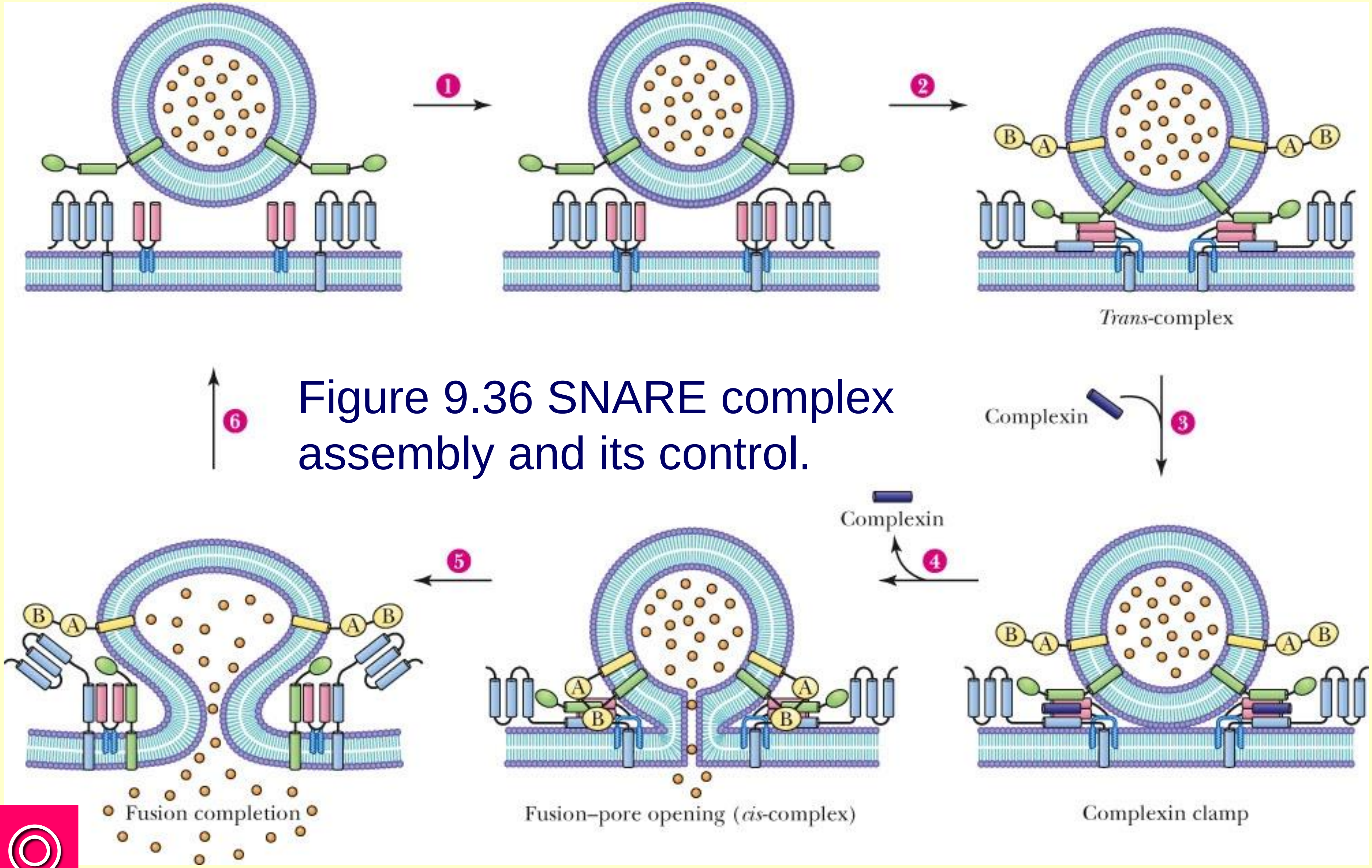
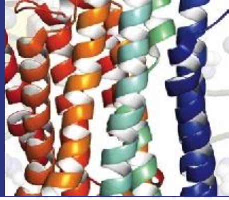


Figure 9.36 SNARE complex assembly and its control.

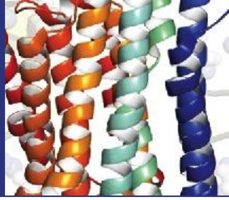
9.6 What is Passive Diffusion?



No special proteins needed

- Transported species simply moves down its concentration gradient - from high [c] to low [c]
- Be able to use Eq. 9.1 and 9.2
- High permeability coefficients usually mean that passive diffusion is not the whole story

9.7 How Does Facilitated Diffusion Occur?



ΔG negative, but proteins assist

- Solutes only move in the thermodynamically favored direction
- But proteins may "facilitate" transport, increasing the rates of transport
- Understand plots in Figure 9.39
- Two important distinguishing features:
  - solute flows only in the favored direction
  - transport displays saturation kinetics

9.7 How Does Facilitated Diffusion Occur?

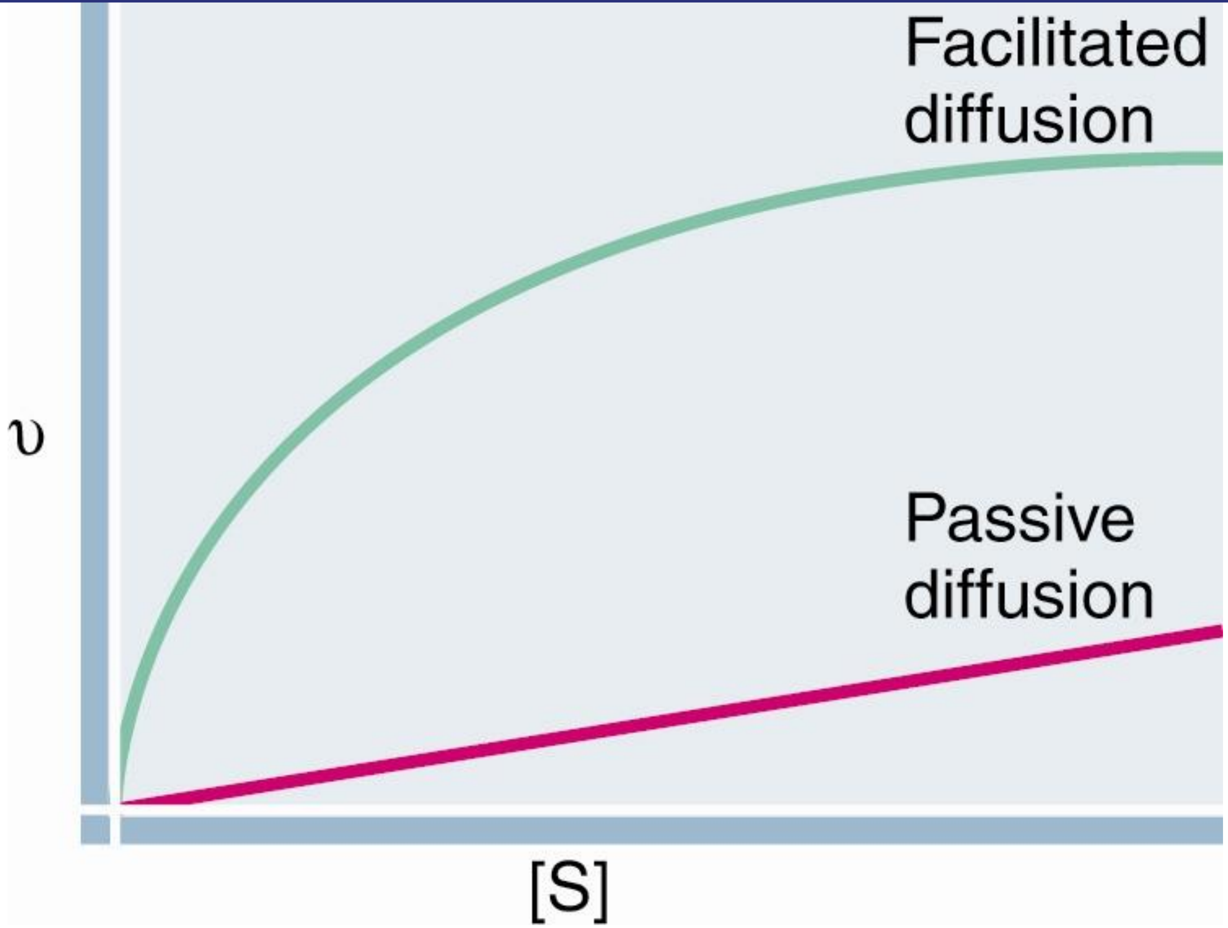
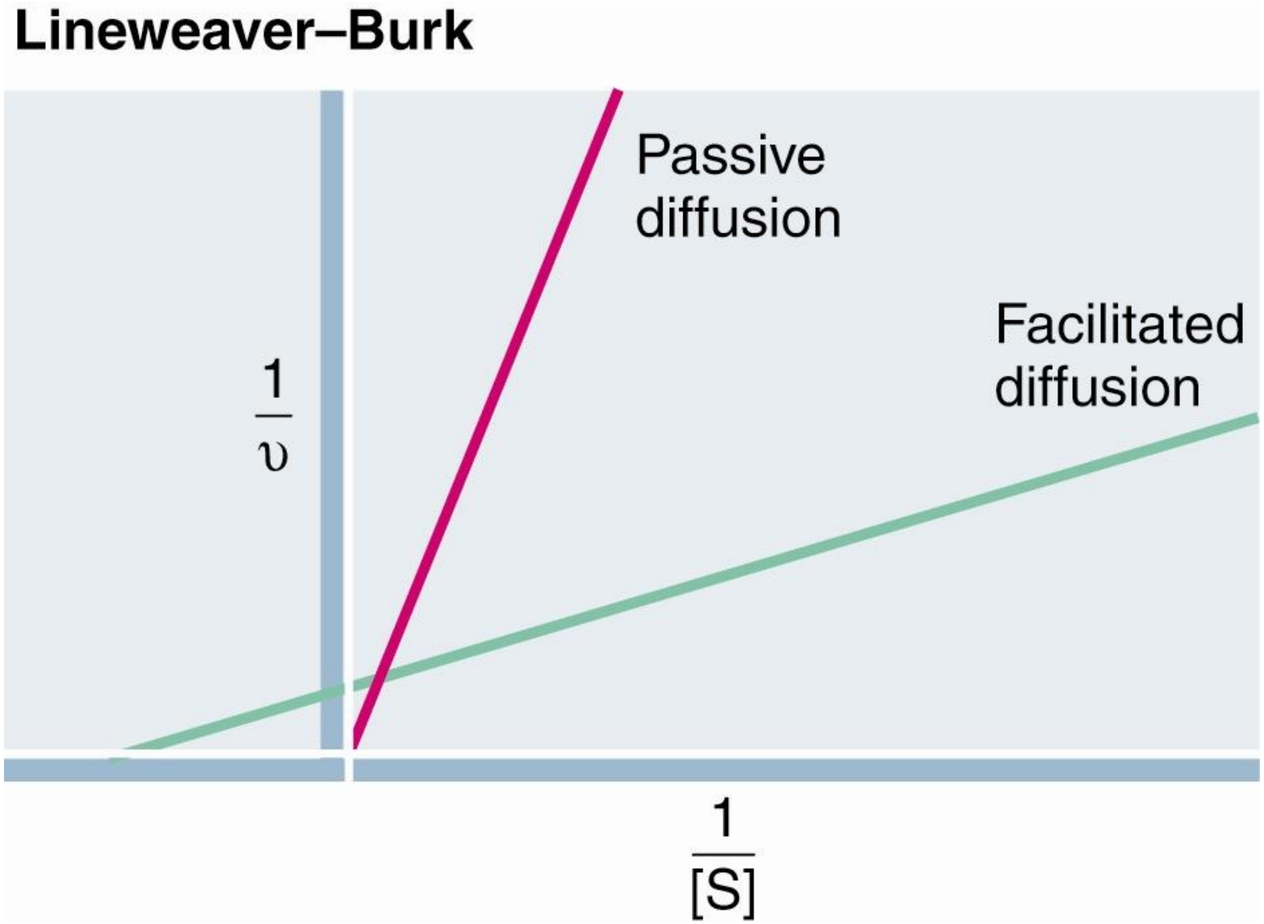


Figure 9.39 Passive diffusion and facilitated diffusion may be distinguished graphically. The plots for facilitated diffusion are similar to plots of enzyme-catalyzed processes, and they display saturation behavior.

9.7 How Does Facilitated Diffusion Occur?



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9.7 How Does Facilitated Diffusion Occur?

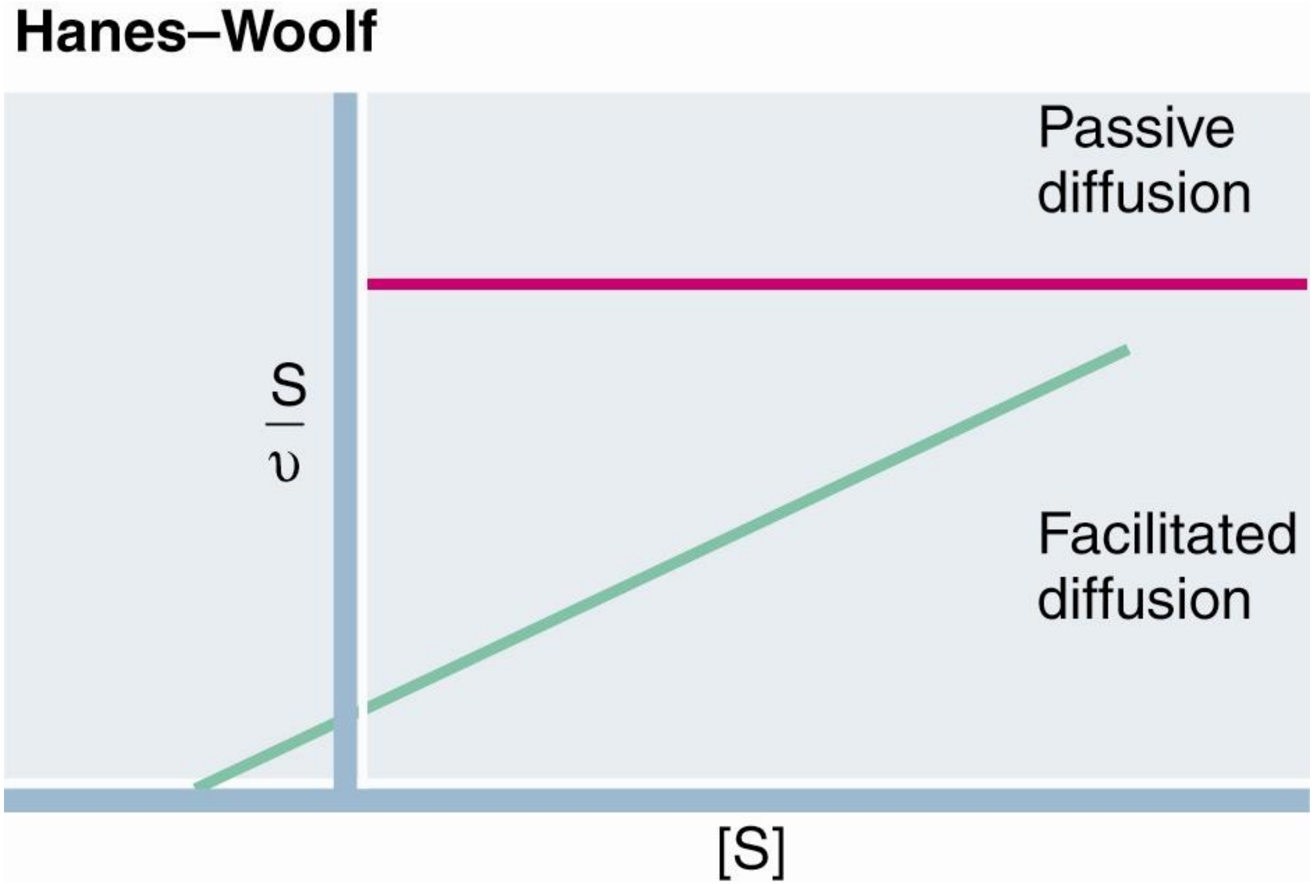


Figure 9.39 Passive diffusion and facilitated diffusion may be distinguished graphically. The plots for facilitated diffusion are similar to plots of enzyme-catalyzed processes, and they display saturation behavior.

Membrane Channel Proteins Facilitate Diffusion of Various Solutes

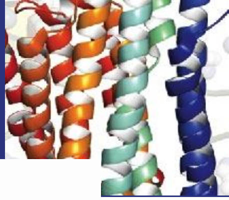
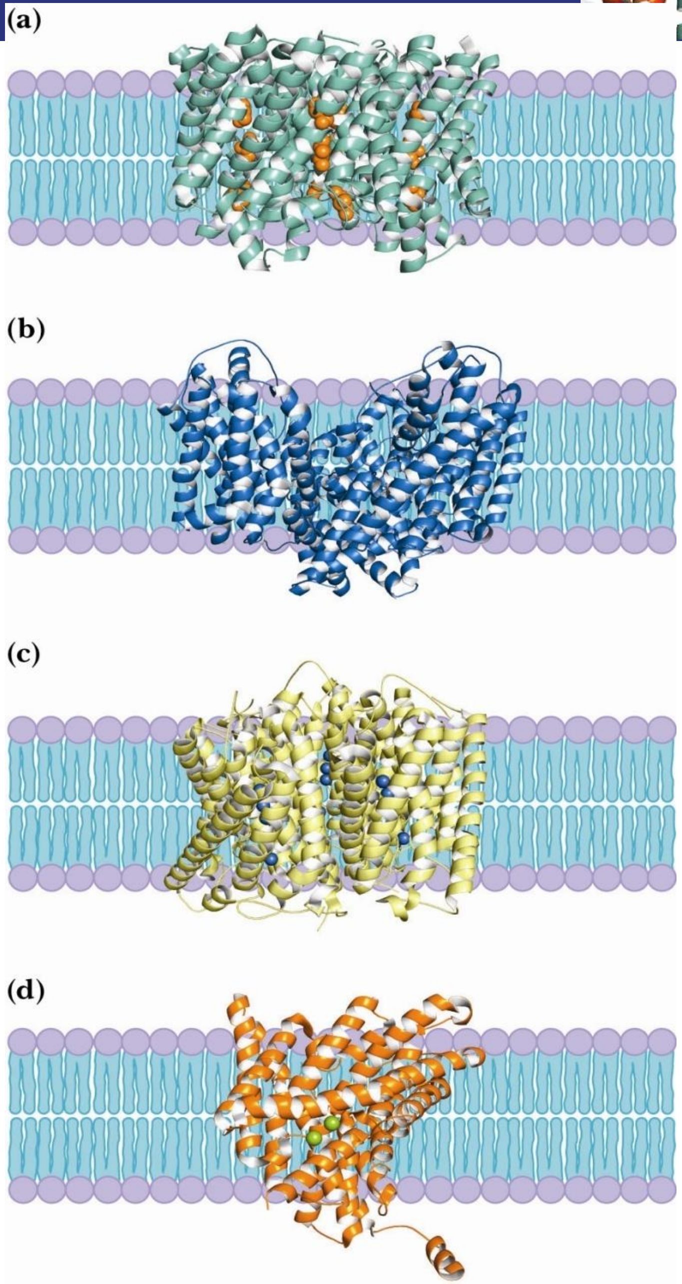


Figure 9.40 The structures of channel proteins that transport:

a) Glycerol
b) Glutamate
c) Ammonia
d) Chloride



Membrane Channel Proteins Facilitate Diffusion of Various Solutes

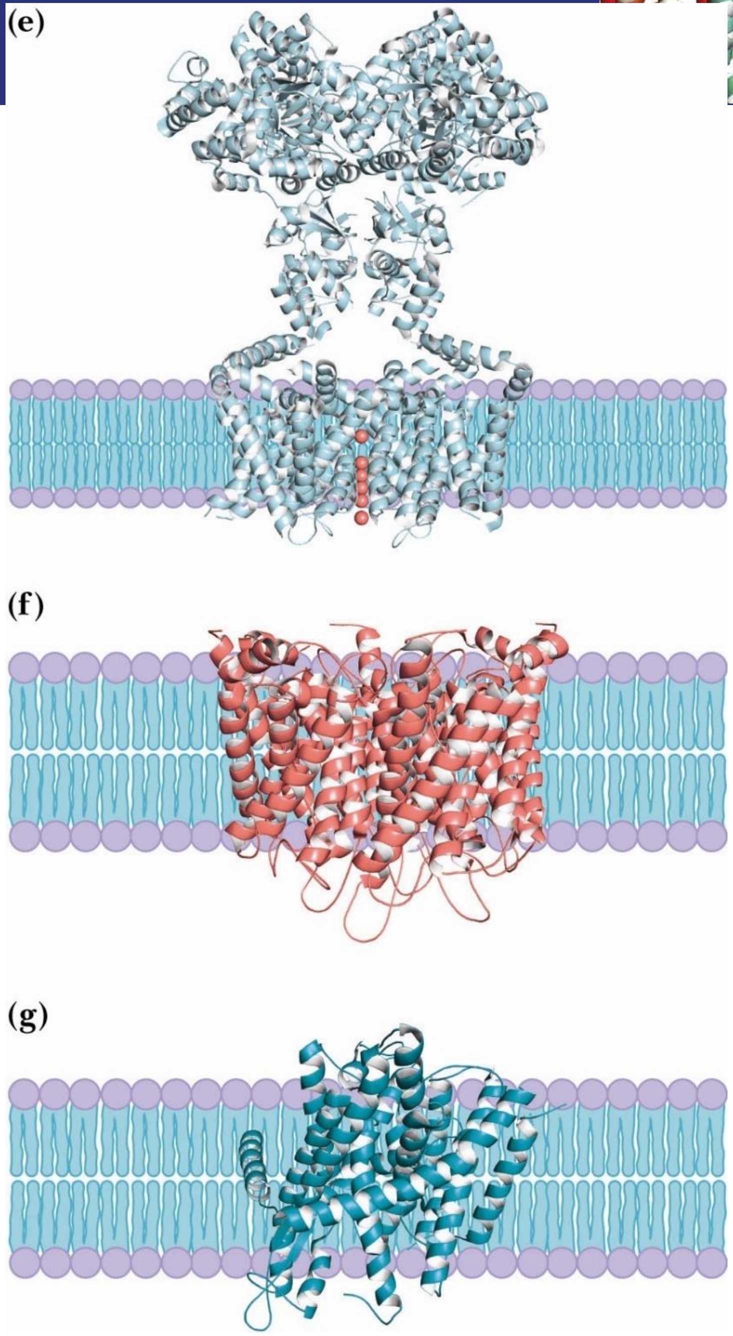


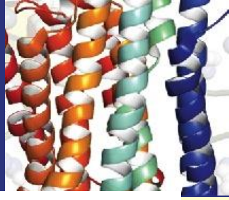
Figure 9.40 The structures of channel proteins that transport:

e) Potassium

f) Water

g) Proteins

Membrane Channels Can be Formed in a Variety of Ways



Single channel pores can be formed from dimers, trimers, tetramers or pentamers of protein subunits.

Multimeric assemblies in which each subunit has its own pore are known.

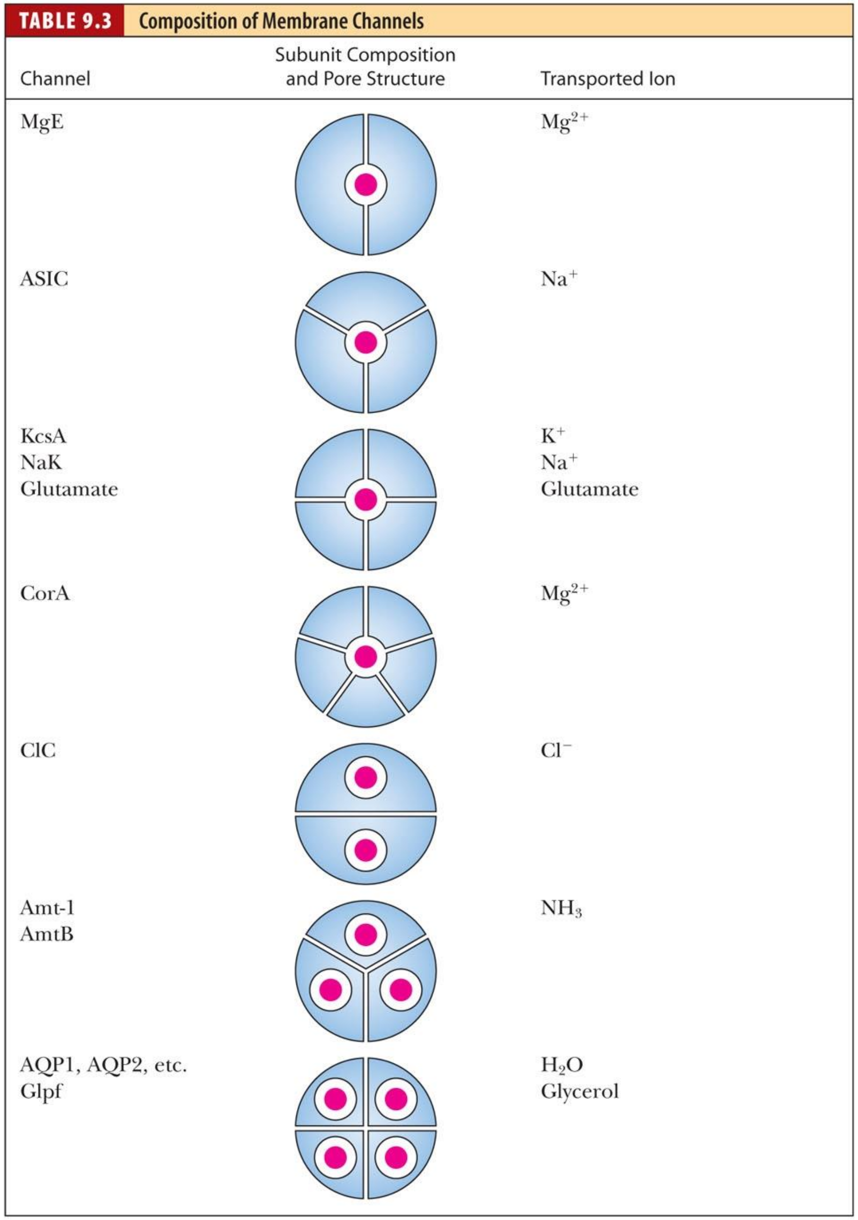


TABLE 9.3 Composition of Membrane Channels

Channel	Subunit Composition and Pore Structure	Transported Ion
MgE		Mg^{2+}
ASIC		Na^+
KcsA NaK Glutamate		K^+ Na^+ Glutamate
CorA		Mg^{2+}
ClC		Cl^-
Amt-1 AmtB		NH_3
AQP1, AQP2, etc. Glpf		H_2O Glycerol

Potassium Channels Combine High Selectivity with High Conduction Rates

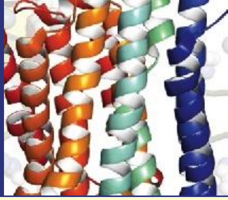
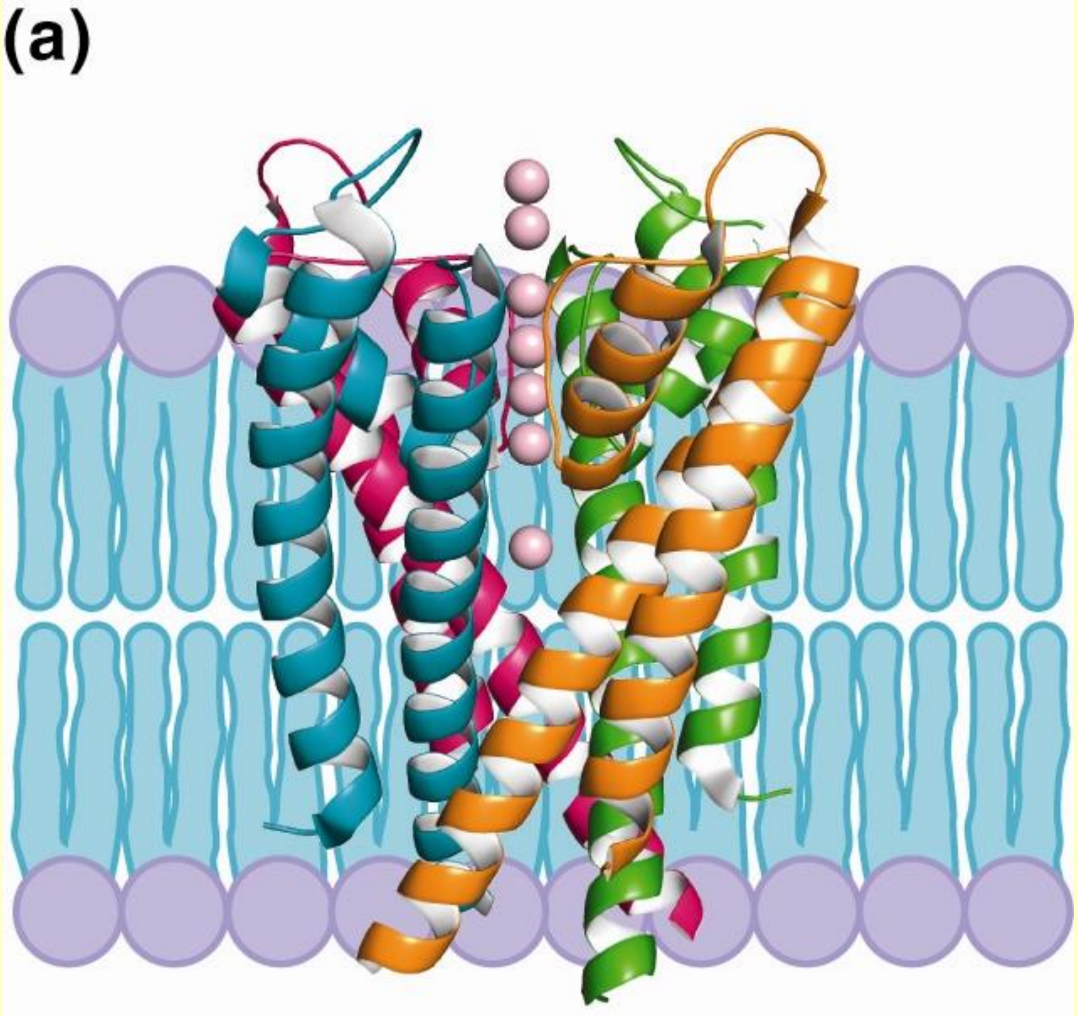
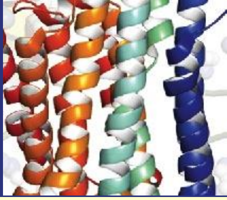


Figure 9.41 Structure of the KcsA potassium channel from *S. lividans*.

The four identical subunits of the channel, which surround a central pore, are shown in different colors.



Potassium Channels Combine High Selectivity with High Conduction Rates



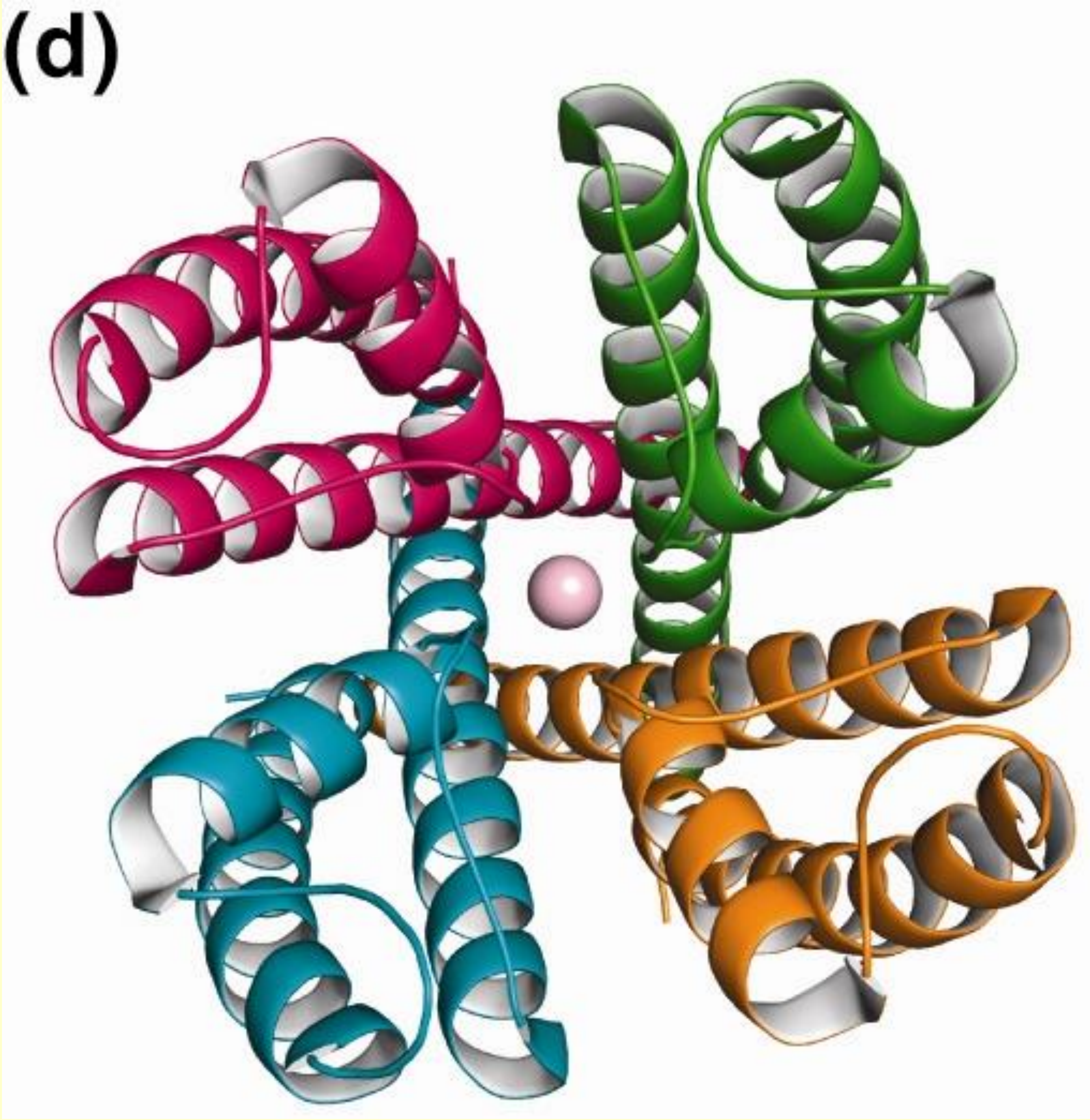
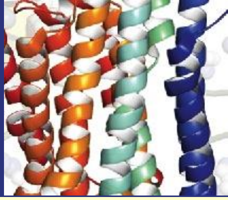


Figure 9.41 Structure of the KcsA potassium channel from *S. lividans*.

The tetrameric channel, as viewed through the pore.

Potassium Channels Combine High Selectivity with High Conduction Rates



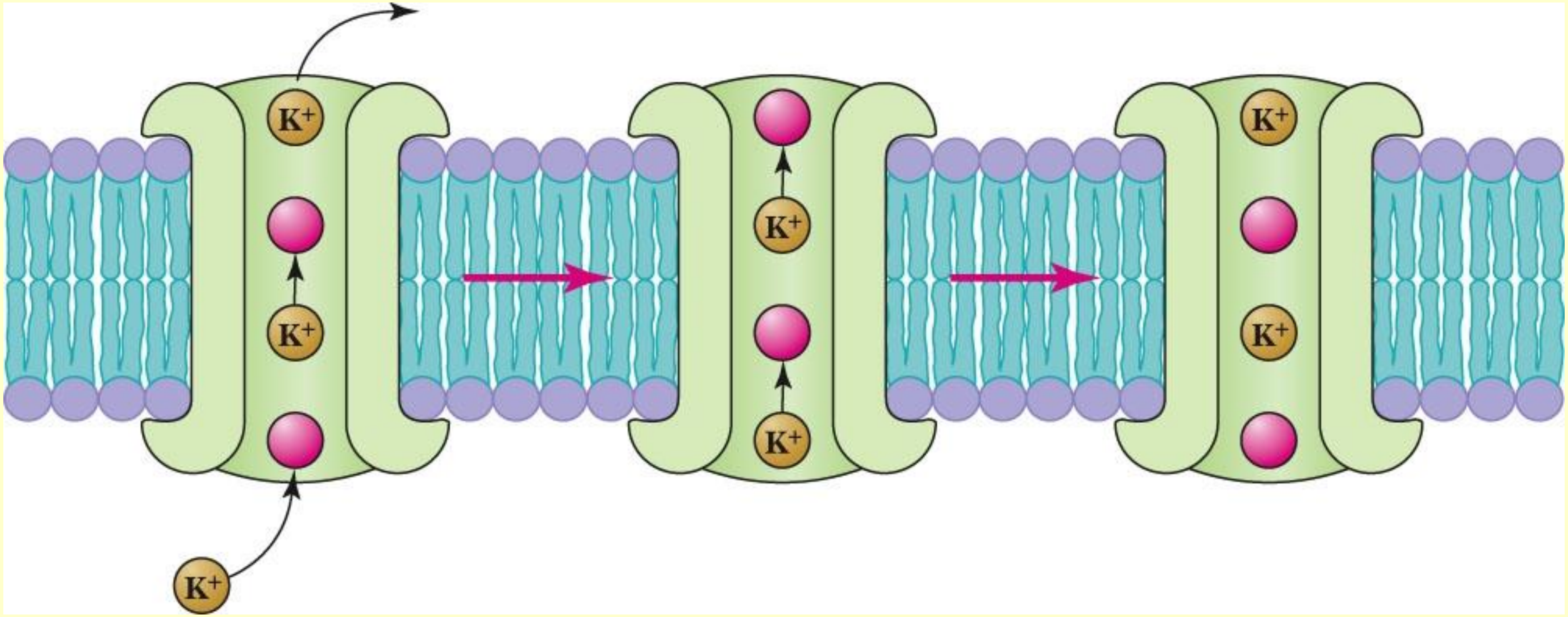
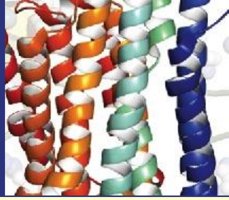


Figure 9.42 Model for outward and inward transport through the KcsA potassium channel. The selectivity filter in the channel contains four K^+-binding sites, only two of which are filled at any time.

The KcsA channel is gated by intracellular pH. It is closed at neutral pH, open at low pH.



Helix bending and rearrangement deep in the membrane opens K$^+$ channels.

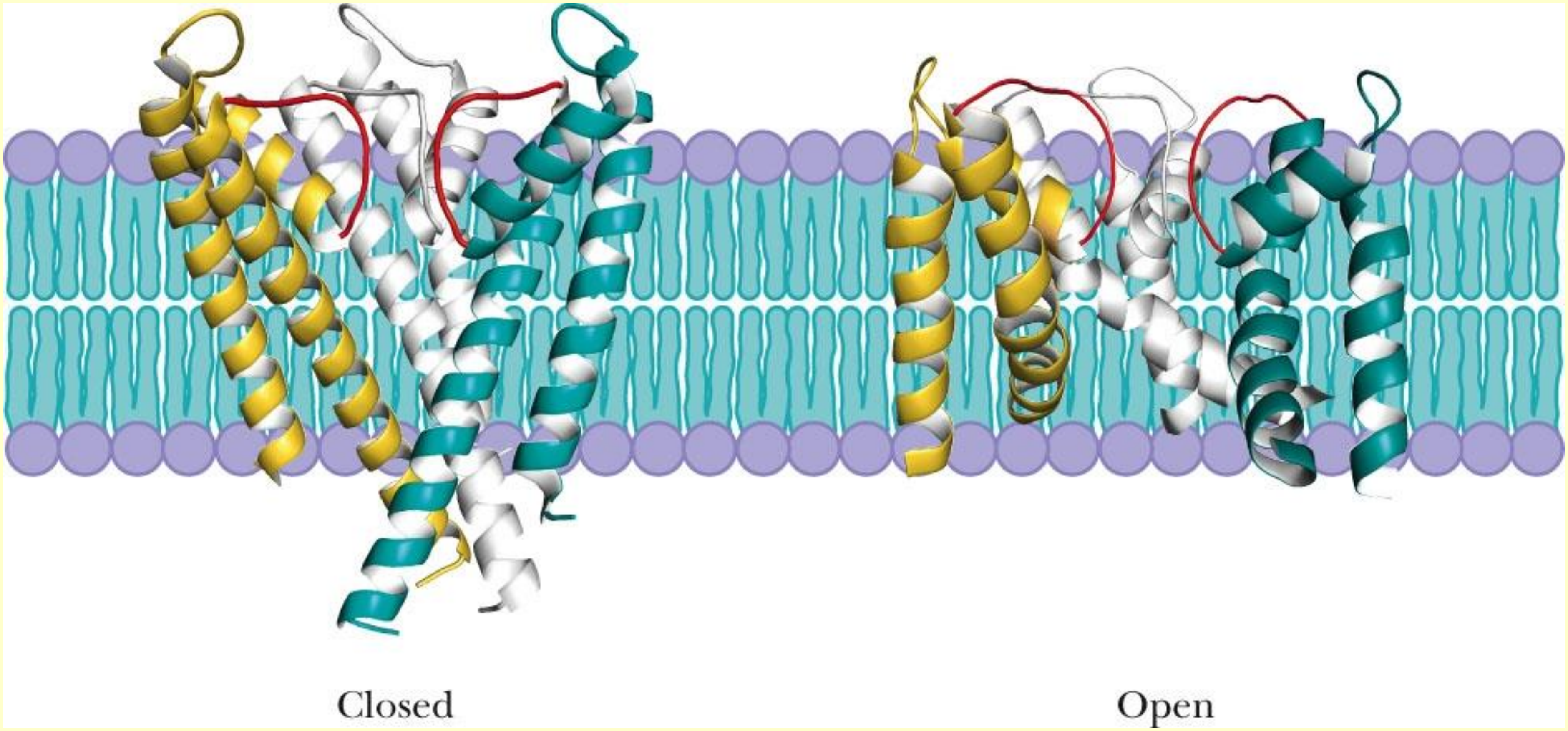
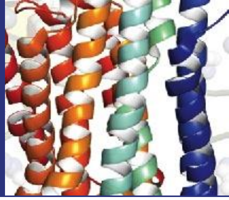


Figure 9.43 The closed and open states of the potassium channel.

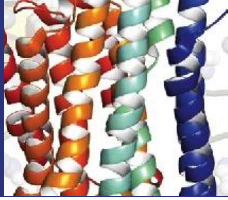
9.8 How Does Energy Input Drive Active Transport Processes?



Energy input drives transport

- Some transport must occur such that solutes flow against their thermodynamic potential
- Energy input drives such transport
- Energy source and transport machinery are "coupled"
- Energy source may be ATP, light or a concentration gradient

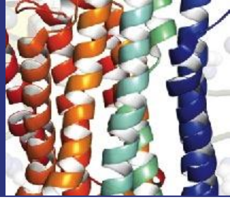
The Sodium Pump



aka Na,K-ATPase

- Large protein - 120 kD and 35 kD subunits
- Maintains intracellular Na^+ low and K^+ high
- Crucial for all organs, but especially for neural tissue and the brain
- ATP hydrolysis drives Na^+ out and K^+ in
- Alpha subunit has ten transmembrane helices with large cytoplasmic domain

Na$^+$,K$^+$-ATPase Uses ATP Energy to Drive Sodium and Potassium Transport



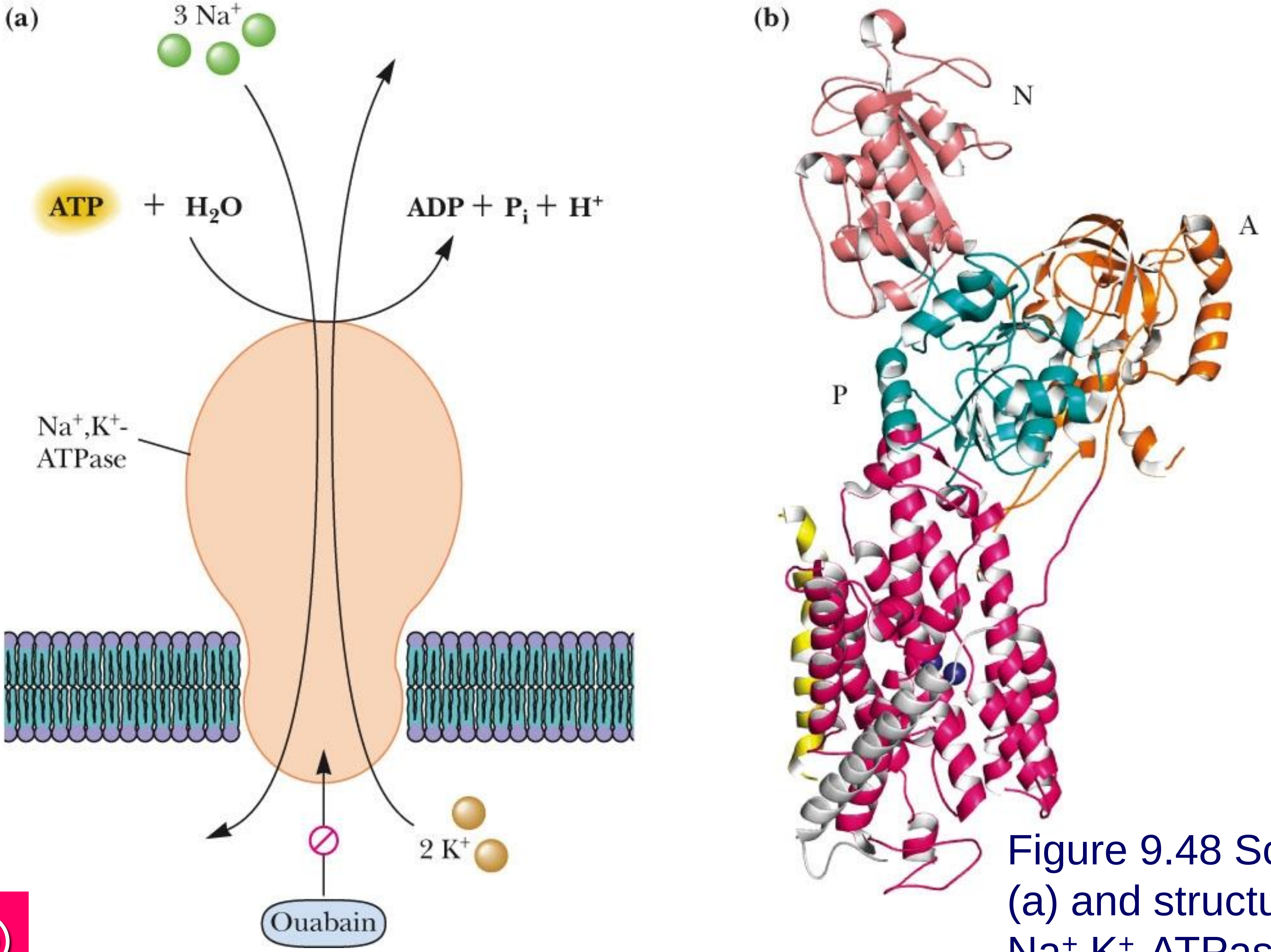
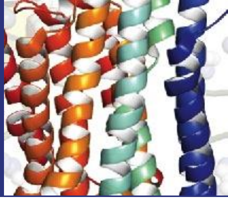


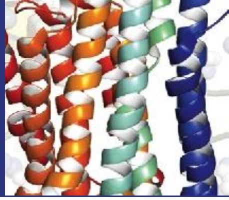
Figure 9.48 Schematic (a) and structure (b) of Na$^+$,K$^+$-ATPase

Na,K Transport



- Hypertension in humans involves apparent inhibition of sodium pump
- Inhibition in cells lining blood vessel walls results in Na^+ and Ca^{2+} accumulation by the cells and narrowing of the vessels to create hypertension.
- Studies show this inhibitor – the hypertensive agent - to be ouabain!

Na^+,K^+-ATPase is Inhibited by Cardiotonic Steroids



毛地黄毒苷配基

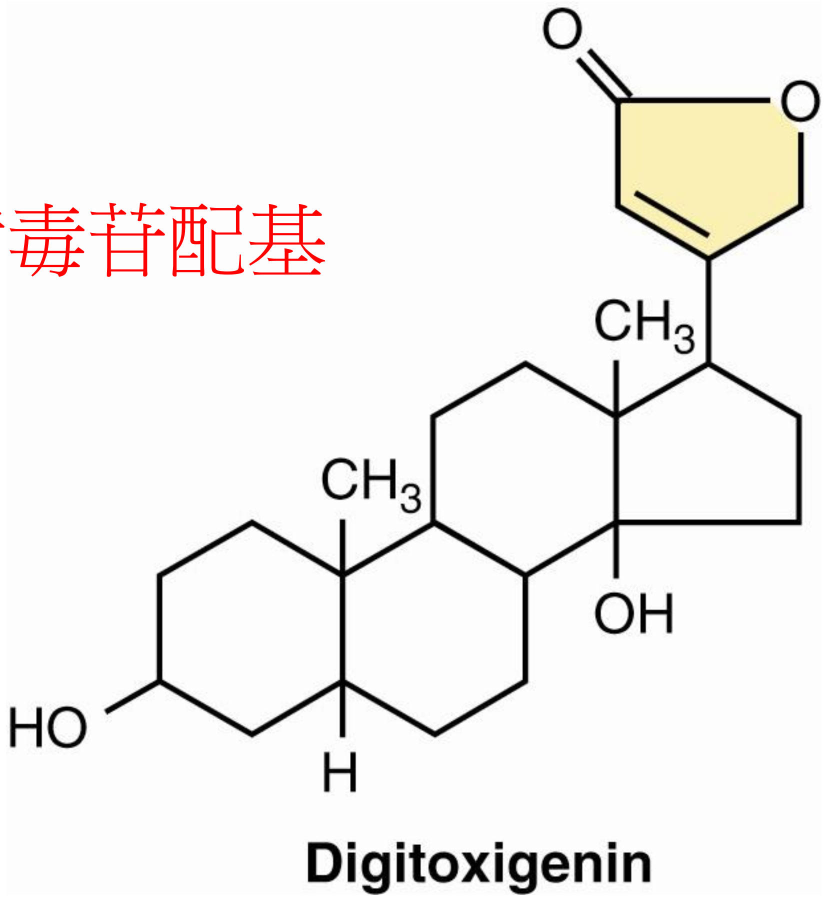
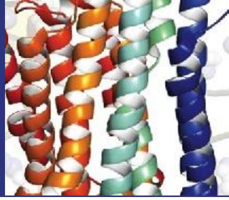
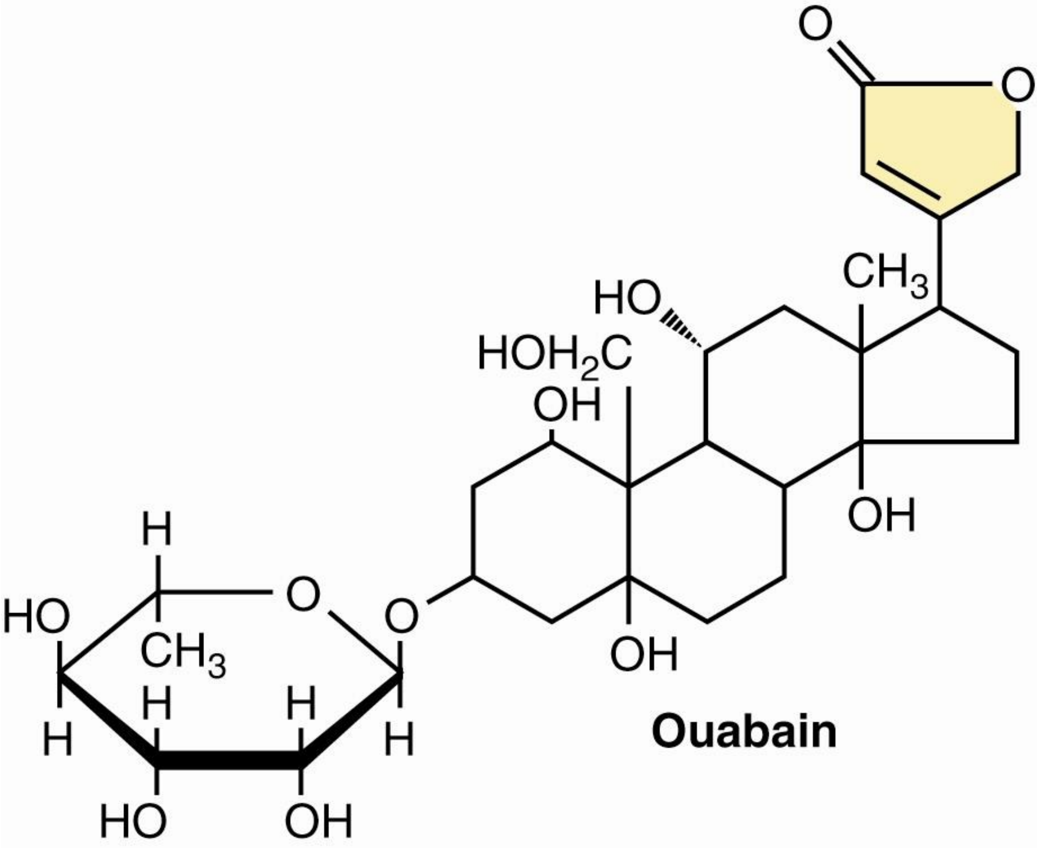


Figure 9.50 The structure of digitoxigenin, one of the class of cardiotonic steroids. With a sugar esterified at C-3, the molecule is referred to as a “cardiac glycoside”.

Na^+,K^+-ATPase is Inhibited by Cardiotonic Steroids





Ouabain

Figure 9.50 The structure of ouabain, one of the class of cardiotonic steroids. With a sugar esterified at C-3, the molecule is referred to as a “cardiac glycoside”.

Cardiac glycosides occur in several plants

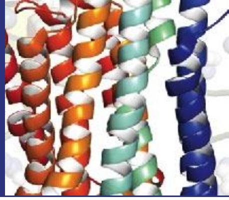
The colorful oleander shrub contains toxic cardiac glycosides. Cardiac glycosides are produced by a number of plants, including foxglove, lily of the valley, milkweed (a favorite food of monarch butterflies), and oleander.

(a) Oleander

Monarch Butterflies Are Toxic to Birds, Due to the Cardiac Glycosides in Their Exoskeleton

(b) Monarch butterfly

Calcium Transport Is Accomplished in the Sarcoplasmic Reticulum by Ca^{2+}-ATPase



A process akin to Na,K transport

- Calcium levels in resting muscle cytoplasm are maintained low by Ca^{2+}-ATPase - a Ca^{2+} pump
- Calcium is pumped into the sarcoplasmic reticulum (SR) by a 110 kD protein that is very similar to the alpha subunit of Na,K-ATPase
- Aspartyl phosphate E-P intermediate is at Asp-351 and Ca^{2+}-pump also fits the E_1-E_2 model

Calcium Transport Is Accomplished in the Sarcoplasmic Reticulum by Ca^{2+}-ATPase

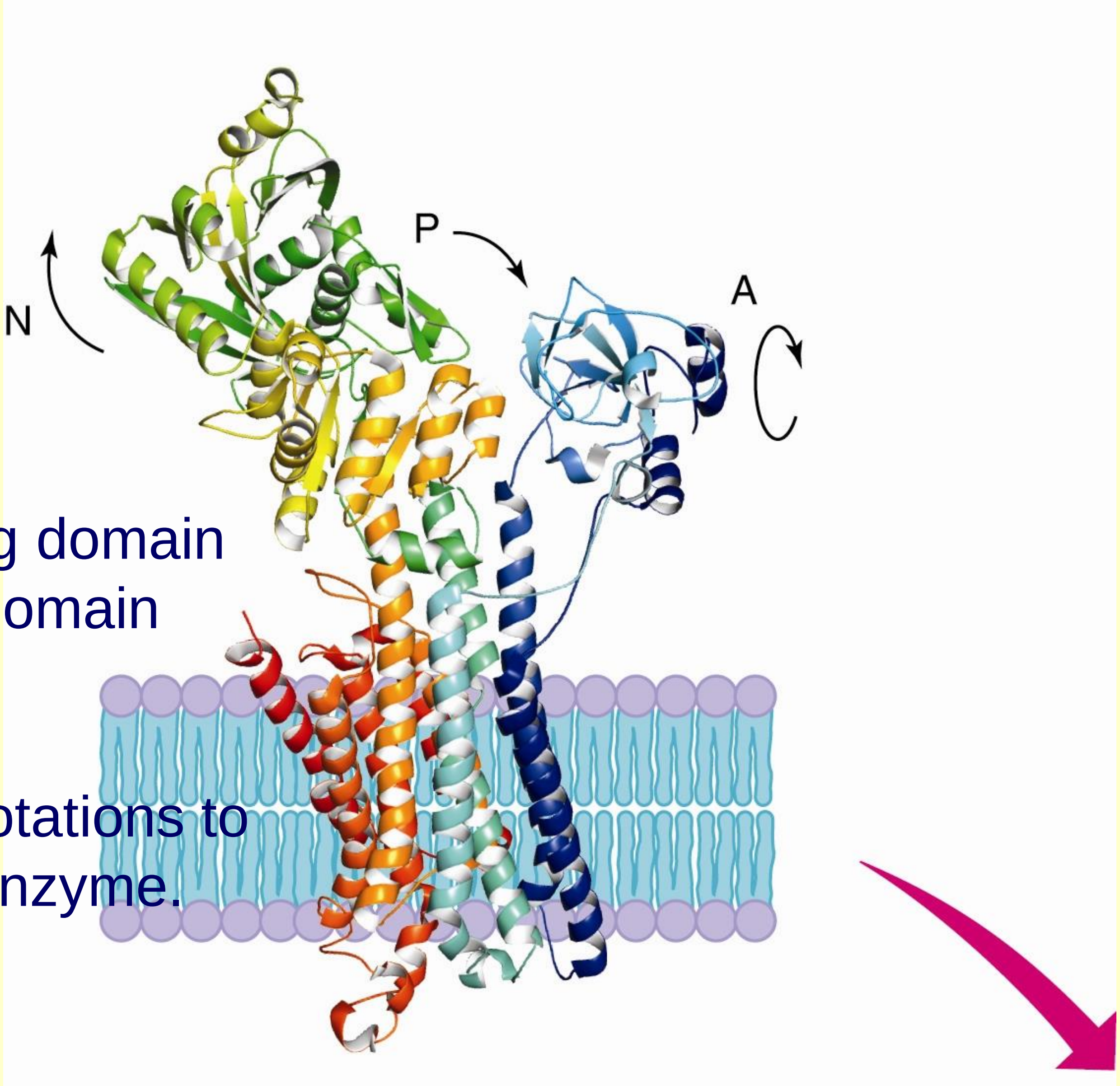
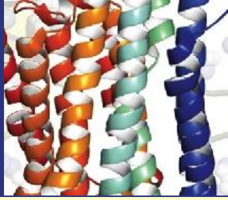


Figure 9.51 This structure corresponds to the $E_1 \cdot 2Ca^{2+}$ state of the Ca^{2+}-ATPase.

N = nucleotide-binding domain
P = phosphorylation domain
A = actuator domain

The arrows indicate rotations to the next state of the enzyme.

Calcium Transport Is Accomplished in the Sarcoplasmic Reticulum by Ca^{2+}-ATPase



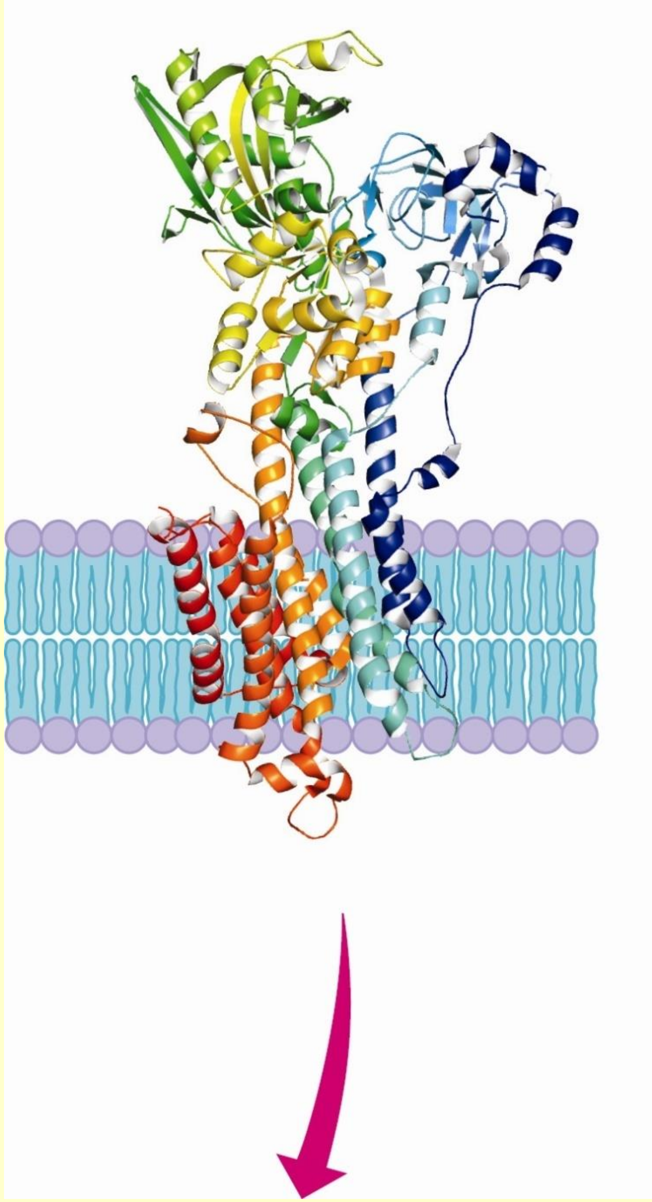


Figure 9.51 This structure corresponds to the $E_1 \cdot ATP$ state of the Ca^{2+}-ATPase.

Calcium Transport Is Accomplished in the Sarcoplasmic Reticulum by Ca^{2+}-ATPase

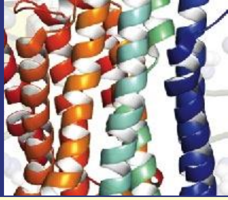
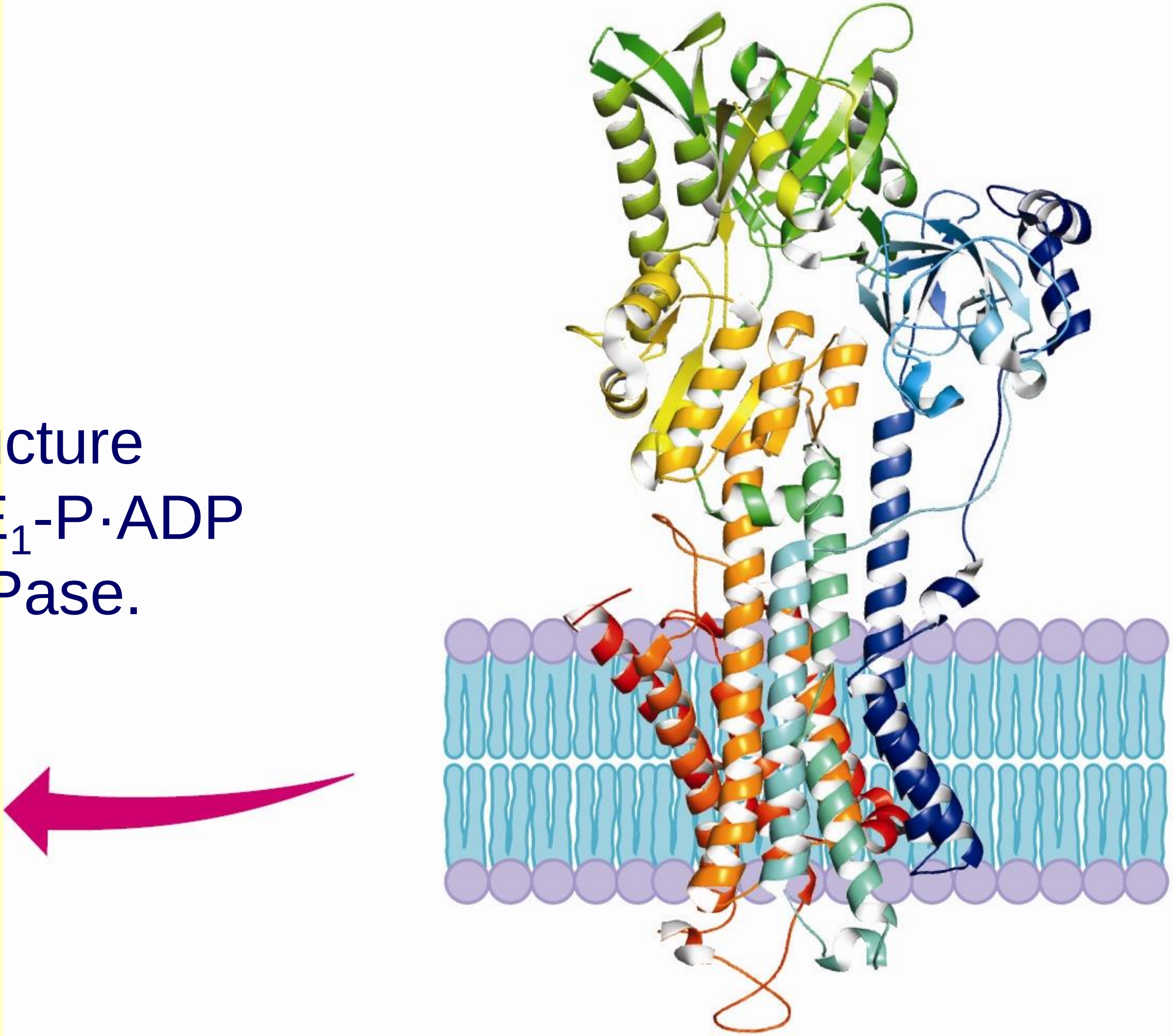
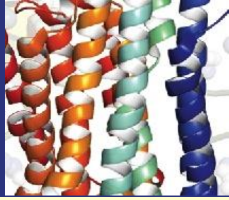


Figure 9.51 This structure corresponds to the E_1-P·ADP state of the Ca^{2+}-ATPase.



Calcium Transport Is Accomplished in the Sarcoplasmic Reticulum by Ca^{2+}-ATPase



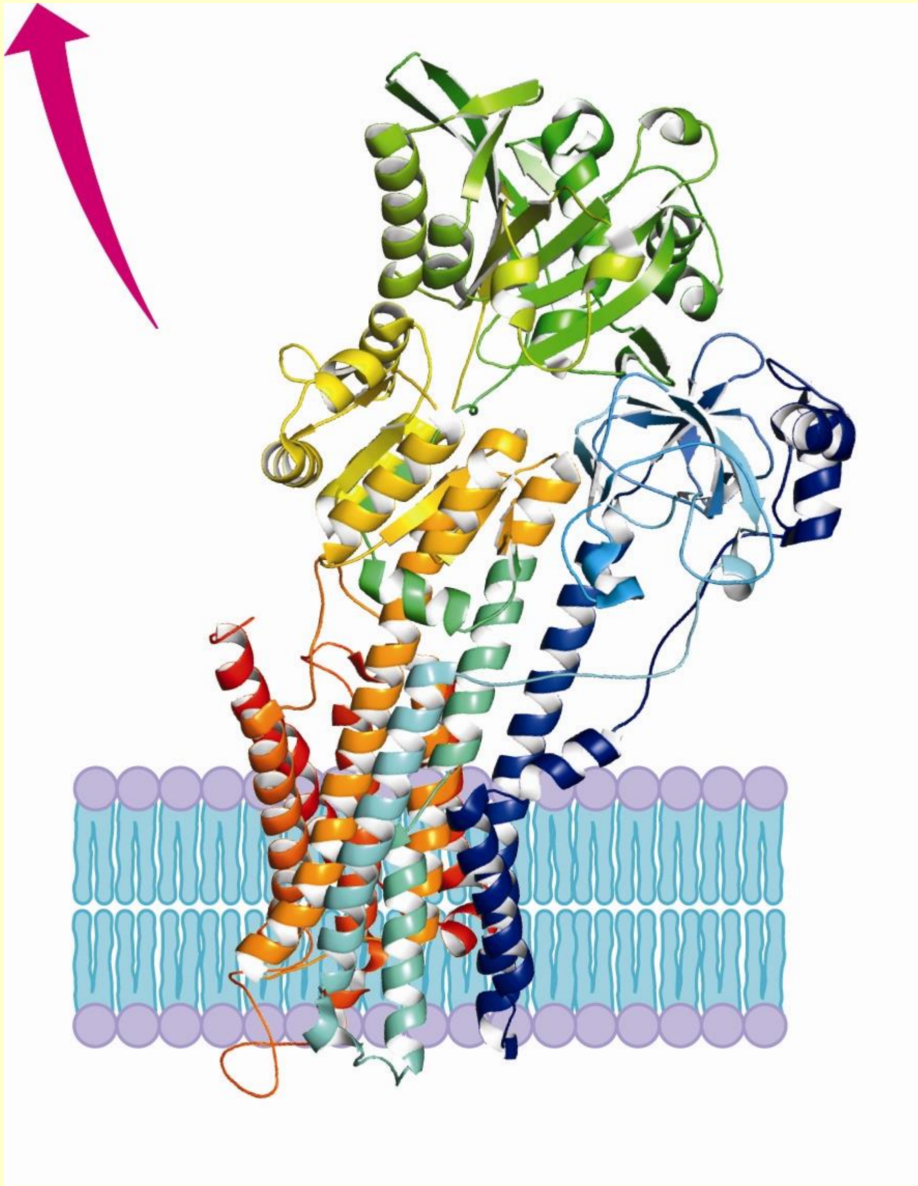
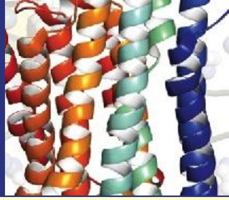


Figure 9.51 This structure corresponds to the $E_2 \cdot P_i$ state of the Ca^{2+}-ATPase.

Calcium Transport Is Accomplished in the Sarcoplasmic Reticulum by Ca^{2+}-ATPase



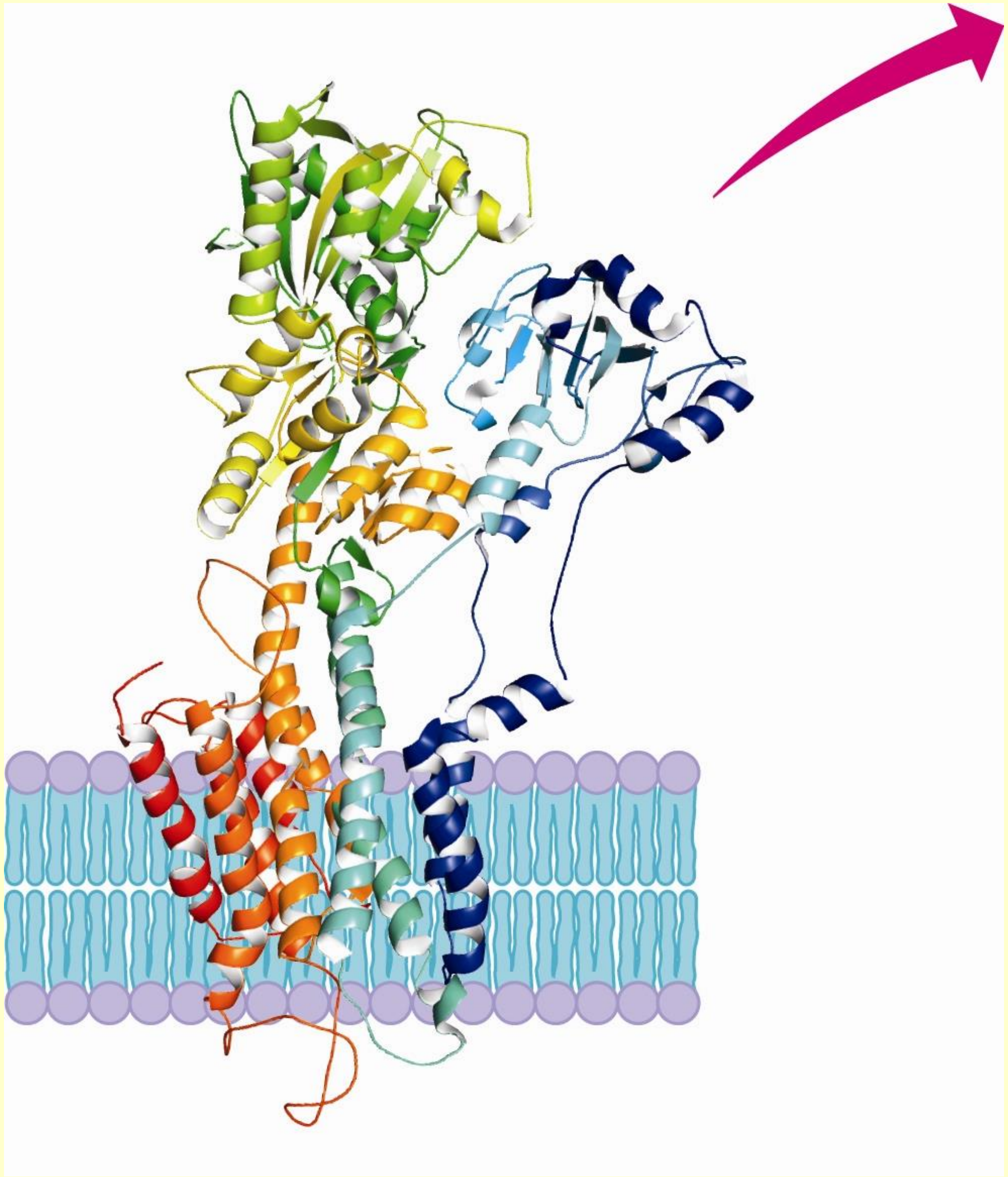
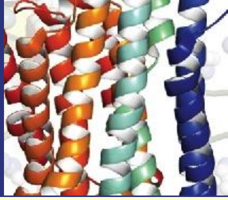


Figure 9.51 This structure corresponds to the E_2 state of the Ca^{2+}-ATPase.

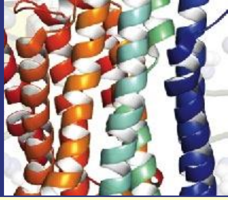
The Gastric H^+,K^+-ATPase



The enzyme that keeps the stomach at pH 0.8

- The parietal cells of the gastric mucosa (lining of the stomach) have an internal pH of 7.4
- H^+,K^+-ATPase pumps protons from these cells into the stomach to maintain a pH difference across a single plasma membrane of 6.6!
- This is the largest known transmembrane gradient in eukaryotic cells

The Gastric H^+,K^+ -ATPase Maintains the Low pH of the Stomach



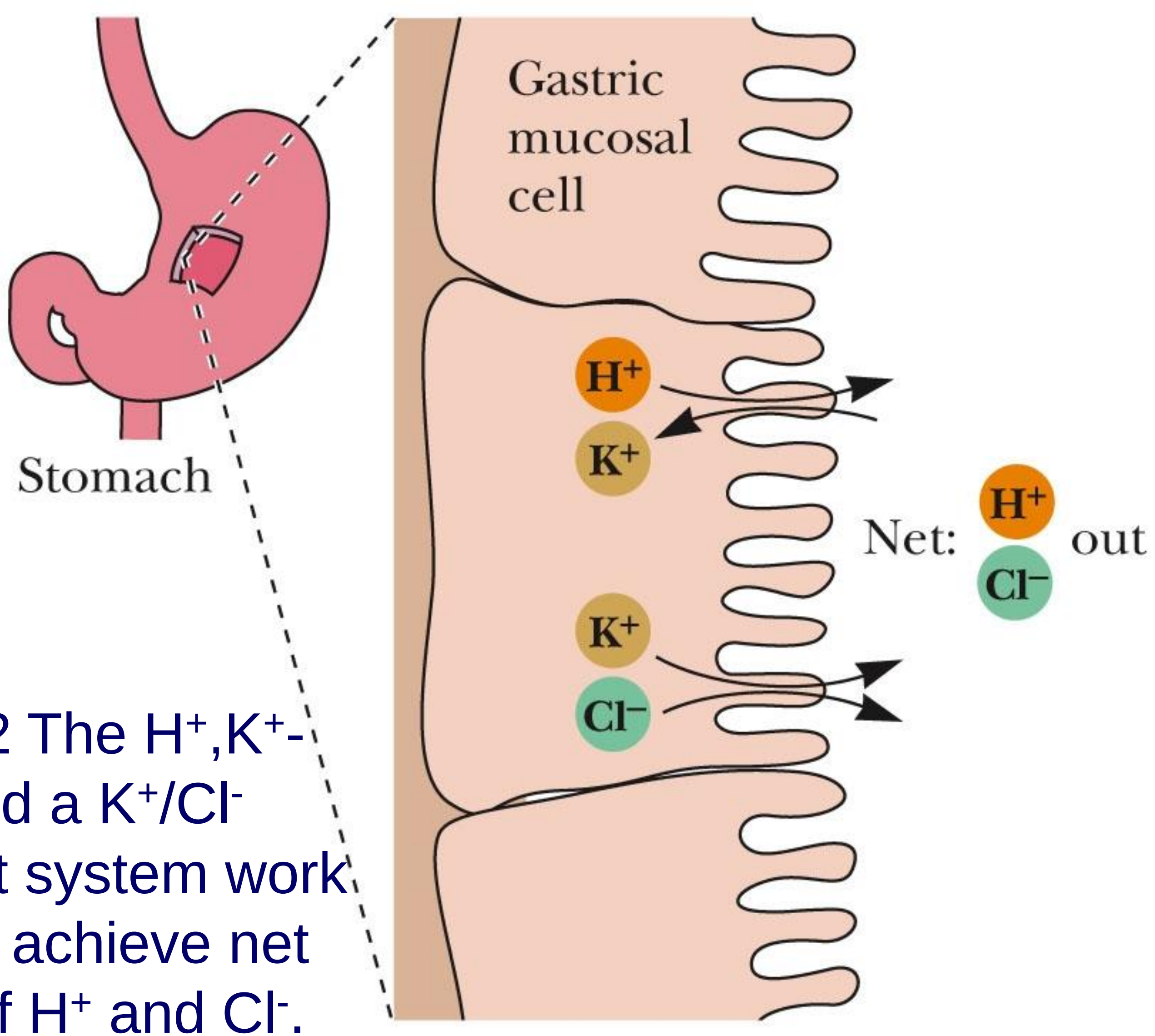
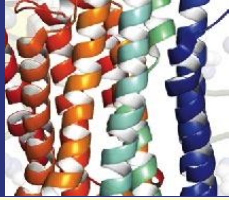


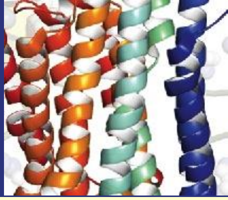
Figure 9.52 The H^+,K^+-ATPase and a K^+/Cl^- cotransport system work together to achieve net transport of H^+ and Cl^-.

The Gastric H^+,K^+-ATPase



- H^+,K^+-ATPase is similar in many respects to Na^+,K^+-ATPase and Ca^{2+}-ATPase
- All three enzymes form covalent E-P intermediates
- All three have similar sequences for the large (α) subunit

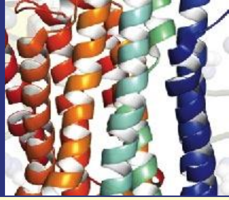
Bone Remodeling by Osteoclast Proton Pumps



How your body takes your bones apart:

- Bone material undergoes ongoing remodeling
  - osteoclasts tear down bone tissue
  - osteoblasts build it back up
- Osteoclasts function by secreting acid into the space between the osteoclast membrane and the bone surface - acid dissolves the Ca^{2+}-phosphate matrix of the bone
- An ATP-driven proton pump in the membrane does this

Bone Remodeling by Osteoclast Proton Pumps



- Vacuolar (V-type) ATPases are found in vacuoles, lysosomes, endosomes and other organelles
- A vacuolar ATPase is present in osteoclasts (multinucleate cells that break down bone during normal bone remodeling)
- This osteoclast ATPase secretes acid (protons) into the space between the bone surface and the cell
- This transport of protons out of the osteoclast lowers the pH of the extracellular space near the bone to about 4, dissolving the crystalline hydroxyapatite lattice of the bone

Bone Remodeling by Osteoclast Proton Pumps

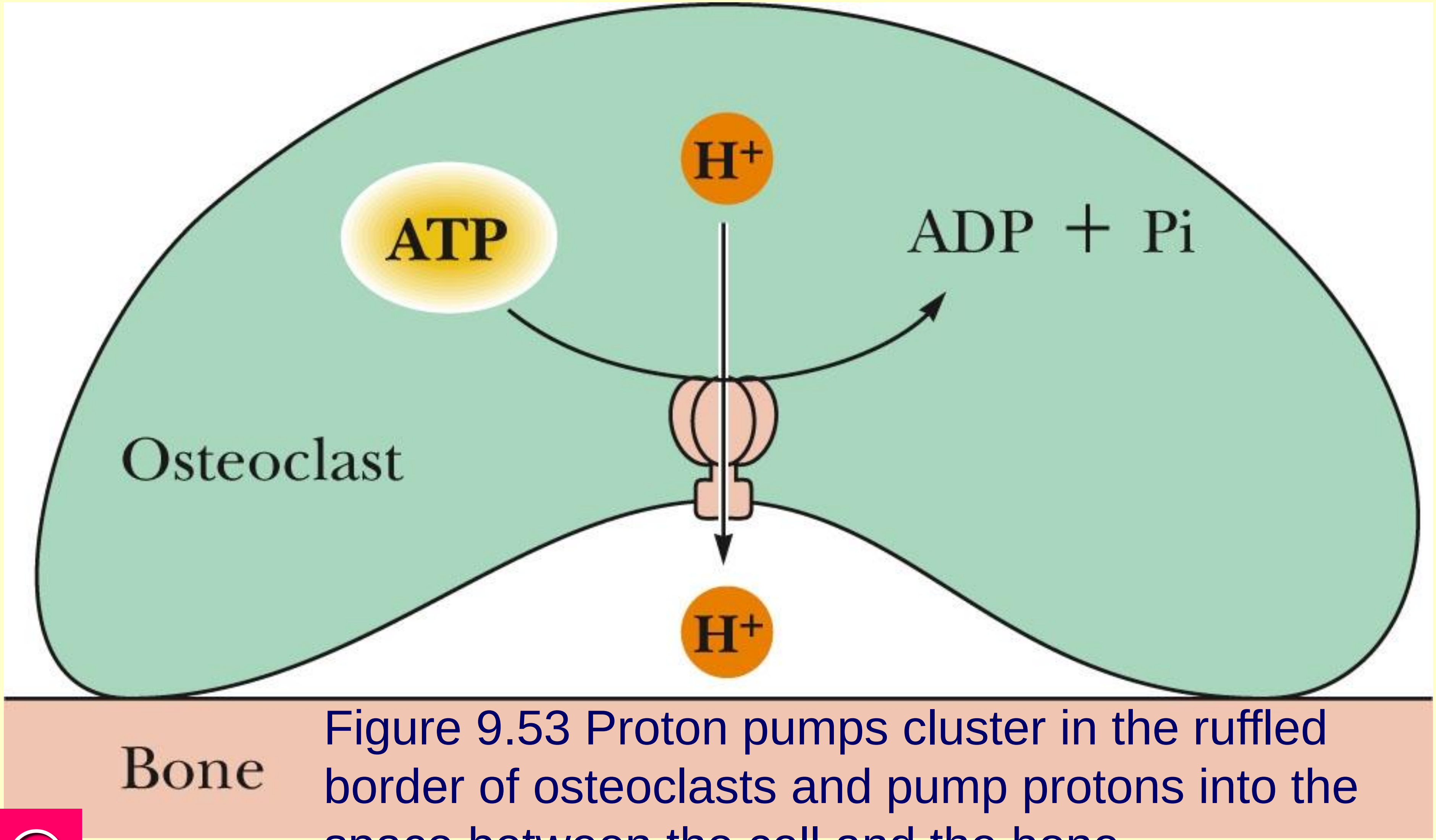
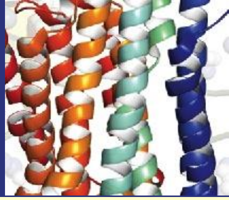


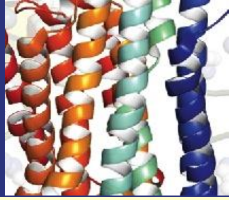
Figure 9.53 Proton pumps cluster in the ruffled border of osteoclasts and pump protons into the space between the cell and the bone.

ABC Transporters Use ATP to Drive Import and Export Functions and Multidrug Resistance



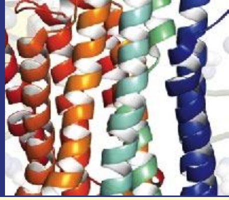
- Cells “clean house” with membrane transporters known as **multidrug resistance (MDR)** pumps
- MDR pumps are designed to recognize foreign organic molecules in cells and pump them out
- In bacteria, these pumps use the hydrolytic energy of ATP to import nutrients into the cell
- At least five families of influx and efflux pumps are known
- Among these are the **ABC transporters,** which export therapeutic drugs from cancer cells

ABC Transporters Use ATP to Drive Import and Export Functions and Multidrug Resistance



- All ABC transporters consist of two transmembrane domains (TMDs) which form the pore and two cytosolic nucleotide-binding domains (NBDs) that bind and hydrolyze ATP
- Bacterial ABC transporters are multimeric, but eukaryotic ABC pumps are monomeric

ABC Transporters Use ATP to Drive Import and Export Functions and Multidrug Resistance



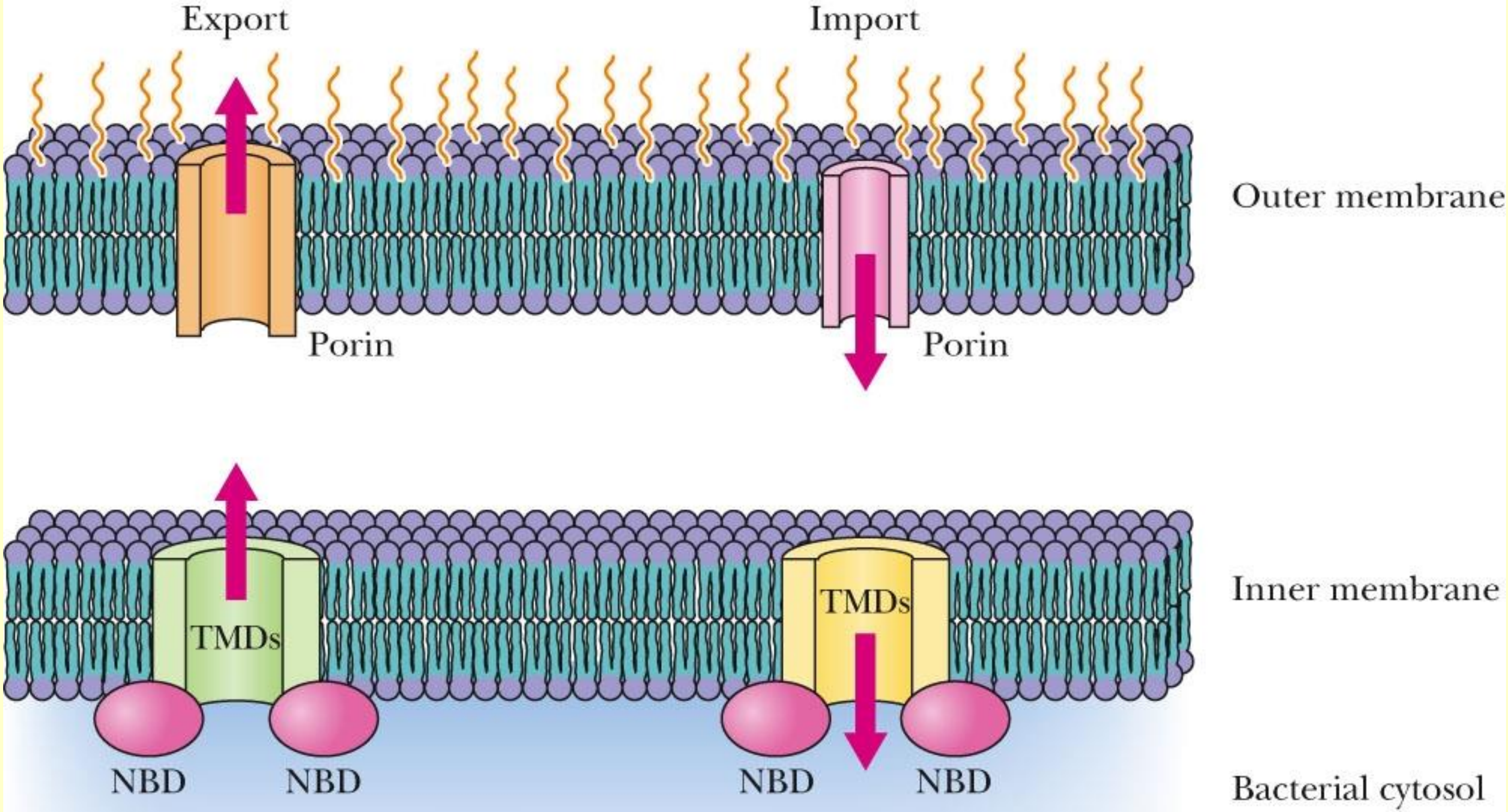


Figure 9.54 Influx pumps in the inner membrane of Gram-negative bacteria bring nutrients into the cell; efflux pumps export cellular waste products.

ABC Transporters Use ATP to Drive Import and Export Functions and Multidrug Resistance

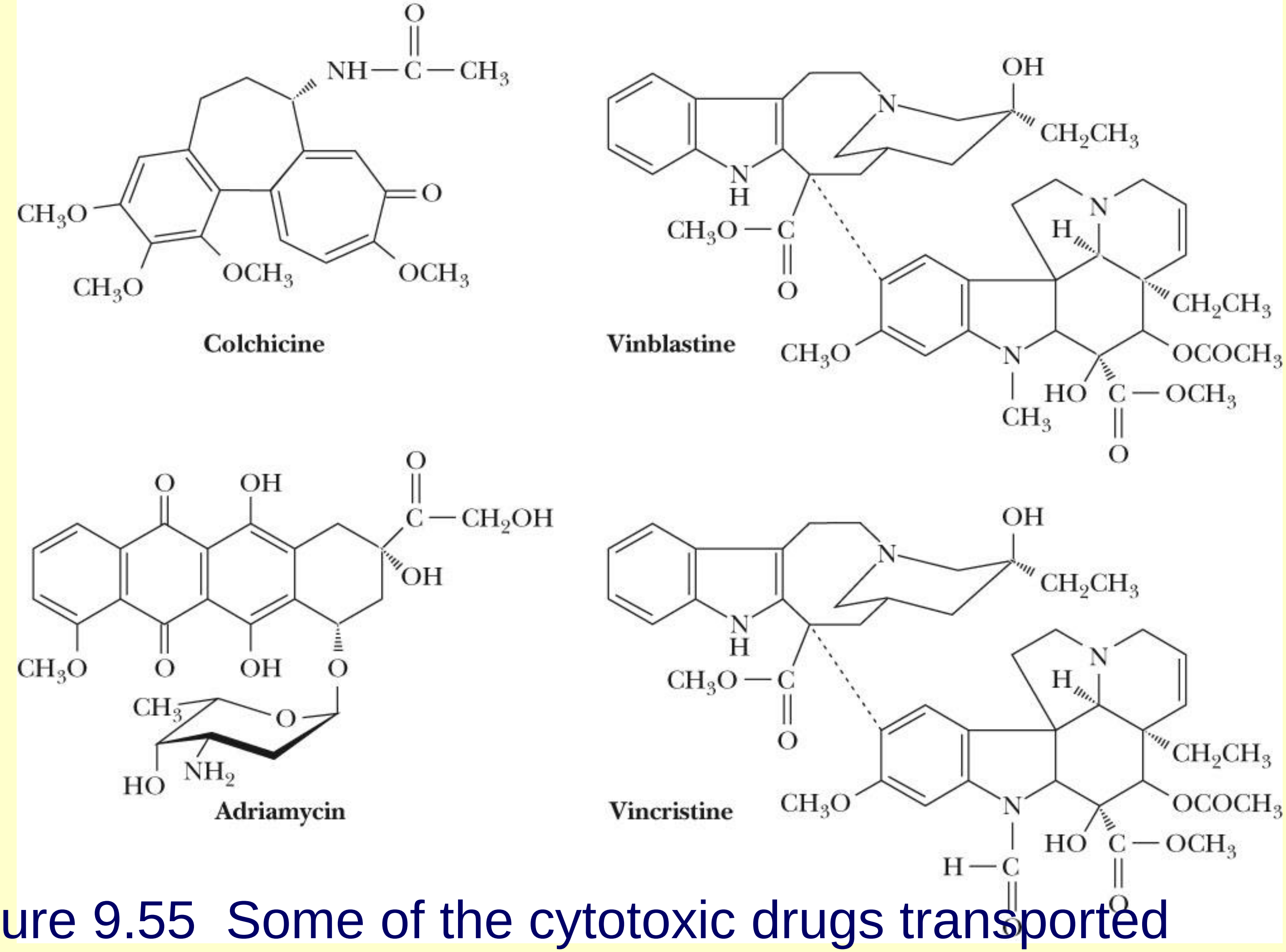


Figure 9.55 Some of the cytotoxic drugs transported by the MDR ATPase.

ABC Transporters Use ATP to Drive Import and Export Functions and Multidrug Resistance

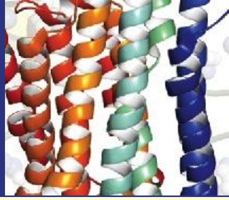
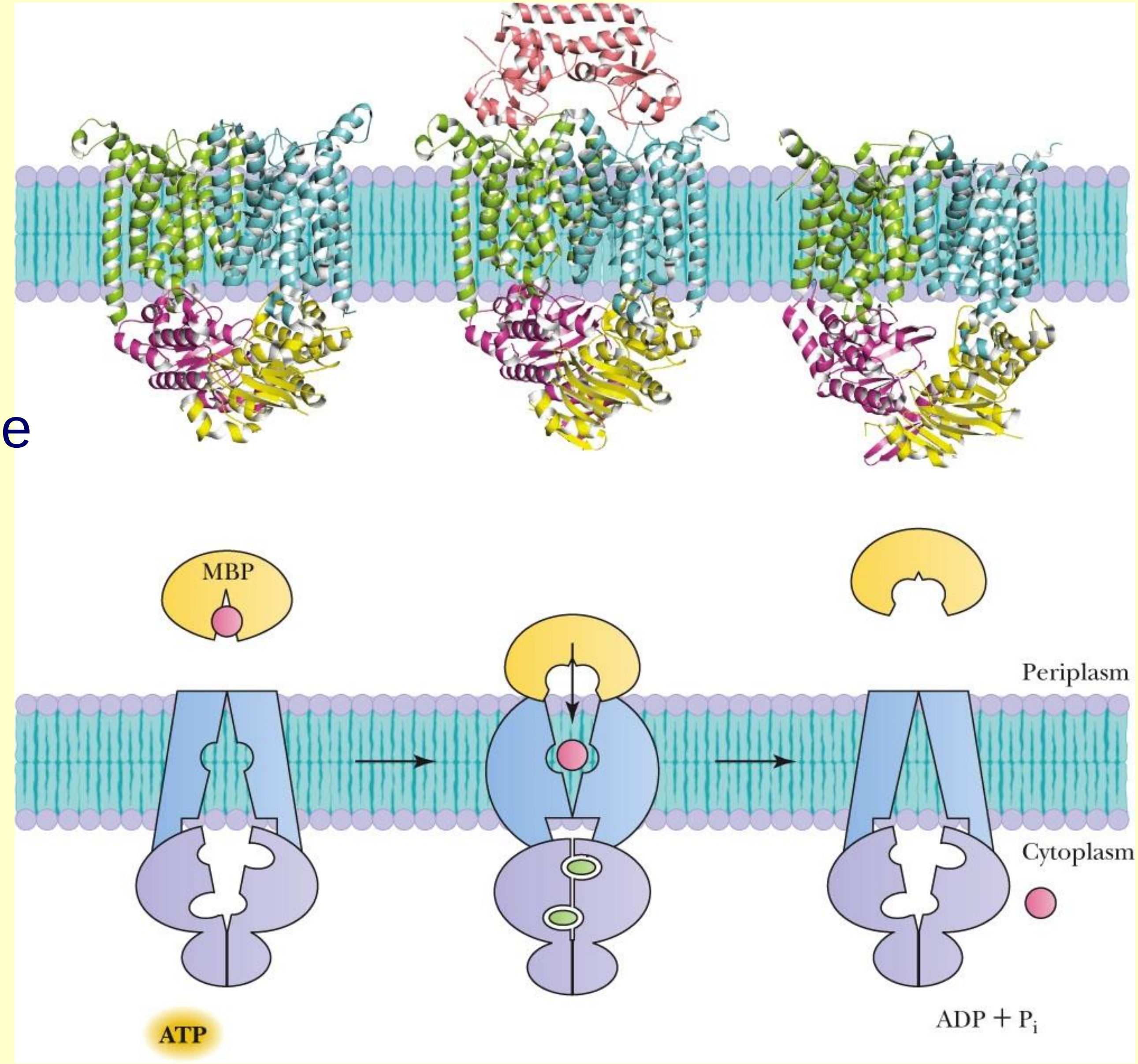
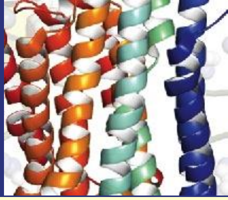


Figure 9.56 Several ABC transporters are shown in different stages of their transport cycles.



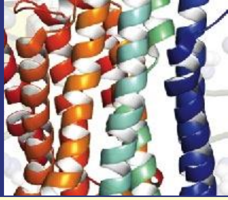
9.9 How Are Certain Transport Processes Driven by Light Energy?



Bacteriorhodopsin is a light-driven proton pump

- *Halobacterium halobium*, the salt-loving bacterium, carries out normal respiration if O_2 and substrates are plentiful
- But when substrates are lacking, it can survive by using bacteriorhodopsin and halorhodopsin to capture light energy
- Purple patches of *H. halobium* are 75% bR and 25% lipid - a "2D crystal" of bR - ideal for structural studies

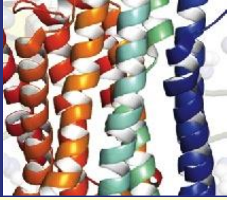
9.9 How Are Certain Transport Processes Driven by Light Energy?



Protein opsin and retinal chromophore

- Retinal is bound to opsin via a Schiff base linkage
- The Schiff base (at Lys^{216}) can be protonated, and this site is one of the sites that participate in H^+ transport
- The carboxyl groups of Asp^{85} and Asp^{96} also serve as proton binding sites during transport
- These Asp residues lie in hydrophobic environments; their carboxyl pK_a values are near 11.

9.9 How Are Certain Transport Processes Driven by Light Energy?



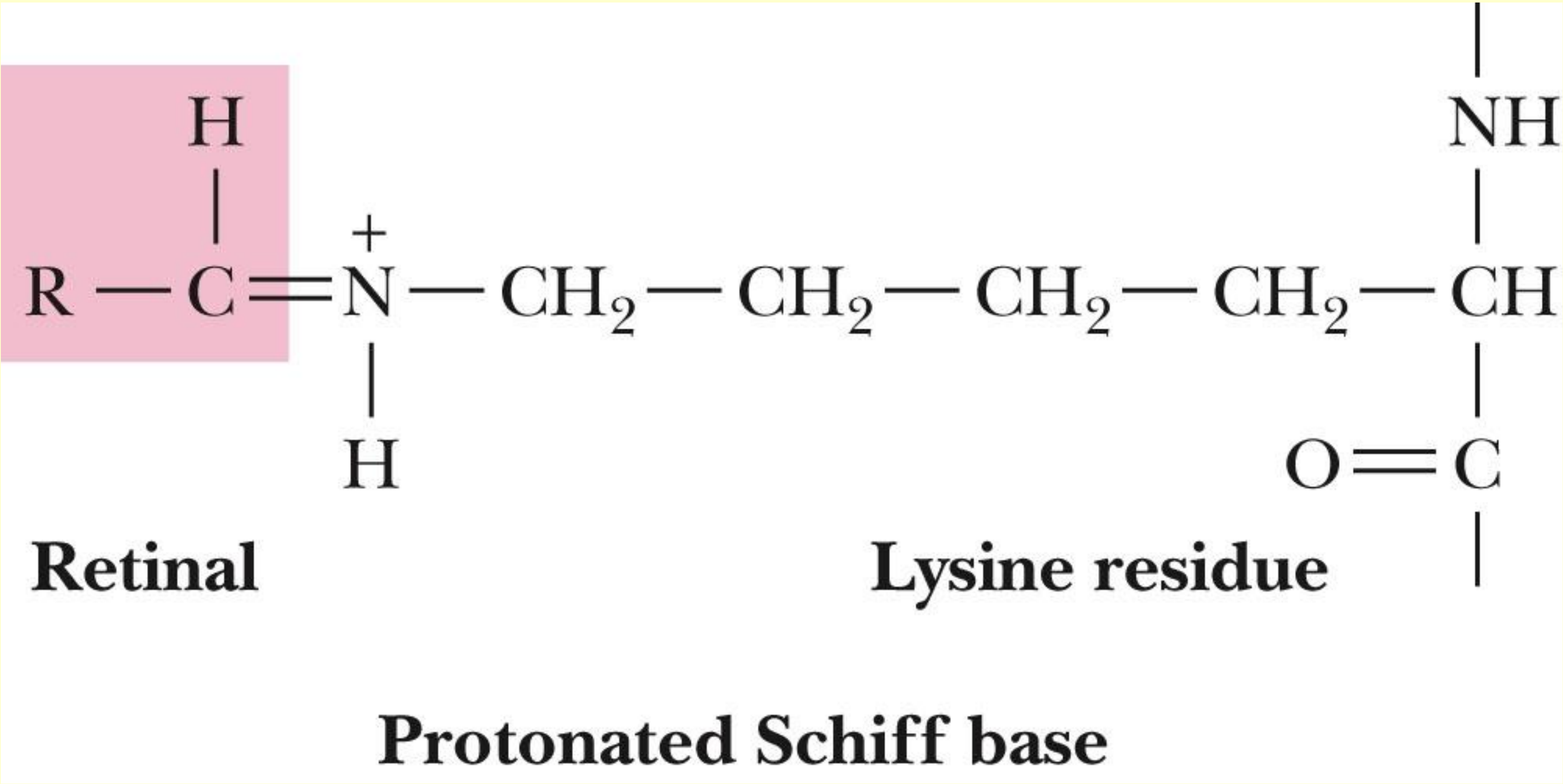
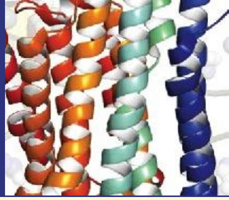


Figure 9.57 The Schiff base linkage between the retinal chromophore and Lys^{216}.

9.9 How Are Certain Transport Processes Driven by Light Energy?



- Lys^{216} is buried in the middle of the 7-TMS structure of bR, and retinal lies mostly parallel to the membrane and between the helices
- Light *absorption* converts retinal from *all-trans* to *13-cis* configuration, triggering conformation changes that induce pK$_a$ changes
- This facilitates proton transfers from Asp^{96} to the Lys Schiff base to Asp^{85} and net proton transport across the membrane
- See Figure 9.58

A Proton Transport Model

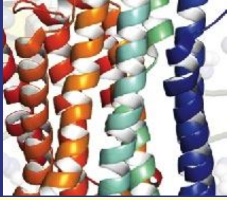
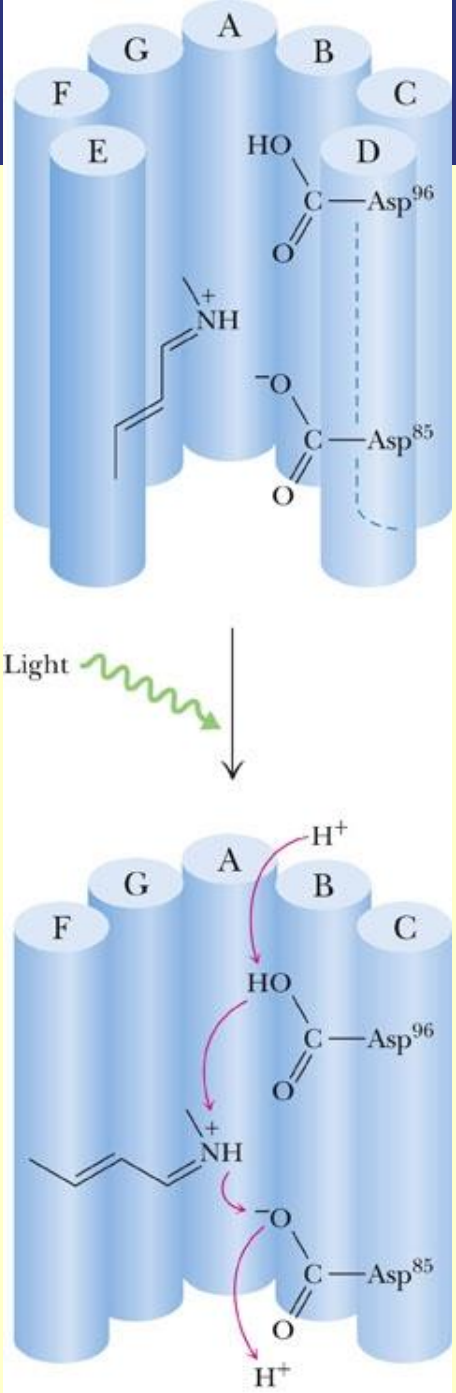
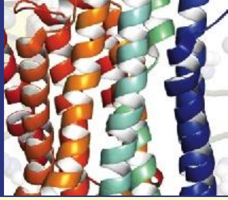


Figure 9.58 The mechanism of proton transport by bacteriorhodopsin. The hydrophobic environments of Asp^{85} and Asp^{96} raise the pKa values of their side chain carboxyl groups, making it possible for these carboxyls to accept protons as they are transported across the membrane.

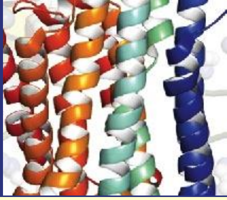


9.10 How Is Secondary Active Transport Driven by Ion Gradients?



- The gradients of H^+, Na^+ and other cations and anions established by ATPases can be used for **secondary active transport** of various substrates
- Many amino acids and sugars are accumulated by cells in transport processes driven by Na^+ and H^+ gradients
- Many of these are **symports**, with the ion and the transported amino acid or sugar moving in the same direction
- In **antiport** processes, the ion and the transported species move in opposite directions

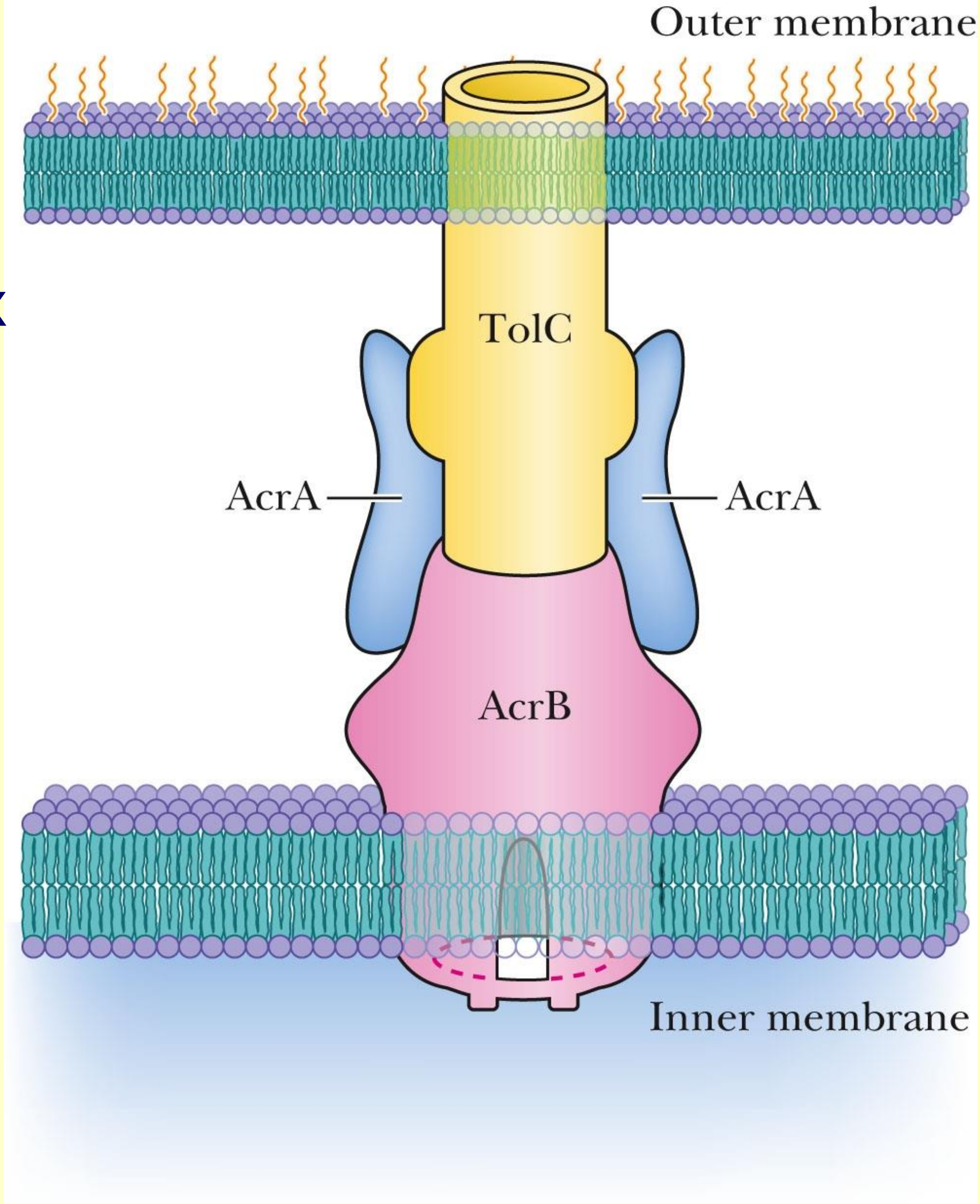
AcrB is a Secondary Transport System



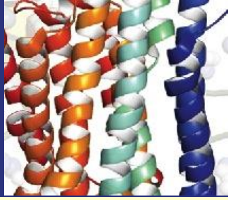
- AcrB is the major MDR transporter in *E. coli*
- It is responsible for pumping a variety of molecules
- AcrB is part of a **tripartite complex** that bridges the *E. coli* inner and outer membranes and spans the entire periplasmic space
- AcrB works with AcrA and TolC to transport drugs and other toxins from the cytoplasm across the entire cell envelope and into the extracellular medium

AcrB is a Secondary Transport System

Figure 9.59 A tripartite complex of proteins comprises the large structure in *E. coli* that exports waste and toxin molecules. The transport pump is AcrB, embedded in the inner membrane.



AcrB is a Secondary Transport System



- AcrB is a secondary active transport system and a **H^+-drug antiporter**
- As protons flow spontaneously inward through AcrB in the *E. coli* inner membrane, drug molecules are driven outward
- Remarkably, the three identical subunits of AcrB adopt slightly different conformations, denoted loose (L), tight (T), and open (O)
- These three conformations are three consecutive states of a transport cycle
- As each monomer cycles through L, T, and O states, drugs enter tunnel, are bound and then exported

AcrB is a Secondary Transport System

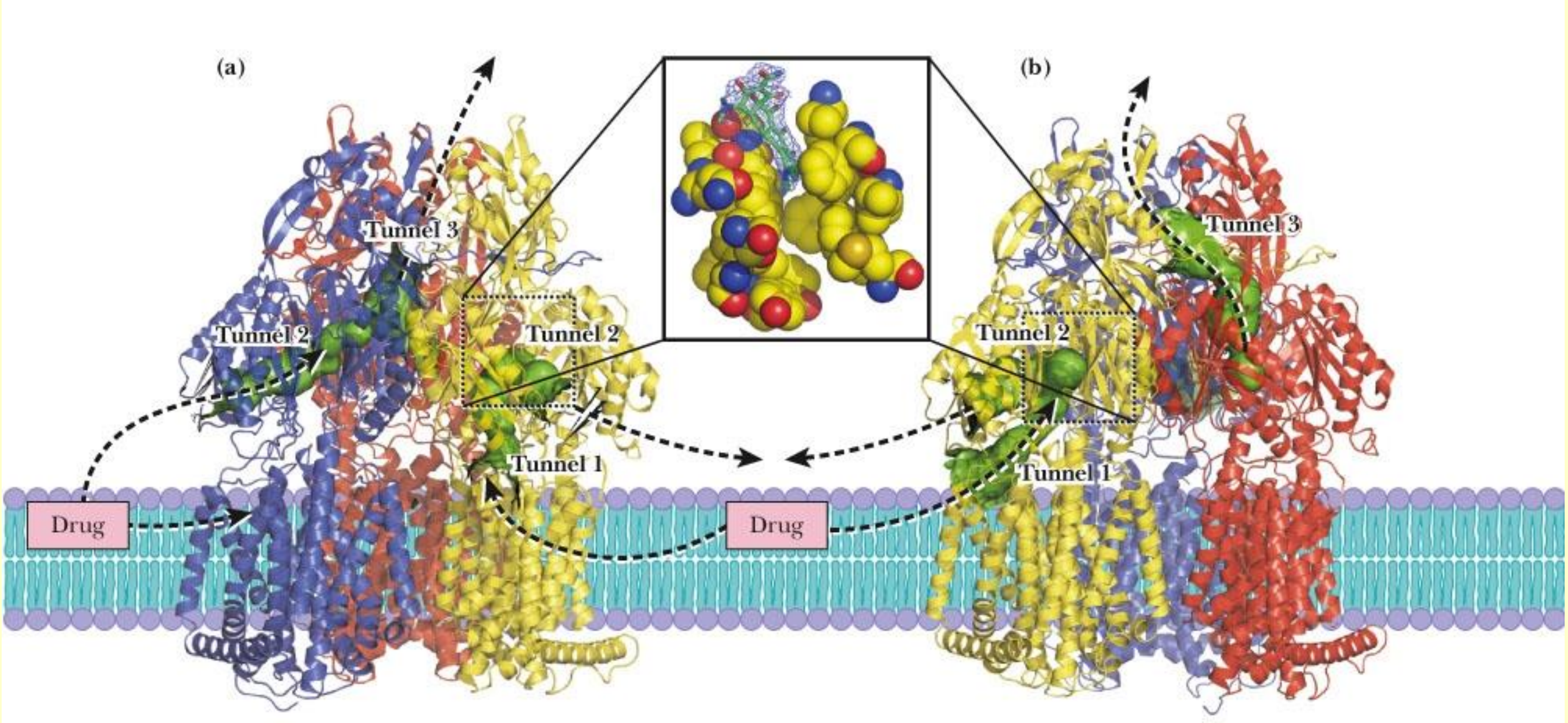


Figure 9.60 In the AcrB trimer, the three identical subunits adopt three different subunits. Possible transport paths of drugs through the tunnels are shown in green.

AcrB is a Secondary Transport System

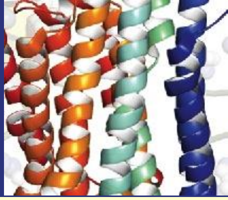


Figure 9.61 A model for drug transport by AcrB involves three different conformations.