

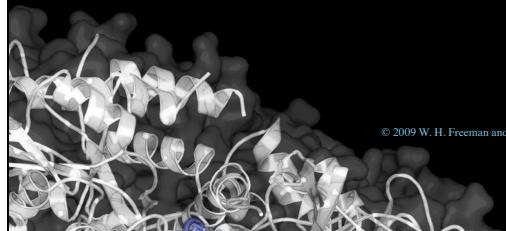
LEHNINGER
PRINCIPLES OF BIOCHEMISTRY

David L. Nelson
Michael M. Cox

FIFTH EDITION

Lecture Connections

11 | Membranes



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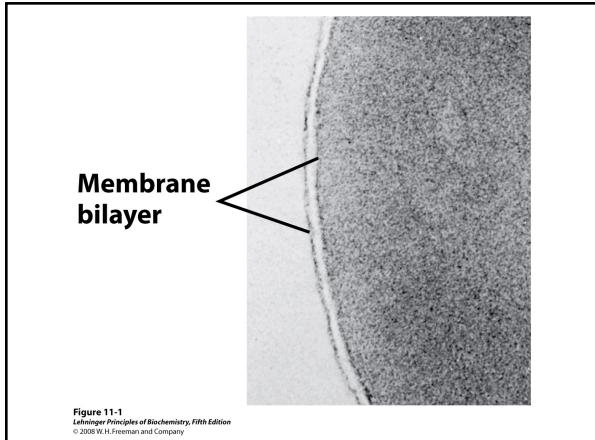


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Why membranes?

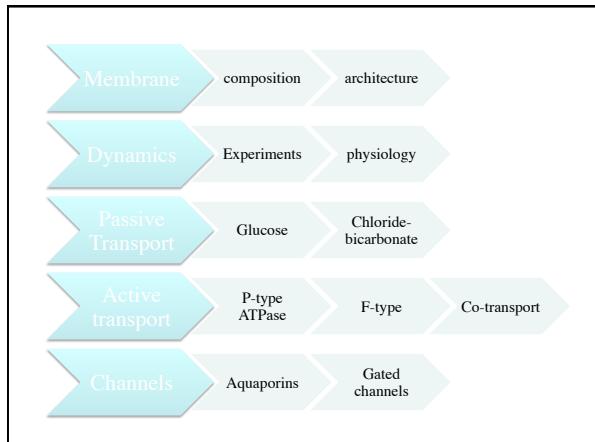
- Self and non-self
- Organize complex reaction to 2D space
- Flexible, self-sealing, selectivity permeability
- Communication
- Transport

CHAPTER 11

Membranes

Key topics:

- The function of biological membranes
- The structure and composition membranes
- Dynamics of membranes
- Structure and function of membrane proteins
- Transport across biological membranes



What are Membranes?

- Complex lipid-based structures that form pliable sheets
- Composed of a variety of lipids and proteins
- Some membrane lipids and proteins are glycosylated
- All cells have the cell membrane, which separates the cell from its surrounding
- Eukaryotic cells have various internal membranes that divide the internal space into compartments

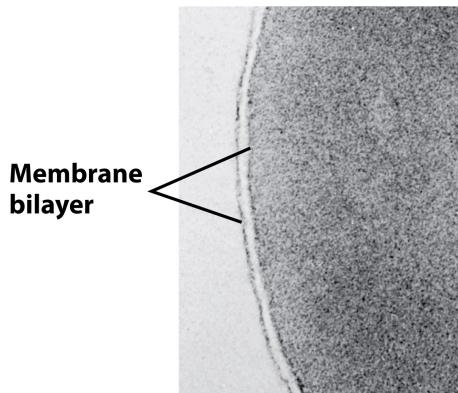
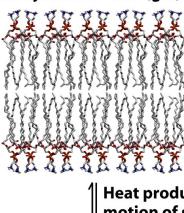


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Functions of Membranes

- Define the boundaries of the cell
- Allow import and export
 - Selective import of nutrients (e.g. lactose)
 - Selective export of waste and toxins (e.g. antibiotics)
- Retain metabolites and ions within the cell
- Sense external signals and transmit information into the cell
- Provide compartmentalization within the cell
 - separate energy-producing reactions from energy-consuming ones
 - keep proteolytic enzymes away from important cellular proteins
- Produce and transmit nerve signals
- Store energy as a proton gradient and support synthesis of ATP

(a) Paracrystalline state (gel)



Heat produces thermal motion of side chains (gel → fluid transition)

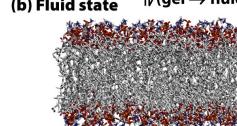
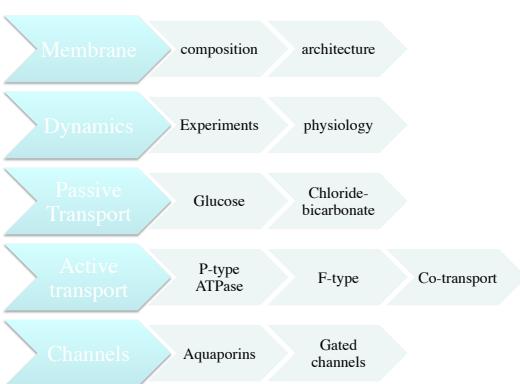
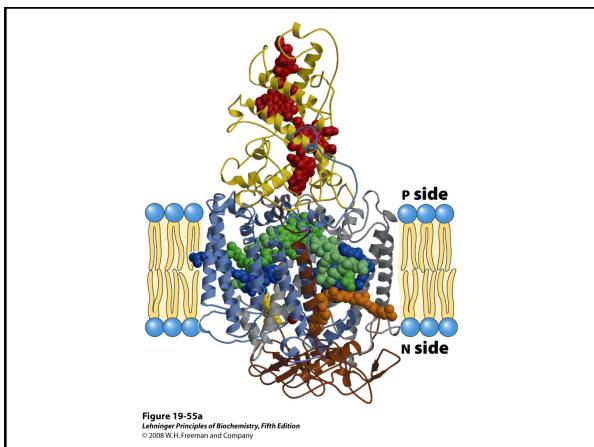


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Functions of Proteins in Membranes

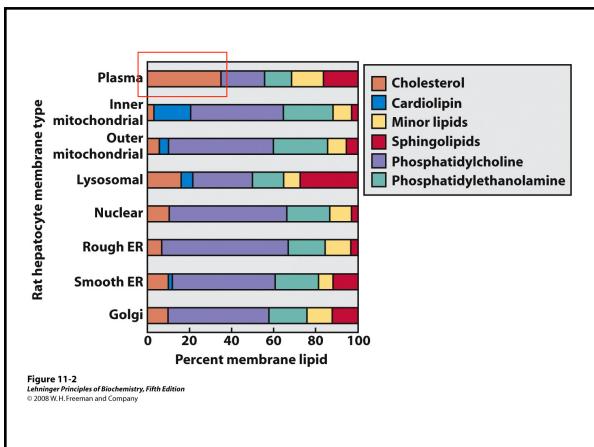
- Receptors: detecting signals from outside
 - Light (opsin)
 - Hormones (insulin receptor)
 - Neurotransmitters (acetylcholine receptor)
 - Pheromones (taste and smell receptors)
- Channels, gates, pumps
 - Nutrients (maltoporin)
 - Ions (K-channel)
 - Neurotransmitters (serotonin reuptake protein)
- Enzymes
 - Lipid biosynthesis (some acyltransferases)
 - ATP synthesis (F_0F_1 ATPase/ATP synthase)





Membrane Composition is Highly Variable within the Cell

- Lipid composition is different in different organelles of the same cell
 - cholesterol predominant in the plasma membrane, virtually absent in mitochondria
 - galactolipids abundant in plant chloroplasts but almost absent in animals
 - linked to the evolution of eukaryotes?



The Composition of Membranes

Lipid composition is different in different organisms and in different tissues of the same organism:

- Ratio of lipid to protein varies
- Type of phospholipid varies
- Abundance and type of sterols varies, prokaryotes lack sterols

TABLE 11-1 Major Components of Plasma Membranes in Various Organisms				
	Components (% by weight)		Sterol type	Other lipids
	Protein	Phospholipid		
Human myelin sheath	30	30	19	Cholesterol Galactolipids, plasmalogens
Mouse liver	45	27	25	Cholesterol —
Maize leaf	47	26	7	Sitosterol Galactolipids
Yeast	52	7	4	Ergosterol Triacylglycerols, sterol esters
Paramecium (ciliated protist)	56	40	4	Stigmasterol —
<i>E. coli</i>	75	25	0	— —

Note: Values do not add up to 100% in every case, because there are components other than protein, phospholipids, and sterol; plants, for example, have high levels of glycolipids.

Table 11-1
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Common Features of Membranes

- Sheet-like flexible structure, 30-100 Å (3-10 nm) thick
- Main structure is composed of two leaflets of lipids (**bilayer**)
 - Except of archaebacteria: monolayer of bifunctional lipids
- Form spontaneously in aqueous solution and are stabilized by non-covalent forces, esp. hydrophobic effect
- Protein molecules span the lipid bilayer
- Asymmetric
 - Some lipids are found preferably "inside"
 - Some lipids are found preferably "outside"
 - Carbohydrate moieties are always outside the cell
 - Electrically polarized (inside negative ~ -60mV)
- Fluid structures: 2-dimensional solution of oriented lipids

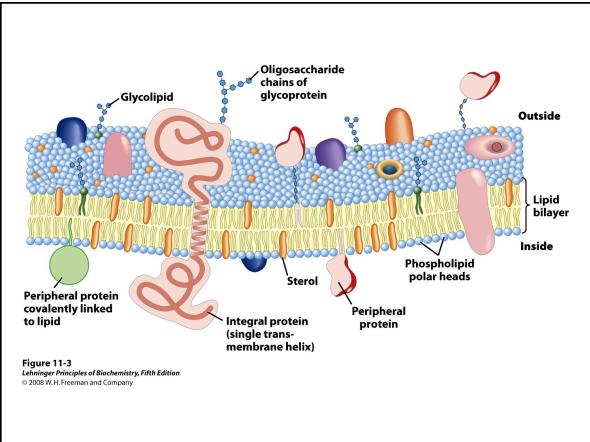


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Membrane Bilayer

- The membrane bilayer consists of a **two leaflets of lipid monolayers**
 - Hydrophilic head groups interact with water
 - Hydrophobic fatty acid tails are packed inside
 - One leaflet faces the cytoplasm
 - Another leaflet faces the extracellular space or the inside of membrane-enclosed organelle

Micelle

- Forms in the solution of **amphipathic** molecules that have larger head than tail
 - Fatty acids
 - Sodium dodecyl sulfate
- Each micelle has from a few dozen to few thousand lipid molecules
- Aggregation occurs when the concentration of molecules is higher than a certain threshold

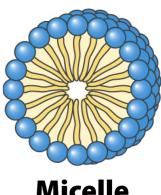
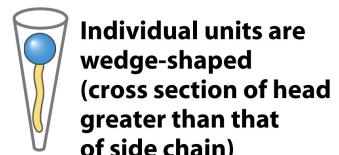


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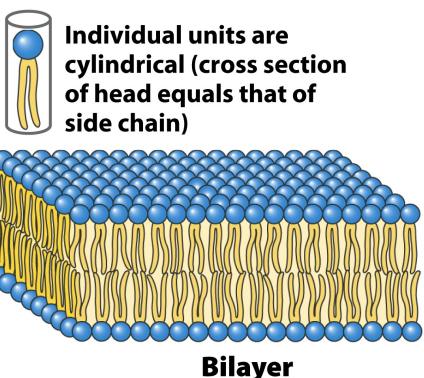


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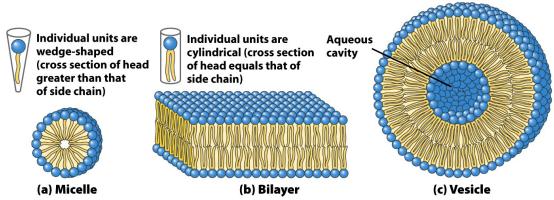


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Vesicle (Liposome)

- Small bilayers will spontaneously seal into spherical vesicles
- Vesicle membranes can contain artificially inserted proteins
- The central aqueous cavity can enclose dissolved molecules
- They are useful artificial carriers of molecules (e.g. drugs)
- Vesicles fuse readily with cell membranes or with each other

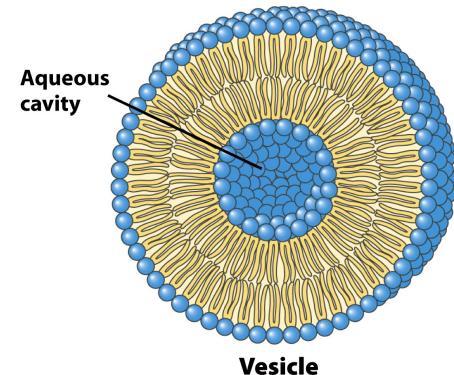
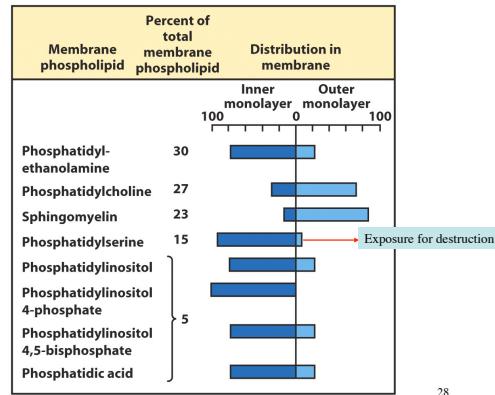


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Two Leaflets have Different Lipid Compositions

- Bilayer is asymmetric
- Outer leaflet is often more positively charged
- Phosphatidylserine outside has a special meaning:
 - Platlets: Activates blood clotting
 - Other cells: Marks the cell for destruction



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Fluid Mosaic Model of Membranes

- Proposed in 1972 by Singer and Nicholson (UCSD)
- Lipids form a viscous, two-dimensional solvent into which proteins are inserted and integrated more or less deeply
- Integral proteins are firmly associated with the membrane, often spanning the bilayer
- Peripheral proteins are weakly associated and can be removed easily
 - Some are non-covalently attached
 - Some are linked to membrane lipids

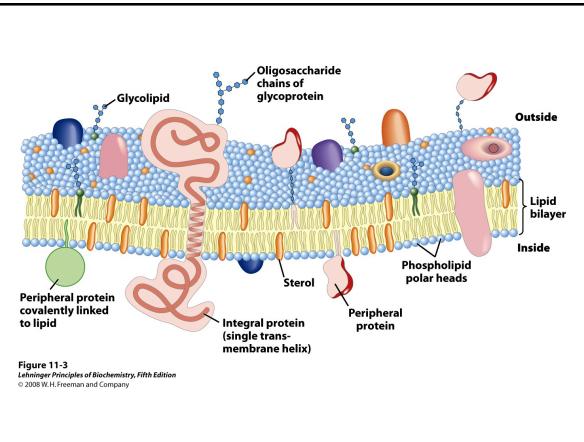


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The Fluid Mosaic Model: Details

Three Classes of Membrane Proteins

1. Integral membrane proteins

- Contain hydrophobic regions embedded in the hydrophobic lipid bilayer
- Usually span the bilayer completely
- Bacteriorhodopsin has seven membrane-spanning α -helices

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2 Peripheral membrane proteins

- Associated with membrane through charge-charge or hydrogen bonding interactions to integral proteins or membrane lipids
- More readily dissociated from membranes than covalently bound proteins
- Change in pH or ionic strength often releases these proteins

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3 Lipid-anchored membrane proteins

- Tethered to membrane through a covalent bond to a lipid anchor as:
 - (1) Protein amino acid side chain ester or amide link to fatty acyl group (e.g. myristate, palmitate)
 - (2) Protein cysteine sulfur atom covalently linked to an isoprenoid chain (**prenylated proteins**)
 - (3) Protein anchored to glycosylphosphatidylinositol

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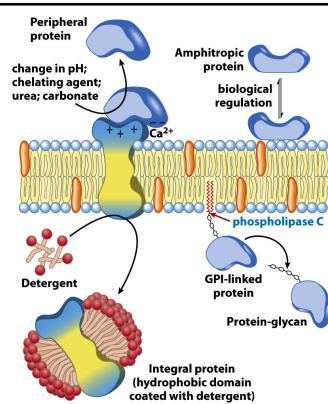
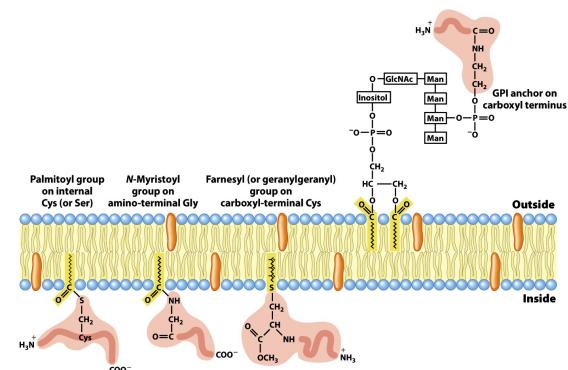


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Lipid-linked Membrane Proteins

Lipid Anchors

- Some membrane proteins are lipoproteins; they contain a covalently linked lipid molecule
 - Long chain fatty acids
 - Isoprenoids
 - Sterols
 - Glycosylated phosphatidylinositol (PGI)
- The lipid part can become part of the membrane
- The protein is now anchored to the membrane
 - reversible process
 - allows targeting of proteins
 - Some, such as GPI anchors are found only on the outer face of plasma membrane



Farnesylation of Proteins

- Proteins can be targeted to the inner leaflet of the plasma membrane by **farnesylation**
- Primary sequence of the protein contains a **signature for farnesylation**: CaaX
 - C is a conserved Cys,
 - 'a' is usually an aliphatic amino acid,
 - 'X' is Met, Ser, Glu, or Ala
- This reaction is catalyzed by **farnesyl transferase**
- Non-farnesylated proteins do not go to the membrane and are inactive
 - Promising cancer therapy (onco-Ras)

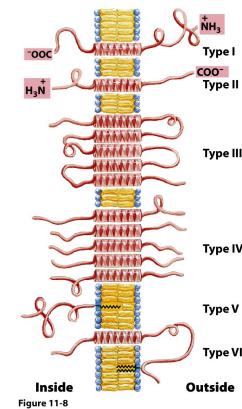
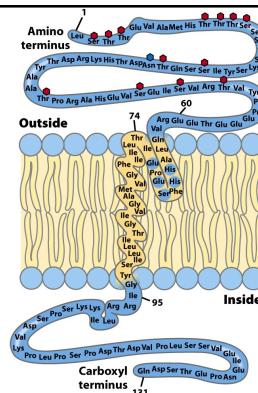
Topology of Integral membrane proteins

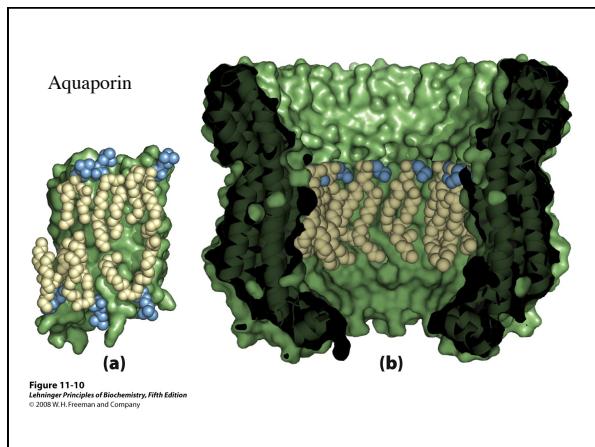
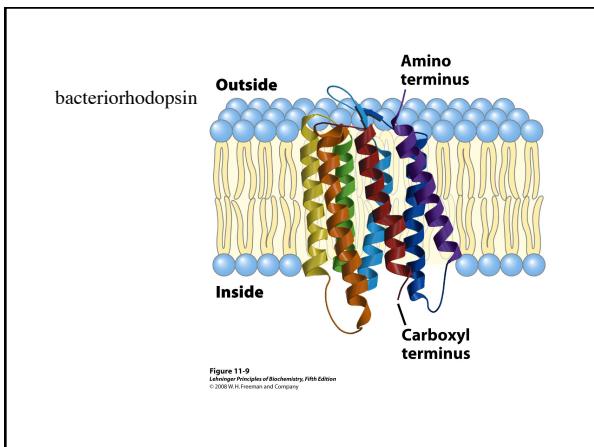
- Topology of membrane protein
 - Localization of protein domains relative to the bilayer
- Methods that reveal topology
 - Polar chemicals (e.g. modify lysine)
 - Protease protection
 - Bioinformatics

Glycophorin

How many amino acids in transmembrane (TM) region?

This TM is an alpha-helix





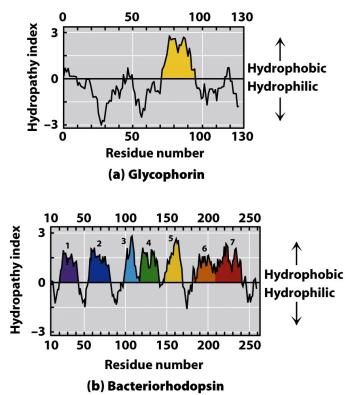
Bioinformatic method for the prediction of TM- hydropathy plot

- Side chain of TM domain are mostly hydrophobic
- Hydrogen bond donor and acceptor have been satisfied in α -helix
- One amino acid span $\sim 1.5 \text{ \AA}$ in α -helix

Bioinformatic method for the prediction of TM

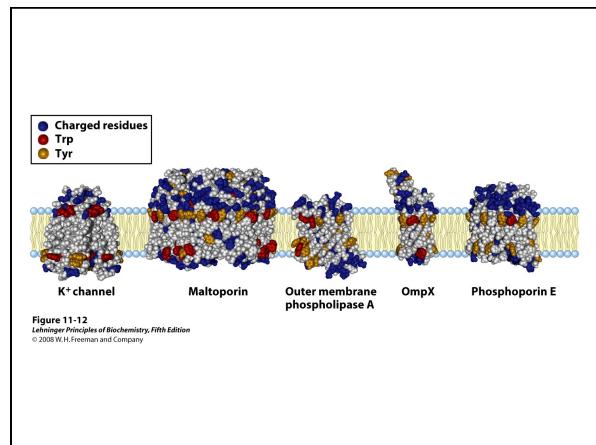
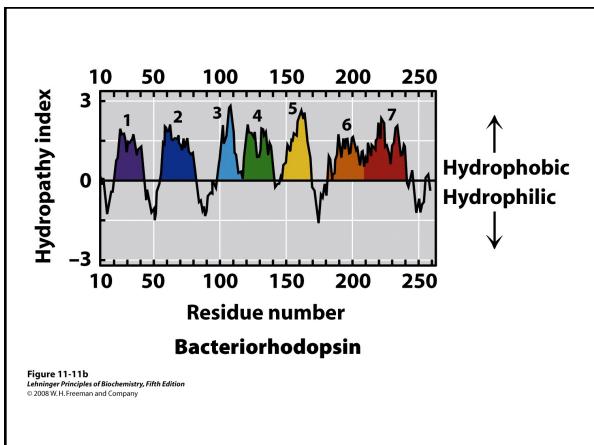
- Roughly 20 amino acids required for TM with α -helix
- Trp /Tyr locate in interface
- Positive-inside rule

Hydropathy plots



Glycophorin

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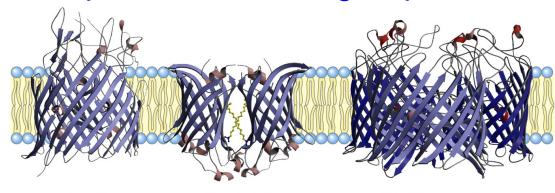
Please notice their characters

- Depending on the “angle” that TM relate to membrane, TM can be longer than 20 amino acids
- Trp and Tyr are located in the interface
- Charged amino acids within TM is possible

β-strands have un-satisfied H-bond donor/acceptor

- To maximize H-bond formation for the transmembrane regions
 - More than ~20 β-strands form a β-barrel
 - The side chains outside of β-barrel are hydrophobic
 - Polar side chain inside β-barrel
 - Length of a single TM β-strands is less than 20 amino acids. Why?

β-barrel forms larger “pore”



Membrane Structure in Archaea

- Unique glycerol chirality in phospholipids
 - L-glycerol in archaea
 - D-glycerol in bacteria
- Unique fatty acids
 - Branched isoprene chains in archaea
 - Unbranched fatty acid chains in bacteria
- Unique linkages
 - Ether linkages in archaea
 - Ester linkages in bacteria
- Membrane topology
 - Monolayer in some archaea
 - Bilayer in all bacteria

Lipid Monolayer in Archaea

- Sulfolobus solfataricus* and relatives:
 - Volcanic hot springs
 - Temperatures 75 – 80 °C
 - Acidic environment: pH 2 – 3
- Better membrane stability by:
 - Isoprenoid **tetraethers** with unique alcohols

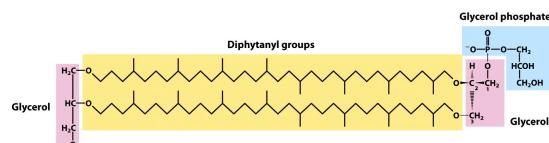
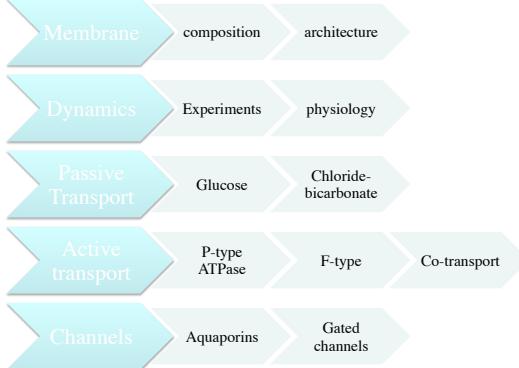


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Physical Properties of Membranes

- Dynamic and flexible structures
- Can exist in various phases and undergo phase transitions
- Not permeable to large polar solutes and ions
- Permeable to small polar solutes and nonpolar compounds
- Permeability can be artificially increased by chemical treatment
 - When we want to get DNA into the cell

Organisms can Adjust the Membrane Composition

- Membrane fluidity is determined by the ratio of unsaturated to saturated fatty acids
- To maintain constant fluidity, cells need more saturated fatty acids at higher temperature

	Percentage of total fatty acids*			
	10 °C	20 °C	30 °C	40 °C
Myristic acid (14:0)	4	4	4	8
Palmitic acid (16:0)	18	25	29	48
Palmitoleic acid (16:1)	26	24	23	9
Oleic acid (18:1)	38	34	30	12
Hydroxymyristic acid	13	10	10	8
Ratio of unsaturated to saturated†	2.9	2.0	1.6	0.38

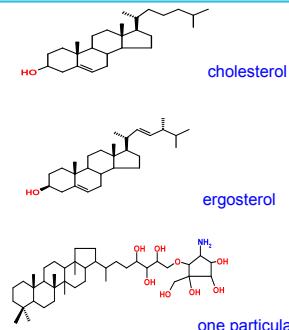
Source: Data from Marr, A.G. & Ingraham, J.L. (1962) Effect of temperature on the composition of fatty acids in *Escherichia coli*. *J. Bacteriol.* 84, 1260.

*The exact fatty acid composition depends not only on growth temperature but on growth stage and growth medium composition.

†Ratios calculated as the total percentage of 16:1 plus 18:1 divided by the total percentage of 14:0 plus 16:0. Hydroxymyristic acid was omitted from this calculation.

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Sterols and Hopanols Increase Membrane Rigidity and Permeability



- Cell membranes of many eukaryotes contain **sterols**
 - Cholesterol in animals
 - Phytosterols in plants
 - Ergosterol in fungi
- Cell membranes of aerobic prokaryotes contain **hopanols**

Membrane Phases

- Depending on their composition and the temperature, lipid bilayer can be in gel or fluid phase
 - Gel phase: individual molecules do not move around
 - Fluid phase: individual molecules can move around
- Heating causes phase transition from the gel to fluid
- Under physiological conditions, membranes are more fluid-like than gel-like

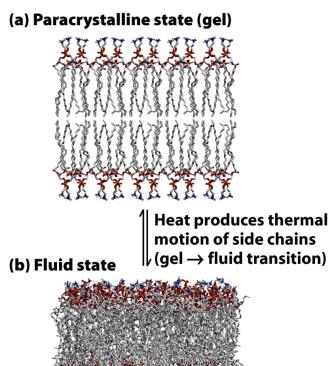


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Uncatalyzed transbilayer (“flip-flop”) diffusion

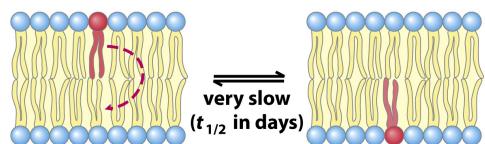


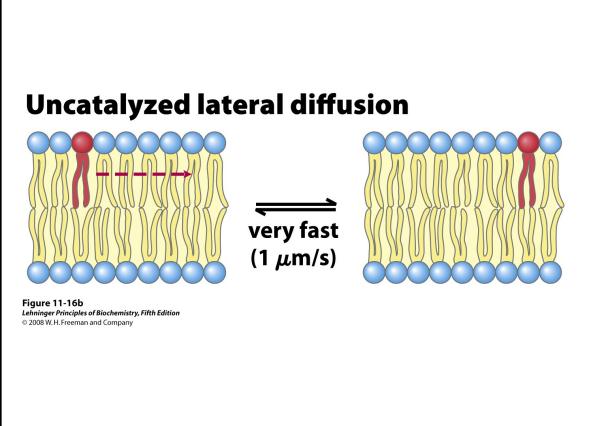
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Membrane Dynamics: Lateral Diffusion

- Individual lipids undergo fast lateral diffusion within the leaflet

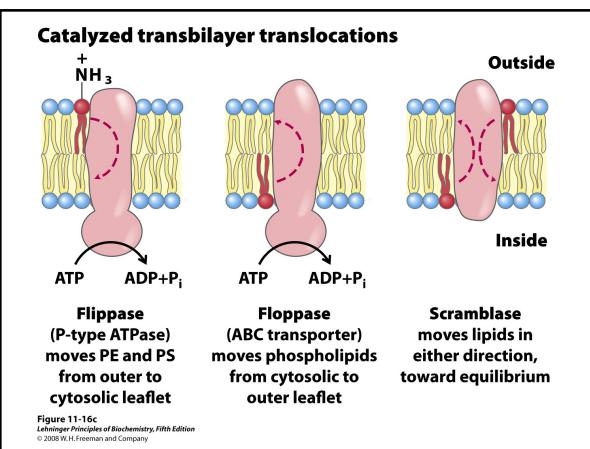
Membrane Dynamics: Transverse Diffusion

- Spontaneous flips from one leaflet to another are rare because the charged head group, which is normally well-solvated, must transverse the apolar region



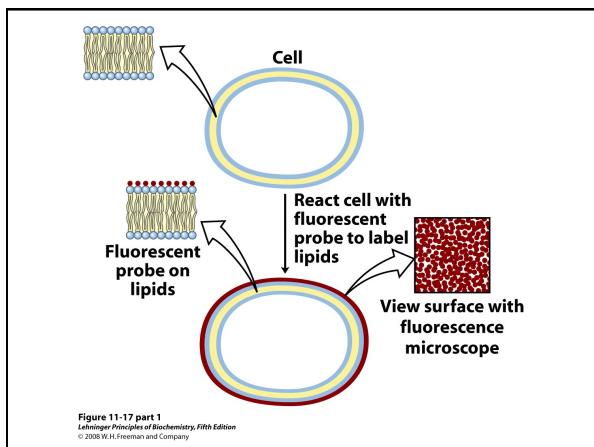
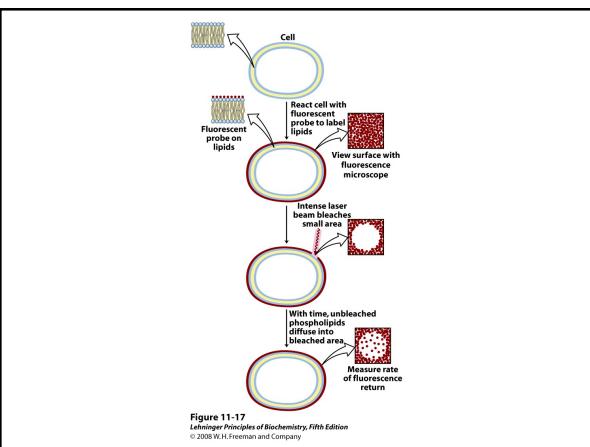
Membrane Diffusion: Flippases

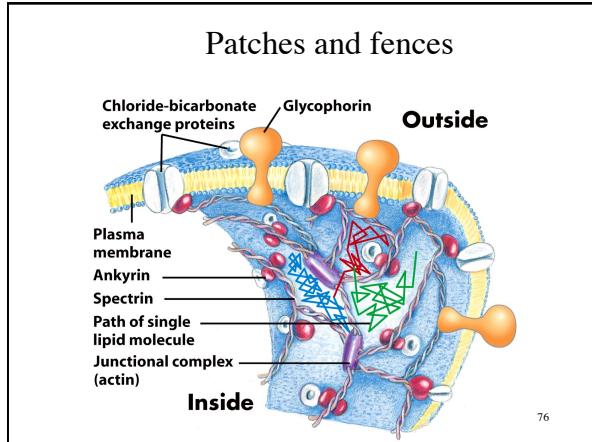
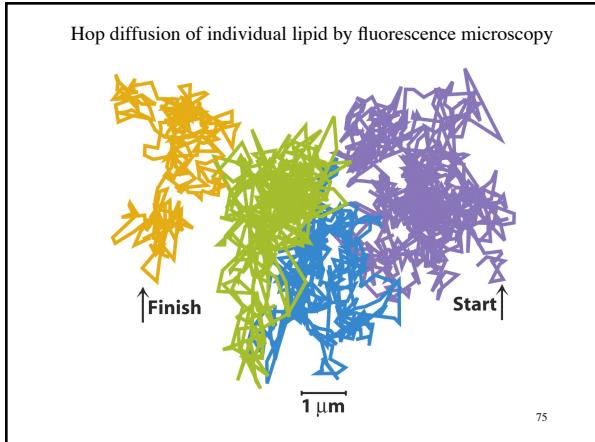
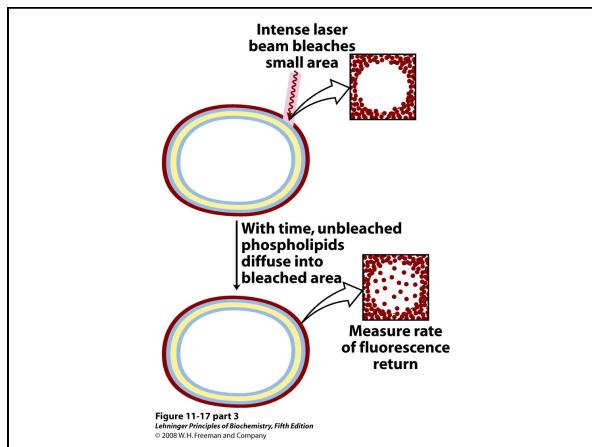
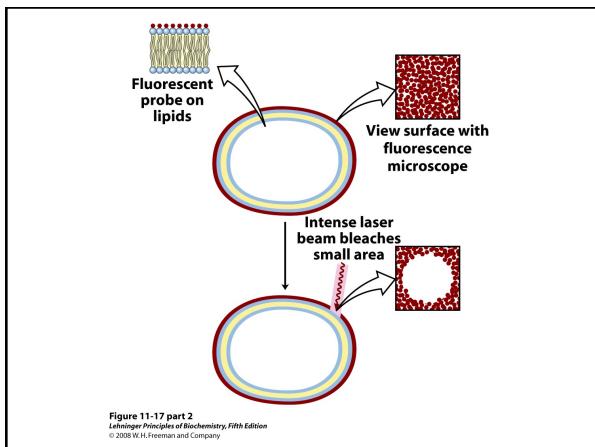
- Special enzymes—flippases—catalyze transverse diffusion
- Some flippases use energy of ATP to move lipids against the concentration gradient



Study of Membrane Dynamics: FRAP

- Fluorescence Recovery After Photobleaching (FRAP) allows to monitor lateral lipid diffusion by monitoring the rate of fluorescence return
- From the rate of return of lipids, the diffusion coefficient of a lipid in the leaflet can be determined
- Rates of lateral diffusion are high (up to 1 $\mu\text{m/sec}$):
 - a lipid can circumnavigate *E.coli* cell in one second

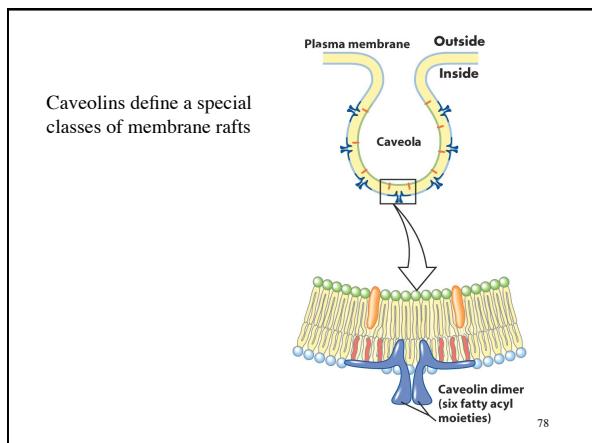




The significance of the presence of lipid rafts

- The “raft” and “sea” domains of the plasma membrane are not rigidly separated; membrane proteins can move into and out of lipid rafts on a timescale of seconds. But in the shorter timescale (microseconds) more relevant to many membrane-mediated biochemical processes, many of these proteins reside primarily in a raft. (p.385)

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Membrane Rafts

- Lipid distribution in a single leaflet is not random and even
- Some regions contain clusters of glycosphingolipids with longer than usual tails
- These regions are more ordered and contain specific doubly- or triply-acylated proteins
- Rafts allow segregation of proteins in the membrane

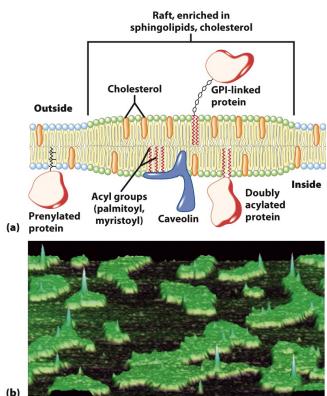


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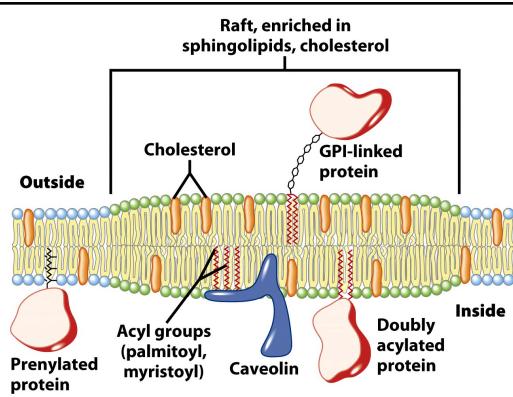


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Lipid rafts are visualized by atomic force microscope (AFM)

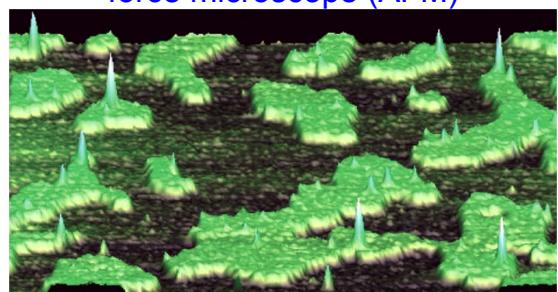
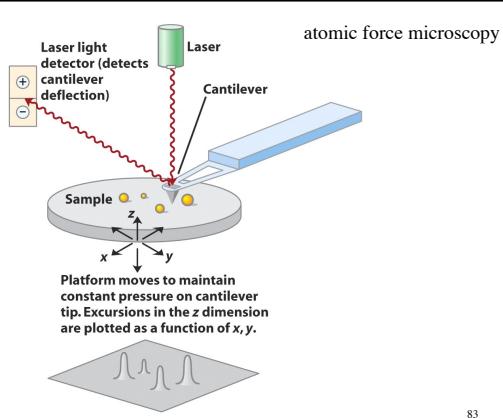
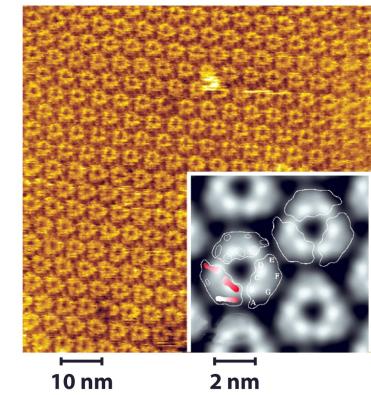


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Membrane Fusion

- Membranes can fuse with each other without losing continuity
- Fusion can be spontaneous or protein-mediated
- Examples of protein-mediated fusion are
 - Entry of influenza virus into the host cell
 - Release of neurotransmitters at nerve synapses

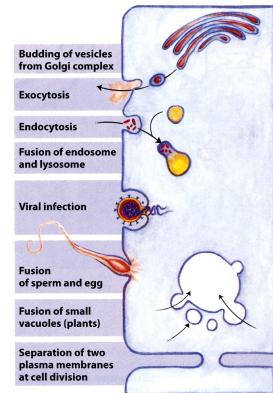
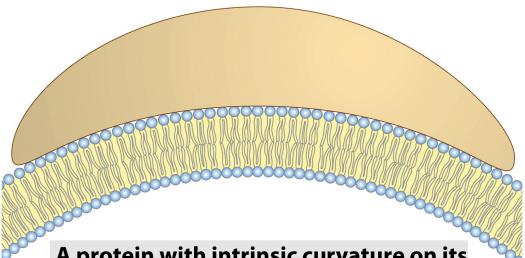


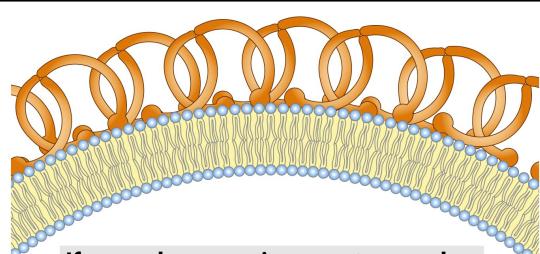
Figure 11-22
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Models for Protein-induced curvature formation



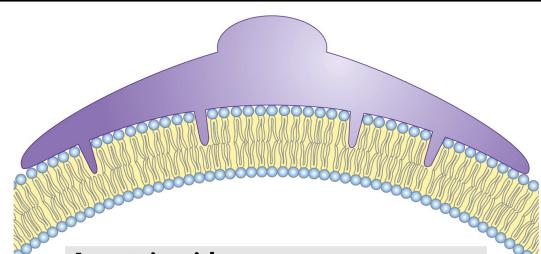
A protein with intrinsic curvature on its surface interacts strongly with a curved membrane surface, allowing both membrane and protein to achieve their lowest energy.

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If a membrane region spontaneously curves, monomeric subunits of certain proteins can polymerize into a superstructure that favors and maintains the curvature.

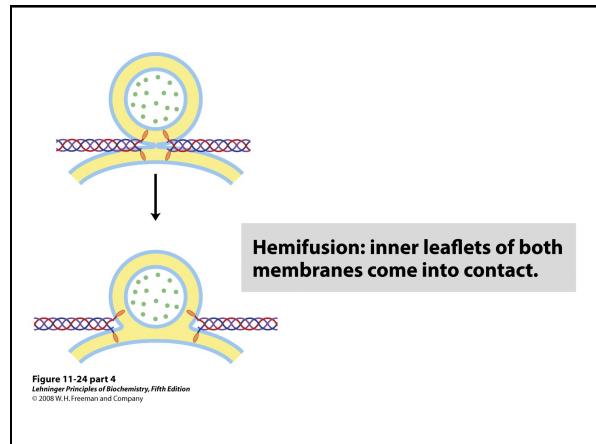
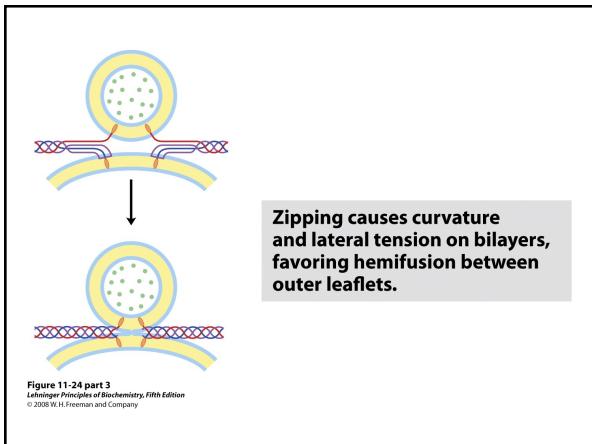
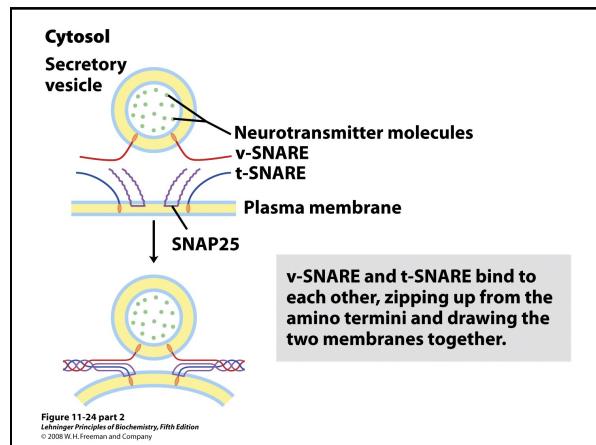
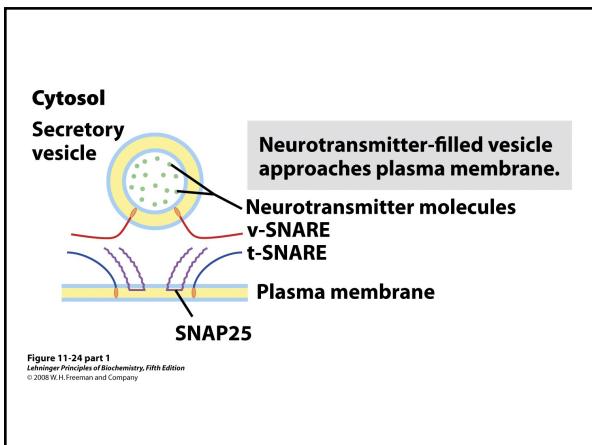
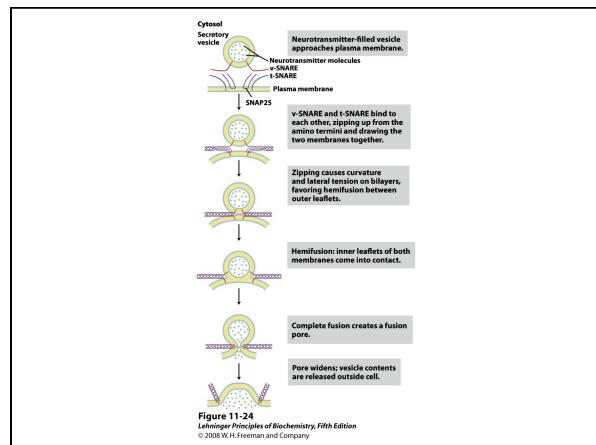
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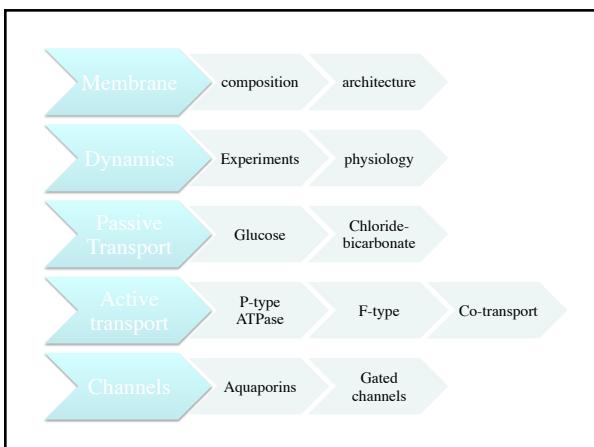
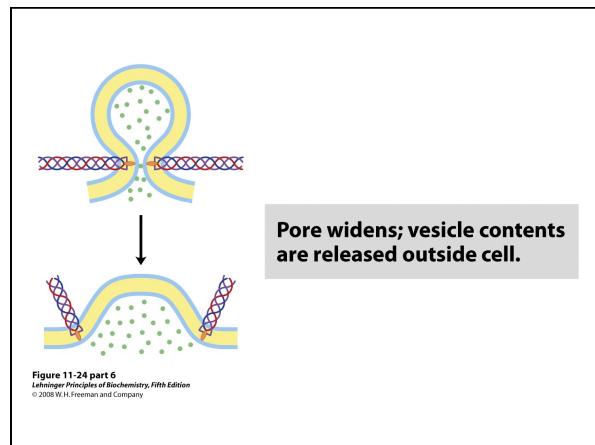
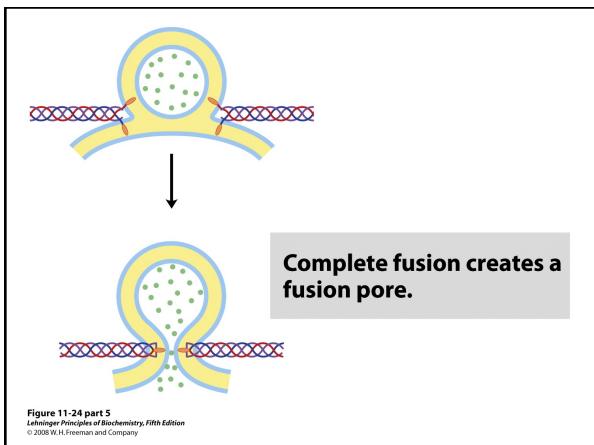


A protein with one or more amphipathic helices inserted into one leaflet of the bilayer crowds the lipids in that leaflet, forcing the membrane to bend.

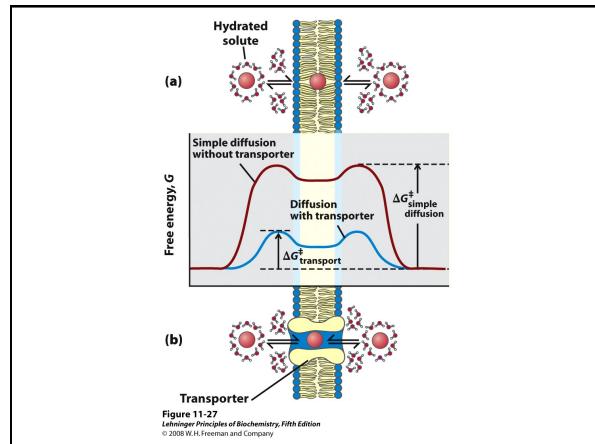
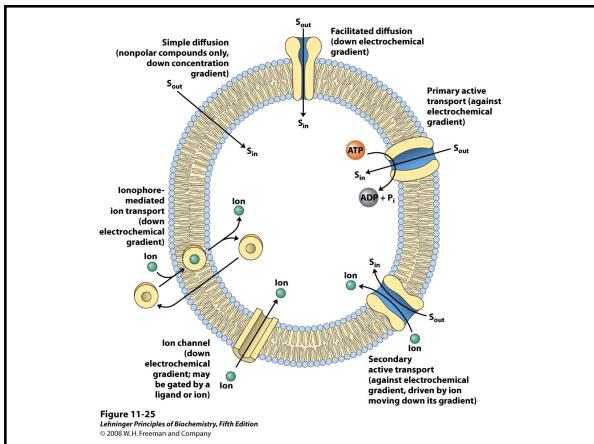
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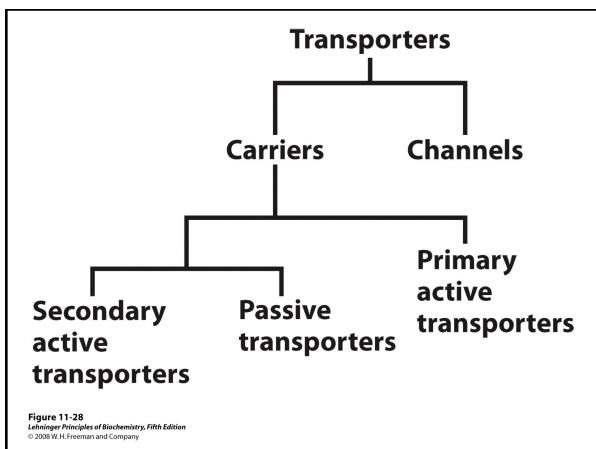
Neurotransmitter Release





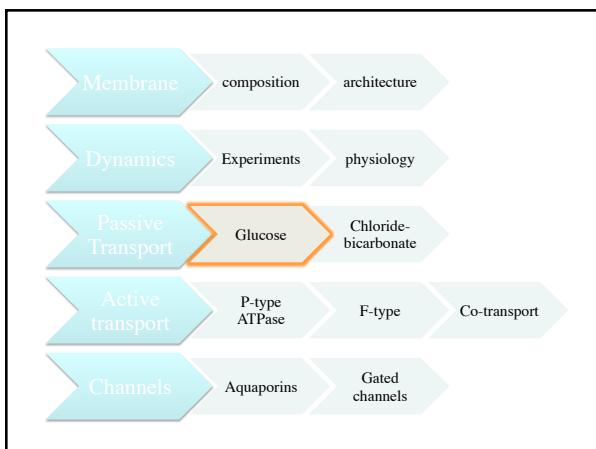
- ## Transport Across Membranes
- Some solutes passively diffuse through the lipid membrane
 - Passive diffusion of polar molecules involves desolvation and thus has a high activation barrier
 - Transport across the membrane can be facilitated by proteins that provide an alternative diffusion path
 - Such proteins are called **transporters** or **permeases**



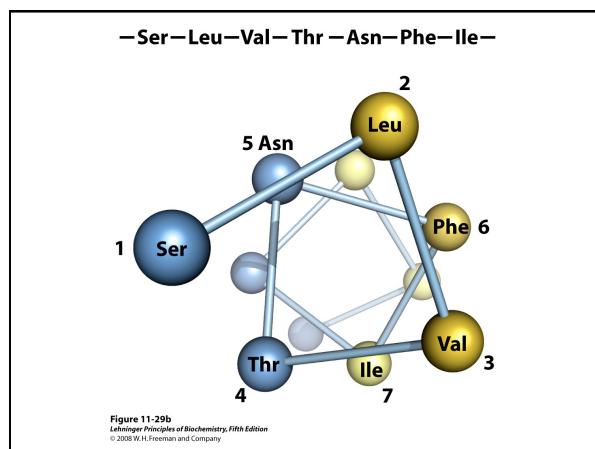
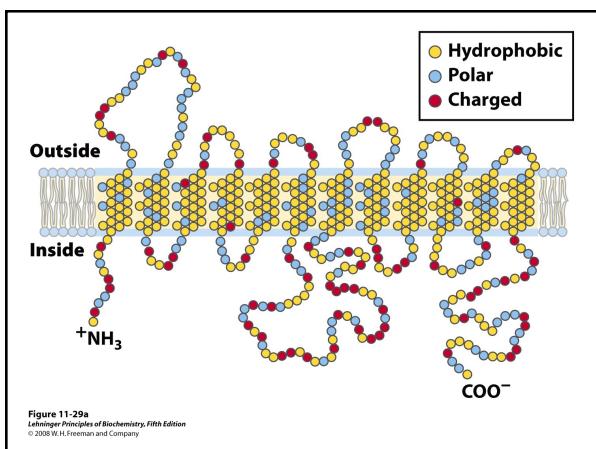


Transporters

- Carriers
 - Bind substrates with specificity
 - Rates are saturable
- Channels
 - Rates approach to the limit of diffusion



Model for Glucose Transport



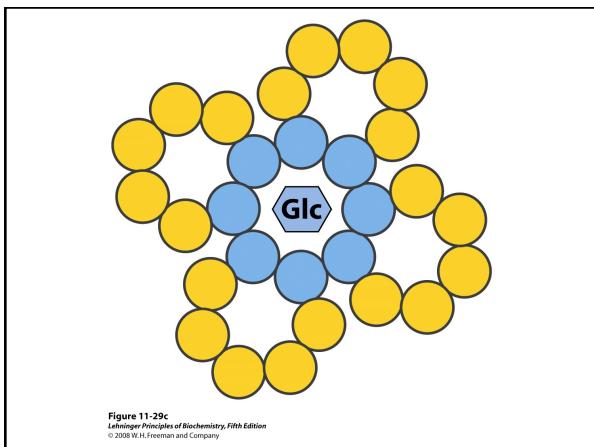


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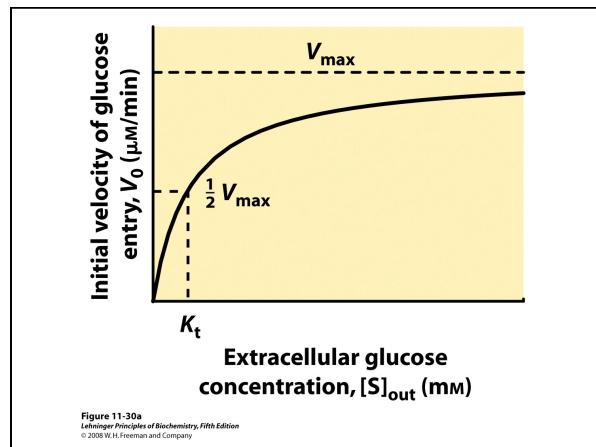


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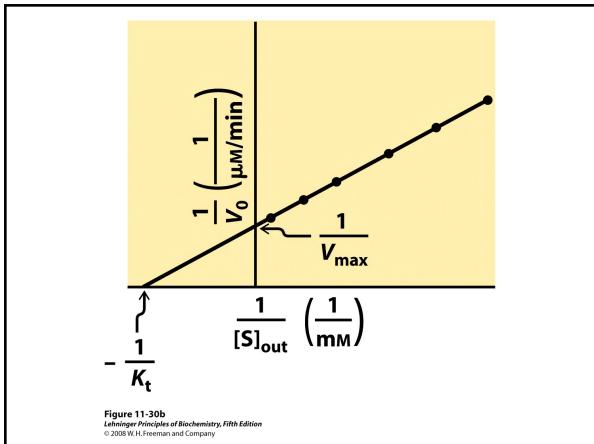


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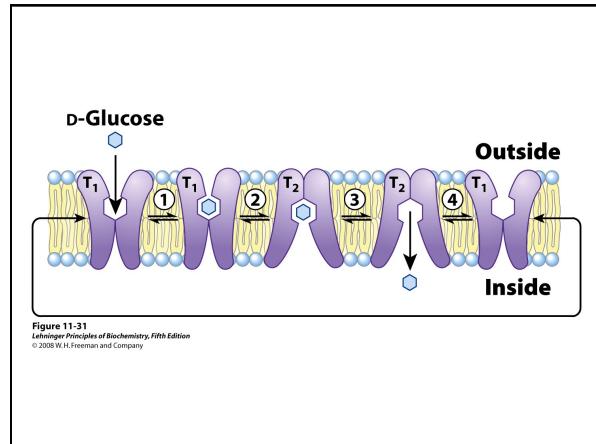


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K_t is related to its physiology function

TABLE 11-3 Glucose Transporters in the Human Genome			
Transporter	Tissue(s) where expressed	Gene	Role*
GLUT1	Ubiquitous	1.5 mM SLC2A1	Basal glucose uptake
GLUT2	Liver, pancreatic islets, intestine	66 mM SLC2A2	In liver, removal of excess glucose from blood; in pancreas, regulation of insulin release
GLUT3	Brain (neuronal)	SLC2A3	Basal glucose uptake
GLUT4	Muscle, fat, heart	5 mM SLC2A4	Activity increased by insulin
GLUT5	Intestine, testis, kidney, sperm	SLC2A5	Primarily fructose transport
GLUT6	Spleen, leukocytes, brain	SLC2A6	Possibly no transporter function
GLUT7	Liver microsomes	SLC2A7	—
GLUT8	Testis, blastocyst, brain	SLC2A8	—
GLUT9	Liver, kidney	SLC2A9	—
GLUT10	Liver, pancreas	SLC2A10	—
GLUT11	Heart, skeletal muscle	SLC2A11	—
GLUT12	Skeletal muscle, adipose, small intestine	SLC2A12	—

*Dash indicates role uncertain.

Table 11-3
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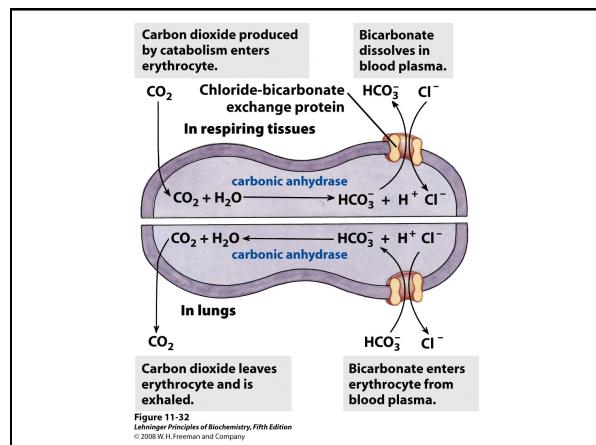


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Three Classes of Transport Systems

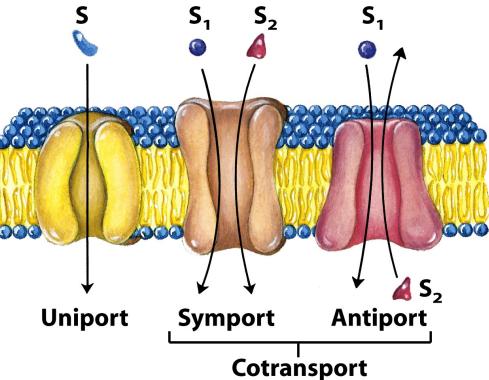


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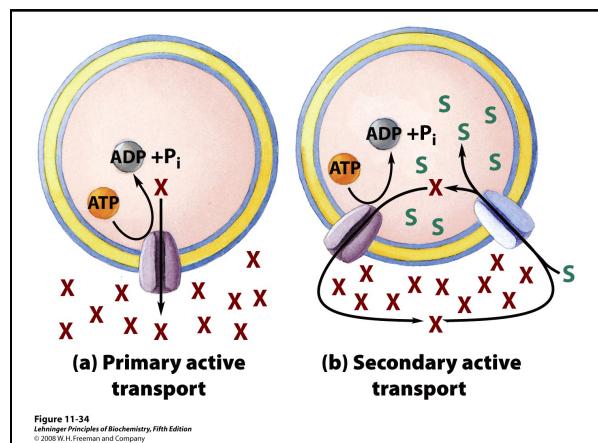
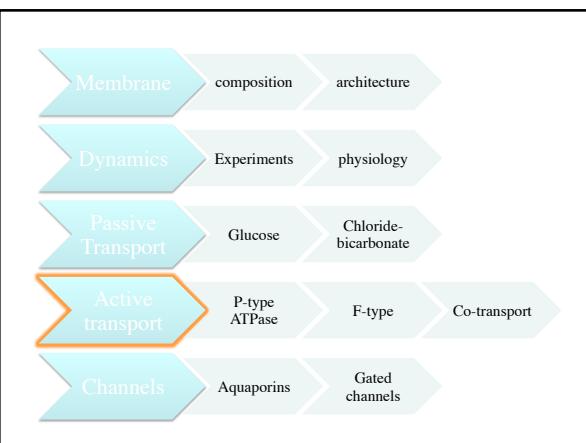


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SERCA, a P-type ATPase

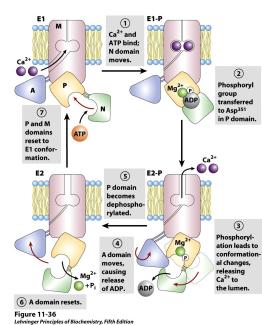


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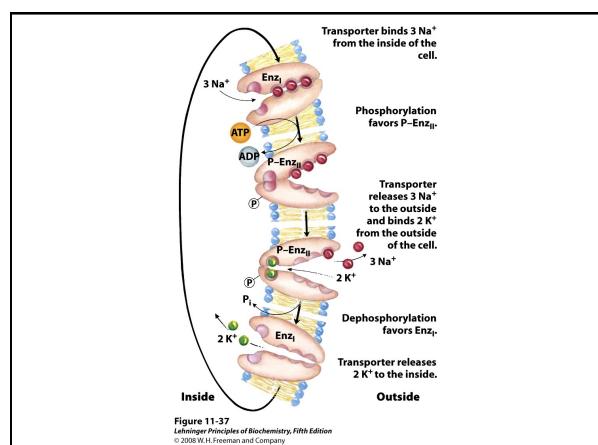
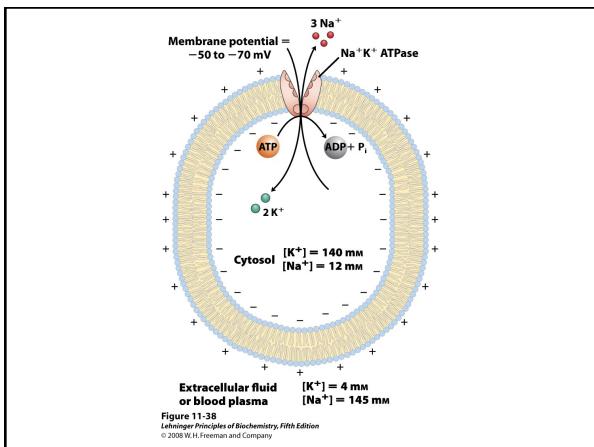


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Proton Transport and Chemical Energy of ATP

- Energy of ATP hydrolysis can be used to drive protons through the membrane
 - pH control in the cell by F-type ATPase
- Energy of the proton gradient can be used to synthesize ATP
 - In chloroplast and mitochondrial membranes by ATP synthase

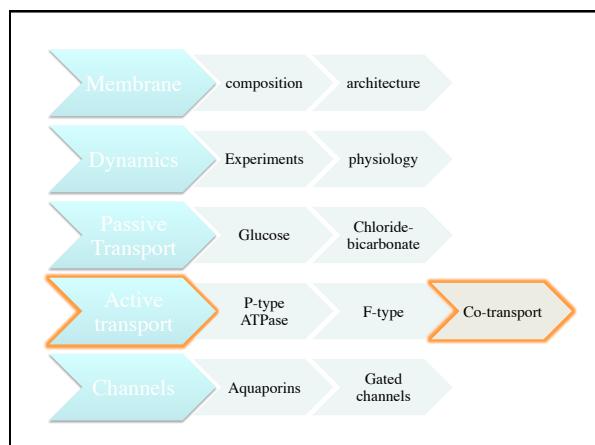
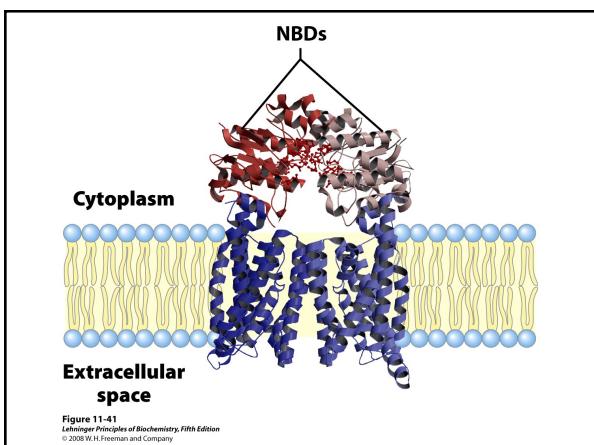
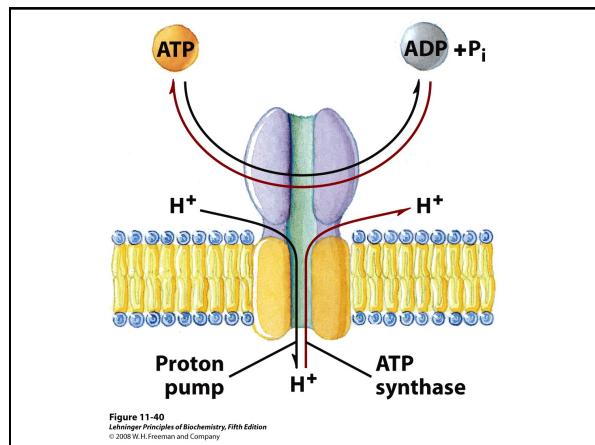
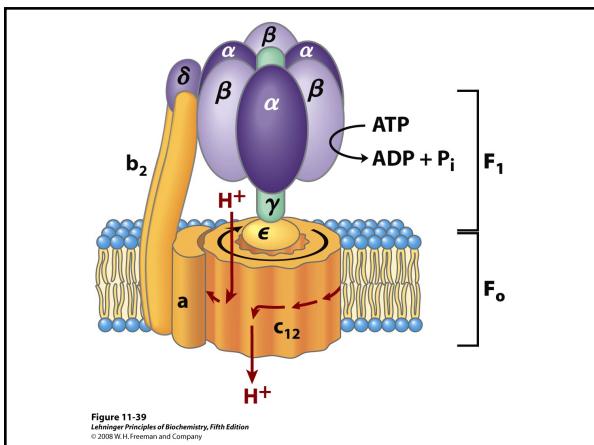
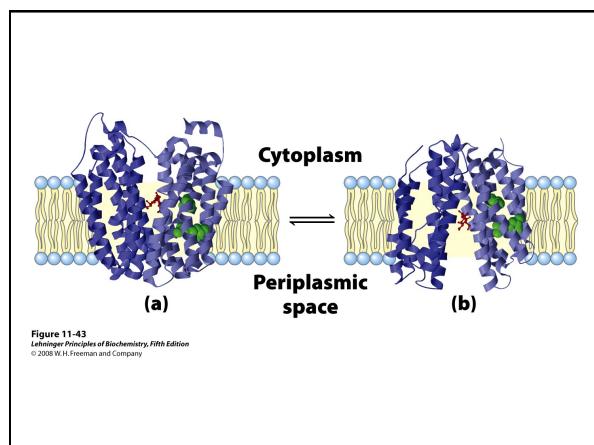
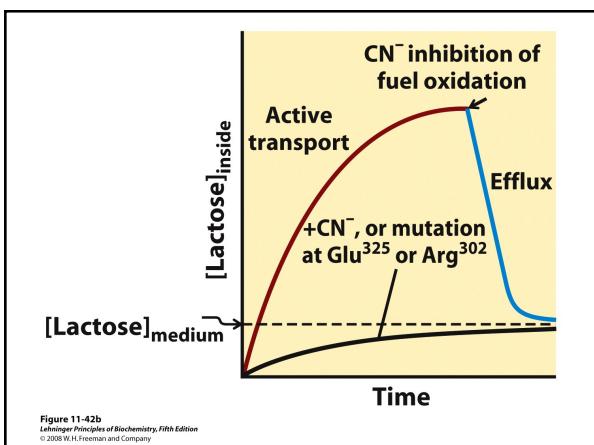
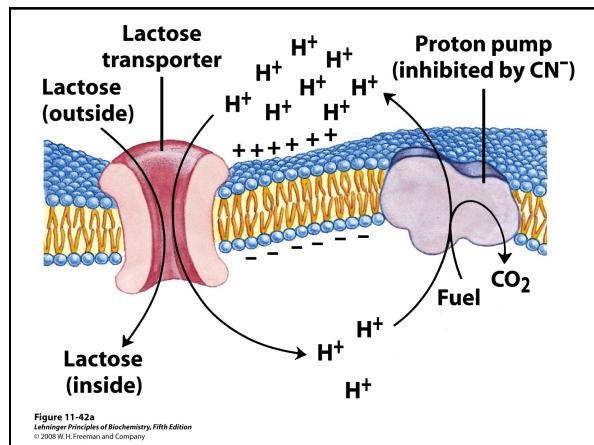
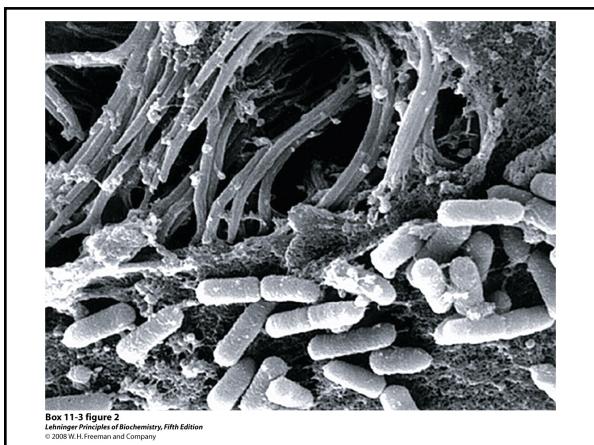
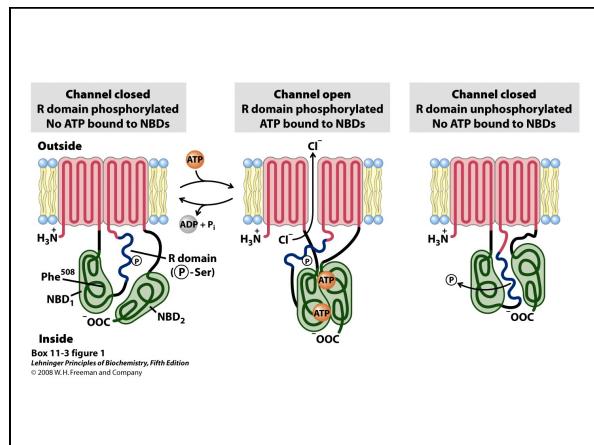
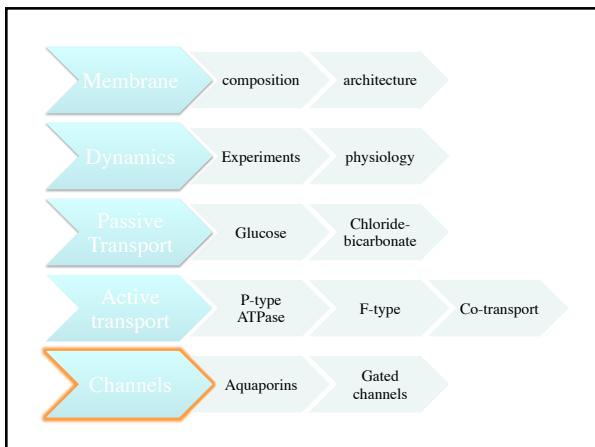
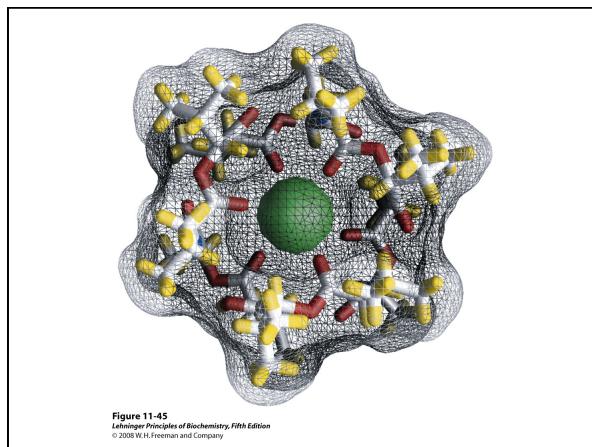
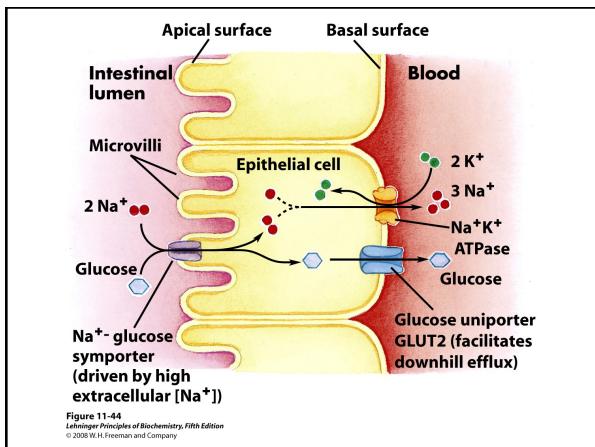


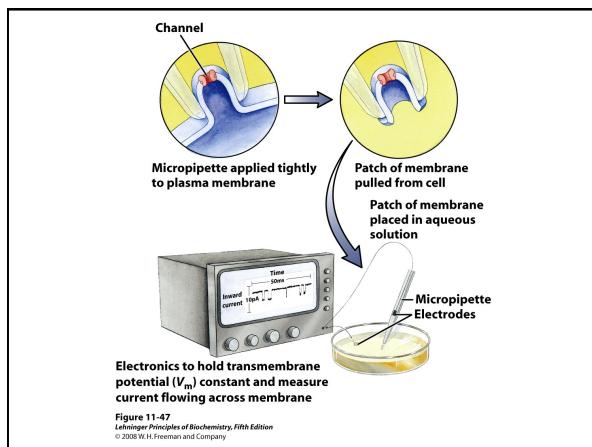
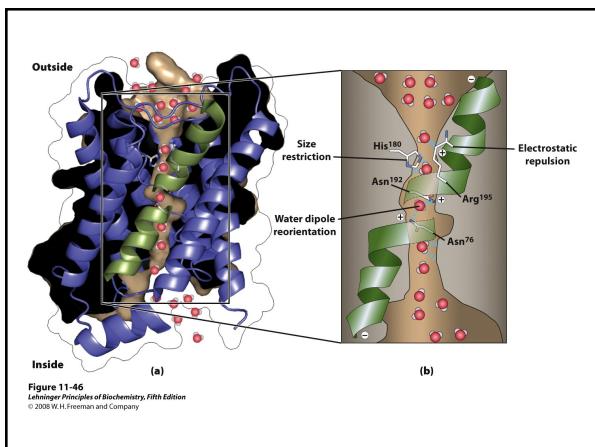
TABLE 11–4 Cotransport Systems Driven by Gradients of Na^+ or H^+			
Organism/ tissue/cell type	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transport
<i>E. coli</i>	Lactose	H^+	Symport
	Proline	H^+	Symport
	Dicarboxylic acids	H^+	Symport
Intestine, kidney (vertebrates)	Glucose	Na^+	Symport
	Amino acids	Na^+	Symport
Vertebrate cells (many types)	Ca^{2+}	Na^+	Antiport
Higher plants	K^+	H^+	Antiport
Fungi (<i>Neurospora</i>)	K^+	H^+	Antiport

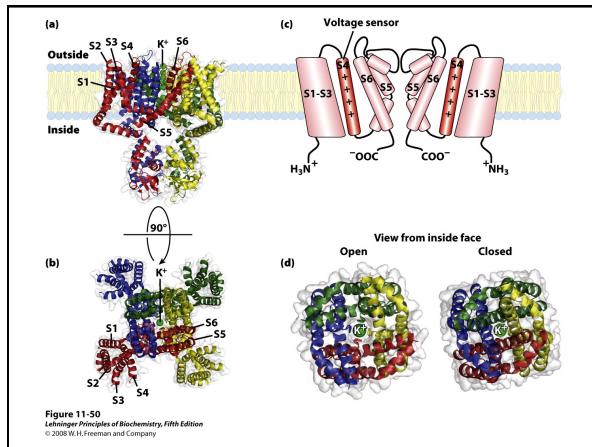
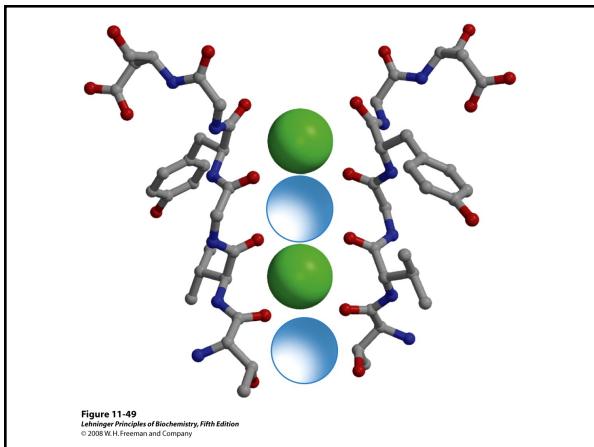
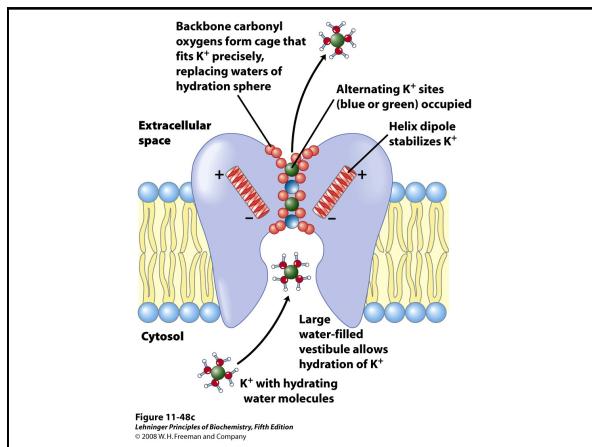
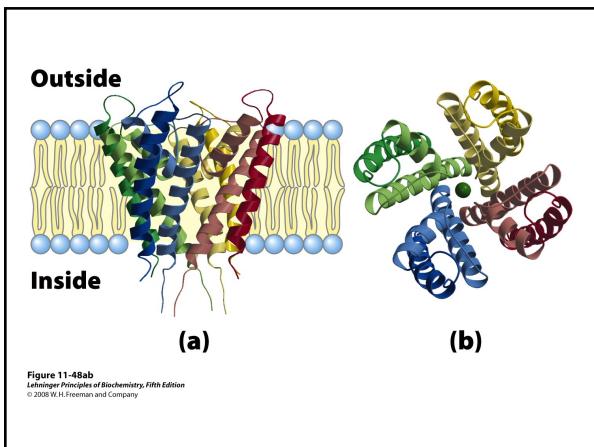
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Aquaporins Allow Rapid Water Passage through Membranes





Chapter 11: Summary

In this chapter, we learned that:

- membranes are composed of various lipids and proteins
- phospholipids form a selectively permeable bilayer
- properties of the bilayer depend on the lipid composition, which varies strongly from
 - organism to organism,
 - from tissue to tissue
 - from organelle to organelle
- membrane proteins play a variety of structural and functional roles, esp. in the transport of solutes across the membrane