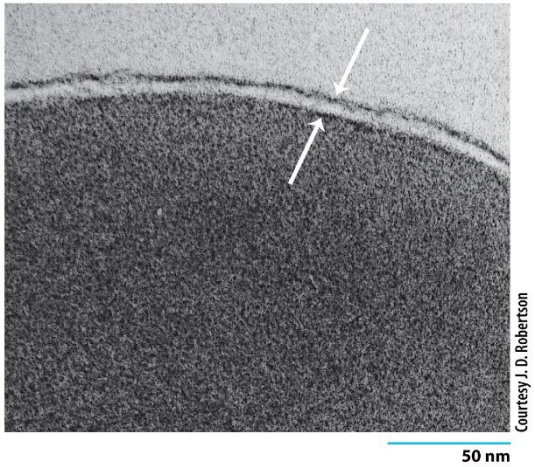
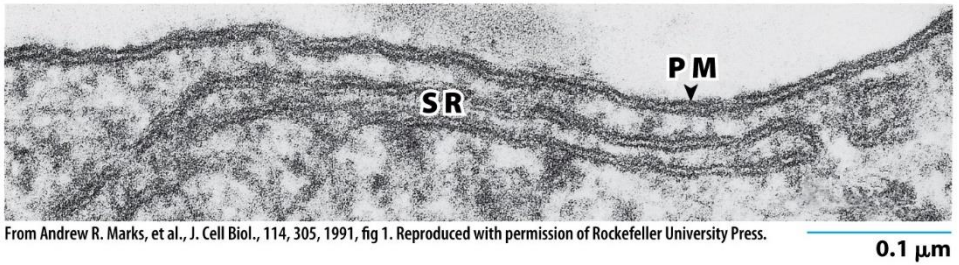
CHAPTER 8

Cellular Membrane

8.1 | Introduction to the Plasma Membrane





The trilaminar appearance of membranes as revealed by electron micrograph of the plasma membrane and sarcoplasmic reticulum.

Plasma membrane: The outer boundary of the cell that separates it from the world is a thin, fragile structure about 5 – 10 nm thick.

Not detectable with light microscope need electron microscope.

The 2 dark-staining layers in the electron micrographs correspond primarily to the inner & outer polar surfaces of the bilayer.

All membranes examined closely (plasma, nuclear or cytoplasmic) from plants, animals or microorganisms have the same ultrastructure.

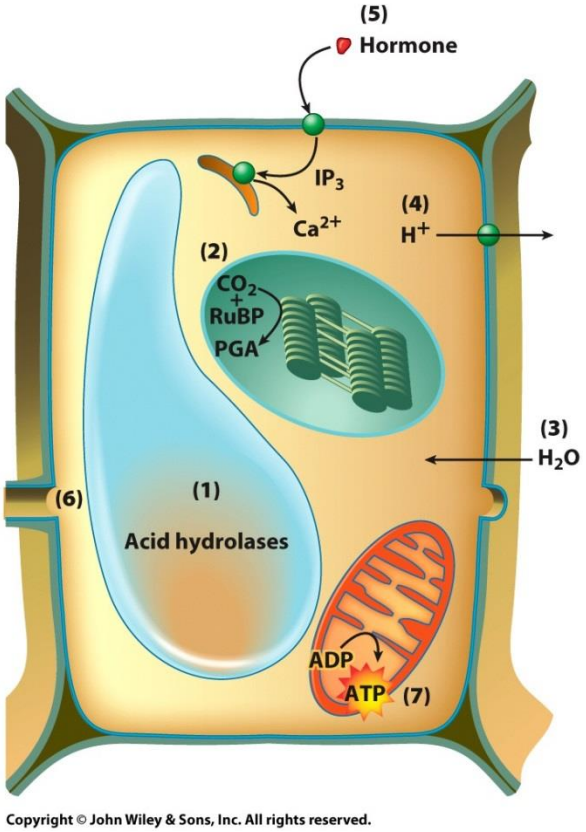
8.1 | Introduction to the Plasma Membrane

An Overview of Membrane Functions

Compartmentalization: Membranes form continuous sheets that enclose intracellular compartments. (acid hydrolases within the vacuole)

Scaffold for biochemical activities: Membranes provide a framework that organizes enzymes for effective interaction. (Carbon fixation)

Selectively permeable barrier: Membranes allow regulated exchange of substances between compartments. (H2O)



A summary of membrane functions in a plant cell.

8.1 | Introduction to the Plasma Membrane

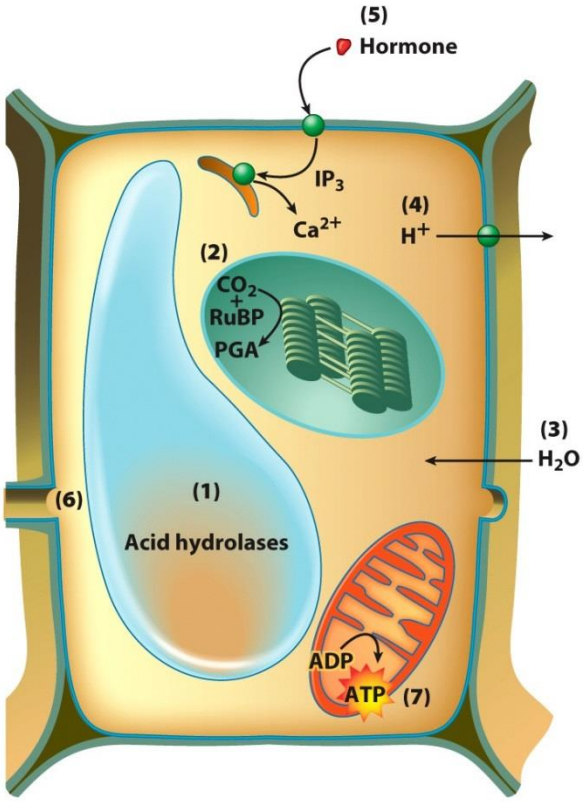
An Overview of Membrane Functions

Transporting solutes: Membrane proteins facilitate the movement of substances between compartments. (H+)

Responding to external signals: Membrane receptors transduce signals from outside the cell in response to specific ligands. (Hormone)

Intracellular interaction: Membranes mediate recognition and interaction between adjacent cells. (Plasmodesmata)

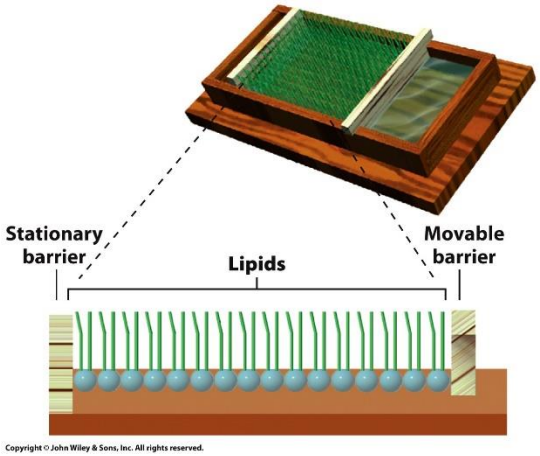
Energy transduction: Membranes transduce photosynthetic energy, convert chemical energy to ATP, and store energy.



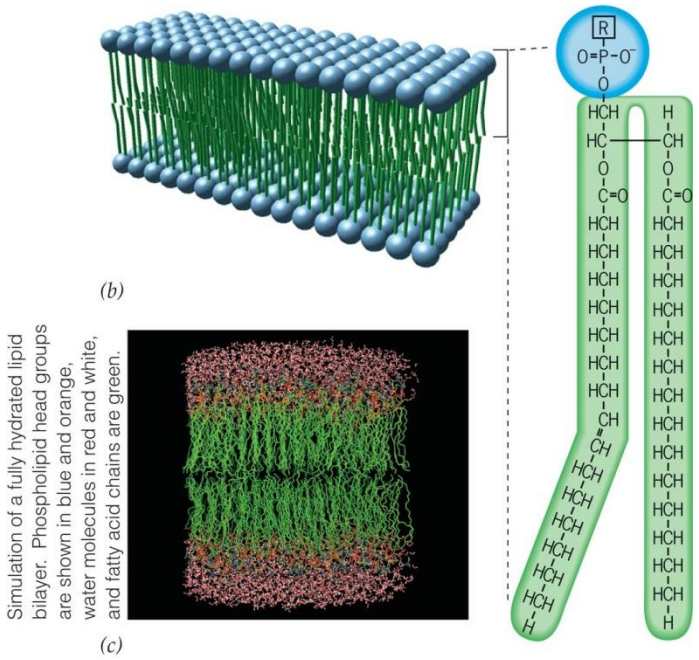
A summary of membrane functions in a plant cell.

8.1 | Introduction to the Plasma Membrane

A Brief History of Studies on Plasma Membrane Structure



Calculating the surface area of a lipid preparation



Membranes were found to be mostly composed of lipids because their dissolving power matched that of oil.

The **lipid bilayer** accounted for the 2:1 ratio of lipid to cell surface area.

The most energetically favored orientation for polar head groups is facing the aqueous compartments outside of the bilayer.

Bimolecular layer of phospholipids with water soluble head groups facing outward

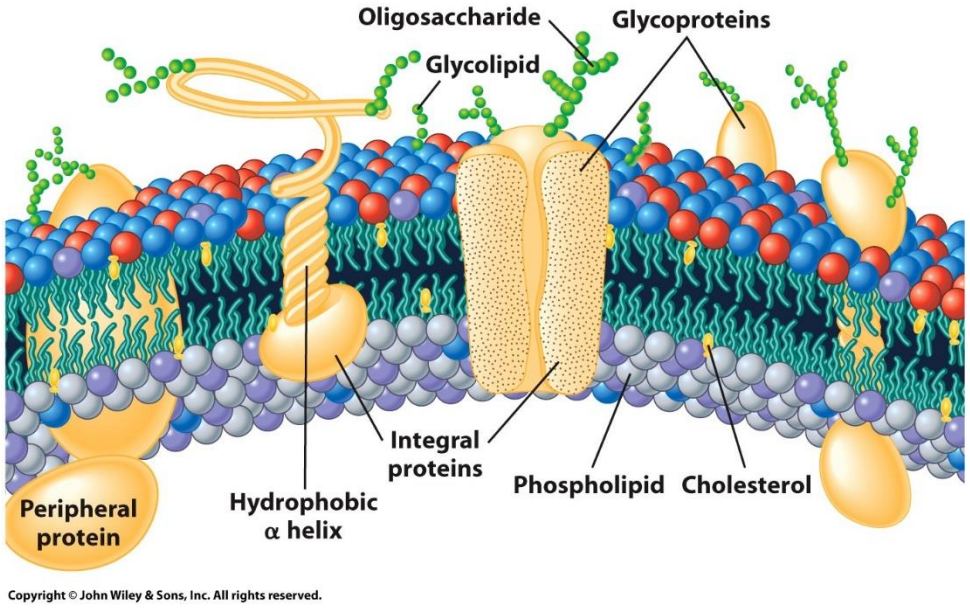
8.1 | Introduction to the Plasma Membrane

A Brief History of Studies on Plasma Membrane Structure

Cell physiologists determined that there must be more to the structure of membranes than simply a lipid bilayer.

Lipid solubility was not the sole determining factor as to whether a substance could penetrate the plasma membrane.

Surface tensions of membranes were calculated to be much lower than those of pure lipid structures; explained by the presence of protein in the membrane.



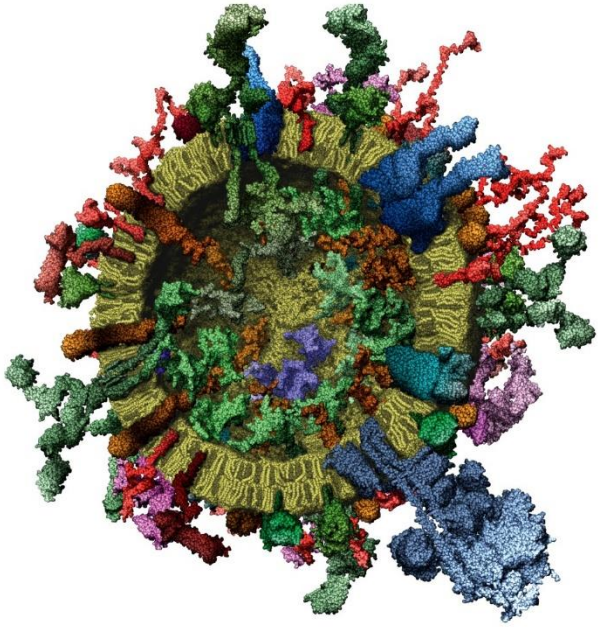
Organization of proteins embedded in the lipid bilayer

8.1 | Introduction to the Plasma Membrane

A Brief History of Studies on Plasma Membrane Structure

Protein is present in the form of individual protein molecules and protein complexes that penetrate a fluid lipid bilayer and extend out into the surrounding aqueous environment.

Due to lipid bilayer fluidity, membranes are dynamic structures in which the components are mobile and capable of coming together for transient interactions.



From Shigeo Takamori, et al, Courtesy of Reinhard Jahn, Cell, 127;841, 2006, reprinted with permission from Elsevier.

Molecular model of the membrane of a synaptic vesicle constructed with various proteins embedded into the lipid bilayer

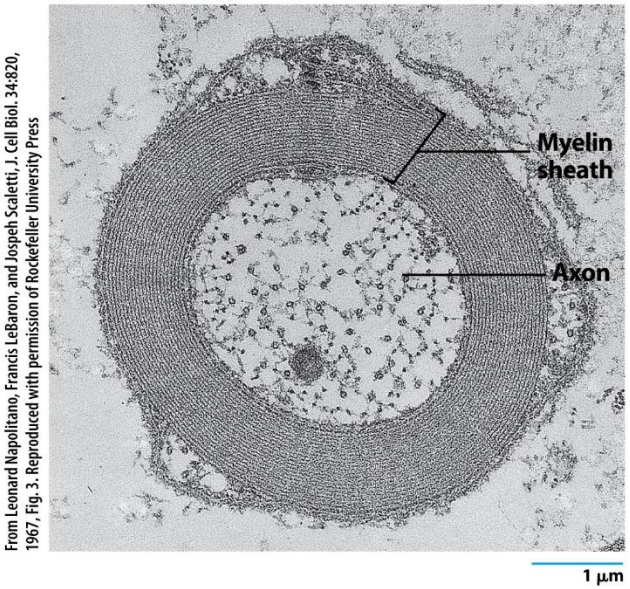
8.2 | The Lipid Composition of Membranes

Membranes are lipid–protein assemblies held together by noncovalent bonds.

The lipid bilayer is a structural backbone and barrier to prevent random movements of materials into and out of the cell.

A unique complement of membrane proteins that contributes to the specialized activities of that cell type.

The ratio of lipid to protein varies, depending on the type of cellular membrane, the type of organism, and the type of cell.



From Leonard Napolitano, Francis LeBaron, and Jospeh Scaletti, J. Cell Biol. 34:820, 1967, Fig. 3. Reproduced with permission of Rockefeller University Press

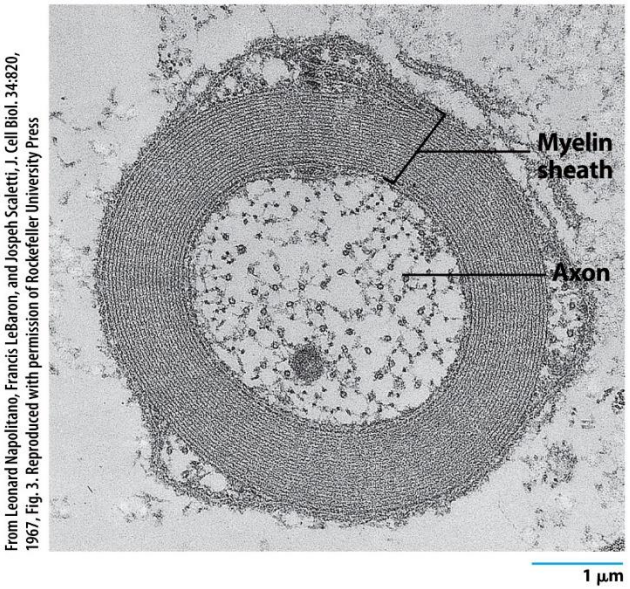
Electron micrograph of a nerve cell axon

8.2 | The Lipid Composition of Membranes

The inner mitochondrial membrane has a very high ratio of protein/lipid, whereas a myelin sheath has a low ratio of protein/lipid.

The inner mitochondrial membrane contains the protein carriers of the electron-transport chain, and relative to other membranes, lipid is diminished.

The myelin sheath acts as electrical insulation for the nerve cell, best carried out by a thick lipid layer of high electrical resistance with a minimal content of protein.



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Electron micrograph of a nerve cell axon

8.2 | The Lipid Composition of Membranes

Membrane Lipids

Membrane lipids are **amphipathic** with three main types:

Phosphoglycerides are diacylglycerides with small functional head groups linked to the glycerol backbone by phosphate ester bonds.

Sphingolipids are ceramides formed by the attachment of sphingosine to fatty acids.

Cholesterol is a smaller and less amphipathic lipid that is only found in animals.

8.2 | The Lipid Composition of Membranes

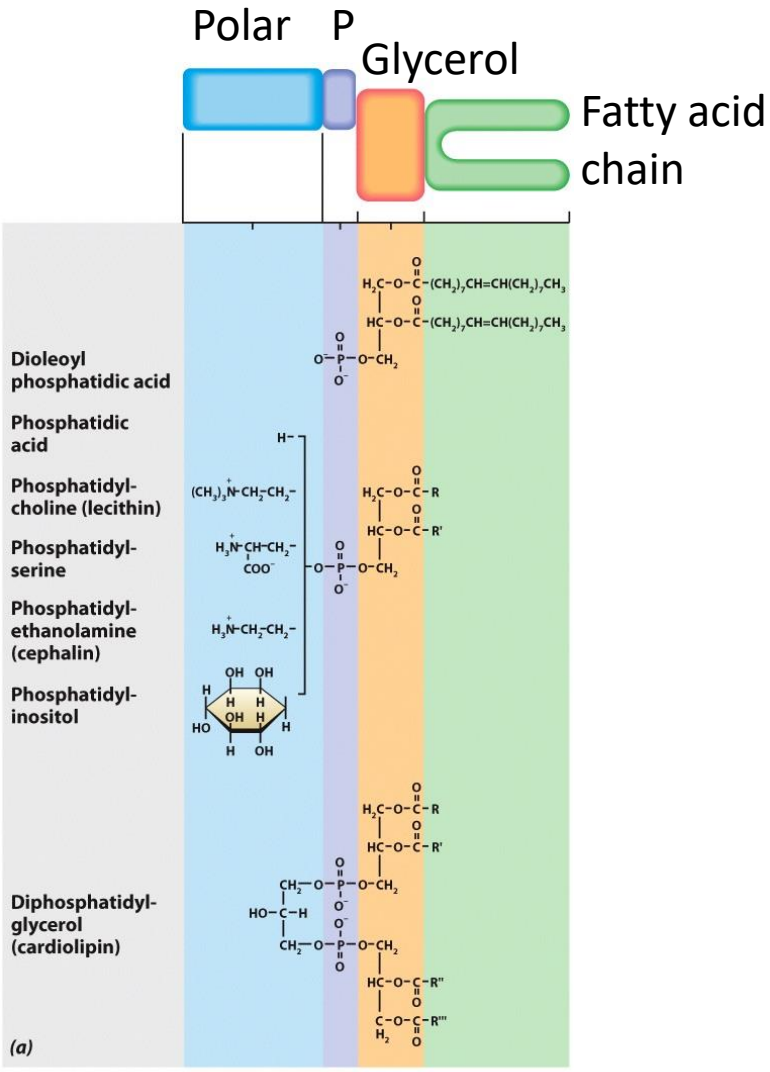
Membrane Lipids: Phosphoglycerides

Lipids with a phosphate group are **phospholipids**. Phospholipids built on a glycerol backbone are called **phosphoglycerides**.

Membrane glycerides are diglycerides; two hydroxyl groups of glycerol are esterified to fatty acids; the third is esterified to a hydrophilic phosphate group.

Most phosphoglycerides have a small hydrophilic group linked to phosphate: choline, ethanolamine, serine or inositol.

This group, together with the negatively charged phosphate, forms a highly water-soluble domain, called the **head group.**



8.2 | The Lipid Composition of Membranes

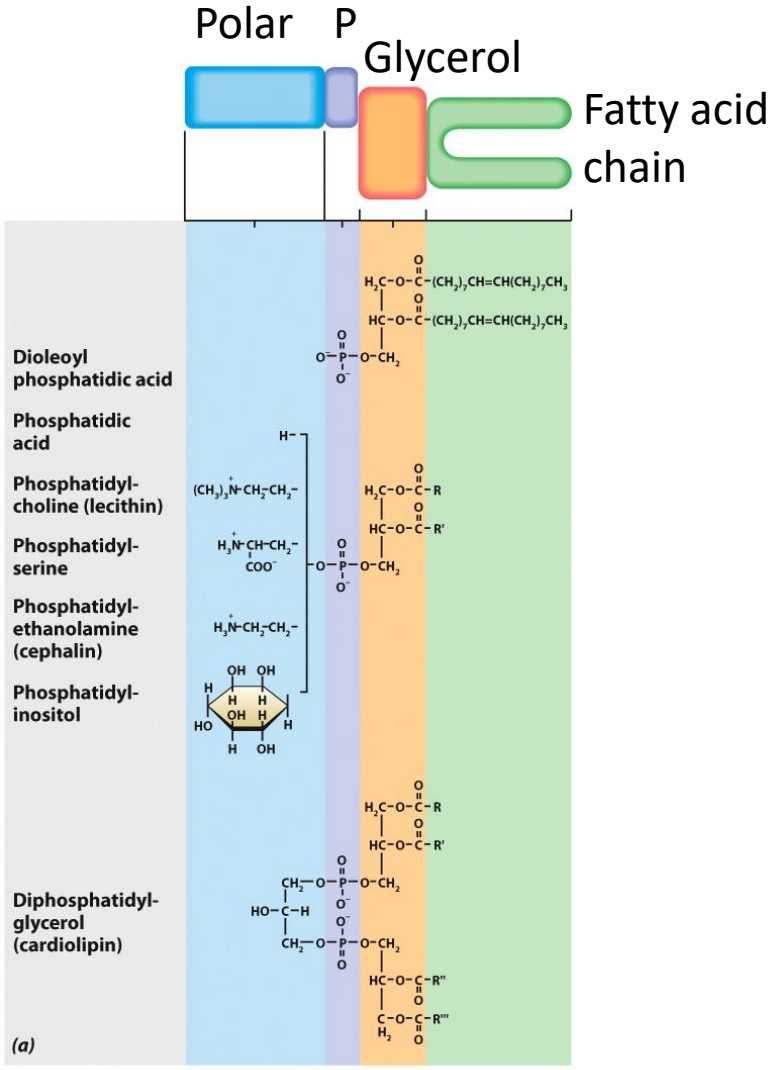
Membrane Lipids: Phosphoglycerides

Fatty acyl chains are hydrophobic, unbranched hydrocarbons approximately 16 to 22 carbons in length.

A fatty acid may be fully saturated, monounsaturated, or polyunsaturated.

Phosphoglycerides often contain one unsaturated and one saturated fatty acyl chain.

With fatty acid chains at one end of the molecule and a polar head group at the other end, all of the phosphoglycerides exhibit a distinct amphipathic character.



8.2 | The Lipid Composition of Membranes

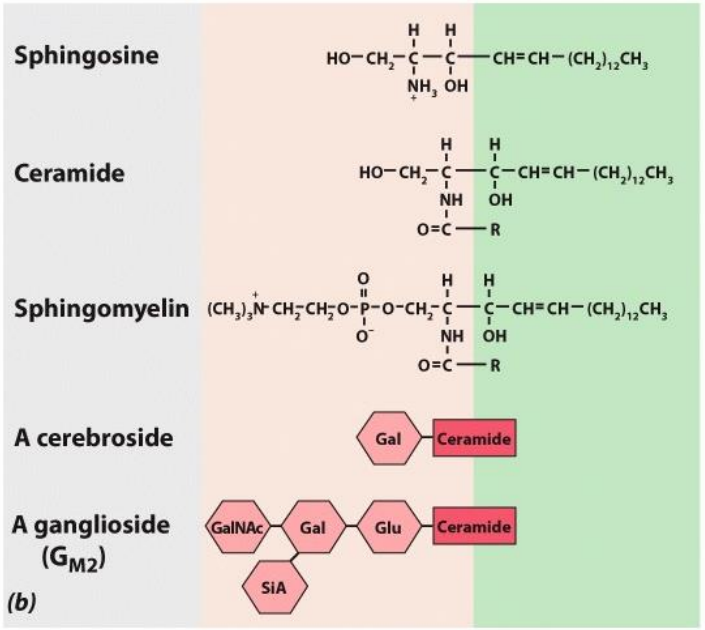
Membrane Lipids: Sphingolipids

Sphingolipids are derivatives of **sphingosine**, an amino alcohol that contains a long hydrocarbon chain.

Sphingolipids consist of sphingosine linked to a fatty acid by its amino group, called a ceramide.

If the substitution is phosphorylcholine, the molecule is sphingomyelin. If the substitution is a carbohydrate, the molecule is a glycolipid.

If the carbohydrate is a simple sugar, the glycolipid is a *cerebroside*; if it is a small cluster of sugars that includes sialic acid, the glycolipid is a *ganglioside*.



The chemical structure of membrane lipids

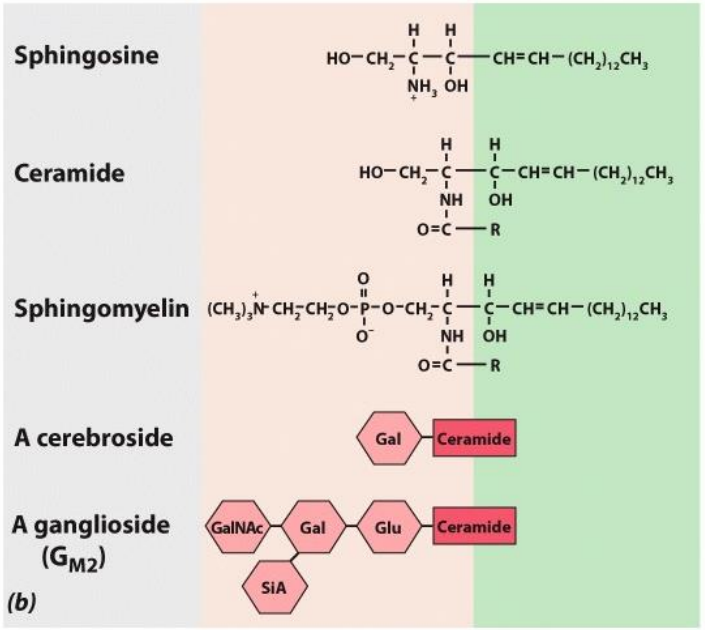
8.2 | The Lipid Composition of Membranes

Membrane Lipids: Sphingolipids

The nervous system is rich in glycolipids; myelin sheaths contain a high content of galactocerebroside.

Mice lacking the enzyme that makes this glycolipid exhibit severe muscular tremors and eventual paralysis. Humans unable to synthesize ganglioside suffer from a serious neurological disease characterized by severe seizures and blindness.

Glycolipids play a role in infectious diseases; toxins that cause cholera and botulism both enter their target cell by first binding to cell-surface gangliosides, as does the influenza virus.



The chemical structure of membrane lipids

8.2 | The Lipid Composition of Membranes

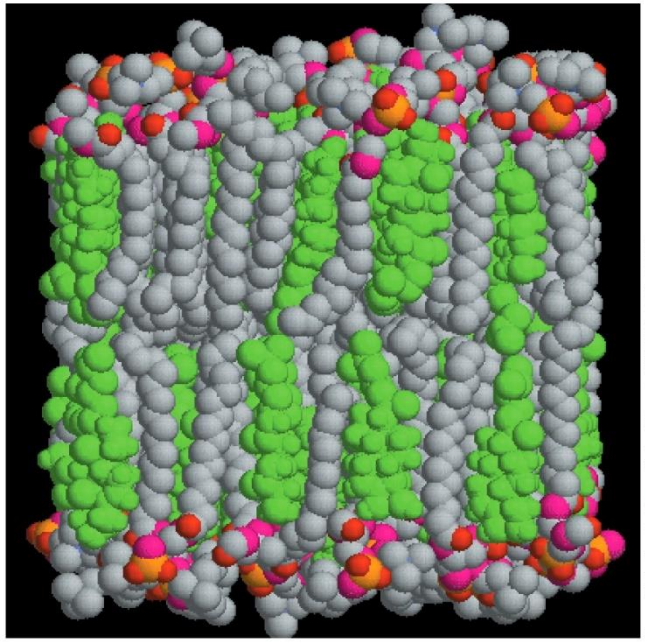
Membrane Lipids: Cholesterol

Cholesterol is a smaller and less amphipathic lipid that is only found in animals.

A sterol that makes up to 50% of animal membrane lipids.

The -OH group is oriented toward membrane surface.

Carbon rings are flat and rigid; interfere with movement of phospholipid fatty acid tails



From H. L. Scott, Curr. Opin. Struct. Biol 12: 499, 2002, Figure 3. © 2002, with permission from Elsevier.

Cholesterol molecules (green) oriented with their small hydrophilic end facing the external surface of the bilayer

8.2 | The Lipid Composition of Membranes

The Nature and Importance of the Lipid Bilayer

TABLE 4.1 Lipid Compositions of Some Biological Membranes*

Lipid	Human erythrocyte	Human myelin	Beef heart mitochondria	*E. coli*
Phosphatidic acid	1.5	0.5	0	0
Phosphatidylcholine	19	10	39	0
Phosphatidyl-ethanolamine	18	20	27	65
Phosphatidylgycerol	0	0	0	18
Phosphatidylserine	8.5	8.5	0.5	0
Cardiolipin	0	0	22.5	12
Sphingomyelin	17.5	8.5	0	0
Glycolipids	10	26	0	0
Cholesterol	25	26	3	0

*The values given are weight percent of total lipid.
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Cells membranes have distinct lipid compositions, differing in lipid types, head groups, and species of fatty acyl chain(s).

Membranes contain hundreds of chemically distinct species of phospholipids.

Lipid composition can determine the physical state of the membrane and influence membrane protein activity. Membrane lipids can be precursors for highly active chemical messengers that regulate cellular function.

8.2 | The Lipid Composition of Membranes

The Nature and Importance of the Lipid Bilayer

The entire lipid bilayer is only about 60 Å (6 nm) thick.

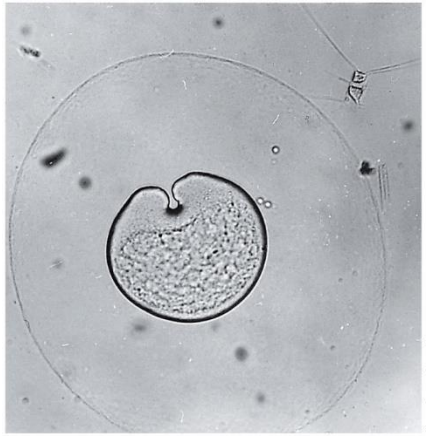
Since hydrocarbon chains of the lipid bilayer not exposed to the aqueous environment, membranes are always continuous, unbroken structures.

Membranes form extensive inter-connected networks within the cell.

Lipid bilayers are flexible, so membranes are deformable and their shape can change, as occurs during locomotion or cell division.

Movement: ruffling of the plasma membrane of a migrating cell

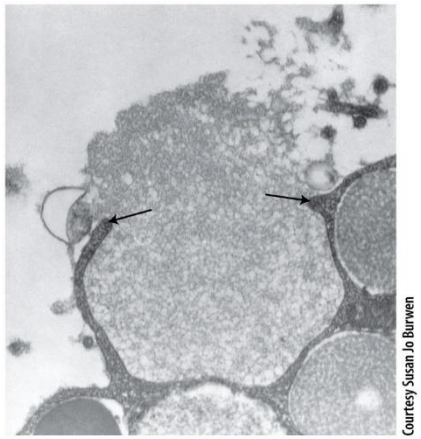
Courtesy Jean Paul Revel



Courtesy Gary Freeman

Division: invagination of the plasma membrane during cell division

Fusion: plasma membranes of sperm and egg unite



Courtesy Susan Jo Burwen

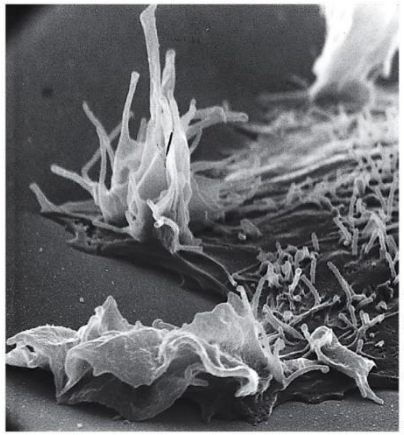
8.2 | The Lipid Composition of Membranes

The Nature and Importance of the Lipid Bilayer

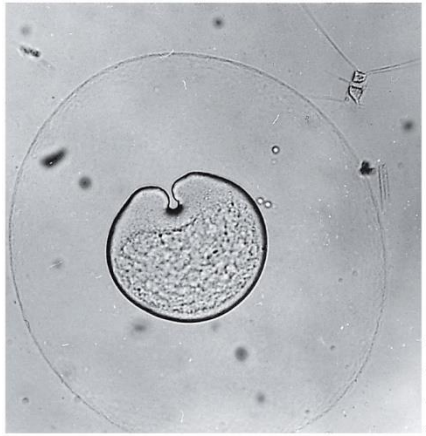
The lipid bilayer facilitates the regulated fusion or budding of membranes, processes in which two separate membranes come together to become one continuous sheet.

The lipid bilayer helps to maintain the proper internal composition of a cell and to separate electric charges across the plasma membrane.

Movement: ruffling of the plasma membrane of a migrating cell



Courtesy Jean Paul Revel



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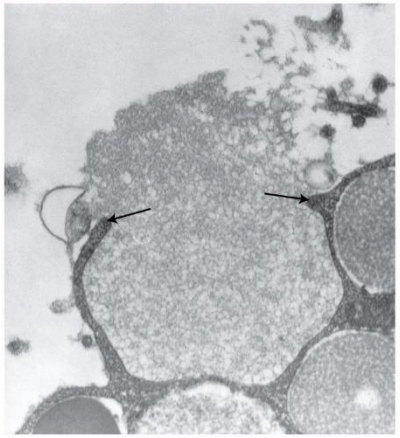
Division: invagination of the plasma membrane during cell division



Courtesy Susan Jo Burwen

Fusion: plasma membranes of sperm and egg unite

8.2 | The Lipid Composition of Membranes

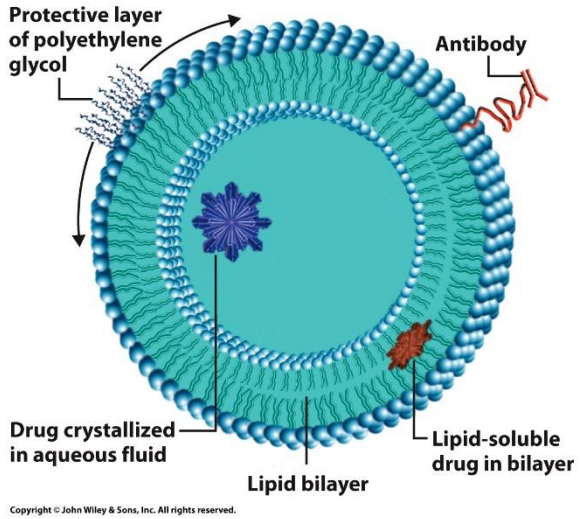
The Nature and Importance of the Lipid Bilayer

The lipid bilayer can self-assemble, demonstrated easily *in vitro*.

Phospholipid molecules assemble spontaneously to form the walls of fluid-filled spherical vesicles, called liposomes.

Liposomes have proven invaluable in membrane research.

Membrane proteins can be inserted into liposomes and their function studied in a much simpler environment than that of a natural membrane.



Liposomes: synthetic vesicles

8.2 | The Lipid Composition of Membranes

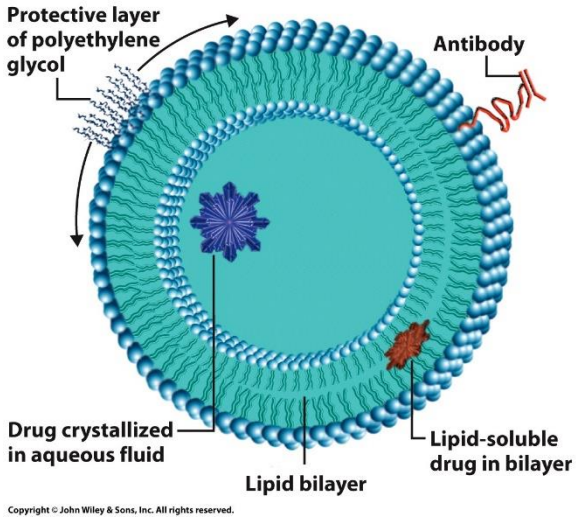
The Nature and Importance of the Lipid Bilayer

Liposomes are vehicles to deliver drugs or DNA within the body; they can be linked to the liposome wall or contained at high concentration within its lumen.

These walls are constructed to contain specific proteins for selective binding to the surfaces of particular target cells.

Stealth liposomes contain an outer polymer coating that protects the liposomes from immune destruction.

Caelyx, a stealth liposome containing doxorubicin, is an accepted therapy for treatment of metastatic breast cancer.



Liposomes: synthetic vesicles

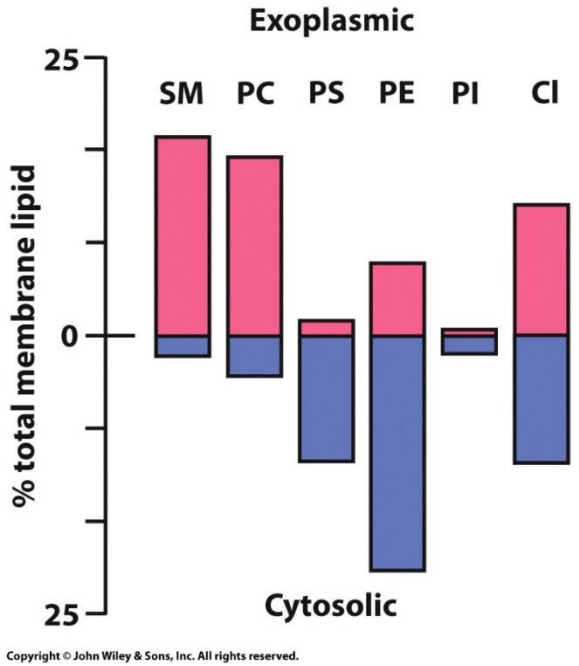
8.2 | The Lipid Composition of Membranes

The Asymmetry of the Membrane Lipids

The lipid bilayer consists of two distinct leaflets that have a distinctly different lipid composition.

Compared to the inner leaflet, the outer leaflet has a relatively high concentration of PC (and sphingomyelin) and a low concentration of PE and PS.

The lipid bilayer is composed of two semi-stable, independent monolayers having different physical and chemical properties.



SM: sphingomyelin
PC: phosphatidylcholine
PS: phosphatidylserine
PE: phosphatidylethanolamine
PI: phosphatidylinositol
Cl: cholesterol

8.2 | The Lipid Composition of Membranes

The Asymmetry of the Membrane Lipids

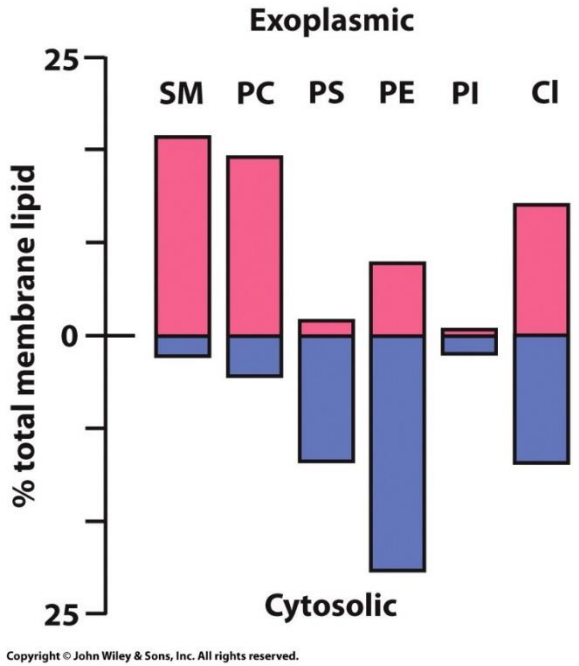
Glycolipids are in the outer leaflet where they act as receptors for ligands.

PE (inner) promotes membrane curvature for membrane budding and fusion.

PS (inner) has a negative charge to bind positively charged lysine and arginine residues on adjacent proteins.

PS (outer) on aging lymphocytes marks the cells for destruction by macrophages.

PI (inner) can be phosphorylated, which converts the lipid into a phosphoinositide for signal transduction pathways.



SM: sphingomyelin
PC: phosphatidylcholine
PS: phosphatidylserine
PE: phosphatidylethanolamine
PI: phosphatidylinositol
Cl: cholesterol

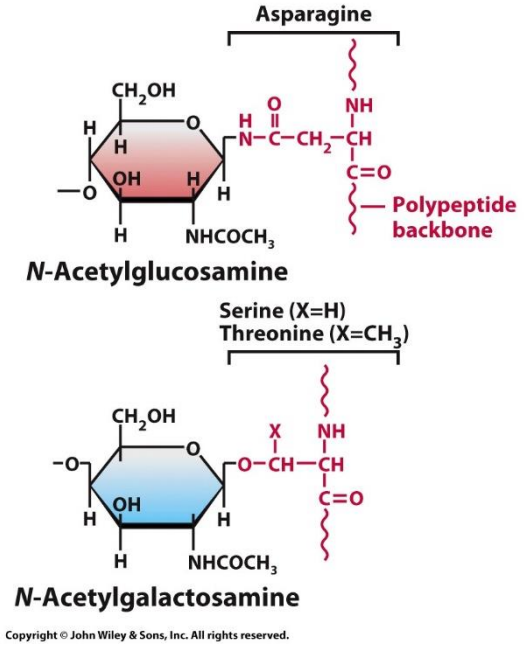
8.3 | Membrane Carbohydrates

Plasma membranes of eukaryotic cells have carbohydrate (2-10% by weight), with 90% glycoproteins and 10% glycolipids.

Carbohydrates face outward into the extracellular space, and internal cellular membranes faces away from the cytosol.

Glycoproteins have short, branched hydrophilic **oligosaccharides** (<15 sugars per chain) with extensive variability in composition and structure.

Oligosaccharides may be attached to several different amino acids by two types of linkages: N-linkages and O-linkages.



Two types of linkages that join sugars to a polypeptide chain

8.3 | Membrane Carbohydrates

Carbohydrate projections play an important role in mediating the interactions of a cell with its environment and sorting of proteins to different cellular compartments.

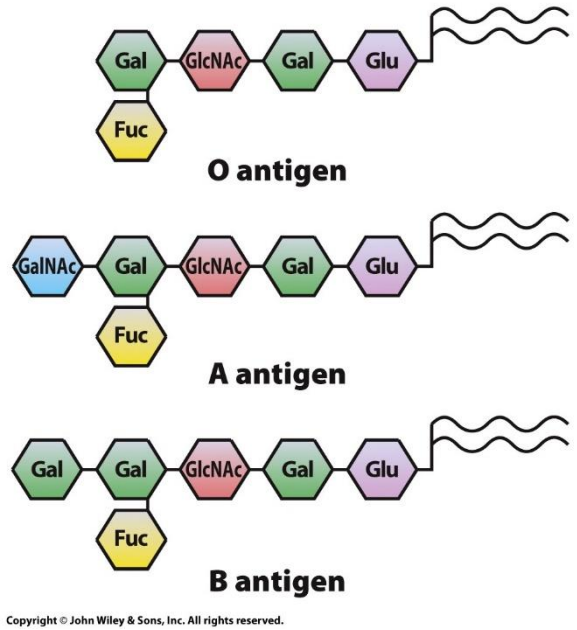
Glycolipid carbohydrates of the red blood cell plasma membrane determine whether a person's blood type (A, B, AB, or O).

A: Enzyme adds N-acetylgalactosamine to the end of the chain.

B: Enzyme adds galactose to the chain terminus.

AB: Both enzymes present.

O: Lack enzymes capable of attaching either terminal sugar.



Blood-group antigens

8.4 | Membrane Proteins

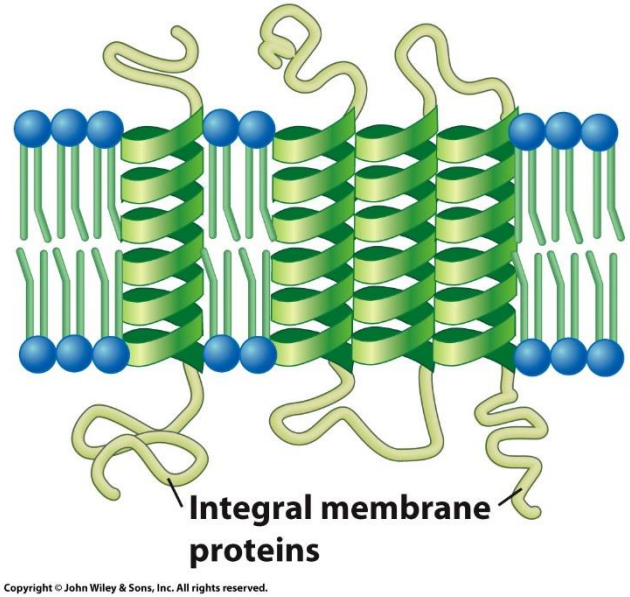
Membrane proteins attach to the bilayer asymmetrically, giving the membrane a distinct "sidedness"

Membrane proteins grouped into 3 classes:

Integral proteins: Penetrate and pass through lipid bilayer; make up 25 -30% of all encoded proteins and 60% of current drug targets.

Amphipathic- hydrophilic domains anchor them in the bilayer and hydrophilic regions form functional domains outside of the bilayer.

Channel proteins have hydrophilic cores that form aqueous channels in the membrane-spanning region.



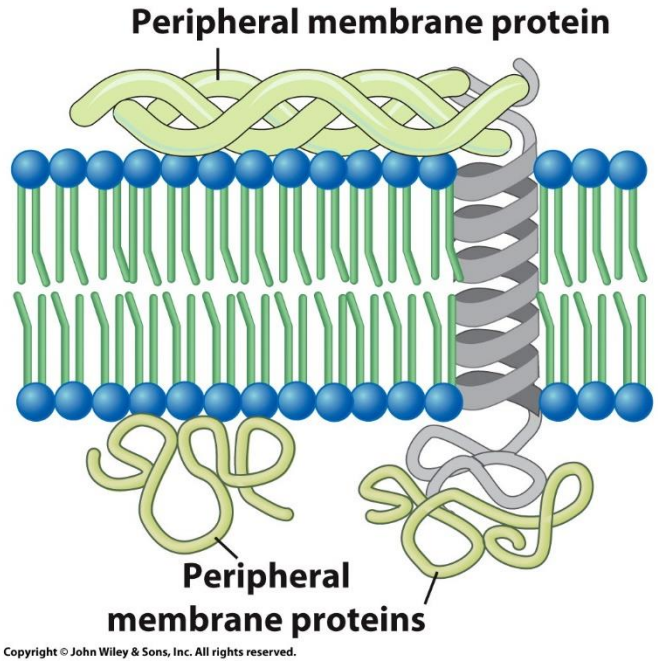
Integral proteins

8.4 | Membrane Proteins

Membrane proteins can be grouped into three distinct classes:

Peripheral proteins: Attached to the membrane by weak bonds and are easily solubilized.

Located entirely outside of bilayer on either the extracellular or cytoplasmic side; associated with membrane surface by non-covalent bonds.



Peripheral proteins

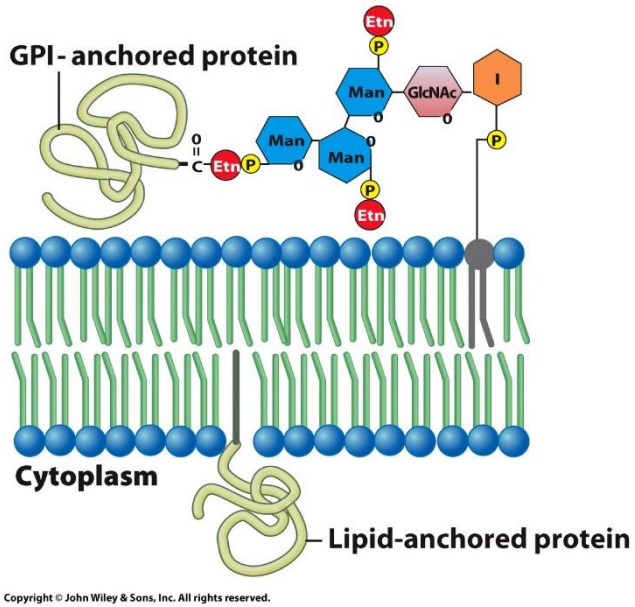
8.4 | Membrane Proteins

Membrane proteins can be grouped into three distinct classes:

Lipid-anchored membrane proteins are distinguished both by the types of lipid anchor and their orientation.

Glycophosphatidylinositol **(GPI)-linked proteins** found on the outer leaflet can be released by inositol-specific phospholipases.

Some inner-leaflet proteins are anchored to membrane lipids by long hydrocarbon chains.



Lipid-anchored proteins

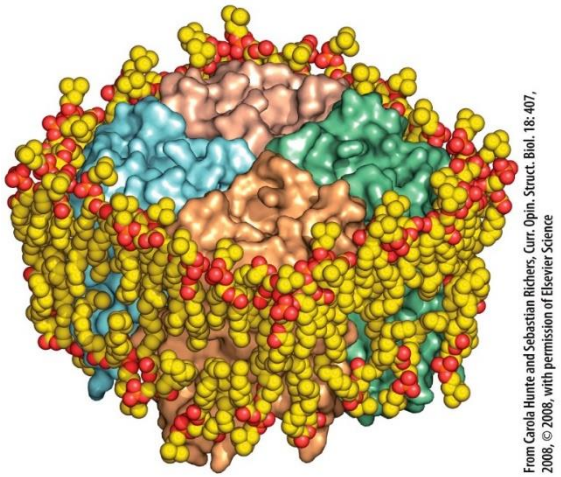
8.4 | Membrane Proteins

Integral Membrane Proteins

Integral membrane proteins function as receptors that bind ligands, channels or transporters to move ions/solutes across the membrane, or agents that transfer electrons during photosynthesis and respiration.

They are amphipathic, having both hydrophilic and hydrophobic portions; the hydrophobic transmembrane domains form van der Waals interactions with the fatty acyl chains of the bilayer.

This preserves the permeability barrier of the membrane, since the protein is anchored within the bilayer and has direct contact with surrounding lipid molecules.



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Driven by van der Waals forces between amino acids and lipids, proteins can be surrounded by a closely applied shell of lipid molecules.

8.4 | Membrane Proteins

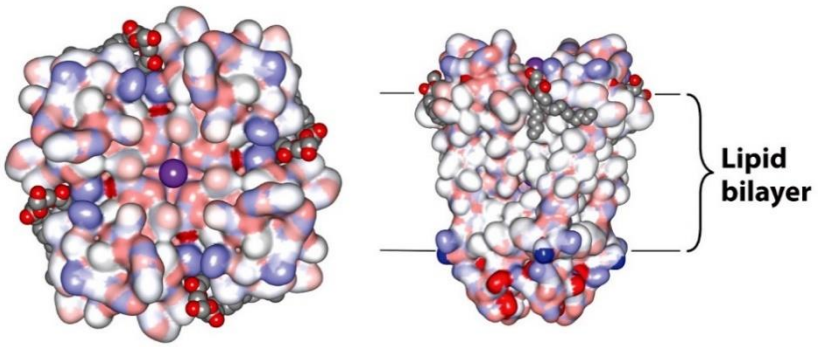
Integral Membrane Proteins

Most lipid molecules that make contact with a transmembrane domain are rapidly exchanged with other lipid molecules in the bilayer.

Specific sites on membrane proteins can form important functional interactions with specific lipid molecules.

Anionic lipid molecules can bind at subunit interfaces of a tetrameric KcsA K^+ channel.

The channel does not open normally in a bilayer that lacks these specific lipid molecules.



From A.G. Lee, Trends Biochem. Sci. 36:497, 2011, © 2011; with permission from Elsevier.

Views of a tetrameric membrane protein, the bacterial K+ channel

8.4 | Membrane Proteins

Integral Membrane Proteins

Protein domains that project into either the cytoplasm or extracellular space tend to be like the globular proteins, with hydrophilic surfaces that interact with water-soluble substances.

Transmembrane domains can be devoid of water molecules, while others allow the water to penetrate deeply.

Large families of membrane proteins contain an interior channel with key hydrophilic residues at strategic locations that provides an aqueous passageway through the lipid bilayer.

Integral proteins need not be fixed structures but may be able to move laterally within the membrane.

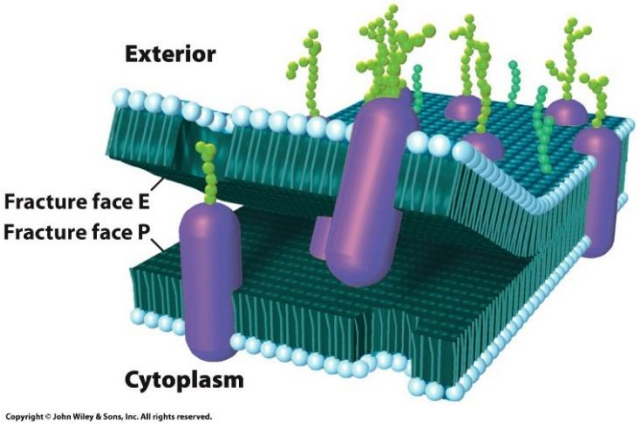
8.4 | Membrane Proteins

Integral Membrane Proteins

The concept that proteins penetrate through membranes was derived primarily from the results of freeze-fracture replication.

Tissue is frozen solid and then struck with a knife blade, which often causes a fracture plane between the two leaflets of the lipid bilayer.

Deposited metals on the exposed surfaces form a shadowed *replica*, viewed by electron microscopy.



Ectoplasmic vs protoplasmic

8.4 | Membrane Proteins

Peripheral Membrane Proteins

Peripheral proteins associate with the membrane by weak electrostatic bonds, and can usually be solubilized by extraction with high-concentration salt solutions.

The best studied are located on the cytosolic surface of the plasma membrane, where they form a fibrillar network that acts as a membrane "skeleton" to provide mechanical support to the membrane and to function as an anchor for integral membrane proteins.

Other cytosolic peripheral proteins act as enzymes, specialized coats, or factors that transmit transmembrane signals.

Peripheral proteins typically have a dynamic relationship with the membrane, being recruited or released as needed.

8.4 | Membrane Proteins

Lipid-Anchored Membrane Proteins

Numerous peripheral membrane proteins have a glycosyl-phosphatidylinositol linkage that embeds them in the outer leaflet of the lipid bilayer, so are called GPI-anchored proteins.

Examples include the normal cellular scrapie protein PrP^C, as well as various receptors, enzymes, and cell-adhesion proteins.

Another group of proteins is anchored on the cytoplasmic side of the plasma membrane within the inner leaflet of the lipid bilayer by one or more long hydrocarbon chains.

Two proteins associated with the plasma membrane in this way (Src and Ras) are implicated in the transformation of a normal cell to a malignant state.

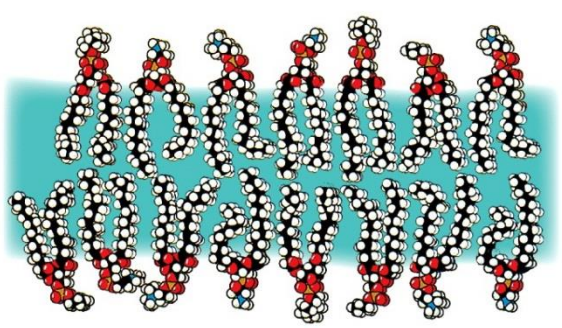
8.6 | Membrane Lipids and Membrane Fluidity

Physical state of the membrane lipid is described by its fluidity or viscosity.

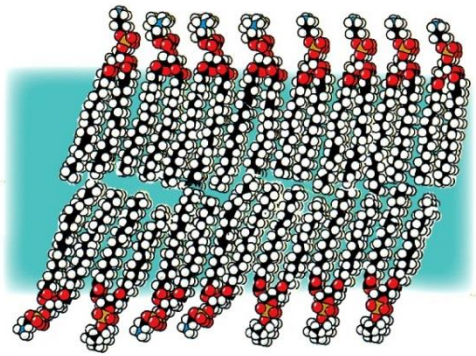
If the temperature of the bilayer is kept relatively warm (37°C), the lipid exists in a relatively fluid state, and is described as a two-dimensional liquid crystal.

Molecules retain a specified orientation; the long axes are parallel, yet individual lipids can rotate around their axis or move laterally within the plane.

If the temperature is slowly lowered to the transition temperature, the lipid is converted to a frozen crystalline gel and movement is greatly restricted.



(a)



(b)

Structure of the lipid bilayer depends on the temperature: above and below the transition temperature.

8.6 | Membrane Lipids and Membrane Fluidity

TABLE 4.2 Melting Points of the Common 18-Carbon Fatty Acids

Fatty acid	*cis* Double bonds	M.p. (°C)
Stearic acid	0	70
Oleic acid	1	13
Linoleic acid	2	−9
Linolenic acid	3	−17
Eicosapentanoic acid (EPA)*	5	−54

*EPA has 20 carbons.

Saturated fatty acids resemble a straight, flexible rod, while cis-unsaturated fatty acids have crooks in the chain at the sites of a double bond. Saturated chains pack together more tightly than those containing unsaturated chains.

The greater the degree of unsaturation of the fatty acids of the bilayer, the lower the temperature before the bilayer gels.

The shorter the fatty acyl chains, the lower its melting temperature.

Cholesterol abolishes sharp transition temperatures and creates a condition of intermediate fluidity; it increases membrane durability and decreases membrane permeability.

8.6 | Membrane Lipids and Membrane Fluidity

The Importance of Membrane Fluidity

Fluidity allows for interactions to occur; clusters of membrane proteins can assemble at particular sites and form specialized structures. Molecules can come together, carry out a necessary reaction, and move apart.

Cellular processes, including cell movement, cell growth, cell division, formation of intercellular junctions, secretion, and endocytosis, depend on the movement of membrane components and would probably not be possible if membranes were rigid, non-fluid structures.

8.6 | Membrane Lipids and Membrane Fluidity

Maintaining Membrane Fluidity

Internal temperatures of most organisms can fluctuate with the temperature, so cells respond by altering phospholipid composition. If the temperature is lowered, cells can remodel membranes to make them more cold resistant.

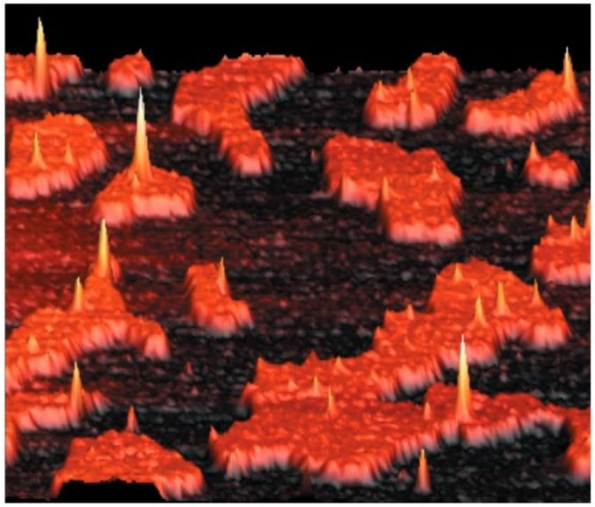
Remodeling is accomplished by (1) desaturating single bonds in fatty acyl chains to form double bonds, and (2) reshuffling chains between different phospholipid molecules to make ones that have two unsaturated fatty acids.

Desaturation is catalyzed by desaturases, while reshuffling is accomplished by phospholipases, which split the fatty acid from the glycerol backbone, and acyl- transferases, which transfer fatty acids between phospholipids.

In addition, the cell changes the types of phospholipids being synthesized in favor of ones containing more unsaturated fatty acids.

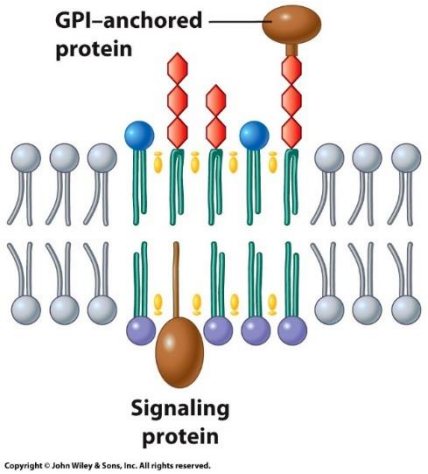
8.6 | Membrane Lipids and Membrane Fluidity

Lipid Rafts



Sphingomyelin organizing into orange-colored rafts

Schematic model of a lipid raft



The outer leaflet of plasma membrane contains specialized regions.

Cholesterol and sphingolipids tend to pack together to form highly ordered microdomains forming **lipids rafts** that float within the more fluid and disordered environment.

They provide a favorable environment for cell-surface receptors and GPI-anchored proteins.

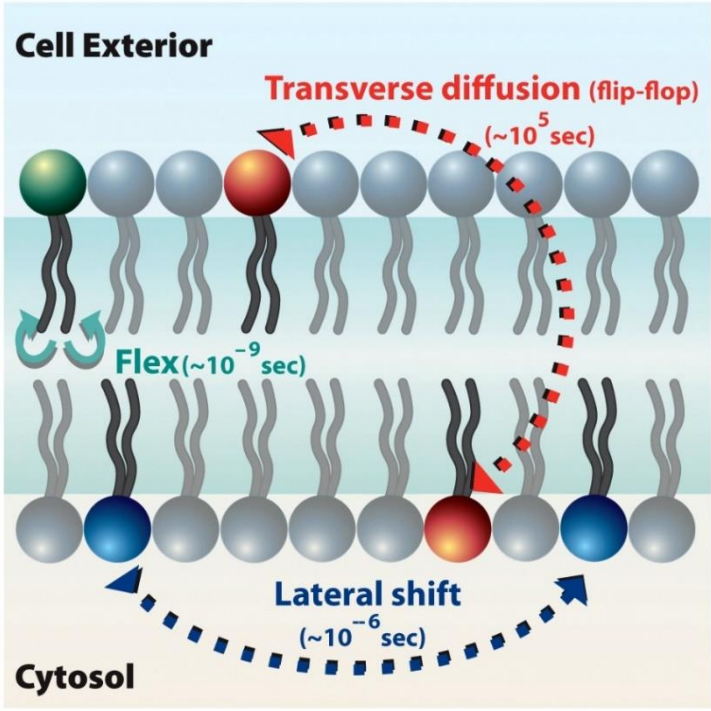
8.7 | The Dynamic Nature of the Plasma Membrane

A phospholipid can move laterally within the same leaflet with considerable ease.

A phospholipid can diffuse from one end of a bacterium to the other end in a second or two, but it takes hours to days to move across to the other leaflet.

To flip-flop, the hydrophilic head of the lipid must pass through the internal hydrophobic sheet of the membrane.

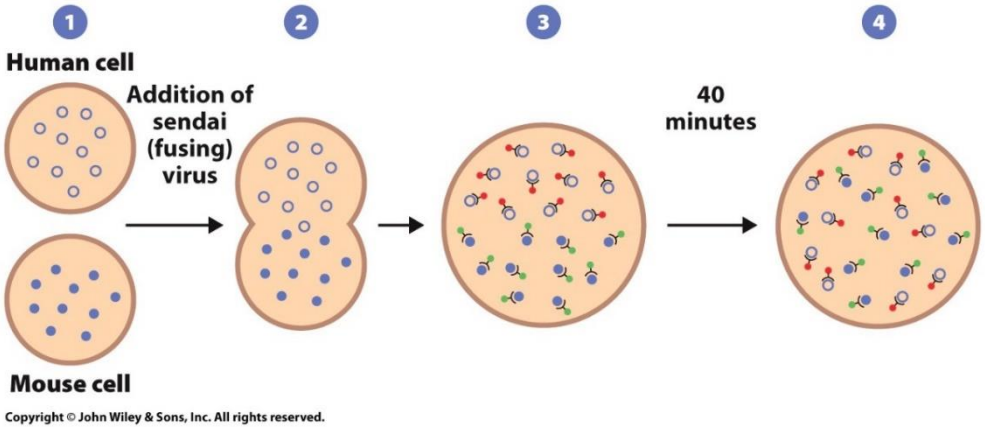
Flippases are enzymes that move certain phospholipids from one leaflet to the other.

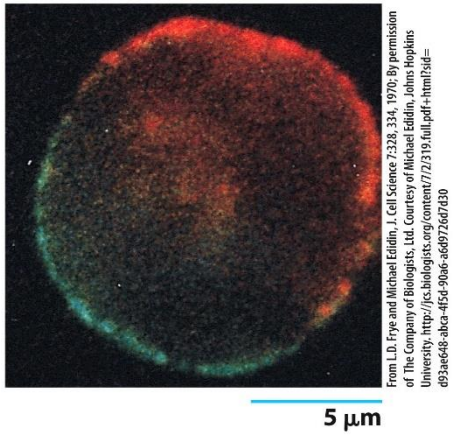


The possible movements of phospholipids in a membrane

8.7 | The Dynamic Nature of the Plasma Membrane

The Diffusion of Membrane Proteins after Cell Fusion





Cell fusion to reveal mobility of membrane proteins: fusion of human and mouse cells

Cell fusion is a technique whereby two different types of cells, or cells from two different species, can be fused to produce one cell with a common cytoplasm and a single, continuous plasma membrane.

Cell fusion be induced by certain viruses, or with polyethylene glycol.

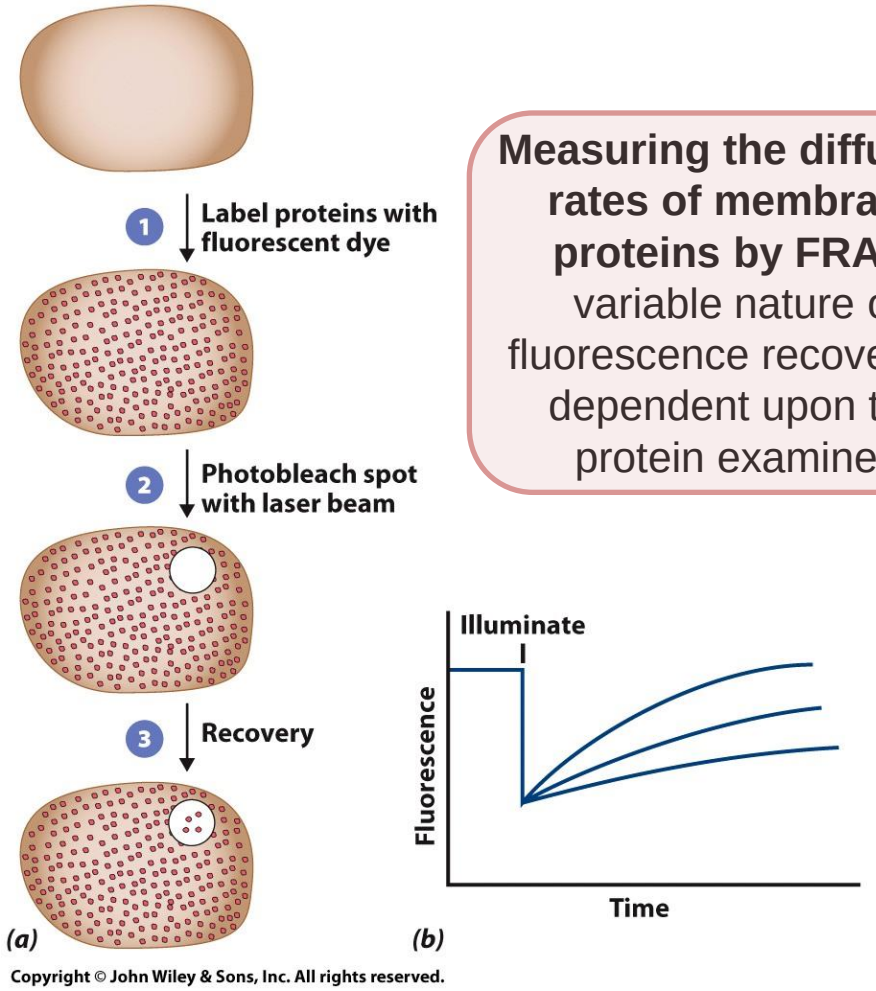
Labeled proteins have shown that membrane proteins can move between fused cell.

8.7 | The Dynamic Nature of the Plasma Membrane

Restrictions on Protein and Lipid Mobility

Proteins can be labeled and tracked by **fluorescence recovery after photobleaching (FRAP)** and **single particle tracking (SPT)**.

Proteins can be immobile, mobile in a directed manner, or exhibit random movement.



Measuring the diffusion rates of membrane proteins by FRAP: variable nature of fluorescence recovery is dependent upon the protein examined

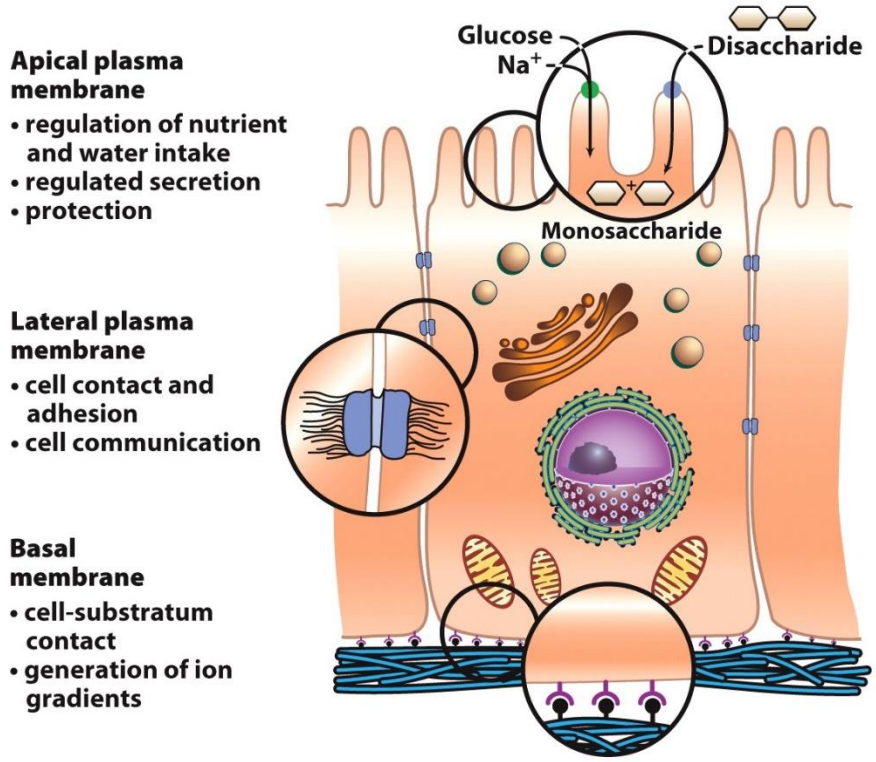
8.7 | The Dynamic Nature of the Plasma Membrane

Membrane Domains and Polarity

Most membranes vary in protein composition and mobility.

Epithelial cells of the intestinal wall or kidney tubules are highly polarized whose surfaces carry out different functions.

The apical plasma membrane absorbs substances from the lumen; the lateral plasma membrane interacts with neighboring epithelial cells; the basal membrane adheres to an underlying basement membrane.



Differentiated functions of the plasma membrane of an epithelial cell.

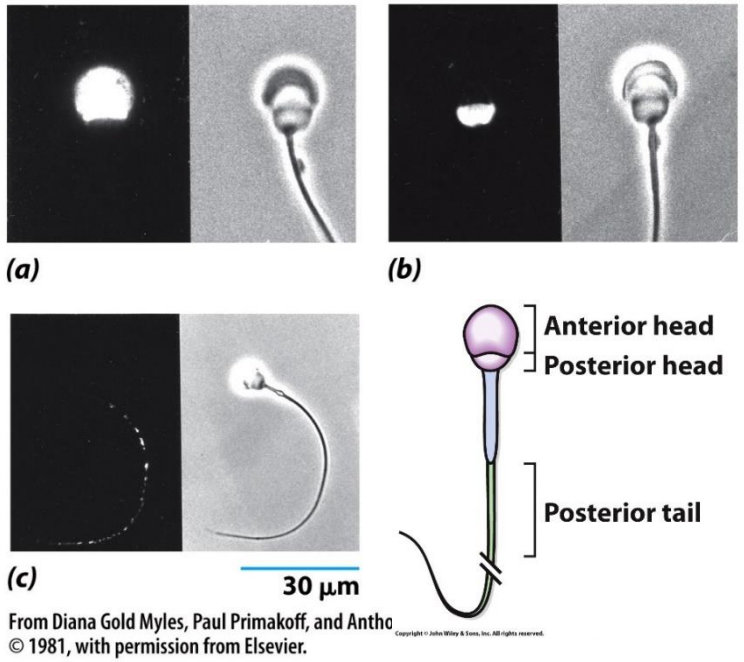
8.7 | The Dynamic Nature of the Plasma Membrane

Membrane Domains and Polarity

Sperm may have the most highly differentiated structure with a head, midpiece, and tail, each having its own specialized functions.

A sperm is covered by a continuous plasma membrane that consists of a mosaic of different types of localized domains.

Antibodies can detect and reflect the distribution of different proteins within the plasma membrane.



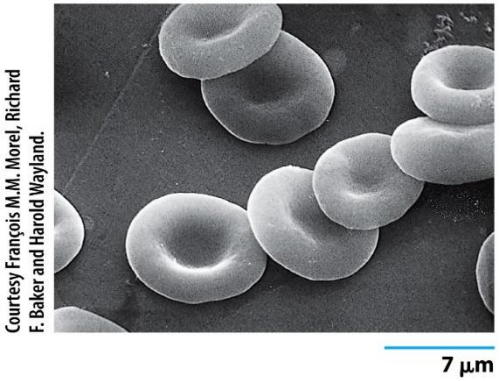
Differentiation of the mammalian sperm plasma membrane as revealed by fluorescent antibodies.

8.8 | The Red Blood Cell: An Example of Plasma Membrane Structure

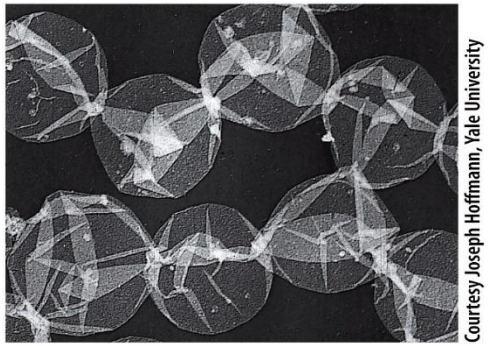
The plasma membrane of the human erythrocyte (red blood cell) is the most studied and best understood.

Homogeneous preparation of membrane "ghosts" can be prepared by *hemolysis* for isolation of intact membranes.

Membrane proteins can be purified and characterized by fractionation using SDS-PAGE electrophoresis.

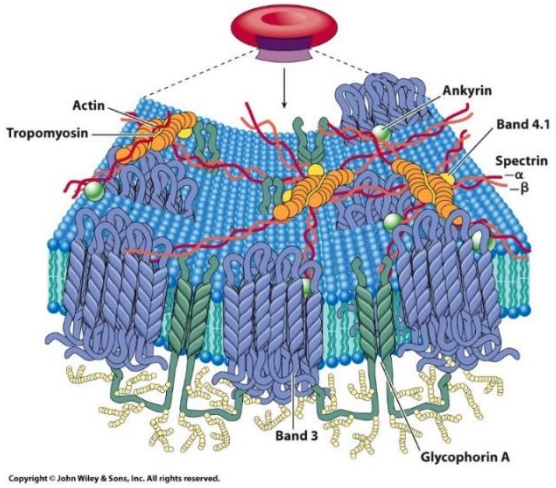


SEM of human erythrocytes and membrane ghosts

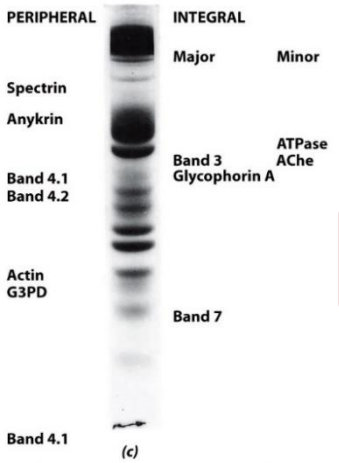


8.8 | The Red Blood Cell: An Example of Plasma Membrane Structure

Integral Proteins of the Erythrocyte Membrane



Erythrocyte plasma membrane model viewed from the internal surface



From V.T. Marchesi, H. Furthmayr, and M. Tomita, Annu. Rev. Biochem., vol. 45; © 1976 by Annual Reviews, Inc.

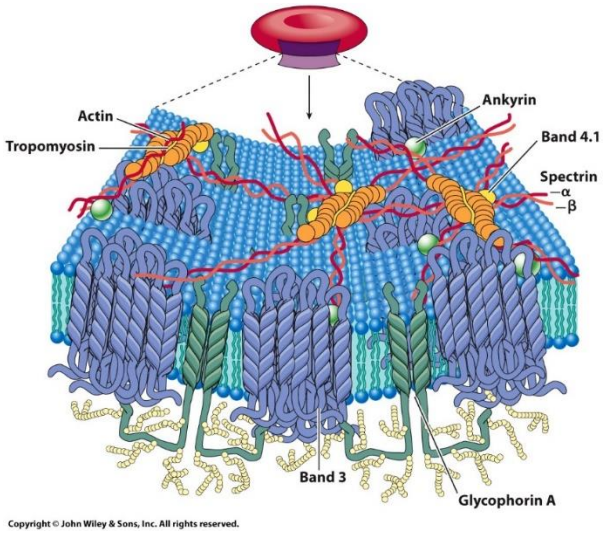
SDS–PAGE of membrane proteins

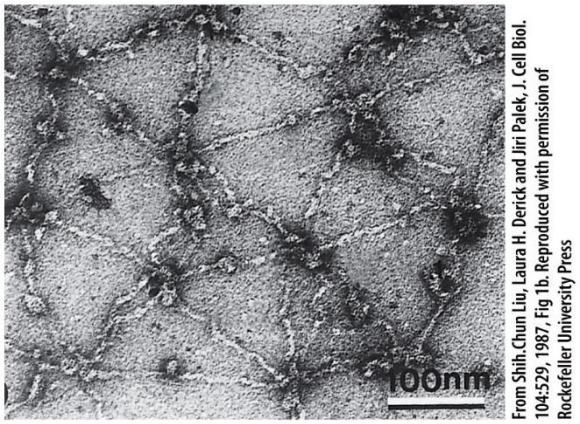
Band 3 is composed of two *homodimers* of a glycoprotein that exchanges Cl^- and HCO_3^- across the red cell membrane.

Glycophorin A is a dimer with 16 oligosaccharide chains bearing negative charges that may prevent red cells from clumping.

8.8 | The Red Blood Cell: An Example of Plasma Membrane Structure

The Erythrocyte Membrane Skeleton





From Shih, Chun Liu, Laura H. Derick and Jiri Palek, J. Cell Biol. 104:529, 1987, Fig 1b. Reproduced with permission of Rockefeller University Press

EM: inner membrane skeleton proteins

The major component of the internal membrane skeleton is *spectrin*.

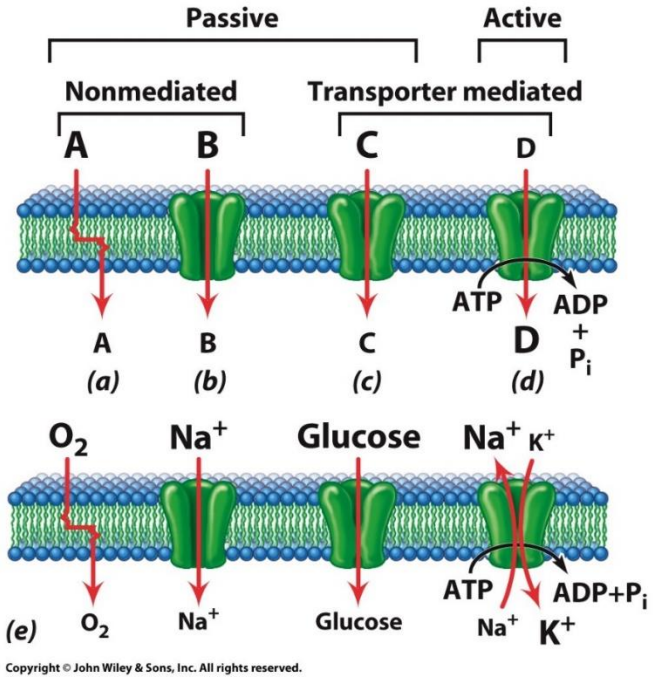
Spectrin molecules are attached to the membrane surface by non-covalent bonds to *ankyrin*, a peripheral membrane protein which is non-covalently bonded to band 3.

Spectrin is linked to other cytoplasmic proteins, such as *actin* and *tropomyosin*, which maintains the integrity of the membrane.

8.9 | Solute Movement Across Cell Membranes

Selective permeability allows for separation and exchange of materials across the plasma membrane

Substances move across membranes by:
1) simple diffusion through the lipid bilayer;
2) simple diffusion through an aqueous, protein-lined channel;
3) diffusion facilitated by a protein transporter;
4) active transport, requires an energy-driven protein "pump" to move substances against a concentration gradient



Four basic mechanisms by which solute molecules move across membranes

8.10 | Diffusion Through the Lipid Bilayer

Diffusion of Substance Through Membranes

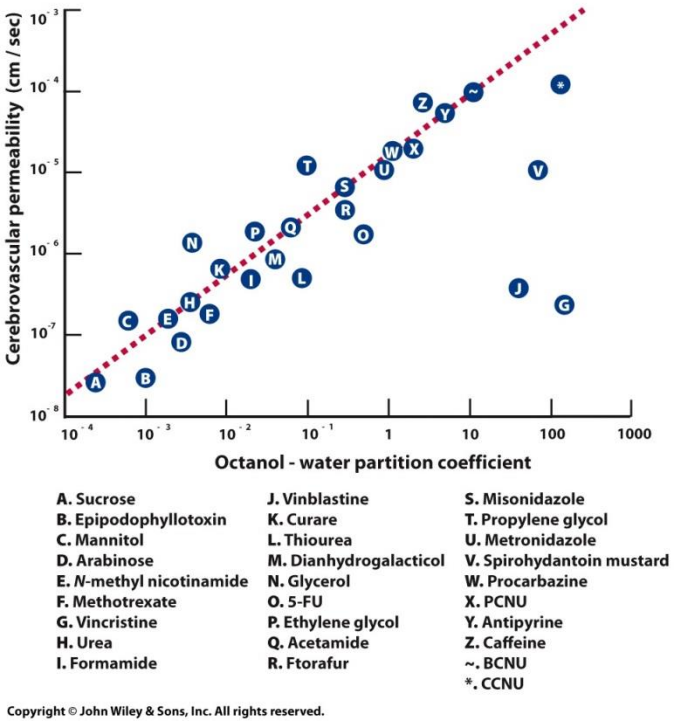
Diffusion requires both a concentration gradient and membrane permeability.

Lipid permeability is determined by the molecular size, polarity, and **partition coefficient**, the ratio of solubility in a nonpolar solvent to that in water.

Small molecules penetrate the lipid bilayer more rapidly than larger ones.

Polar molecules, like sugars and amino acids, have poor membrane penetration.

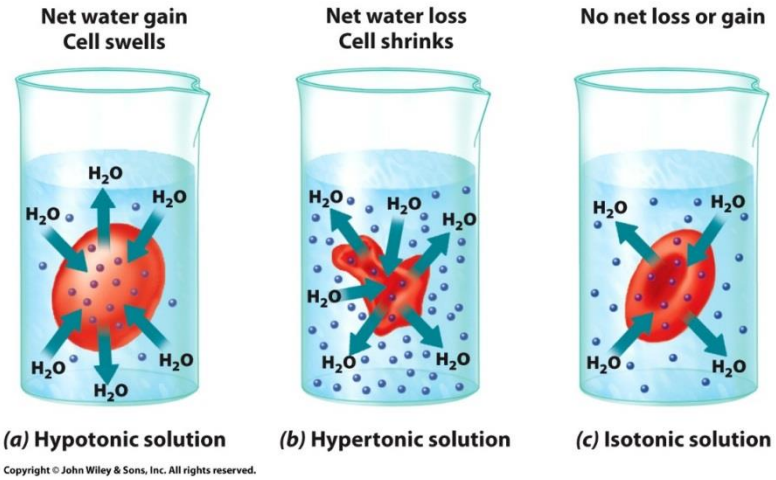
The greater the lipid solubility, the faster the penetration.



The relationship between partition coefficient and membrane permeability.

8.10 | Diffusion Through the Lipid Bilayer

The Diffusion of Water Through Membranes



The effects of differences in the concentration of solutes on opposite sides of the plasma membrane

Diffusion of water through a semipermeable membrane is called **osmosis**.

Water diffuses from areas of lower solute concentration to areas of higher solute concentration.

Cells swell in **hypotonic** solution, shrink in **hypertonic** solutions, and remain unchanged in **isotonic** solutions.

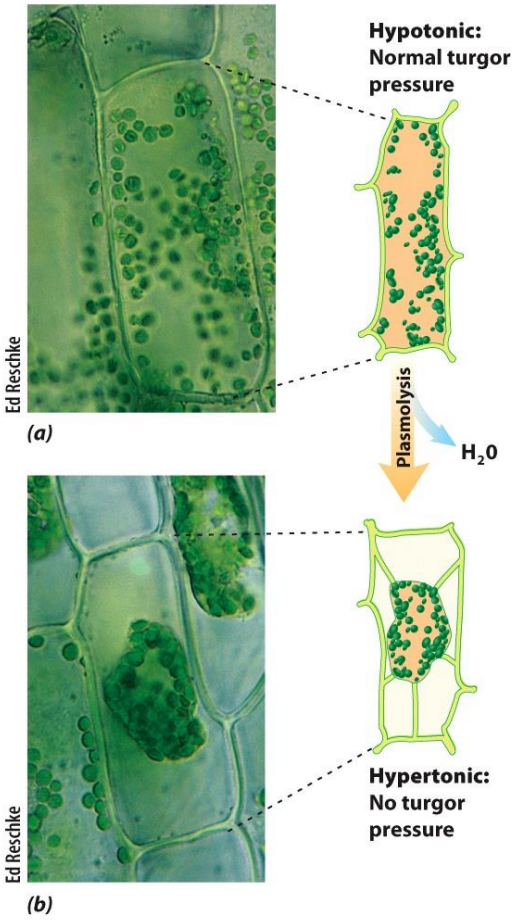
8.10 | Diffusion Through the Lipid Bilayer

The Diffusion of Water Through Membranes

Plants utilize osmosis in different ways as they are usually hypertonic compared to their fluid environment.

There is a tendency for water to enter the cell, causing it to develop an internal (*turgor*) pressure that pushes against its surrounding wall.

In hypertonic solutions the plant cell undergoes **plasmolysis,** and the plant loses it support and wilts.



The effects of osmosis on a plant cell

8.10 | Diffusion Through the Lipid Bilayer

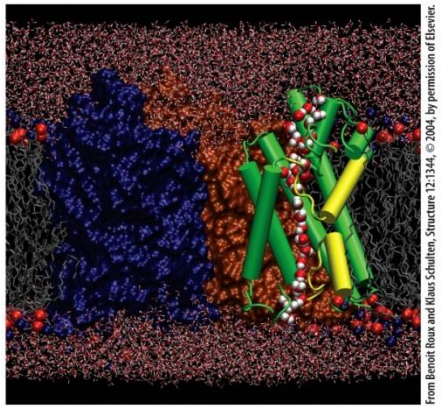
The Diffusion of Water Through Membranes

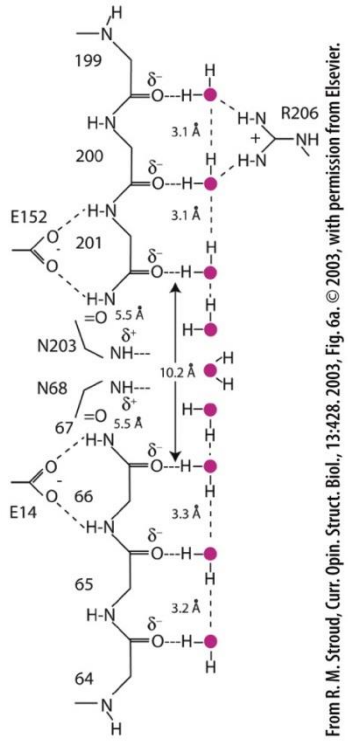
A family of small integral proteins, **aquaporins**, allow the passive movement of water across the plasma membrane.

It's central channel is lined primarily by hydrophobic amino acid residues and is highly specific for water molecules.

Water molecules pass in single file while H^+ ions are not able to penetrate these open pores.

A pair of positive charged residues (N203 and N68) attract the oxygen atom of each water molecule to prevent hydrogen bonds.





Passage of water molecules through an aquaporin channel

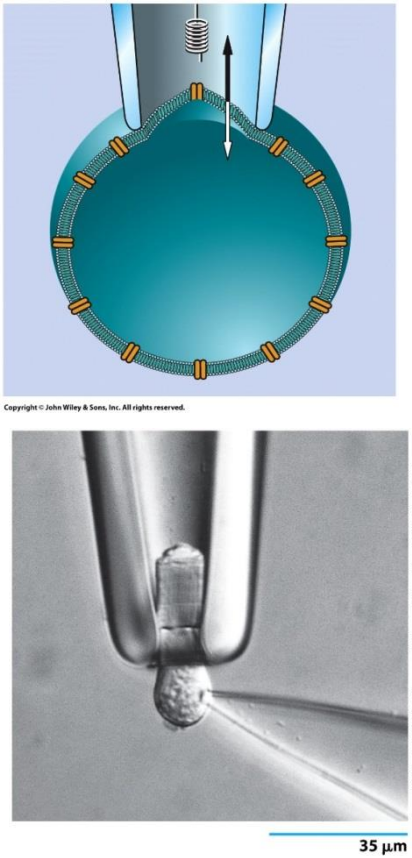
8.11 | The Diffusion of Ions through Membranes

Ions cross membranes through **ion channels**.

Ion channels are selective and bidirectional, allowing diffusion in the direction of the electrochemical gradient.

Superfamilies of ion channels have been characterized by patch-clamping experiments.

The voltage across the membrane can be maintained (*clamped*) at any value, and the current originating in the small patch of membrane surrounded by the pipette can be measured.



Measuring ion conductance by patch-clamp recording

8.11 | The Diffusion of Ions through Membranes

Most ion channels can exist in either an open or a closed conformation, and are called gated. The three major categories of gated channels are:

1. **Voltage-gated channels:** Conformational state depends on the difference in ionic charge on the two sides of the membrane.

2. **Ligand-gated channels:** Conformational state depends on the binding of a specific molecule (ligand), which is usually not the solute that passes through the channel. Ligand-gated channels can either open or close after ligand binding to the outer or inner surface of the channel.

3. **Mechano-gated channels:** Conformational state depends on mechanical forces (e.g., stretch tension) that are applied to the membrane. Specific cation channels can be opened by stereocilia movement on the hair cells of the inner ear in response to sound or motions of the head.

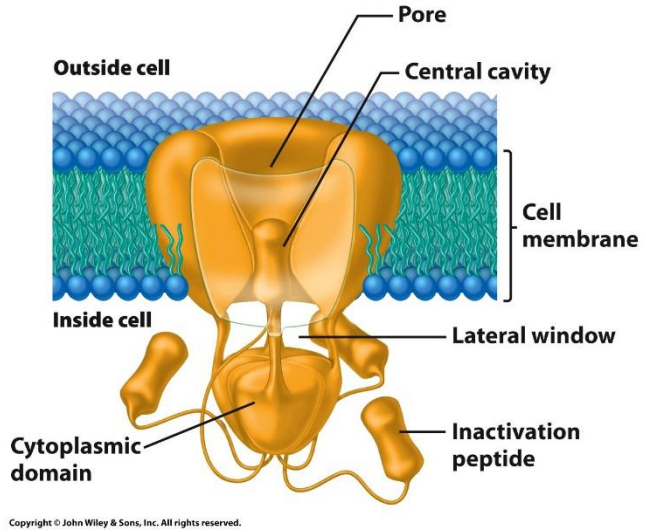
8.11 | The Diffusion of Ions through Membranes

Once opened, more than 10 million K^+ ions can pass through per second.

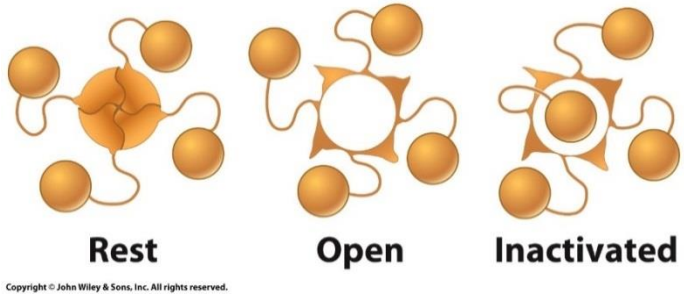
After the channel is open for a few milliseconds, the movement of K^+ ions is "automatically" stopped by a process known as inactivation.

Channel inactivation occurs by movement of a small inactivation peptide that dangles from the cytoplasmic portion of the protein.

Can exist in three different states: open, inactivated, and closed.



Conformational states of a voltage-gated K^+ ion channel



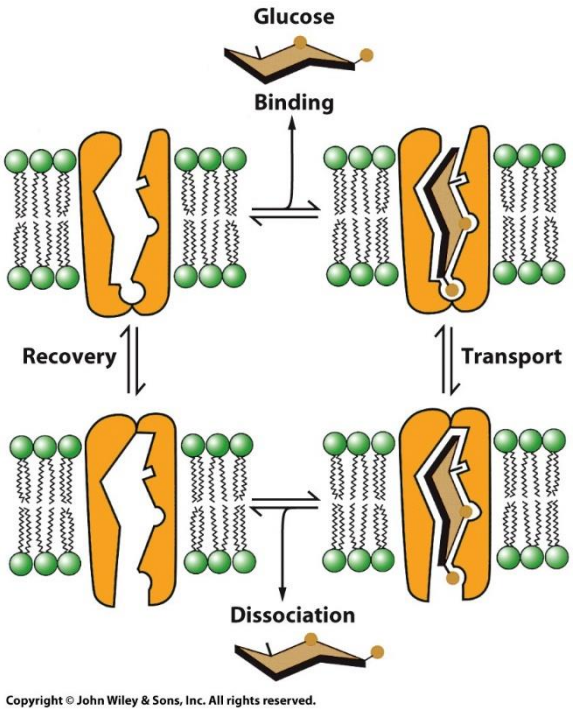
8.12 | Facilitated Diffusion

In many cases, the diffusing substance binds selectively to a membrane-spanning protein, called a facilitative transporter.

Solute binding triggers a conformational change to expose it to the other membrane surface and diffuse down its concentration gradient.

Facilitated transporters can mediate the movement of solutes in both directions and depends on the relative concentration of the substance on both sides.

Facilitated diffusion is similar to an enzyme-catalyzed reaction since it is specific for the molecules transported.



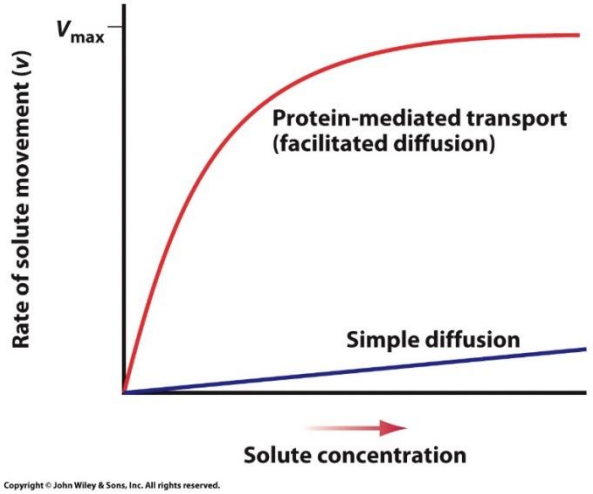
Schematic model of facilitated diffusion

8.12 | Facilitated Diffusion

Transporters can be regulated and exhibit saturation-type kinetics; they can only move hundreds to thousands of molecules per second across the membrane.

It is important in transporting polar solutes, like sugars and amino acids, that do not penetrate the lipid bilayer.

The glucose transporter (**GLUT**) is an example of facilitated diffusion.



Kinetics of facilitated diffusion compared to simple diffusion

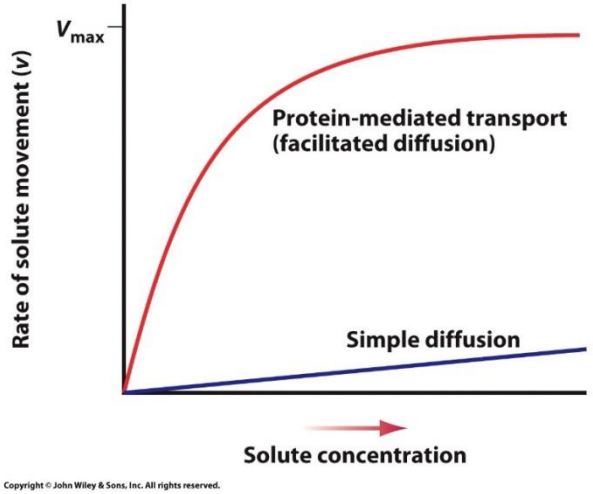
8.12 | Facilitated Diffusion

Insulin plays a key role in maintaining proper blood sugar levels.

An increase in blood glucose levels triggers the secretion of insulin, which stimulates the uptake of glucose.

When insulin levels are low, responsive cells contain relatively few glucose transporters, GLUT4, on their plasma membrane.

Rising insulin levels stimulates the movement of transporters to the cell surface where they can bring glucose into the cell.



Kinetics of facilitated diffusion compared to simple diffusion

8.13 | Active Transport

Cells maintain an imbalance of ions across the plasma membrane, which cannot occur by either simple or facilitated diffusion.

Gradients are generated by active transport, which depends on integral membrane protein "pumps" to bind a solute and move it across the membrane in a process driven by changes in the protein's conformation.

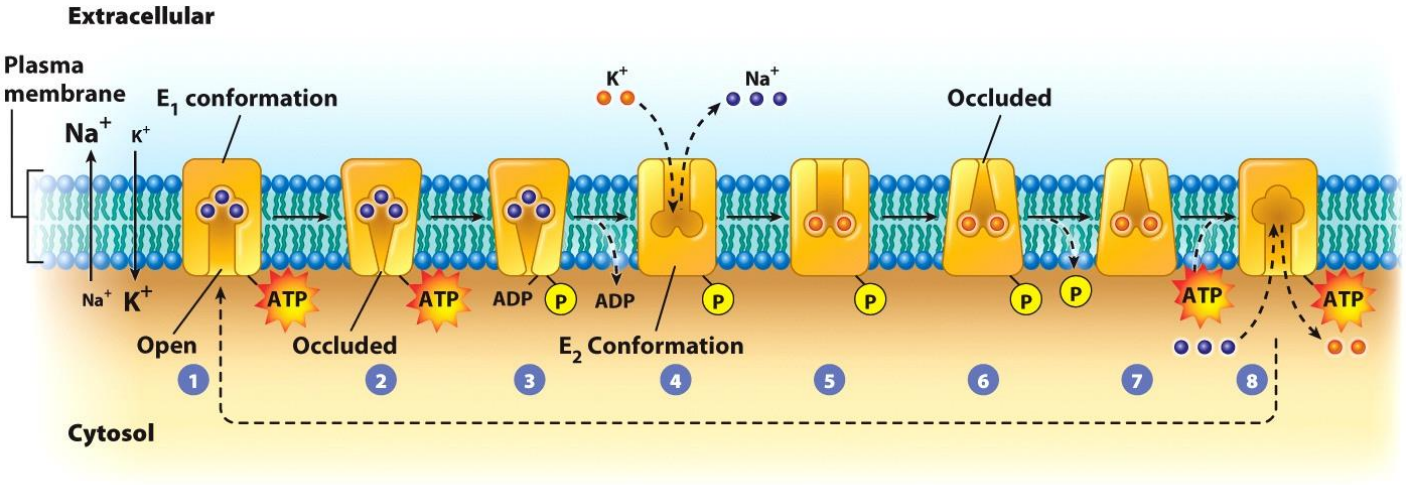
Coupled energy input is needed like ATP hydrolysis, absorbance of light, electron transport, or the flow of other substances down their gradients.

TABLE 4.3 Ion Concentrations Inside and Outside of a Typical Mammalian Cell

	Extracellular concentration	Intracellular concentration	Ionic gradient
Na^+	150 mM	10 mM	15x
K^+	5 mM	140 mM	28x
Cl^-	120 mM	10 mM	12x
Ca^{2+}	10^{-3} M	10^{-7} M	10,000x
H^+	$10^{-7.4}$ M (pH of 7.4)	$10^{-7.2}$ M (pH of 7.2)	Nearly 2x

8.13 | Active Transport

Primary Active Transport: Coupling Transport to ATP Hydrolysis



The Na^+/K^+ ATPase (*sodium-potassium pump*)requires K^+ outside, Na^+ inside, and is inhibited by ouabain.

The ratio of Na^+:K^+ pumped is 3:2.

The ATPase is a P-type pump, in which phosphorylation causes changes in conformation and ion affinity that allow transport against gradients.

8.13 | Active Transport

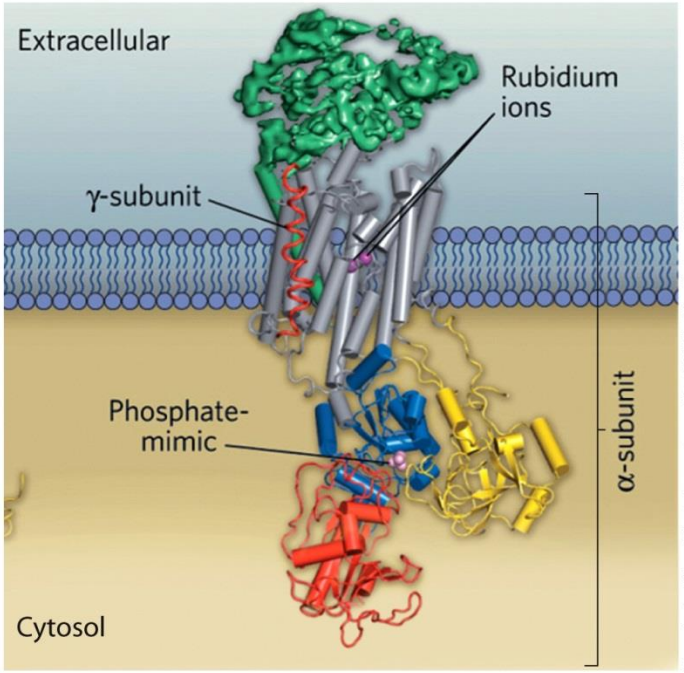
Primary Active Transport: Coupling Transport to ATP Hydrolysis

E1 conformation: Ion binding sites are accessible to the inside of the cell.

E2 conformation: Ion binding sites are accessible to the outside of the cell.

The change in protein structure between the conformations alternately exposes ion binding sites to the opposite membrane.

The sodium–potassium pump is found only in animal cells.



From Ayako Takeuchi, et al., courtesy of David C. Gadsby, Nature 450:958, 2007, © 2007 reprinted by permission from Macmillan Publishers Ltd.

The Na^+/K^+-ATPase pump: A model of the E2 conformation

8.13 | Active Transport

Other Primary Ion Transport Systems

The best studied P-type pump is the Ca^{2+}-ATPase, present in the ER to actively transport Ca^{2+} out of the cytosol into the lumen of this organelle.

Unlike P-type pumps, *V-type* pumps utilize the energy of ATP without forming a phosphorylated protein intermediate.

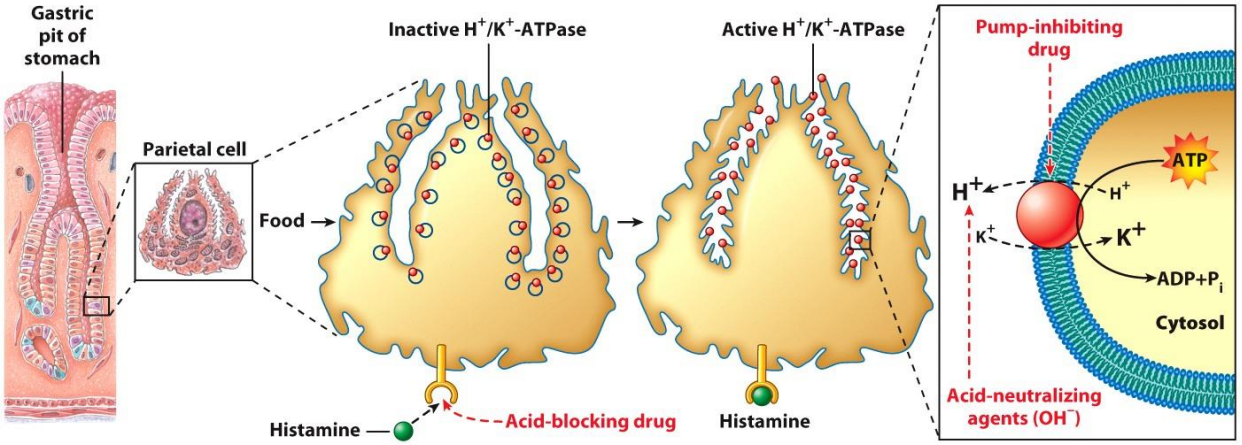
V-type pumps actively transport H^+ across the walls of cytoplasmic organelles (e.g. lysosomes) and vacuoles to maintain a low pH.

A V-type pump in the plasma membranes of kidney tubules helps maintain the body's acid–base balance by secreting protons into the forming urine.

Another diverse group of proteins that actively transport ions is the *ATP-binding cassette* (*ABC*) *transporters*, so called because all of the members of this superfamily share a homologous ATP-binding domain.

8.13 | Active Transport

Other Primary Ion Transport Systems



Control of acid secretion in the stomach

The stomach contains the P-type pump, the H/K-ATPase, which secretes a solution of concentrated acid (up to 0.16 N HCl) into the stomach chamber.

When food enters the stomach, this pump moves to the apical cell surface in parietal cells, fuses with the plasma membrane, and secretes acid.

Prilosec prevents heartburn by inhibiting this pump. Zantac, Pepcid, and Tagamet block a receptor on the surface of the parietal cells, thereby stopping the cells from becoming activated by the hormone.

8.13 | Active Transport

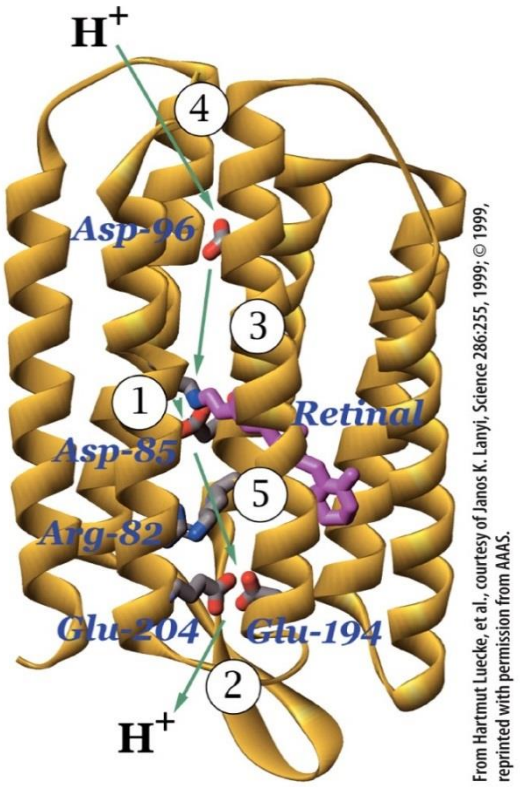
Using Light Energy to Actively Transport Ions

Halobacterium salinarium is an archae-bacterium that uses *bacteriorhodopsin,* as a light-driven proton pump.

Bacteriorhodopsin contains retinal, also present in rhodopsin, the light-absorbing protein of the rods of the retina.

Absorption of light energy by retinal induces conformational changes to cause a proton to move from retinal, through a channel to the cell exterior.

This generates a steep H+ gradient across the plasma membrane.



Bacteriorhodopsin: a light-driven proton pump

8.13 | Active Transport

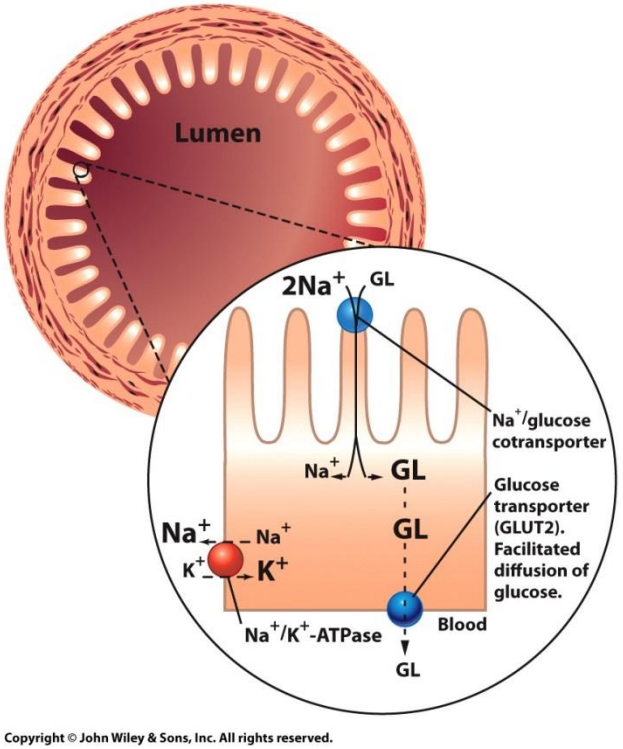
Co-Transport: Coupling Transport to Existing Ion Gradients

Potential energy stored in ionic gradients is utilized to perform work, including the transport of other solutes.

The movement of glucose across the apical plasma membrane of the epithelial cells, against a concentration gradient, occurs by cotransport with sodium ions.

Na+ concentration is kept low by a Na+/K+-ATPase pump.

Diffusion of sodium ions down a concentration gradient drives the cotransport of glucose molecules into the cell against a concentration gradient.



Secondary transporter: the Na+ gradient helps to transport glucose by a Na+/glucose co-transporter

8.13 | Active Transport

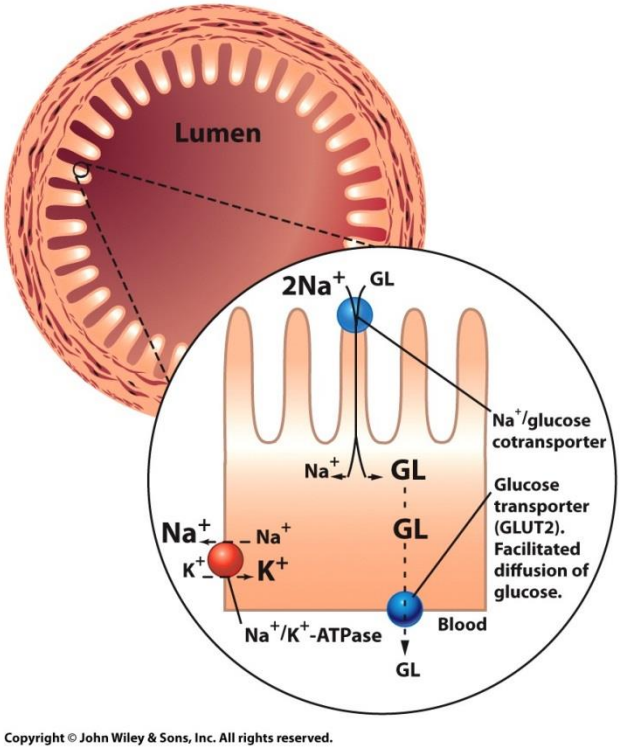
Co-Transport: Coupling Transport to Existing Ion Gradients

Glucose molecules are driven by secondary active transport.

The transport protein, the Na+/glucose cotransporter, moves two Na+ and one glucose molecule with each cycle.

Once inside, the glucose molecules diffuse through the cell and are moved across the basal membrane by facilitated diffusion.

The Na+/glucose cotransporter is capable of transporting glucose into a cell against a concentration gradient greater than 20,000-fold.



Secondary transporter: the Na+ gradient helps to transport glucose by a Na+/glucose co-transporter

8.13 | Active Transport

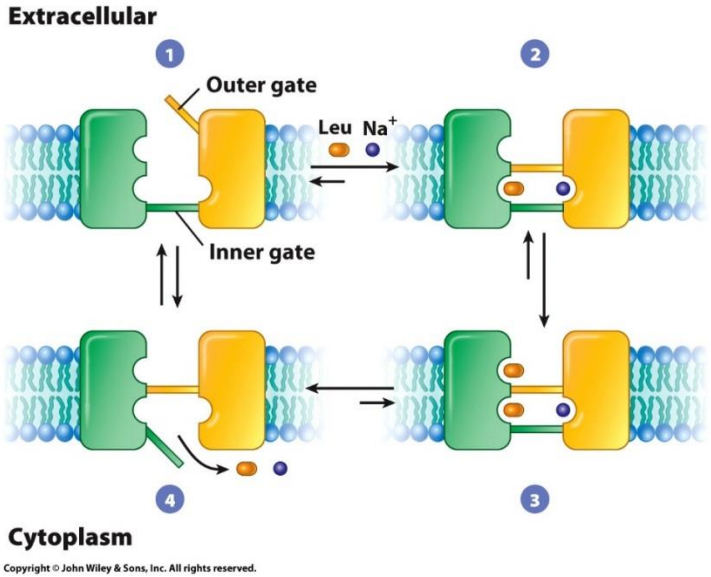
Co-Transport: Coupling Transport to Existing Ion Gradients

Secondary active transport of glucose is an example of symport, two transported species moving in the same direction.

Antiporters or exchangers move two transported species in opposite directions.

Cells can maintain a proper cytoplasmic pH by coupling the inward movement of Na^+ with the outward movement of H^+.

During the transport cycle, the protein's binding sites gain alternating access to the cytoplasm and the extracellular space.



Secondary transporter: the Na+ gradient helps to transport leucine into bacteria