Biological Membranes and Transport

Objectives:
I. Know the structure and functions of cell membranes.
   A. What lipids are present in cellular membranes?
   B. Describe the function of the lipids in membranes.
   C. Describe the lipid composition of the outer leaflet compared to the inner leaflet.
   D. Describe membrane fluidity
      1. Lateral diffusion versus “Flip-Flop”.
         a) Flipases, Floppases, Scramblases.
      2. Factors that effect membrane fluidity.
         a) saturated versus unsaturated fatty acids.
         b) cholesterol
   E. Describe the fluid mosaic model of a cell membrane.
II. Compare and contrast the structures and possible functions for integral (intrinsic) membrane proteins, peripheral (extrinsic) membrane proteins, and lipid-anchored membrane proteins.
III. Describe the structure and function of the cytoskeleton.
   A. Microfilaments - e.g., actin
   B. Intermediate filaments - e.g., spectrin
   C. Microtubules - e.g, tubulin
IV. The Glycocalyx.
   A. Structure
   B. Function
V. Define and describe cellular adhesion molecules (CAM’s).
   A. Compare the structure and function of the cellular adhesion molecules.
VI. Describe the extracellular matrix.
   A. Types of molecules present in the extracellular matrix and their probable function.
   B. Describe, compare, and contrast the possible interactions between the CAM’s and the extracellular matrix proteins.
   C. Describe, compare, and contrast the possible interactions between the CAM’s and the extracellular proteoglycans.
VII. How do molecules (polar versus nonpolar) cross cellular membranes?
VIII. Define the terms osmosis, simple diffusion, channels (pores), passive transport, facilitated diffusion, and active transport.
   A. What types of molecules move across the cell membrane by simple diffusion?
      1. Driving force of molecular/ionic movement by simple diffusion.
      2. Concentration effects versus electrical gradient.
   B. What types of molecules move across the cell membrane by channels or pores?
      1. Describe the different types of channels or pores.
      2. Describe the kinetics of movement through channels or pores.
   C. What types of molecules move across the cell membrane by passive transport or facilitated diffusion?
      1. Describe the kinetics of movement by passive transport.
   D. What types of molecules move across the cell membrane by active transport?
      1. Describe the different types of active transport.
2. Describe the kinetics of movement by active transport.

E. Which forms of movement across the cell membrane can act against a concentration gradient; can be used to develop a concentration gradient?

F. Compare and contrast the functions of a Uniport, Symport, and an Antiport.

The Fluid Mosaic Model of Cell Membranes

Membranes define the external boundary of all cells and separates compartments within the eukaryotic cell. Biological membranes are not passive barriers, they serve a wide variety of complex biological functions.

A typical membrane is a complex mixture of phospholipids (phosphoglycerides & sphingomyelin), glycosphingolipids (cerebrosides, globosides, & gangliosides), and cholesterol. Membranes are composed of two layers of lipid arranged into a bilayer. The polar heads of these amphipathic lipids are arranged on the external and internal surfaces of the bilayer where they interact with the extracellular and intracellular water, respectively. The polar heads interact with each other and with cellular water by dipole-dipole, dipole-ion, hydrogen bonding, and electrostatic interactions. The hydrophobic tails of the lipids interact with each other in the interior of the bilayer by hydrophobic interactions (London Dispersion Forces). Glycosphingolipids are only found on the outer leaflet of the membrane. On the outer leaflet of the bilayer the hydrophilic sugars of the glycosphingolipids interact with extracellular water and with molecules of the extracellular matrix.

Proteins associate with the lipid bilayer in four possible ways. INTEGRAL MEMBRANE PROTEINS or INTRINSIC MEMBRANE PROTEINS are embedded in and pass completely through the membrane. These proteins contain one or more highly hydrophobic domains that interact with the hydrophobic tails of the membrane lipids. Some intrinsic proteins contain a single hydrophobic region of about 20 amino acids. These hydrophobic amino acids adopt an α-helical structure and this region of secondary structure is embedded in the membrane. The amino and carboxyl terminal regions of the protein fold into extracellular and intracellular domains. Other intrinsic proteins contain from 7 to 20 helical segments that are embedded in the lipid bilayer. These helical regions are usually arranged in a cylindrical bundle. The surface of the helical bundle that is in contact with the hydrophobic tails of the lipids is comprised of hydrophobic amino acids. The interior surfaces of the bundle, the surfaces of the helices that interact with each other usually contain polar amino acids. Polar molecules can pass through, can be transported through these “channels” lined with polar amino acids. The third type of structural motif common to intrinsic membrane proteins is the β barrel in which 20 or more transmembrane segments form β sheets that line a cylinder. The exterior
surface of the β barrel, the surface that is in contact with the hydrophobic tails of the lipids, is comprised of hydrophobic amino acids. The interior surfaces of the barrel, the surfaces of the β structures that interact with each other usually contain polar amino acids. Polar molecules can pass through, can be transported through these “channels” lined with polar amino acids. Many of the intrinsic membrane proteins are Glycoproteins. The extracellular domains of these proteins contain either O-linked, N-linked, or both types of polysaccharides.

The second class of proteins associated with biological membranes are the PERIPHERAL MEMBRANE PROTEINS or EXTRINSIC MEMBRANE PROTEINS. The extrinsic proteins are loosely bound to one of the two faces of the bilayer. Polar interactions (dipole-dipole, dipole-ion, hydrogen bonds, and electrostatic interactions) between the protein and the polar heads of the membrane lipids bind these proteins to the membrane surface. Alternatively, the extrinsic protein can be bound to the intracellular or extracellular domain of an intrinsic membrane protein. Peripheral proteins associated with the outer leaflet of the membrane are often glycoproteins.

The third class of membrane associated proteins are the LIPID-ANCHORED PROTEINS or the LIPID-LINKED PROTEINS. Proteins of this type are covalently linked to a fatty acid, a hydrophobic isoprenoid (a terpene) molecule, or to a phosphoglyceride. The linked hydrophobic molecule is embedded in the membrane. The protein can be linked to the lipid and anchored to the membrane in one of four ways.

1. The protein can be covalently linked by a thioester bond between a cysteine side chain and the carboxyl group of one of the fatty acids. The fatty acids is embedded in the membrane.

2. The linkage can be an amide bond between the fatty acid myristate that is embedded in the membrane and the α-amino group of an N-terminal glycine of selected proteins.
3. The protein can be linked to an isoprenoid molecule, either FARNESYL or GERANYLGERANYL, by a thioether bond between the isoprene and the side chain of a carboxyl-terminal cysteine residue. The hydrophobic isoprene is embedded in the membrane. These first three linkages are found only on the cytosolic face (inner leaflet) of the membrane.

4. The C-terminal amino acid of the protein can be anchored in the membrane by Glycosylated
derivatives of Phosphatidylinositol (GIP). The carboxyl group of the C-terminal amino acid is amide linked to ethanolamine. The hydroxyl group of ethanolamine is esterified to phosphate and the phosphate is esterified to a heteropolysaccharide. The reducing end of the heteropolysaccharide is glycosidically linked to the inositol moiety of a phosphatidylinositol embedded in the membrane. Proteins with this type of lipid anchor are found only on the extracellular face (outer leaflet) of the membrane.

The hydrophobic interactions between the lipid bilayer and a single hydrocarbon chain linked to a protein is barely strong enough to anchor the protein. Other interactions (hydrogen bonds and/or salt bridges) between the protein and the polar head groups of the membrane lipids probably aid in stabilizing the attachment. The weaker interaction between the lipid-linked proteins and membrane lipids, when compared to intrinsic or extrinsic membrane proteins, allows the lipid-linked proteins to be more mobile in the membrane allowing them to migrate more rapidly along the cell surface.

AMPHITROPIC PROTEINS exist as cytosolic proteins as well as membrane associated proteins. When associated with the membrane they can interact with intrinsic membrane proteins or with one or more types of lipid in the membrane. In many cases the reversible association/dissociation is mediated by phosphorylation/dephosphorylation, ligand binding, or exposure of some binding site and it is often the response to some signal molecule.

### Lipid Composition - In vs. Out

<table>
<thead>
<tr>
<th>Lipid</th>
<th>% of Human Erythrocyte Membrane</th>
<th>% Inner Leaflet vs. % Outer Leaflet</th>
<th>% of Human Myelin</th>
<th>% Inner Leaflet vs. % Outer Leaflet</th>
<th>% of Heart Mitochondrial Membrane</th>
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<tbody>
<tr>
<td>Phosphatidate</td>
<td>1.5</td>
<td>70/30</td>
<td>0.5</td>
<td>75/25</td>
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<td>Phosphatidylcholine</td>
<td>19</td>
<td>25/75</td>
<td>10</td>
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<td>39</td>
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<td>Phosphatidylethanolamine</td>
<td>18</td>
<td>75/25</td>
<td>20</td>
<td>75/25</td>
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<tr>
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<td>75/25</td>
<td>1</td>
<td>80/20</td>
<td>7</td>
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<tr>
<td>Phosphatidylserine</td>
<td>8.5</td>
<td>85/15</td>
<td>8.5</td>
<td>87/13</td>
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<tr>
<td>Cardiolipin</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Sphingomyelin</td>
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<td>12.5/87.5</td>
<td>8.5</td>
<td>10/90</td>
<td>0</td>
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<td>0/100</td>
<td>26</td>
<td>0/100</td>
<td>0</td>
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<tr>
<td>Cholesterol</td>
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<td>45/55</td>
<td>26</td>
<td>35/65</td>
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The relative proportions of protein and lipid vary with the type of membrane, reflecting the diversity of biological roles. Each kingdom, each species, each tissue or cell type, and the organelles of each cell type have a characteristic set of membrane lipids and membrane bound proteins. The different membrane proteins reflect the functional diversity of the different cell types and/or organelles. In many cases, the adaptive advantages of distinct combinations of membrane lipids and membrane proteins are understood; in other cases, the functional significance of these combinations remains to be discovered.

The cell membrane, the plasma membrane has a definite sidedness, a definite orientation. The outer leaflet can be distinguished from the inner leaflet by its characteristic phospholipid composition. The Cerebrosides, Globosides, and Gangliosides are found only in the outer leaflet of cellular membranes. These molecules are seldom, if ever, found in the membranes of organelles. For most mammalian cells, the outer leaflet has a high proportion of Phosphatidylcholine and Sphingomyelins, whereas the inner leaflet is rich in Phosphatidylserine, Phosphatidylinositol, and Phosphatidylethanolamine.

Fluidity

The entire membrane is in constant motion. The lipids in each leaflet undergo LATERAL DIFFUSION very rapidly. A lipid molecule in one leaflet can easily diffuse within that leaflet, traveling “around” the cell in a short period of time. TRANSVERSE DIFFUSION or “FLIP-FLOP”, the movement of lipids from one leaflet to the other is very slow. It is slow because the polar head of the amphipathic lipid must move through the nonpolar central region of the membrane in order to flip between surfaces. Even though the rate of “flip-flop” is slow it would eventually destroy the characteristic phospholipid composition of the individual membrane leaflets. The initial asymmetry of the membrane is achieved and maintained Flippases and Floppases. Flippase Enzymes, with energy supplied from ATP, moves phosphatidylethanolamine and phosphatidylserine from the outer leaflet to the inner. Floppases, by an ATP driven process, move phospholipids from the inner leaflet to the outer. These enzymes are responsible for moving phosphatidylcholine and the sphingolipids to the outer leaflet. Scrambleases move phospholipids in either direction down their concentration gradient. These enzymes destroy the “sidedness” of the membrane. The loss of the “sidedness” is a harbinger to apoptosis and cell death.

The associated proteins can also move within the lipid environment of the membrane. Lipid linked membrane proteins move the most rapidly, since they are the least tightly tethered. Movement of peripheral proteins along the surface of the membrane is only slightly slower. Intrinsic protein motion is the slowest, taking minutes or longer to diffuse along the surface of the membrane.

Several factors control the fluidity of the cell membrane. The ratio of saturated fatty acids to unsaturated fatty acids is of primary importance. At room temperature saturated fatty acids are solids, whereas the unsaturated fatty acids are oily liquids. The “cis” double bonds of unsaturated fatty acids kinks the molecule which makes it more difficult for the molecule to “pack” into a solid. Increasing the number of unsaturated fatty acids in the membrane increases the fluidity of the membrane. Membranes of organisms living in cold climates contain a larger proportion of unsaturated fatty acids.

Cholesterol in the plasma membrane is a second factor that modulates fluidity. Cholesterol has two effects of membrane fluidity. It is inserted in the membrane such that the rigid planar ring system is in the hydrophobic region of the membrane and the hydroxyl group on carbon three is exposed to the polar head
groups and to intracellular or extracellular water. This rigid planar structure stabilizes the extended conformation of the saturated fatty acids in the membrane. Pure lipids exhibit a sharp transition from the liquid to the solid state. Cholesterol in the cell membrane broadens the temperature at which the liquid to solid transition occurs.

The Cytoskeleton

The Cytoskeleton gives the cell mechanical strength, increases the rigidity of the cell membrane, allows cells to change shape, plays a role in the movement of organelles and vesicles within the cell, and at the extreme, changes in the cytoskeleton structure directs the migration of cells. The Cytoskeleton is a complex, dynamic arrangement of protein fibers constantly dissociating and reforming. It is associated with the inner leaflet of the cell membrane. In many cell types the cytoskeleton responds to outside stimuli, moving vesicles to the membrane for secretion and/or moving/rearranging intrinsic membrane proteins. Three types of protein fibers make up the cytoskeleton:
1. The MICROFILAMENTS are the smallest diameter fibers. They are composed of polymerized ACTIN. ACTIN monomer is a globular protein, the polymer is fibrous with a plus (+) end and a minus (-) end. Polymerization is driven by ATP hydrolysis and occurs at the (+) end of the filament. Microfilaments are involved in cytoplasmic streaming, ameboid movement, and muscle contraction.

2. The second type of fibers are the INTERMEDIATE FILAMENTS. There are six different types of intermediate filament proteins, SPECTRIN is one example. Intermediate Filaments are flexible, strong, and stable polymers that provide the cell with mechanical support and strength. A network of Intermediate Filaments extend from a meshwork around the nucleus to points on the plasma membrane. This complex network of fibers is anchored to the inner surface of the membrane by protein-protein interactions between the filaments and the inner domains of intrinsic membrane proteins and/or between the fibers and peripheral proteins bound to the inner membrane surface. The only function of some of the peripheral membrane proteins is to serve as anchor points for the cytoskeleton, they are Scaffold Proteins. In the cartoon of the red cell cytoskeleton (above) the small actin polymers (microfilaments) are Junctional Complexes, points where several spectrin fibers (intermediate filaments) come together on the inner membrane surface. Ankyrin and Band 4.1 are scaffold proteins. Ankyrin forms a bridge between spectrin and intrinsic membrane proteins and Band 4.1 mediates the interaction of spectrin with the actin junctional complexes. Under certain conditions, changes in the Intermediate Filament network aids or directs the movement of integral membrane proteins along the cell surface.

3. MICROTUBULES are the largest type of fiber in the cytoskeleton. They are composed of α-TUBULIN and β-TUBULIN. β-TUBULIN binds GTP (a G-protein) followed by α-TUBULIN and then this complex binds to the plus (+) end of the microtubule. The (+) end is the growing end moving toward the cells periphery, Microtubules dissociate from the (-) end. Microtubule associated proteins (MAPs) control the stability; promoting or preventing assembly. The MAPs guide assembly toward specific cellular locations. ATP dependent motor proteins KINESIN and DYNEIN move cargo, vesicles or organelles, along the microtubules. KINESINS move vesicles along the microtubules of the cytoskeleton away from the centrosome, away from the microtubule organizing center (MTOC). Kinesin moves vesicles from the Golgi to the cell periphery using the cytoskeleton as the roadway. DYNEINS move vesicles along the microtubules of the cytoskeleton toward the centrosome, toward the MTOC, toward the interior of the cell. Microtubules are also part of the mitotic spindle, cilia, and flagella.

Extracellular Surface of Membranes
The Glycocalyx

The outside surface of the cell membrane, the outer leaflet, is coated with carbohydrates. This is the GLYCOCALYX. The glycocalyx is composed of the polysaccharides attached to the extracellular domains of intrinsic membrane proteins, the polysaccharides of extrinsic membrane proteins present on the outer leaflet, and the carbohydrates of the cerebrosides, globosides, and gangliosides. The glycocalyx plays a role in Contact Inhibition (the cessation of cell division when cells contact each other) and it interacts with the Extracellular Matrix, the complex mixture of connective tissue proteins, proteoglycans, and other molecules that
exists outside and between cells.

Cell Adhesion Molecules on the Extracellular Surface of Membranes

Several families of integral membrane proteins provide specific points of attachment between cells and between cells and the extracellular matrix.

1. **Integrins** are heterodimeric proteins composed of two dissimilar subunits (one α & one β) anchored through the plasma membrane by a single α helical region in each subunit. The extracellular domains of the α and β subunits form specific binding sites for extracellular proteins like collagen and fibronectin. Integrins are not merely glue; they also function as receptors and signal transducers. For example; Integrins take part in platelet aggregation at the site of a wound, tissue repair, immune cell activity, and tumor invasion.

2. **Cadherins** contain four extracellular Ca\(^{2+}\)--binding domains, the most distal interacts with identical cadherin molecules on adjacent cells mediating cell-cell binding.

3. **Immunoglobulin-like Proteins** (N-CAM, Neuronal Cell Adhesion Molecule; P-CAM, Platelet Cell Adhesion Molecule; etc.) can interact with their identical counterparts on another cell or interact with an integrin on an adjacent cell thereby mediating cell-cell binding.

4. **Selectins** are a subset of the Lectin Proteins. They have extracellular domains that, in the presence of calcium ions, bind to specific polysaccharides on the surface of neighboring cells and/or bind to specific polysaccharides in the extracellular matrix. Selectins present on the surface of leukocytes (platelets, lymphocytes, monocytes, macrophages) and the endothelial cells that line blood vessels
play an important role in localizing white cells to the site of damage (e.g., bacterial invasion) and in hemostasis.

The Extracellular Matrix

The Extracellular Matrix is an interlocking meshwork of fibrous proteins and heteropolysaccharides. Its main protein component is collagen. Other fibrous proteins, such as fibronectin, laminin, vitronectin, elastin, fibrin, and fibrinogen are also present. These other proteins contain numerous different binding domains that allow them to interact with other components of the extracellular matrix. For example, fibronectin contains binding domains for collagen, heparin, fibrinogen, and laminin. The protein “fibers” of the extracellular matrix are enclosed by a network of proteoglycans. Proteoglycans are a large family of glycoproteins whose carbohydrate moieties are predominantly glycosaminoglycans and by mass there is more carbohydrate than protein. Some of these molecules are intrinsic membrane proteins that are part of the glycocalyx (syndecan), others are strictly components of the extracellular matrix. Proteoglycans typically contain one or two types of glycosaminoglycans O-linked to serine residues that are part of ser-gly repeats present on the protein. These molecules can also contain small O-linked or complex type N-linked polysaccharides. Proteoglycans function by interacting with a variety of other molecules through their glycosaminoglycan components and through specific domains on the polypeptide. For example, a specific intracellular domain on the protein part of syndecan associates with actin of the cytoskeleton and the heparin sulfate of Syndecan binds to fibronectin in the extracellular matrix. The fibronectin
binds to other glycosaminoglycans and/or proteins of the extracellular matrix and a three dimensional matrix is born. Over 2200 different protein-protein and protein-glycosaminoglycan interactions have been mapped to date in the extracellular matrix.

Tasks of the extracellular matrix include:
1. interconnection and anchoring of cells,
2. structural support of tissues and organs.
3. migration and localization of cells especially during embryonic development.
4. the proteoglycans may modulate cell growth processes by binding and concentrating growth factors in the extracellular matrix creating a pool of growth factors for the cells to use. Several proteoglycan core proteins have Epidermal Growth Factor domains (e.g., VERSICAN) that can interact with growth factor receptors to modulate cell growth.
5. the very polar nature of the extracellular matrix slows the diffusion / migration of polar molecules within the extracellular space allowing cells to more efficiently / effectively “capture” them for use.

Membrane Transport

The lipid bilayer of the cell is a Selectively Permeable Barrier. The nonpolar tails of the membrane lipids form a nonpolar core in the bilayer that prevents or severely inhibits the movement of polar and charged molecules across the membrane. Small nonpolar molecules such as O₂, and CO₂, can freely move down a concentration gradient and cross the cell membrane by diffusion. The small polar molecule, urea, can apparently fit in the space between lipids tails and diffuse across the cell membrane. Water by a similar mechanism can osmose across the membrane.

Small ions (Na⁺, K⁺, Cl⁻, HCO₃⁻) diffuse slowly across the cell membrane. The movement of these ions is driven by two forces, the concentration gradient that exists across the membrane and the potential (voltage) gradient across the membrane. A typical plasma membrane has a potential of -60 mV (negative inside). The potential across the cell membrane pulls cations (Na⁺) into the cell and pushes anions (Cl⁻) out of the cell.

The majority of polar molecules must be transported across the cell membrane with the aid of proteins embedded in the membrane. Many of the intrinsic membrane proteins act as transporters for polar and charged molecules. There are three types of transporters. Each of these transporters functions by a different mechanism. The three types of transporters are:
1. **CHANNELS or PORES**
2. **PASSIVE TRANSPORTERS**
3. **ACTIVE TRANSPORTERS**

Transporters are also categorized by how many molecules they move and the direction of movement:

1. **UNIPORTS** carry a single polar molecule.
2. **SYMPORTS** require two molecules for function. It carries two polar molecules simultaneously across the membrane. The two molecules are transported in the same direction.
3. **ANTIPORTS** likewise carry two ligands across the membrane. An antiport carries these two molecules in opposite directions. One is carried “in” while the other is transported “out”.

**Channels or Pores**

**CHANNELS or PORES** are protein lined “holes” through the membrane. Channels are typically intrinsic proteins with multiple α-helical segments passing through the membrane. The polar amino acids of the helixes serve as a polar environment through which the polar molecule or ion can migrate. Since the polar molecule does not bind to the channel, this type of transporter cannot be saturated with the transported molecule. Channels only work down a concentration gradient, they allow molecules to move from a region of high concentration to a region of lower concentration. Molecules move through pores at a rate near the theoretical maximum rate for unrestricted diffusion. Channels are somewhat specific with respect to the molecules allowed to pass through. For example, the channel can be lined with negatively charged amino acid side chains to prevent the transport of anions and the diameter of the channel can restrict movement to molecules to a particular size or shape.

The **AQUAPORINS** provide channels for rapid movement of water across membranes in a variety of tissues. Red blood cells and kidney tubule cells contain many copies of the aquaporins. In many instances the opening to the channel is GATED so that it can be opened or closed when necessary. There are four types of gating mechanisms - **LIGAND GATED**, **SIGNAL GATED**, **VOLTAGE GATED**, and **MECHANOSENSITIVE (STRETCH-ACTIVATED) CHANNELS**.
**Ligand Gated Channels** are opened or closed when a specific extracellular signal molecule (stimuli) binds to the gate domain of the protein. The Acetylcholine Receptor is an example of a ligand gated channel. When the neurotransmitter acetylcholine binds to this channel, it opens allowing Na⁺ or Ca²⁺ to enter the post synaptic cell.

**Signal Gated Channels** are opened when an intracellular second messenger molecule such as cAMP or Ca²⁺ binds to the gate domain.

**Voltage Gated Channels** are opened or closed by a change in membrane potential, by a change in voltage across the membrane. Cell membranes in general, and membranes of nerve cells in particular, maintain a small voltage or "potential" across the membrane in its normal or resting state. In the rest state, the inside of the cell membrane is negative with respect to the outside (typically -60 to -70 millivolts). The Na⁺ ion channel and the K⁺ ion channel in neuronal membranes are examples of voltage gated channels. They open when the potential across the neuronal membrane changes.

**Mechanosensitive (Stretch-Activated) Channels** open or close in response to mechanical forces that arise from local stretching or compression of the membrane around them; e.g., when their cells swell or shrink or when some mechanical force impinges upon the membrane. Such channels are believed to underlie the sensation of touch and they are believed be involved in the transduction of acoustic vibrations into the sensation of sound.

**Passive Transport or Facilitated Diffusion**

**Passive Transport (Facilitated Diffusion)** requires a binding interaction between the molecule to be transported (ligand) and the transport protein. Since the molecule to be transported must physically bind to the transport protein, passive transport exhibits **Saturation Kinetics** similar to the saturation kinetics observed with enzymes. A plot of transport rate versus ligand concentration results in a rectangular hyperbolic plot and the rate of transport approaches a limiting value, a **Vmax**, at very high concentrations of the transported molecule. In the **Oscillating Banana Hypothesis** of transport, ligand binding to the transport protein induces a conformational change in the transporter and the change in conformation carries the molecule from one side of the membrane to the other. Once on the other side of the membrane the molecule is released and the transporter assumes its original conformation. No outside energy is required for passive transport and it only functions down a concentration gradient. Passive transporters move molecules from a region of high concentration to a region of low concentration. The rate of transport is dependent upon the magnitude of the concentration gradient. As the difference in concentration approaches the equilibrium concentration the rate of transport decreases, and at equilibrium the rate of transport into the cell equals the rate of transport out. Examples of passive transporters include the twelve glucose (monosaccharide) transporters [GluT]:

- **GluT1**, is ubiquitous and under normal physiological conditions it transports glucose at a basal rate. It is specific for D-glucose having a **Kt** of 1.5 mM. (Kt is the equivalent of Km - it is the ligand concentration that results in 1/2 maximal transport velocity.) For the close analogs D-mannose and D-galactose the **Kt** values are 20 mM and 30 mM, respectively; for L-glucose the **Kt** is greater than 3000 mM.
- **GluT2**, under normal physiological conditions it transports glucose into or out of hepatocytes, intestinal epithelial cells, and pancreatic islets.
- **GluT3**, under normal physiological conditions it transports glucose at a basal rate into brain and other nervous tissue.
• GluT4, under normal physiological conditions it transports glucose into muscle, adipose, heart, and other insulin dependent cells.
• GluT5, under normal physiological conditions it is the primary fructose transporter.

All of these transporters carry glucose (monosaccharides) from a region of high concentration to a region of low concentration and all of them are Uniports.

Active Transport

Passive diffusion directly through the membrane, movement through channels, and facilitated diffusion are simple systems in the sense that the transported molecules flow down hill energetically, the transported molecules flow from a region of high concentration to a region of low concentration. The cell also needs to transport molecules against a concentration gradient, it needs to move molecules from a region of low concentration to a region of high concentration. Transport systems of this type must be driven by the expenditure of energy. The transport can be driven by the hydrolysis of ATP, it can be driven by light, it can be driven by metabolic reactions, or it can be driven by the energy stored in an ion gradient. PRIMARY ACTIVE TRANSPORT is driven by ATP hydrolysis. SECONDARY ACTIVE TRANSPORT is driven by some energy source other than ATP.

Primary active transport requires energy in the form of ATP to accomplish the movement of molecules across the membrane against a concentration gradient. The transport protein must bind the molecule(s) or ion(s) to be transported along with ATP before primary active transport can occur. Since a binding interaction between transport protein and ligand is required, primary active transport exhibits SATURATION KINETICS. The Na⁺/K⁺ ATPase, present on most cells, is an example of a primary active transporter. The mechanism for this transporter is as follows:

1. Three sodium ions in the cytosol bind to exposed sodium ion sites on the primary transporter ATPase.
2. Once the Na⁺ is bound ATP binds and the ATPase activity of the transporter transfers a phosphate from ATP to an aspartate residue on the transporter forming a high energy mixed anhydride intermediate and inducing a conformational change that oscillates the banana and results in the release of sodium ions into the extracellular space.
3. Two potassium ions outside the cell bind to exposed potassium ion sites on the ATPase.
4. Binding of K⁺ causes dephosphorylation of the transporter. Upon dephosphorylation, the ATPase is converted to the “low energy” form, it undergoes a conformational change that oscillates the banana.
and releases potassium ions into the cell.
5. The three sodium ion sites on the ATPase are again exposed, and the process repeats.

Secondary active transport also requires an energy source to accomplish the movement of polar molecules
across the cell membrane. In secondary active transport the energy for transport is supplied primarily from an ion gradient across the cell membrane. However, it can be driven by light or by metabolic reactions. When ion gradients are the driving force, the ion is usually Na⁺, H⁺, or Cl⁻. Secondary active transporters display SATURATION KINETICS and they move molecules against a concentration gradient. The INTESTINAL GLUCOSE TRANSPORTER (the Na⁺-Glucose symporter) present on the luminal side of intestinal epithelial cells lining the small intestine is an example of a secondary active transporter. This transporter is used to absorb glucose from the gut after a meal. In the lumen of the gut the concentration of Na⁺ outside of the cell is higher than the concentration inside the cell. When this transporter is functioning, glucose binds to the transport protein first. Glucose binding to the transport molecule opens binding sites for two Na⁺. Two Na⁺ ions bind to these sites and the presence of both glucose and Na⁺ on the transport protein induces a conformation change in the transport protein. The conformational change carries both glucose and Na⁺ into the cell, across the membrane. The Na⁺ that enters the cell with glucose is pumped back out by the Na⁺/K⁺ ATPase (the primary active transporter), thus maintaining the Na⁺ ion concentration gradient. If the Na⁺ ion gradient were to dissipate, glucose transport would cease. The intestinal glucose transporter only functions when glucose is present. In the absence of glucose, Na⁺ cannot bind to the transporter thereby inhibiting the transport of Na⁺.