

## Introduction to Membrane Physiology

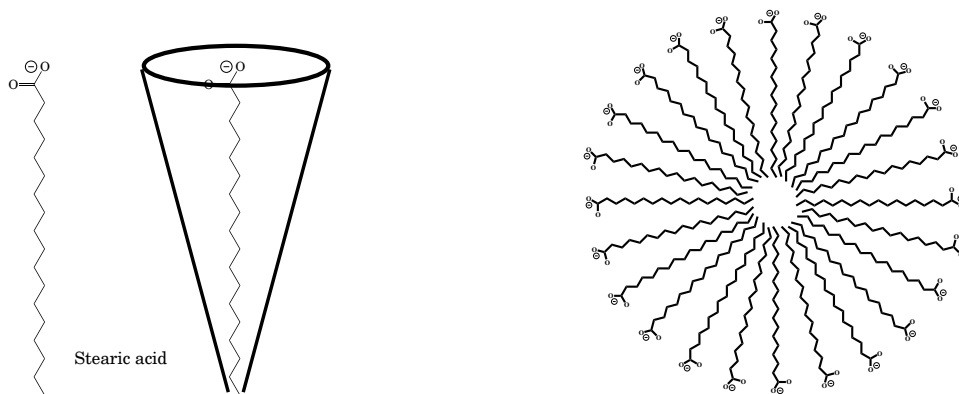
### **Lipids in aqueous solutions**

Some lipids, and especially those comprised of saturated long-chain fatty acids, are solid at room temperature. The main difference between “fats” and “oils” in biological terms is that “fats” are solid while “oils” are liquid at room temperature, although both are largely comprised of triacylglycerols. Butter, which is solid at room temperature is comprised of triacylglycerols that contain ~75% saturated and 25% unsaturated (predominantly monounsaturated) acyl chains. In contrast, olive oil contains triacylglycerols with ~15% saturated, 70% monounsaturated, and 15% polyunsaturated fatty acyl chains.

Oils, being liquids, can be mixed with water; however, the two components tend to rapidly separate out into regions containing only water and only the oil. This is predominantly due to the hydrophobic effect; the water is most stable when it minimizes its contact with the hydrophobic molecules in the oil.

Single lipid molecules have a tendency to aggregate. However, aggregation is associated with a decrease in entropy for the lipid molecules. Therefore, at low concentrations of lipid, individual lipid molecules may remain in solution. As the lipid concentration increases, the hydrophobic effect becomes more important and tends to push the carbon backbones of the lipid molecules together.

If the lipid contains a hydrophilic (and especially a charged) head group, as is found in fatty acids, the polar group will attempt to remain in contact with the aqueous solution. This results in the formation of a spherical aggregate called a **micelle**.



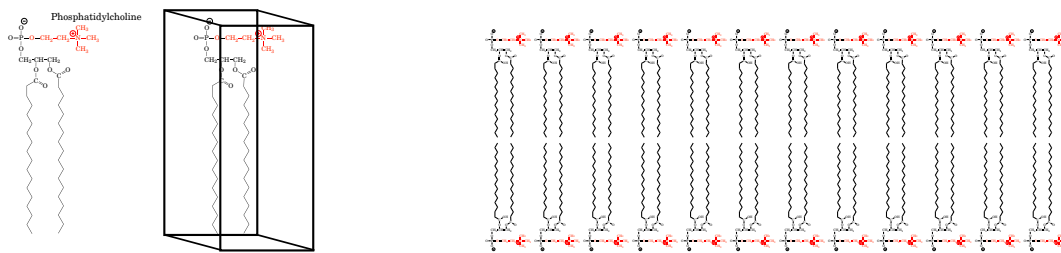
The shape of a micelle is a consequence of the shape of its lipid constituents. Free fatty acids result in spherical micelles, because the van der Waals surface of a free fatty acid is essentially conical (note that the carboxylic acid at the end is slightly more bulky than the hydrocarbon tail, and, more importantly, is negatively charged, which limits the possible packing density of the carboxyl end of the fatty acid).

Micelle formation is cooperative, because it requires the presence of enough amphipathic lipid molecules to form the spherical structure. At low concentrations micelles cannot form. Once a high enough concentration occurs, essentially all of any added lipid will also form micelles. The limiting concentration is called the

“**critical micellar concentration**” (CMC); the CMC is a function of the properties of the lipid molecules, and will be different for each type of amphipathic lipid.

Both the CMC and the size of the micelle depend on the length of the acyl chain and the nature of the head group. Longer acyl chains result in a lower CMC, because both the hydrophobic effect and the strength of van der Waals interactions between the lipids are greater for longer hydrocarbon chains.

Phospholipids have a van der Waals surface that is similar to a rectangular solid. As a result, phospholipids try to form larger micelles. However, this presents a problem, because it usually results in empty space somewhere in the particle. A much more favorable arrangement is the formation of a planar bilayer structure, which allows the optimum packing of the phospholipids.

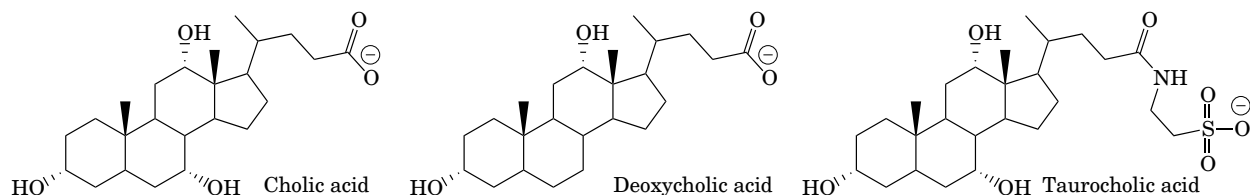


This presents a minor problem in that the ends of the plane must have some protection from the aqueous solvent. Alternatively, the structure can form a large bilayer sphere, with the planar structure slowly bending around to enclose a significant volume. This is how the plasma membrane of a cell is constructed.

## Emulsifiers

An emulsion is a mixture of two (or more) materials that are ordinarily immiscible. When a mixture of oil and water is agitated vigorously, the mixture will form an emulsion. However, in most cases, the oil and water will rapidly separate back into a two-phase system if the shaking stops. An emulsifier is a compound that assists in stabilizing an emulsion. Detergents are amphipathic molecules (such as fatty acids and related molecules) that act as emulsifiers by forming micelles and trapping more hydrophobic molecules in the non-polar center of the micelle.

Digesting fat requires the ability to emulsify the fat so that it can be absorbed. Animals use detergents, and especially the cholesterol-derived bile acids, to solubilize the dietary fat to begin the absorption process. Three examples of bile acids are shown below.



## Saponification

Another method for forming an emulsion is to convert a hydrophobic molecule into an amphipathic molecule. Hydrolysis at high pH results in release of free fatty acids and glycerol from any type of fatty acid ester, including membrane phospholipids and the triacylglycerols found in oils and fats. Soap is produced by addition of sodium hydroxide or potassium hydroxide to oil or membranes. Agents such as sodium hydroxide and hydrolytic enzymes are emulsifiers.

## Bilayers

If a system contains large amounts of lipid, the lipid must either form many small micelles or must form a smaller number of large micelles. Unless the system contains large amounts of totally non-polar lipid, it cannot make large spherical micelles, because the polar head groups are unstable unless they are exposed to the aqueous exterior.

If the amphipathic lipid and water are in a container open to the air, the lipids will tend to form a monolayer on the water surface. This maximizes the amount of polar head group that can interact with the water, and completely hides the hydrophobic tails from the water.

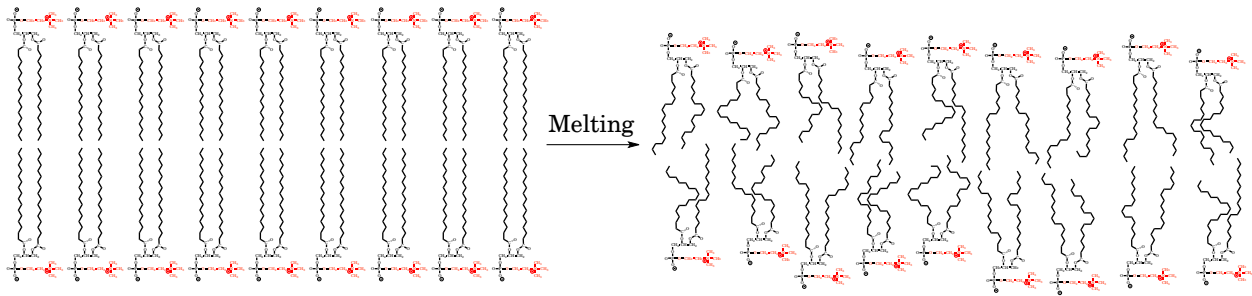
The inside of an organism, however, has few open “surfaces”. Forming a bilayer has the same result as a surface monolayer; the polar groups face out into aqueous phase, while the hydrophobic tails face away from the water. The bilayer also results in a discontinuity: the hydrophobic region in the center of the bilayer can act as a barrier to separate one aqueous environment from another. Barriers are important; they act to keep valuable material inside and potentially deleterious materials outside of a cell.

A bilayer is stabilized by a variety of interactions. The most important, especially for acyl chains greater than about 14 carbons, is the hydrophobic effect, which tends to keep the phospholipids in close proximity. In addition, the head groups of the phospholipids form hydrogen bonding and ionic interactions with one another, and van der Waals interactions stabilize the hydrophobic center of the bilayer.

## Membrane Fluidity

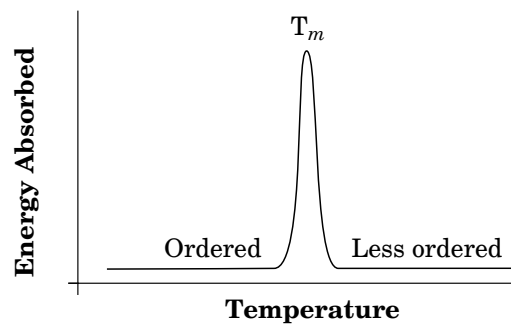
Membrane fluidity is defined based on the ability of molecules to move within or across the membrane. If the membrane “freezes” molecules cannot move, and the membrane becomes an impermeable barrier. The term “freezing” has connotations that are not completely accurate: a better description is that a phase transition can occur which results in an ordered, immobile two-dimensional structure.

The melting temperature ( $T_m$ ) of a membrane is the temperature at which the phase transition occurs. Below the  $T_m$ , the lipids are highly organized. The tight packing of acyl chains results in a minimal surface area and maximal thickness. Above the  $T_m$ , the lipids are more mobile and less tightly packed, and the average thickness of the membrane decreases.



### Experimental determination of phase transitions

As heat is added to a membrane, the hydrogen bonds and van der Waals packing interactions destabilize. As more heat is added, entropic effects overcome stabilizing forces, causing a phase transition. Phase transitions require an additional energy input to cause the same increase in temperature (see the graph below).



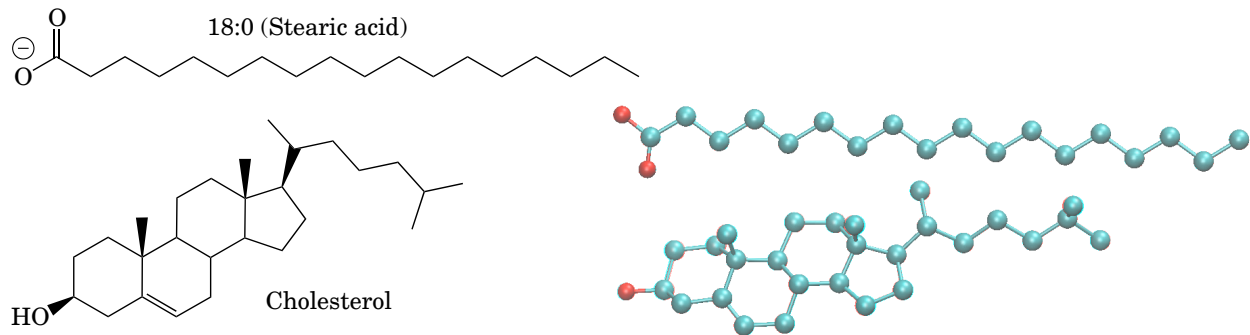
In a simple membrane of uniform composition, adding heat results in a sharp transition, because the melting process shows a high degree of cooperativity due to simultaneous disruption of many favorable packing interactions. The transition temperature and the sharpness of the peak depend on the composition of the membrane, with the uniformity in types of lipids, chain length, and degree of saturation all having a role. In addition, the interactions between the head groups are also important in determining  $T_m$ . The  $T_m$  of the bilayer increases with chain length, and decreases with increasing amounts of unsaturated fatty acids. The  $T_m$  of the vast majority of biological membranes is below the normal operating temperature of the organism; in other words, most organisms have “melted” membranes.

### Cholesterol

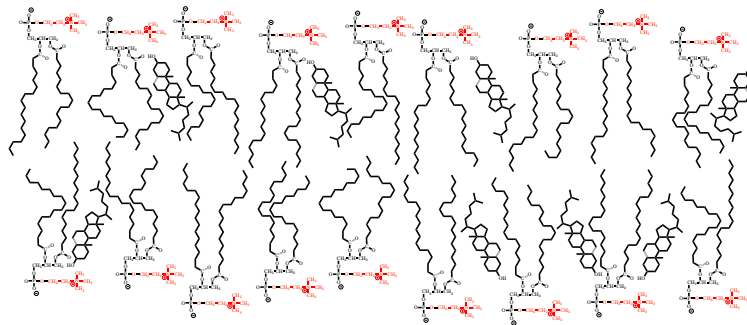
Cholesterol is an important membrane component in animals (plants do not use cholesterol, although plants do have structurally related compounds such as ergosterol). Cholesterol is a major component of membranes, comprising 25-50% of the total lipid by weight. Cholesterol is hydrophobic except for its single hydroxyl group.

The cholesterol hydroxyl orients the molecule, with the hydroxyl close to the surface of the bilayer. With its side-chain extended, cholesterol is slightly shorter than a

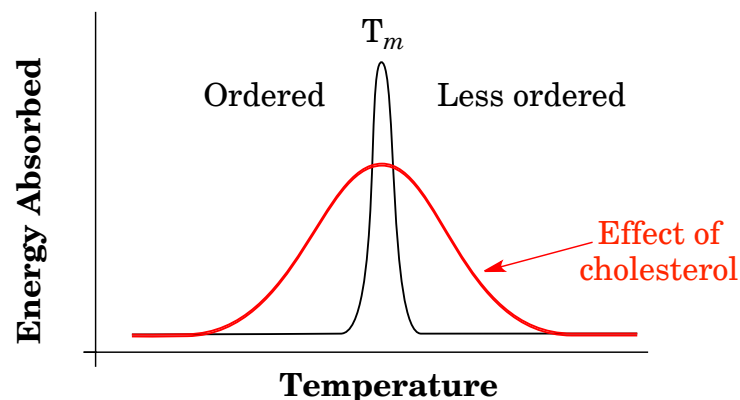
typical, fully extended, acyl chain. In the diagrams below, note the relative lengths of cholesterol and stearic acid.



Cholesterol tends to decrease packing density for saturated acyl chains, and to increase packing density for unsaturated acyl chains. The cholesterol ring structure is rigid; only the side chain is somewhat free to rotate. Cholesterol acts as a **modulator** of membrane fluidity: large amounts of cholesterol decrease fluidity (due to the rigid ring structure, which hinders mobility of nearby acyl chains, especially near the membrane surface), but cholesterol also prevents tight packing of lipid acyl chains.



The usual net effect of cholesterol addition to a membrane is a broadening of the phase transition, although with little change in the  $T_m$ . The presence of cholesterol thus tends to act as a mechanism for localizing the region in which the phase transition occurs, or (at high enough concentration) for completely preventing the phase transition from occurring.



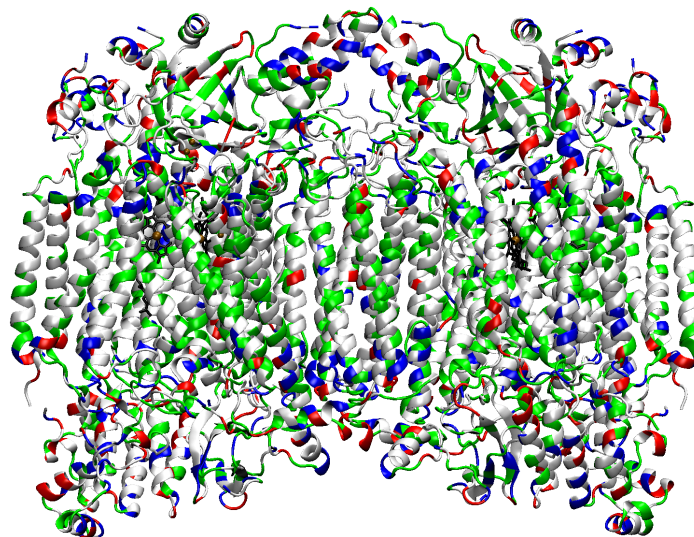
## Membrane proteins

Biological membranes contain proteins in addition to lipids. These proteins can be grouped into three classes: peripheral, integral, and lipid linked.

**Peripheral membrane proteins** bind to the surface of a membrane via ionic or polar **non-covalent** interactions with the head groups. Peripheral membrane proteins can be removed from the membrane fairly easily, usually by a change in the ionic strength or pH, although in some cases, disruption of the cell is necessary. Annexins, proteins that are found in all multicellular organisms, provide an example of peripheral membrane proteins. Annexins form non-covalent interactions with the head groups of membrane phospholipids in the presence of calcium; if the calcium concentration decreases, the annexins dissociate from the membrane.

**Integral membrane proteins** are tightly associated with the membrane. Removal of an integral protein requires disruption of the membrane, usually with detergents or other saponifying agents (*e.g.*, NaOH). The extramembrane regions of an integral membrane protein generally have amino acid compositions similar to those of soluble proteins, because these regions are exposed to the aqueous environment. However, integral proteins also have one or more transmembrane regions; these transmembrane segments have exposed hydrophobic surfaces in order to interact with the non-polar acyl chains of the membrane lipids. These exposed hydrophobic surfaces mean that membrane proteins are somewhat difficult to study in solution; as a result, membrane proteins are generally less well understood than globular proteins.

The transmembrane segments of an integral membrane protein are frequently formed from  $\alpha$ -helices, because this secondary structural element satisfies the hydrogen bonding requirements of the peptide backbone atoms. The structure below was determined for bovine cytochrome *c* oxidase (from PDB ID 2OCC), a large protein with more than 3500 residues. Note the band of hydrophobic residues (colored light grey in this drawing) across the center of the structure. Note also the  $\alpha$ -helical secondary structure of this region of the protein.



Transmembrane proteins may have their N- and C-termini on the same side, or on opposite sides of the membrane. The peptide backbone may cross the membrane once or (as shown above) several times.

Membrane proteins associated with the plasma membrane often have carbohydrate groups covalently attached to the protein and are therefore termed **glycoproteins**. The carbohydrate groups are attached during post-translational modifications of the protein either to serine hydroxyls (O-linked carbohydrate) or to the nitrogen of the asparagine side-chain amide ((N-linked carbohydrate). The carbohydrate modifications are located on the **outside** of the membrane.

**Lipid-linked proteins** are the final class of membrane proteins. Lipid-linked proteins are essentially soluble proteins covalently bonded to lipids. The lipid forms favorable interactions with the membrane interior (and unfavorable interactions with water); lipid-linked proteins are therefore tightly associated with the membrane. Lipid-linked proteins may have functions that require membrane proximity, or may be released by hydrolytic enzymes in response to a signal.

The bond to the lipid can be formed in one of several ways. **Myristoylation** typically occurs when the free  $\alpha$ -amino group of an N-terminal glycine forms an amide bond to the carboxyl of a free myristic acid (myristate is a 14-carbon fatty acid). Fatty acids of varying sizes can also form ester bonds to the side chains of serine or threonine residues, or thioester bonds to cysteine side chains. **Crosslinked-glycoproteins** form covalent bonds between their carbohydrate groups and the head groups of some types of phospholipids (this is the case for thyroglobulin and acetyl cholinesterase).

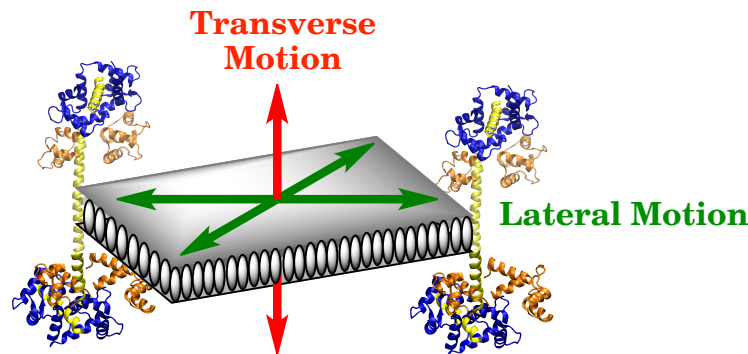
Finally, **prenylated proteins** exhibit thioether bonds to terpene derivatives such as farnesyl or geranylgeranyl groups. Prenylation occurs in proteins where the last four residues are Caax (with C = cysteine, a = aliphatic, and x = any residue). After the thioether is formed, the last three residues are hydrolyzed, and the C-terminus is carboxymethylated to form the neutral methyl ester of the carboxylate. Examples of prenylated proteins include the monomeric G-proteins (also known as ras proteins), and the  $\gamma$ -subunits of the heterotrimer G-proteins.

### **Lateral motion within membranes**

In 1970, Frye and Edidin performed an experiment that demonstrated the ability of membrane proteins to undergo free lateral movement within membranes. They raised antibodies against mouse and human cell surface proteins and labeled the antibodies with fluorescent dyes (using a red fluorescent molecule for the human, and a green fluorescent marker for the mouse). They then fused a human and a mouse cell. Initially, the fused cell had a red half and a green half; over time the colored regions merged, until the entire surface of the fused cell had both red and green markers. Other scientists performed similar experiments by adding fluorescent tags to lipid molecules of different cells and fusing the cells, showing that, like the membrane proteins, the lipids diffused laterally through the membrane.

### Fluid mosaic model

Singer and Nicholson (1972) proposed, based on the Frye-Edidin experiment and similar data, the **fluid mosaic model** for biological membranes. The membrane was known to consist of protein and lipid. Their model proposed that **transverse movement in membrane is constrained** and that it was difficult to cross membranes or to move molecules in or out of the membrane. The model also proposed that the membrane is fluid, and that both lipid and protein molecules can move **laterally** within the plane of the membrane.



The fluid mosaic model has several consequences. A bilayer made of uniform phospholipid molecules is fairly rigid, especially if all acyl chains in those phospholipids are saturated. The model therefore proposed an explanation for the observation that biological membranes are typically comprised of a variety of phospholipids, and of other lipid molecules (especially cholesterol): the non-uniform population of molecules acts to maintain the fluidity of the membrane. The model also proposed that membrane proteins exist in a two-dimensional “solution” and are therefore capable of moving and possibly interacting with one another.

In real biological systems, lateral mobility can be modified by intracellular and extracellular events. An example of this is an experiment using a fluorescent-tagged protein hormone. When the hormone was allowed to bind to its cell-surface receptor, the receptor molecules were observed to be scattered across the surface of the cell. Over the course of a few minutes, however, the fluorescent signal moved to a few small regions in the membrane, suggesting that the cell was forcing the receptor proteins to aggregate. The receptor proteins are thus moving laterally in the membrane in a non-random way, presumably because of events occurring within the cell.

### Transverse motion

In contrast to the lateral mobility observed in both model systems and real cells, the movement of lipids from one **side** (termed “**leaflet**”) of the membrane to the other is extremely limited. In cells, the lipid compositions of the inner and outer leaflet are often quite different. This is possible because the exchange of phospholipids from one leaflet to the other is very slow ( $t_{1/2} = \sim 10$  days).

Enzymes called **translocases**, which use the energy derived from ATP-hydrolysis to specifically move lipids from one side of the membrane to the other, create the

asymmetrical arrangement of the membrane phospholipids. As a result of the action of these enzymes, the plasma membrane outer leaflet of most human cells contains glycolipids and phosphatidylcholine, while the inner leaflet contains primarily phosphatidylserine and phosphatidylethanolamine, and some phosphatidylinositol.

Membrane proteins also have extremely limited transverse motion. For example, the plasma membrane contains a number of glycoproteins; the carbohydrate groups of these molecules always face the extracellular space.

Some membranes also contain “**flipases**”, which are passive exchangers that *decrease* the asymmetrical orientation of the membrane lipids.

### **Membrane transport**

The barrier function of membranes is extremely important for living organisms. However, while the membranes must act as barriers, some transverse movement of molecules must also occur; cells must be capable of importing nutrient materials and must be able to export waste products. In addition, cells need to control the osmotic pressure differential between the inside and outside of the cell. Finally, cells frequently use ion fluxes across the cell membrane for signaling functions. To achieve these functions, the membrane properties must allow **selective permeability**, in which movement of molecules across the membrane can be controlled.

Three mechanisms are known that provide for the transverse movement of molecules across membranes.

- 1) **Passive diffusion**: some molecules, including water, some non-polar molecules, and some gasses, can cross membranes unassisted.
- 2) **Assisted diffusion**: a variety of proteins specifically allow some molecules to equilibrate across the membrane.
- 3) **Active transport**: some proteins act as energy-dependent **pumps** to force molecules across membranes, generally against an electrochemical gradient.

Hydrophobic molecules are usually assumed to cross membranes relatively easily (although some have some form of assisted transport or pump mechanism). However, polar, and especially charged, molecules have considerable difficulty crossing membranes without assistance. As a result, specific integral membrane proteins mediate most transmembrane movement of polar molecules. We will consider each mechanism for transmembrane movement in turn.

### **Passive diffusion of uncharged molecules**

Most molecules cross bilayers in proportion to their solubility in organic solvents. **Water** is an exception: water molecules cross membranes relatively readily, due to their small size and high concentration in the aqueous medium surrounding the membrane.

For an **uncharged species**, the tendency of a molecule to cross a membrane can be assessed thermodynamically:

$$\Delta G = RT \ln \left( \frac{C_{in}}{C_{out}} \right)$$

where  $C_{in}$  and  $C_{out}$  are the concentrations of the molecule on different sides of the membrane. The difference in concentration is the **concentration gradient**, and the  $\Delta G$  is the **chemical potential gradient** for the system. The chemical potential gradient attempts to drive the molecule from the region of high concentration to the region of low concentration. In this treatment, the sign of the (non-zero)  $\Delta G$  indicates the direction of flux (negative means “into cell”, positive means “out of cell”).<sup>6</sup>

As with the  $\Delta G$  for any thermodynamic system, **the  $\Delta G$  for passive diffusion yields no information about the rate of movement**. A separate equation provides rate information:

$$J_c = -P(C_{out} - C_{in})$$

In this equation,  $J_c$  is the rate of movement of the molecule, with the sign of  $J_c$  indicating the direction of motion (negative means into cell, positive means out of cell),  $C_{out} - C_{in}$  is the concentration gradient, and  $P$  is the permeability coefficient. The permeability coefficient is a function of the membrane composition and of the characteristics of the molecule attempting to cross the membrane.

The permeability coefficient can be expressed as:

$$P = \frac{KD}{x} \text{ and therefore } J_c = \frac{KD}{x} (C_{out} - C_{in})$$

The different terms represent different aspects of the problem of allowing a molecule to cross a membrane. The term  $K$  is the **partition coefficient**, which is a measure of the relative preference of a molecule for hydrophobic or aqueous environments. A hydrophobic molecule usually has a large  $K$ , because it interacts more favorably with the hydrophobic interior of the membrane. Polar molecules have small  $K$ , because they have limited solubility in hydrophobic solvents; this is especially true for charged molecules, in which the low dielectric constant of the membrane interior results in very small values for  $K$ .

The term  $D$  is the **diffusion coefficient**, which is a measure of the mobility of the molecule in a lipid environment. Small molecules are capable of moving through membranes much more readily than large ones, because they need to displace fewer

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<sup>6</sup> **It is important to note** that the formalism used here (*i.e.* a negative  $\Delta G$  corresponding to movement into the cell), is **not** used in all textbooks or in all papers in the scientific literature. In reading other treatments, be aware that formalism used may vary.

lipid molecules in the process. The diffusion coefficient also reflects the relative mobility of the membrane lipids; more rigid membranes exhibit lower diffusion coefficients.

Finally, the term  $x$  is the thickness of the membrane; thicker membranes are more difficult for a molecule to cross. The hydrophobic **core of a biological membrane is ~30-40 Å thick** (the precise thickness depends on the length of the acyl chains, and on other factors such as the amount of protein and cholesterol present, and on the temperature). This thickness is an evolutionary consequence of the equation for passive diffusion; thicker membranes would be too difficult for molecules to cross, while thinner membranes would not provide a strong enough barrier to separate compartments (thinner membranes are also less stable due to a less intense hydrophobic effect and fewer van der Waals interactions in the membrane interior).

The rate of diffusion can vary by several orders of magnitude depending on molecules involved and on the membrane composition.

### Concentration of free ligand

As with all biological processes, diffusion is a function of the **free** ligand in solution on both sides of the membrane. In biological systems, the concentration of the free molecule can be altered: the molecule can bind to another molecule (usually a protein). For example: the free concentration of oxygen inside red blood cells is much lower than the total concentration of oxygen, because most of the oxygen is bound to hemoglobin.

Alternatively, the molecule can be converted into a different molecule. For example, glucose conversion to glucose-6-phosphate lowers the free glucose concentration inside a cell, and therefore increases the concentration gradient for glucose.

Thus, it is possible for the organism to influence the thermodynamics of the diffusion process as necessary to improve the likelihood of spontaneous movement of materials across the membrane.

### Passive diffusion of ions

In the case of charged species, the free energy equation has an extra term that accounts for the movement of a charge across a potential difference:

$$\Delta G = RT \ln \left( \frac{C_{in}}{C_{out}} \right) + Z \mathcal{F} \Delta \Psi$$

In this equation,  $Z$  is the charge on the ion (including both magnitude and sign, so  $\text{Na}^+$  has  $Z = +1$ , while  $\text{HPO}_4^{2-}$  has  $Z = -2$ ),  $\mathcal{F}$  is the Faraday constant (96485 Coulombs/mol = 96.485 kilojoules/volt•mol), and  $\Delta \Psi$  is the potential difference across the membrane (in volts). Note that, in this equation, both  $Z$  and  $\Delta \Psi$  can vary in sign. The direction of the ion flow therefore depends on the concentration

gradient for that specific ion **and** on the electrical effects of the total ion gradient for the membrane.

The convention used here for diffusion for ions is the same as the convention for the diffusion of uncharged molecules: a negative  $\Delta G$  means that it is thermodynamically favorable for the compound to enter the cell, while a positive  $\Delta G$  means that the molecule will spontaneously exit the cell. The sign and magnitude of the  $\Delta G$  define the **electrochemical gradient**.

**Electrochemical equilibrium** is the condition in which the ion concentration gradient is exactly opposed by the potential difference:

$$RT \ln \left( \frac{C_{in}}{C_{out}} \right) = Z \mathcal{F} \Delta \Psi$$

Note that a system for which one molecule is in electrochemical equilibrium is likely to have other molecules that are **not** in equilibrium.

### Membrane potential

Cells nearly always have *slightly* greater number of negative charges inside than outside; this difference in number is too small to detect chemically, but has important electrical consequences. This slight difference in numbers of ions results in the membrane potential. The membrane potential depends on the cell type, and is typically between  $-10$  and  $-120$  millivolts.

### Assisted diffusion

As mentioned earlier, cells have several mechanisms for membrane transport. Some molecules cross membranes using only passive diffusion. However, for most molecules, passive diffusion is too slow to support biological processes. Assisted diffusion techniques allow molecules to move down their electrochemical gradient at much higher rates than would be possible otherwise. Cells use three main types of assisted diffusion: **passive transport**, **ion channels**, and **pores**.

### Passive transport

Passive transporters are proteins that act as enzymes to catalyze the movement of molecules across membranes. These proteins are very similar in many respects to the enzymes that accelerate chemical reactions, although passive transporters only catalyze motion, and do not alter the chemical characteristics of the molecules.

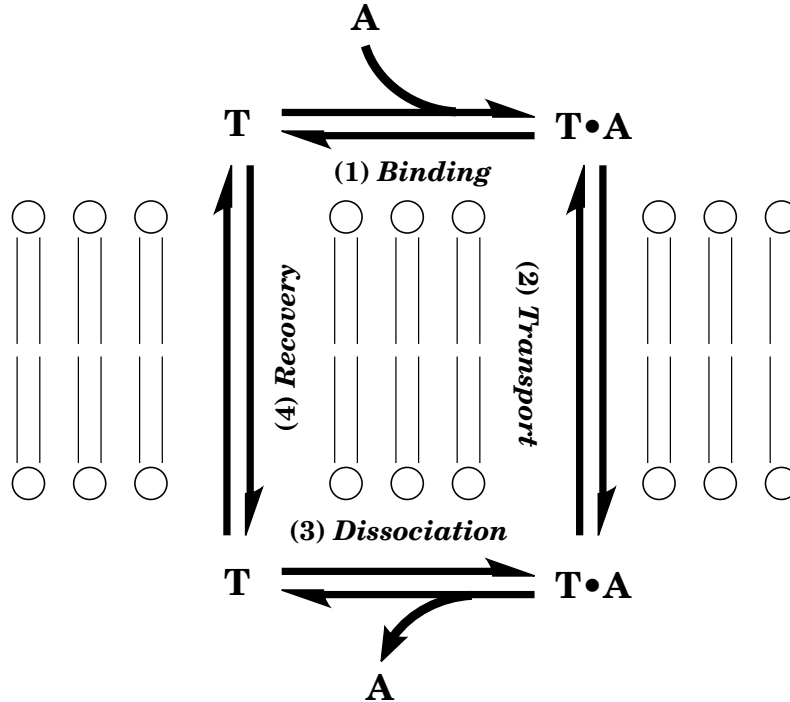
Passive transporters do not create holes in the membrane; instead they act as carriers to move the molecule down its electrochemical gradient. Below is a general model for a passive transporter. The model has several consequences. Note that each step is completely reversible; net transport of **A** will only occur from a region of high to a region of low **A** concentration, and can cross in **either direction**.

- (1) Molecule **A** binds to the transporter **T**
- (2) The complex moves across the membrane (or the transporter undergoes a

conformational change that effectively allows Molecule **A** to cross the membrane)

(3) Release of the **A** molecule

(4) Return of the unoccupied transporter **T** to the other side of the membrane



Note also that, in the model, **A** can only move across the membrane when bound to the transporter. This implies that the transport process has a finite maximum rate, because if all of the transporter molecules have **A** bound, no additional **A** can be transported until some of those **A** molecules dissociate. This contrasts to passive diffusion, in which an infinite concentration gradient theoretically corresponds to an infinite rate of transport; with passive transporters the rate for a given number of transporter molecules has a finite limit.

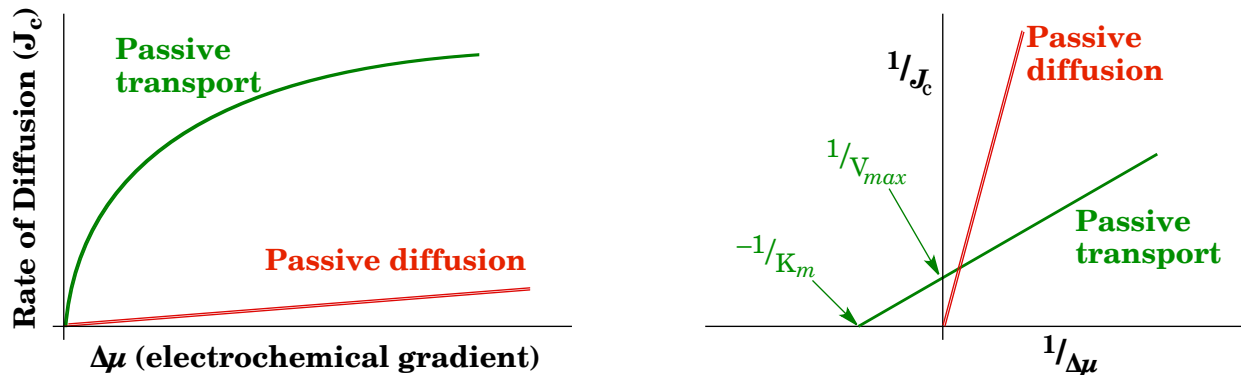
Because the transporters are essentially enzymes, for passive transport the rate of transport is a hyperbolic function of the electrochemical gradient ( $\Delta\mu$ ). In the equation below,  $J_{max}$  is function of both the number of sites (and thus of the concentration of transporter,  $[T]$ ) and of  $k_c$ , the rate constant of transport by each site; as for an enzyme, the  $K_m$  is largely a measure of affinity.

$$J_c = \frac{J_{max} \Delta\mu}{K_m + \Delta\mu} \quad \text{where: } J_{max} = k_c [T]$$

The rate of transmembrane movement can be plotted according to the rate equations for passive diffusion and passive transport. A theoretical plot is shown below left. Note that this plot is not drawn to scale: for most molecules passive diffusion processes are several orders of magnitude slower than passive transport

processes, and the line shown for passive diffusion is therefore much higher than would be true for most molecules.

As with all hyperbolic processes, the equation for passive transport can be linearized by plotting the reciprocal of the rate versus the reciprocal of the electrochemical gradient. (Note that these linear plots have drawbacks, but are also useful.) The passive diffusion curve has its  $x$  and  $y$  intercepts at the origin; this indicates that for passive diffusion,  $J_{max}$  and  $K_m$  are both infinite. In contrast, passive transporters have defined values of  $J_{max}$  and  $K_m$ .



#### Side Note: Mathematical issues related to passive transport

Rectangular hyperbolæ are not symmetrical about the origin. This means that the absolute value of the electrochemical gradient must be plotted for the rate equation in order to obtain meaningful results; plotting negative electrochemical gradients (which may exist physiologically: a negative electrochemical gradient means merely that the intracellular concentration is higher than the extracellular concentration, or that the membrane potential favors exit of the ion from the cell, or both) will result in aberrant values for the rates.

#### Example system: Glucose transporters

Biological systems contain large numbers of different transporter proteins. One important class of transporters found in humans is comprised of several different gene products used to transport glucose; these proteins illustrate a variety of different biochemical features related both to the problem of moving important molecules across membranes and to the problem of allowing different cells to behave according to their differentiated functions. Note that some of the concepts mentioned here depend on other concepts that will be discussed later in the context of glucose metabolism.

Glucose is a polar molecule, and its permeability coefficient for biological membranes is very small. As a result, its rate of entry into cells is far too low to allow normal metabolic functions to proceed. A family of genes called GLUT genes have protein products that are involved in glucose transport; the products of these genes fall into three main classes.

GLUT1 and GLUT3 are the glucose transporters used by most cells. These have a low  $K_m$  (high affinity) for glucose. In cell types that use GLUT1 and GLUT3, glucose transport is regulated by hexokinase activity; if hexokinase activity is high (generally due to low glucose-6-phosphate levels, since glucose-6-phosphate is an inhibitor of hexokinase), then glucose transport is also high. The affinity of the GLUT1 and 3 gene products for glucose is only limiting if glucose levels fall well below the normal physiological range.

The liver contains GLUT2, which has a high  $K_m$  (low affinity) for glucose. As with glucokinase phosphorylation of glucose, the rate of transport by GLUT2 increases considerably as plasma glucose concentration increases. Note that GLUT2 physiologically operates in either direction; it allows glucose entry when plasma glucose levels are high, and allows the exit of glucose when plasma glucose levels are low (or when the rate of gluconeogenesis is high enough to raise intracellular glucose above plasma glucose concentration). Although all of the glucose transporters could, in principle, operate as either importers or exporters of glucose, it is extremely rare for any cells other than those in the intestine, liver, and kidney to export glucose.

GLUT4 is a third type of high affinity glucose transporter, found specifically in muscle and adipose tissue. Muscle can tremendously increase its glucose uptake (either to support exercise, or in response to a sudden increase in plasma glucose levels). This change in glucose flux is due to an increase in the amount of GLUT4 in the plasma membrane. While the concentrations of the GLUT1, 2, and 3 in their cell membranes are essentially constant, the levels of GLUT4 in the cell membrane vary. GLUT4 is normally sequestered in intracellular vesicles; it is translocated to plasma membrane in response to insulin, resulting in enhanced glucose uptake due to an increase in  $J_{max}$ .

Thus, the different glucose transporter gene products have a variety of different properties that are necessary to support the function of the cell types that express them.

### Summary of passive transport

Passive transport differs from passive diffusion in several ways:

1. **Passive transport is much faster than passive diffusion** under physiological conditions (infinite concentration gradients are rare in biological systems).
2. **Passive transport is saturable**, and therefore has a limiting rate, which is a function of the number of transporter molecules and of the rate constant for transport.
3. **Passive transporters are specific**, and only accelerate transport for molecules that fit their binding sites.
4. **Passive transporters are subject to inhibition**: molecules similar to the normal ligand may prevent transport by competitive inhibition, and molecules that inactivate transporters or which remove transporters from membranes may act as non-competitive inhibitors.

## Channels and pores

### Ion channels

Ion channels are proteins that allow specific ions to cross membranes down their electrochemical gradients. Channels are governed by the same thermodynamic principles as passive transporters, but they are functionally distinct. Ion channels are all thought to function by, in effect, creating a small hole across the membrane through which the ion can travel, and have mechanisms that allow the ion to cross the membrane rapidly. They also have the property of being controllable; the channel is not always open, a fact which is critical to normal functioning of cells.

In order to properly understand ion channels, it is first necessary to consider some aspects of ions. Ions dissolve in water because water is a polar molecule. Water is capable of forming partial charges that can interact with an ion; as a result, a **hydration sphere**, a favorable arrangement of water molecules that partially delocalizes the charge of the ion, surrounds ions in aqueous solution. In part because of this, water has a high dielectric constant. In contrast, non-polar environments have little ability to form favorable interactions with ions and exhibit low dielectric constants. The interior of a membrane has a low dielectric constant; ions have little tendency to partition into this hydrophobic environment, and the membrane therefore presents a strong barrier to the transit of ions. Channels must contain mechanisms that allow the ions to overcome the dielectric barrier of the membrane.

### Potassium channels

Potassium channels are members of a large family of related proteins. All known potassium channels are tetrameric proteins (although some have two transmembrane helices per monomer while others have six transmembrane helices per monomer). When the crystal structure of the bacterial potassium channel KscA was solved<sup>†</sup>, it was the first ion channel for which high-resolution structural information was available. The KscA crystal structure has yielded a number of insights into the mechanisms that allow channels to function. Many aspects of the potassium channel crystal structure are believed to apply to all ion channels.

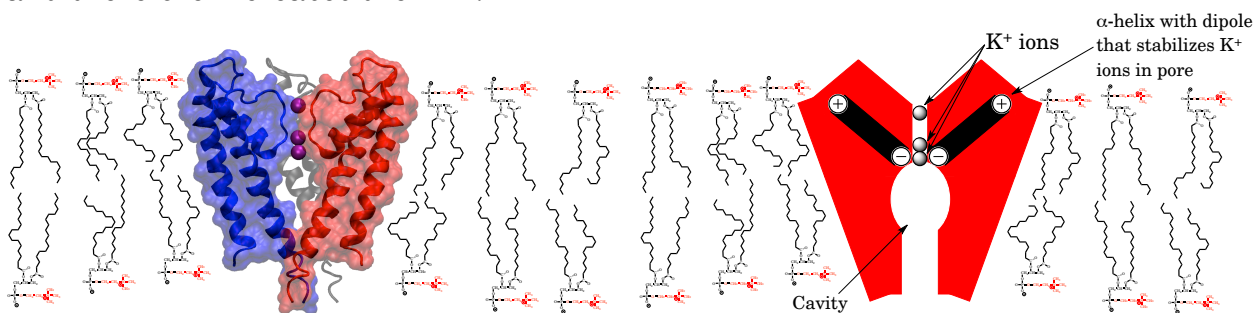
Sodium is featureless sphere 1.90 Å in diameter, while potassium is featureless sphere 2.66 Å in diameter. Although these cations appear very similar, the potassium channel is somehow capable of discriminating between potassium and sodium ions. While it is simple to see how sodium channels might prevent the larger potassium ion from entering, the potassium channel must be able to admit potassium ions while excluding the smaller sodium ions. It is clearly capable of doing this: the potassium flux is >10,000-fold higher than that of sodium. The potassium channel is also highly efficient at allowing potassium to cross the membrane; the potassium flux due to one channel is  $\sim 10^8$  ions per second, a value close to the aqueous solution diffusion limit for potassium ions. Thus, the channel has achieved two competing goals: **high discrimination**, and **high throughput**.

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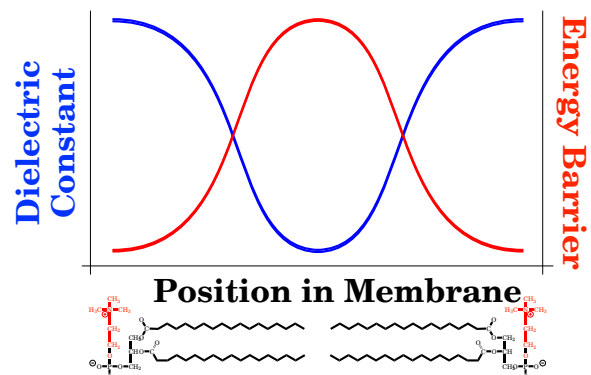
<sup>†</sup> Doyle *et al.*, “The Structure of the Potassium Channel: Molecular Basis of K<sup>+</sup> Conduction and Selectivity” *Science* **280**:69-77 (1998).

For the potassium channel, the discrimination process is made possible by a selection filter: the entrance to the channel exactly fits a dehydrated potassium ion, but sodium is too small to fit well. To allow an ion to enter the channel, the protein must compensate for the positive  $\Delta H$  associated with disruption of the hydration sphere; it does so by forming charge-dipole interactions to the potassium ion. Sodium ions are too small to fit properly, and form much weaker interactions that are insufficiently strong to compensate for the energy cost of removing the sodium hydration sphere.

The requirement for high throughput means that the ionic interactions between the channel and the potassium ion cannot be too strong, because strong bonds would tend to keep the ion in the channel. The binding of the potassium ion is therefore barely sufficient to compensate for the removal of the hydration sphere. A second mechanism for pushing ions through rapidly is to have several sites for ions in the channel (the crystal structure shows three potassium ions; see the cartoon below<sup>7</sup>). Each potassium ion present destabilizes the binding of others by electrostatic repulsion. A third mechanism involves the pore of the channel itself, which is fairly hydrophobic; the hydrophobic lining prevents high affinity interactions with ions and therefore increases the flux.



The final mechanism for increasing the flux involves the presence of a 10 Å diameter cavity in the center of membrane. Ions experience an energy barrier as they attempt to cross membranes, because the membrane interior has a much lower dielectric constant than does the aqueous solution around the membrane (see graph at right). The channel contains a water filled cavity, which results in an increase in the local dielectric constant, and therefore a decrease in the energy barrier.



Channels such as the potassium channel are thus proteins that create a continuous path through the membrane. As should be obvious from the comments above, channels do **not** simply allow any molecule to pass through; instead they have the

<sup>7</sup> The diagram on the left shows the homotetrameric KscA potassium channel structure from pdb ID 1BL8, with the monomer closest to the viewer omitted for clarity. The cartoon on the right shows a simplified version of the structure.

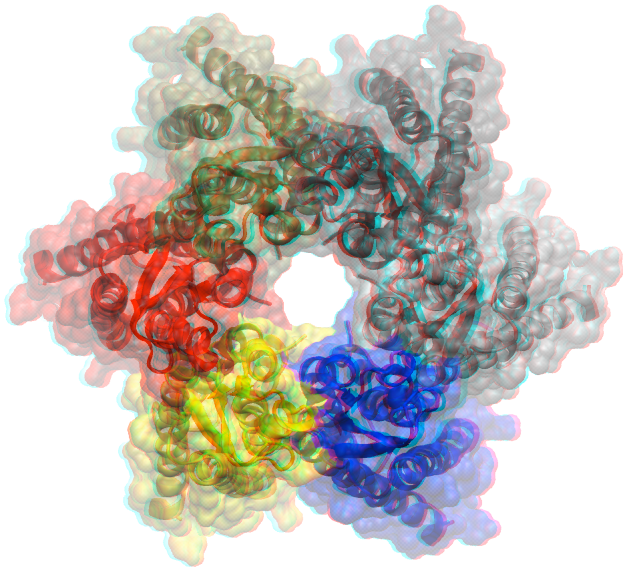
ability to discriminate among molecules. There is one other critically important point: **channels are regulated proteins**, and are not always open. When the channel is closed, the ions do not flow. When the channel is open, the flow through the channel is close to the diffusion limit (and is therefore much faster than transport by typical passive transporters, which often have moderate transport rate constants, and which are usually slowed by the necessity of binding the transported molecule followed by a conformational change by the protein).

Eukaryotic organisms contain channels for several ions. Of these the most important are sodium, potassium, and calcium channels, although a number of others are also present.

Channels are very important for normal functioning. A number of toxins work by affecting channels, with most of these toxins inhibiting the flow of specific ions by binding specific channels. Examples of these toxins include tetrodotoxin, a compound produced by puffer fish that is a highly potent blocker of sodium channels, and several scorpion toxins that are inhibitors of potassium channels. Although many of these toxins are lethal in small amounts, these toxins are also extremely useful for studying the properties of specific channels.

### Gap junctions

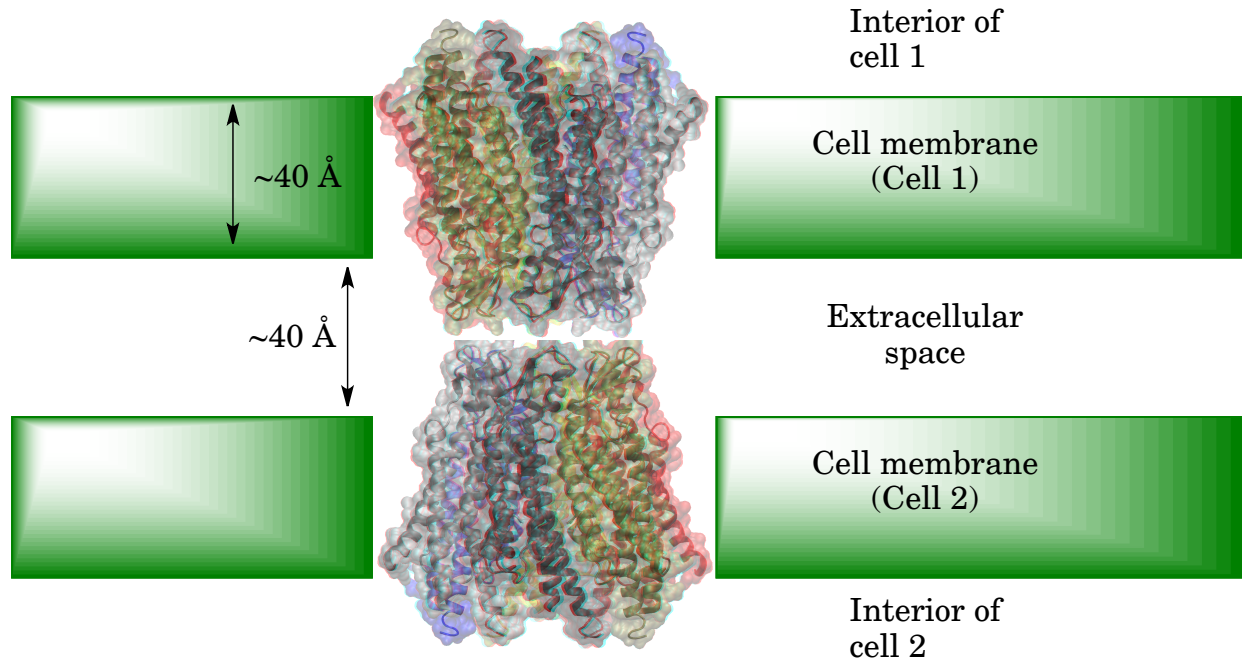
Gap junctions are a different type of structure that forms a hole across membranes. Gap junctions are formed from hexamers of monomers called connexins. Connexins are members of a family of 30-50 kDa proteins. Each connexin monomer is an integral membrane protein with 4 transmembrane  $\alpha$ -helices.



The structure at left is the *Homo sapiens* connexin-26 hexamer (showing each chain in a different color) looking down the opening through the protein. The opening is about 14 Å across (from pdb ID 2ZW3). Note that the solvent accessible surface is superimposed on the ribbon diagram showing the helical secondary structure, with each polypeptide in a different color.

Gap junctions form a pore **between** cells (by interaction of one hexamer from each cell membrane). Both cells must contain the hexamer to form the active gap junction. The function of the gap junction is to allow essentially any small molecule or ions with a molecular weight of less than ~1000 to move between cells. This allows cells to communicate directly. The pore opening can be controlled (so that cells can control the communication, and so that, if one cell dies, the contents of the other do not leak out, killing the second cell also). Although the opening or closing of

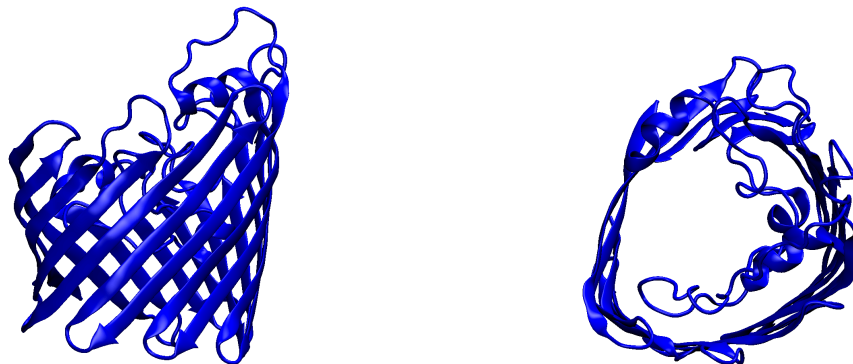
the gap junction can be controlled, the flux through an open gap junction seems to be unregulated.



### Pores

Bacterial outer membranes must allow a reasonable degree of small molecule permeability. The mitochondrial outer membrane also must allow small molecules to enter the intermembrane space freely. In both cases, however, these membranes must present barriers to the passage of proteins. These membranes therefore contain proteins called porins, which form pores across the membrane. The pores vary in size depending on the species; the mitochondrial pores allow molecules with molecular weights less than 10 kDa to cross the membrane freely.

Porins were among the first membrane proteins crystallized. They are thought to be somewhat unusual in that the transmembrane portion of the protein consists of a barrel shaped  $\beta$ -sheet rather than of  $\alpha$ -helices. Two views of the bacterial porin from PDB ID 2POR are shown below; the transmembrane opening is clearly evident in the view on the right.



## Nuclear pore

A membrane surrounds the nucleus of eukaryotic cells. This membrane is important for controlling the movement of molecules in and out of the nucleus. However, many molecules of varying sizes must be able to transit the nuclear membrane. Much of this nuclear transport is mediated by the nuclear pores, which are extremely large complexes (molecular weight ~125 million) comprised of 50 to 100 proteins. The pores allow the transit of large molecules (up to ~60 kDa); in addition, active transport of molecules much larger than 60 kDa is also possible, suggesting that the nuclear pore can undergo large conformational changes.

The nuclear pore complex seems to have a complex active transport mechanism for large variety of different molecules. In spite of the large diameter pore (90 Å to 250 Å, depending on the conditions), most transport in living cells seems to be active, at least for peptides, proteins, and RNA. Many proteins seem to have a nuclear localization signal, an amino acid sequence within the protein that directs that protein to the nucleus. The best characterized sequences are comprised of clusters of several basic residues.

## Ionophores

Living systems thus have a variety of mechanism for moving molecules across membranes, including controlled methods of moving molecules through holes that seem much larger than many proteins. However, transmembrane movement can also be used as a weapon. Ion gradients are very important for normal cellular function; breakdown of the gradient is critically dangerous and potentially lethal.

**Gramicidin A** is an antibiotic. It is a 15 amino acid peptide made from alternating L and D amino acids. It forms a helix (**not** an  $\alpha$ -helix; the structure is actually closer in many respects to a  $\beta$ -sheet) across membranes, with two molecules required to span the bilayer. In the membrane, gramicidin acts as a non-regulated ion channel somewhat selective for potassium.

While gramicidin forms a channel, other ionophores act as soluble ion carriers within the membrane. Examples of these are **valinomycin** and **monensin**, compounds that act as selective carriers for potassium and sodium ions, respectively, and **A23187**, a calcium ionophore.

Ionophores are toxic to cells, because they are capable of disrupting the ion gradients in the cell membranes that are required for normal cellular functions. As with many toxins, ionophores are also extremely useful for studying the effects of ion flow on biological processes.

## Active transporters

Electrochemical gradients contain energy. This energy must come from some source; usually the cell must use metabolite-derived energy to create the gradient. Active transporters (also known as pumps) are the proteins that perform this energy-dependent process of moving molecules across membranes *against* an electrochemical gradient.

## Coupled reactions

In most cases, the pump uses a coupled reaction; the pump obtains energy from one event and uses this energy to drive the movement of the molecule of interest across the membrane. Thermodynamic analysis of this process requires the use of an additional term in the  $\Delta G$  equation we have seen earlier.

$$\Delta G = RT \ln \left( \frac{C_{in}}{C_{out}} \right) + Z\mathcal{F} \Delta \Psi + \Delta G_c$$

Electrochemical gradient

Concentration gradient      Potential gradient

Free energy from coupled reaction

This equation has multiple terms, as shown above. The first term is the concentration gradient, which is due to the difference in concentration of the molecule between the two sides of the membrane. The second term is the potential gradient, which is due to the interaction of charged species with the membrane potential  $\Delta \Psi$  (this term only applies to ions; uncharged molecules have  $Z = 0$ , and therefore have no potential gradient term). The first two terms together are the electrochemical gradient. Note that **it is possible for the signs of the concentration and potential gradients to oppose one another**; thus the magnitude of the electrochemical gradient may be smaller than the individual component gradients.

Unless the overall electrochemical gradient is zero, the molecule **will** move in some direction **spontaneously**. The sign of the electrochemical gradient indicates the direction: **negative means that the molecule will tend to move into the cell**. The magnitude of the electrochemical gradient is the energy stored in the gradient; this is the energy that must be overcome in order to pump a molecule against the gradient, or the energy that is available to drive some other process. In analyzing an active transport mechanism, it is necessary to **compare the magnitude of the electrochemical gradient with the  $\Delta G$  of the coupled reaction**.

## Energy sources

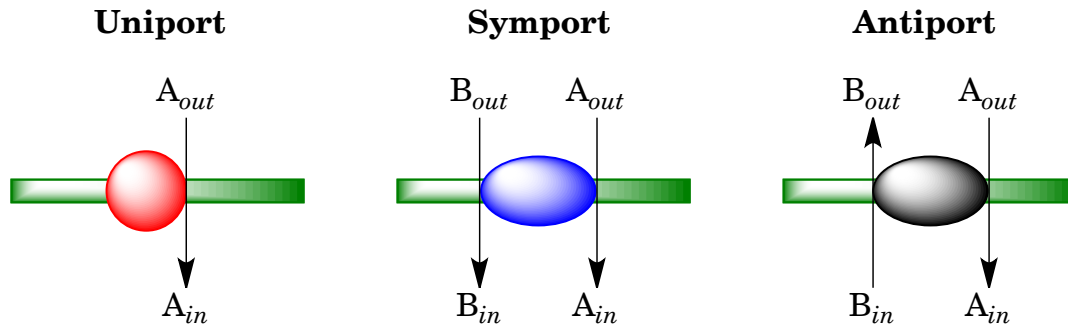
Active transporters have many possible energy sources. One major energy source is the free energy available from chemical reactions such as the **hydrolysis of ATP**. A second major energy source is an **electrochemical gradient**; movement of one molecule down its electrochemical gradient can drive transport of a different molecule against its electrochemical gradient. Finally, energy derived from **light** can be used to move molecules.

## Stoichiometries

**Uniport**: a protein that moves one molecule in one direction at a time. Uniports are frequently passive transporters (*e.g.*, the glucose transporters are uniports), although some pumps also use this stoichiometry.

**Symport:** a protein that moves more than one molecule in the same direction.

**Antiport:** a protein that moves two molecules in opposite directions.



Some symports and antiports use energy from the electrochemical gradient for one molecule to drive transport of a second molecule (especially proteins that use the sodium gradient to pump other molecules), while others are driven by other types of energy sources (such as the Na-K-ATPase pump discussed below, which is an ATP-driven antiport).

### Stoichiometries and ion pumps

If the active transporter moves ions across membranes, the result may or may not affect the membrane potential. An **electrically neutral** pump results in no net movement of charge, because the pump moves the same number of charges in each direction. In contrast, an **electrogenic** pump alters the membrane potential by moving more charges in one direction than in the other.

### ATPase pumps

ATP hydrolysis is a common energy source for pump proteins. ATP is readily available in cells, and the hydrolysis process for ATP releases sufficient energy ( $\Delta G'^{\circ} = -30.5 \text{ kJ/mol}$ ) to allow transport of most molecules.

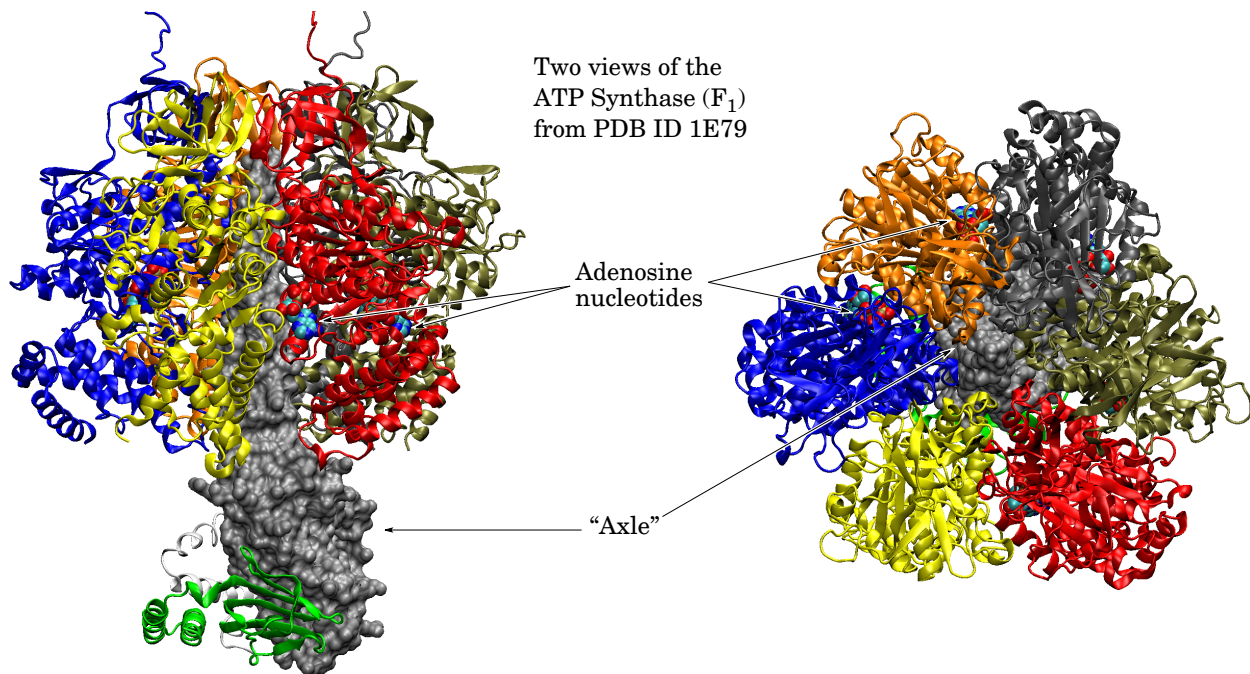
ATPase pumps fall into several classes. **P-type** pumps are the most common class; these are pumps in which the transporter becomes temporarily phosphorylated during the pumping process. A second class are the **V-type** ATPases, of which all known examples are proton pumps; the V-type pumps are ATPase proteins in which the hydrolysis reaction is directly coupled to the pumping process, and for which no phosphorylated intermediate is thought to exist. (The “V” is an abbreviation for “vacuolar”, because these pumps were discovered as proteins that create proton gradients across the vacuolar membrane.) A third class is comprised of the **F-type** ATPases, a small group of proteins distantly related to the V-type ATPases. In multicellular organisms, the F-type ATPase primarily if not exclusively functions as the ATP synthase responsible for oxidative phosphorylation. In bacteria, by contrast, the F-ATPase is reversible. Under aerobic conditions, it acts as an ATP synthase, driven by the proton gradient produced by the electron transport pathway. Under anaerobic conditions, it acts as an ATPase proton pump that generates an electrochemical proton gradient required for other processes (such as nutrient transport and flagellar rotation).

ATPase pumps have a variety of functions. Some of this variety is illustrated by the following descriptions of important members of this category of proteins.

### **F<sub>0</sub>F<sub>1</sub>-ATPase**

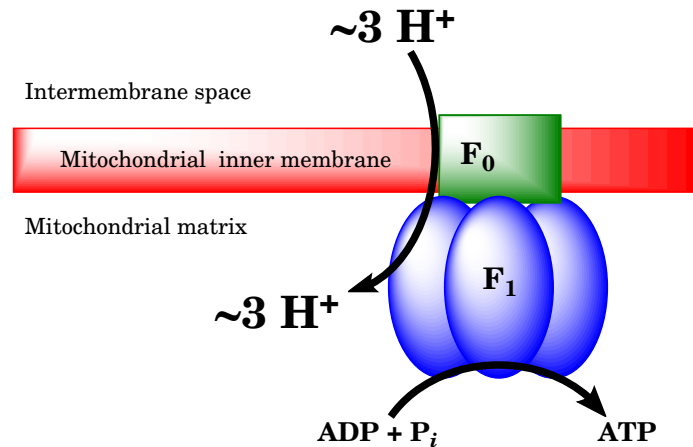
The F-ATPase is a large multisubunit protein, comprised of two main portions. F<sub>0</sub> is an integral transmembrane protein complex, while F<sub>1</sub> is a peripheral membrane protein complex. The F<sub>1</sub> portion of the overall complex is located on the inner face of the eukaryotic mitochondrial inner membrane or on the inner face of the prokaryotic cell membrane. Depending on the method used, the isolation process may result in removal of the F<sub>1</sub> complex from the ATPase structure. The residual F<sub>0</sub> complex acts as a proton passive transporter. The F<sub>1</sub> complex is the part of the overall structure that harnesses the energy of the proton gradient to synthesize ATP.

Crystal structures for the F<sub>1</sub> complex, and (separately) the F<sub>0</sub> complex have been solved. The F<sub>1</sub> complex contains a hexameric arrangement of subunits surrounding a long, narrow central protein. The central protein is thought to rotate relative to the remainder of the complex. In the complete complex, the rotation of the central axle is driven by proton flow through the F<sub>0</sub> complex. The mechanical energy of the rotational motion is converted to chemical energy by formation of ATP. The F-ATPase appears to be somewhat unusual in that the main energy-requiring step is not a chemical reaction, but instead is the dissociation of ATP from the protein. A diagram of the bovine F<sub>1</sub> complex is shown below.



The ATP synthase reaction is thought to require about three protons per ATP produced. Note that this is, at least in part, a consequence of the thermodynamics of the system; the electrochemical gradient experienced by the proton movement does not contain enough energy to allow the synthesis of an ATP molecule as the result of

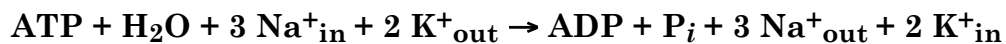
one or two protons crossing the membrane. In other words, the magnitude of the  $\Delta G$  for the transit of a single proton (using the equation for the  $\Delta G$  of an electrochemical gradient) is considerably smaller than the magnitude of the  $\Delta G$  required for ATP production. On the other hand, the enzyme may also require multiple protons for reasons having to do with the reaction mechanism, and the proton stoichiometry gradient may actually reflect both mechanistic and thermodynamic factors.



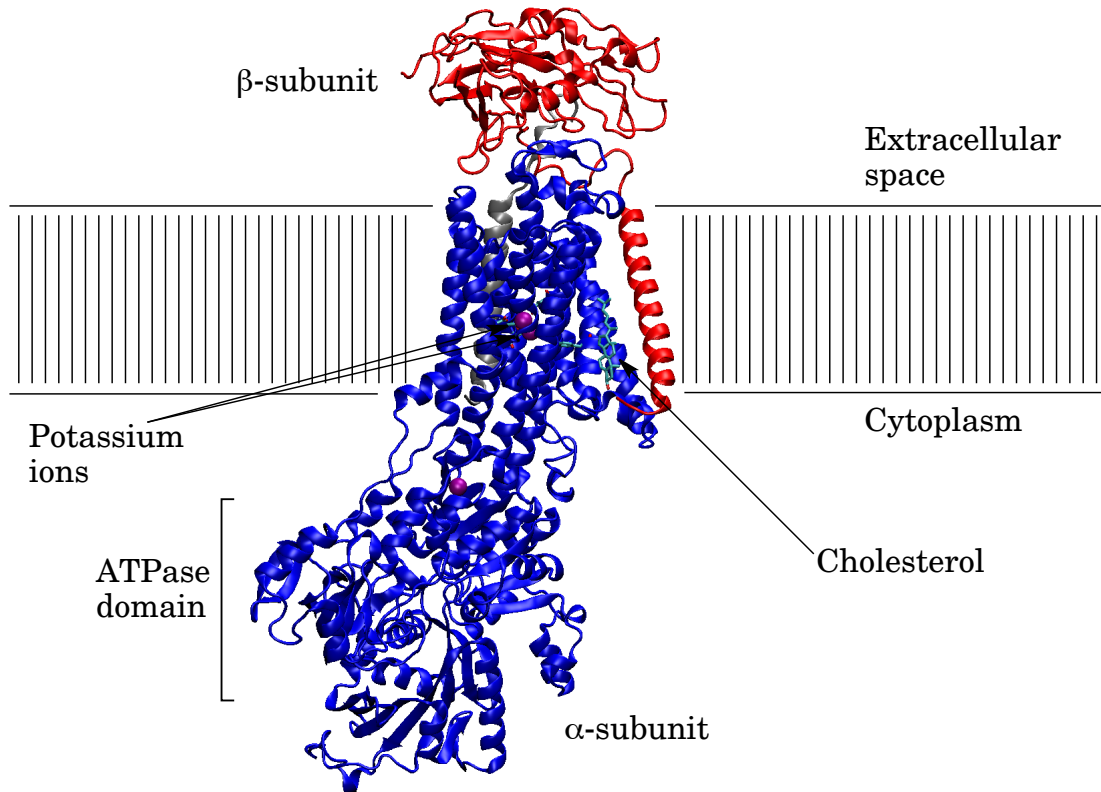
### **Na<sup>+</sup>-K<sup>+</sup>-ATPase pump**

Cells have a gradient of sodium and potassium ions across the cell membrane, with the **sodium** concentration always higher on the **outside**, and the **potassium** concentration always higher on the **inside** of cells. These gradients are important for a number of processes. The gradients are used for a number of energy-dependent processes, especially symport and antiport active transporters. In addition, partially releasing the gradients is a common signaling mechanism. Finally, changes in the sodium gradient magnitude act as a means for cells to compensate for changes in the osmotic pressure of their environment.

Maintaining the asymmetrical arrangement of the two ions requires energy (**20-70% of cell energy requirements** depending on the cell type). This energy is provided by ATP hydrolysis, using the Na<sup>+</sup>-K<sup>+</sup>-ATPase, a **P-type pump** protein. The Na<sup>+</sup>-K<sup>+</sup>-ATPase is electrogenic: it pumps 3 Na<sup>+</sup> out and 2 K<sup>+</sup> in for each ATP hydrolyzed, and therefore creates concentration gradients for these ions as well as a charge separation that is the primary source the cellular membrane potential.

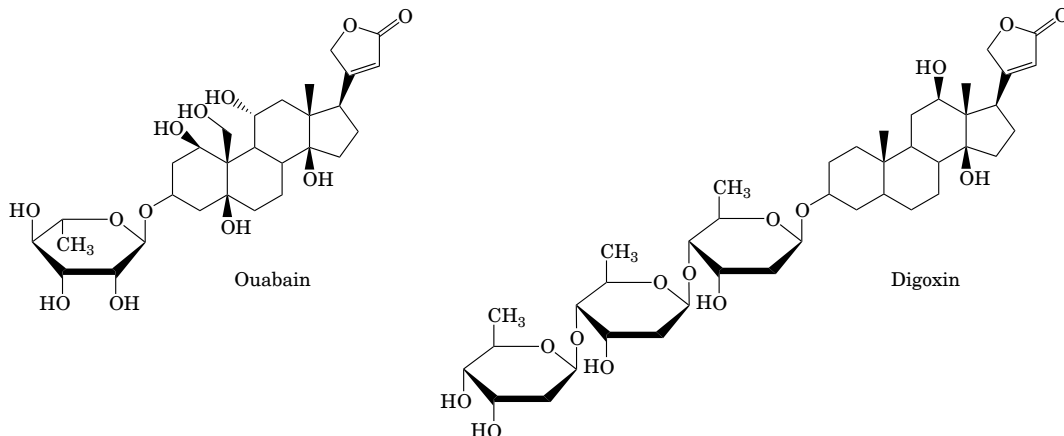


The Na<sup>+</sup>-K<sup>+</sup>-ATPase consists of two types of subunits. The  $\alpha$ -subunit is the transmembrane pump, and contains the ion binding sites and ATPase active site, while the  $\beta$ -subunit is an extracellular glycoprotein probably required for proper folding and localization of the protein. The structure below is from PDB ID 2ZXE.



Why doesn't the Na<sup>+</sup> and K<sup>+</sup> gradient drive the ATPase backwards (and generate ATP, or even simply reverse the gradient without generating energy)? The pump appears to be unidirectional due to kinetic constraints; the multistep process prevents the pump from acting as a passive transporter for sodium or potassium ions.

Cardiotonic steroids and glycosides (*e.g.*, **ouabain**, derived from an African tree called ouabio and **digoxin**, derived from foxglove) bind to the extracellular surface of the α-subunit and inhibit the Na<sup>+</sup>-K<sup>+</sup>-ATPase pump. At low doses, this results in increased use of Ca<sup>2+</sup>-Na<sup>+</sup> antiport and of the Ca<sup>2+</sup>-ATPase in an attempt to maintain the sodium gradient. The result is higher intracellular Ca<sup>2+</sup> levels, which act as a signal to trigger stronger heart muscle contraction.



Foxglove extracts have been used to treat congestive heart failure for centuries. Higher doses of these compounds are toxic; overdose of digoxin is lethal, and ouabain is used in hunting as an arrow tip poison.

In the early 1990s, ouabain and ouabain-like molecules were discovered to be released by the adrenal (in addition to other blood pressure regulators released by this tissue). Some studies indicate that elevated levels of endogenous cardiotonic compounds may be responsible for some types of hypertension.

### **Ca<sup>2+</sup>-ATPase**

Cytosolic calcium levels are normally very low (<1  $\mu\text{M}$ ), while extracellular calcium concentrations are typically maintained at 1-2 mM (in humans, at  $1.2 \pm 0.1$  mM free calcium). This means that cells contain a significant calcium gradient. The gradient is maintained by the Ca<sup>2+</sup>-ATPase, a P-type pump related to Na<sup>+</sup>-K<sup>+</sup>-ATPase. Ca<sup>2+</sup>-ATPase is found in the plasma and endoplasmic reticulum membranes, and is required for moving calcium out of the cytosol, either by pumping calcium ions into the endoplasmic reticulum or by pumping the ions out of the cell. The Ca<sup>2+</sup>-ATPase is a probably a uniport, (and therefore is thought to be electrogenic because it pumps ions in a single direction, although there is some evidence of coordinated pumping with protons) with a stoichiometry of 2 Ca<sup>2+</sup> pumped out per ATP hydrolyzed. The Ca<sup>2+</sup>-ATPase probably contributes to the membrane potential.

The Ca<sup>2+</sup>-ATPase is regulated by calcium concentration. Calmodulin is a highly conserved calcium binding protein that binds calcium at concentrations above 1  $\mu\text{M}$ . Calcium-binding by calmodulin elicits a conformational change in the protein; the calcium-calmodulin complex interacts with a variety of other proteins, including the Ca<sup>2+</sup>-ATPase, and regulates their activities. If the calcium concentration decreases, the calcium dissociates from calmodulin, which inhibits the pump. This prevents the wasteful hydrolysis of ATP when calcium levels have fallen to a sufficiently low level.

The low resting cytosolic calcium concentration is also mediated by the mitochondria. Mitochondria have a negative membrane potential that drives calcium influx via a high  $K_m$  calcium transporter, and also have a Ca<sup>2+</sup>-Na<sup>+</sup> antiport which moves calcium in or out of the mitochondria depending on the sodium and calcium gradients. These mechanisms act in conjunction with the Ca<sup>2+</sup>-ATPase to control cytosolic calcium concentrations.

### **Gastric pumps**

The gastric mucosa uses a H<sup>+</sup>-K<sup>+</sup> P-type ATPase antiport pump to create the acidic environment in the stomach lumen. The free protons come from the action of carbonic anhydrase, which forms carbonic acid from carbon dioxide and water; the carbonic acid then dissociates to release the required protons. The use of carbon dioxide as a source of protons is important because otherwise the pump would raise the intracellular pH as it lowers the extracellular pH. The pump is electrically silent, because it pumps equal numbers of potassium ions and protons.

Histamine, acting through an adenylyl cyclase-coupled histamine receptor, stimulates the H<sup>+</sup>-K<sup>+</sup> ATPase. Cimetidine (a compound sold under the trade name Tagamet) is an anti-ulcer drug; it functions by acting as an antagonist for the gastric histamine receptor, and thereby decreases proton pumping into the stomach.

### **Multi-drug resistance transporter**

Cancer cells that become resistant to one cytotoxic chemotherapeutic agent are frequently resistant to other agents, even if the other agents have completely different mechanisms. The underlying cause of this resistance is thought to be an ATPase pump that exports toxic molecules from cells. The protein has several names: P-glycoprotein, or Multi-Drug Resistance transporter (MDR).

MDR is a member of a superfamily of proteins known as ABC. ABC is an abbreviation for ATP-binding cassette, a conserved structure containing 6 transmembrane helices and an ATP utilization site. MDR has **two ABC** motifs connected together. The ATP binding site is on the inside of the cell membrane (a logical place, since ATP concentrations outside the cell tend to be very low). Unlike the other transporters mentioned thus far, MDR transports a variety of compounds (including large number of cytotoxic chemotherapeutic agents such as vinblastine, vincristine, colchicine, adriamycin, and actinomycin D). The only apparent similarity in these compounds is that they are largely hydrophobic molecules with some amphipathic character.

The stoichiometry of transport by MDR has not been established; most studies indicate that several ATP are hydrolyzed per molecule moved (estimates range from ~2 to ~50). While the reason for this is not entirely clear, it is likely that part of the reason is to allow the generation of the very large concentration gradients necessary to reduce the intracellular concentration of toxins to negligible levels.

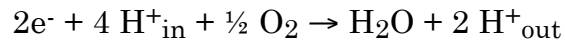
Clearly, this protein did not evolve to allow the survival of cancer cells during chemotherapy. Most MDR-substrate drugs are not orally active, suggesting that these compounds are poorly absorbed in the intestines; the function of this protein may be to pump any of these compounds that diffuse into cells back into the lumen of the intestine. Support for this hypothesis was found in mice in which the MDR gene was deleted; unlike normal mice, MDR-knockout mice can absorb MDR-substrate drugs from their diet. Based on this observation and other evidence, the MDR pump family proteins are thought to be responsible both for efflux of non-polar foreign toxins from cells and for prevention of absorption of these compounds in the digestive system.

### **Electron transport proteins**

While the majority of chemical energy-dependent pump proteins use ATP as their energy, some use other types of chemical energy. Examples of pumps that use other energy sources are Complex I, Complex III, and Complex IV of the electron transport chain. All of these are large multi-subunit protein complexes that act as proton pumps. In eukaryotes, these complexes are found in the mitochondrial inner membrane, while in prokaryotes, the complexes are found in the cell membrane.

Although all of these proteins have been intensively studied, cytochrome *c* oxidase (Complex IV) is probably the best understood.

The crystal structure of cytochrome *c* oxidase has been solved for both bovine and bacterial protein complexes. In both of these widely divergent species, this is a large protein complex that contains several heme and non-heme metal co-factors. The precise stoichiometry for the protein is still under investigation, but it is thought to be:



The electrons used are supplied by the soluble electron carrier cytochrome *c* to the **outside** of cytochrome *c* oxidase. The process results in a net movement of 4 positive charges to outside (translocation of 2 H<sup>+</sup> and utilization of 2 outside negatively charged electrons); it therefore sets up a large potential difference in addition to the proton gradient. The energy for this pumping process is derived from the reduction of oxygen to water.

The mechanism of the cytochrome *c* oxidase pump is still under debate. Although it is known that the pump is not reversible, it is not clear why this is the case.

### **Sodium antiports and symports**

The sodium electrochemical gradient is used as an energy source for many different active transporters. These transporters therefore use the energy of ATP hydrolysis indirectly, since the sodium gradient is ultimately created by the Na<sup>+</sup>-K<sup>+</sup> ATPase. The sodium-dependent pumps use a variety of different stoichiometries.

A major role for the sodium-dependent active transporters is the import of nutrients from the circulation. Many of these nutrient cotransporters are electrogenic, either because they cotransport sodium (which is charged) and a neutral organic compound, or because they transport organic acids in their neutral (protonated) form. Most cells contain Na<sup>+</sup>-dependent symporters for amino acids and TCA cycle intermediates.

The vast majority of cells in multicellular animals use passive transport mechanisms for glucose. However, the kidney has a **Na<sup>+</sup>-glucose symport** for pumping glucose back into the body from the lumen of the kidney tubule. This is necessary to prevent loss of glucose in the urine that would otherwise occur. Note that plasma concentrations of above 10 mM glucose (found only during significant hyperglycemia) result in loss of glucose in the urine because the pump is incapable of handling this level of substrate. The intestine also has a Na<sup>+</sup>-glucose symport to increase the amount of glucose that can be absorbed from the diet.

### **Light driven pumps**

#### **Bacteriorhodopsin**

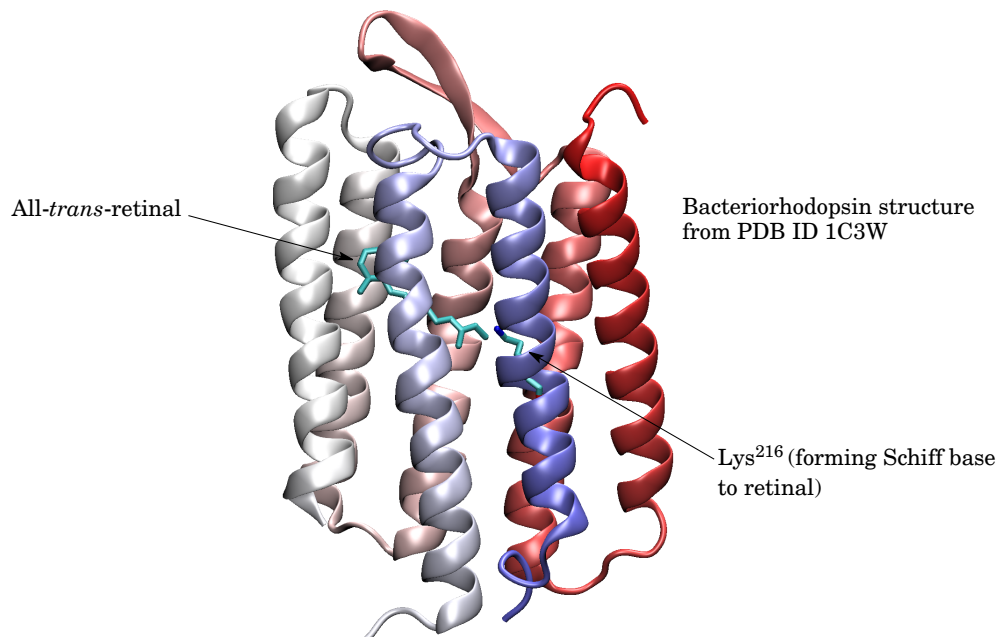
Bacteriorhodopsin is a light-driven proton pump used for a fairly simple version of photosynthesis in the bacterium *Halobacterium halobium*. Under conditions of nutrient availability, *Halobacterium halobium* grows using environmentally

available energy sources. When nutrients are limiting, the bacterium synthesizes bacteriorhodopsin, and uses light to supply metabolic energy.

Bacteriorhodopsin has been studied for many years because it is an extremely useful model system. This is true for several reasons:

1. The halobacteria form two-dimensional liquid crystal patches of bacteriorhodopsin in their cell membrane. These liquid crystals yielded low-resolution structural information at a time when almost no structural information for membrane proteins was available.
2. Although a number of membrane protein crystal structures have been solved in the past few years, bacteriorhodopsin is still a useful model system, because it is a relatively small proton pump (it is a far simpler system than cytochrome *c* oxidase or the other electron transport complexes).
3. Bacteriorhodopsin contains a bundle of seven transmembrane  $\alpha$ -helices, a structure that is found in a large number of other important membrane proteins, and has acted as a model system for studying these proteins.

High resolution bacteriorhodopsin crystal structures have been solved recently, including structures of the protein trapped at different stages during the pumping process. Work using these structures is revealing some details of the proton pumping mechanism. A vastly simplified description of the proposed mechanism suggests that light causes a structural change in retinal (converting the ground state all-*trans* retinal to 13-*cis*-retinal). This change appears to be coupled to a structural change in the protein that results in changes in the  $pK_a$  of some residues in the protein; the sequential changes in affinity for protons then result in proton translocation across the membrane.



### **Additional types of membrane transport**

Other processes involve the movement of molecules across membranes. These processes are far more complicated than the passive and active transport mechanisms, and are somewhat less well understood. These processes will be mentioned very briefly, primarily as a reminder that the processes exist and do involve transmembrane motion.

### **Synthesis of membrane and secreted proteins**

Proteins that will be secreted need to cross the plasma membrane. Proteins that will become integral membrane proteins need to be inserted into appropriate membranes. While aspects of these processes have been worked out, many details remain obscure (especially the mechanism for inserting large protein complexes such as cytochrome *c* oxidase into membranes).

### **Endocytosis and exocytosis**

Endocytosis involves a movement of the membrane such that a part of the extracellular medium is engulfed, and an intracellular vesicle created. The process results in the movement of large amounts of material through the membrane (although, topologically, the vesicle contents remain “outside” the cell). The process has a number of goals, depending on the situation and the cell type. Endocytosis can be a way of internalizing a section of the membrane, or a way of internalizing something from the extracellular medium. Exocytosis is essentially the reverse of the endocytotic process. While some of the cytoskeletal elements involved in endocytotic and exocytotic events have been discovered, the details of the mechanism for controlled membrane fusion and many other aspects remain obscure.

### **Summary**

Membranes create semi-permeable barriers between regions within a cell and between the cell interior and the surroundings. While these barriers are necessary to allow retention of nutrients and other important cellular resources, cells require the ability to move nutrients and waste products across the membrane.

Some materials, including water, due to its high concentration, and non-polar molecules, due to their solubility in the membrane interior, cross membranes in an unassisted fashion called passive diffusion.

Other materials, for which transport is thermodynamically favorable, cross membranes using assisted diffusion. Passive transporters, ion channels, and gap junctions, each of which is a different solution to the problem of transport, mediate assisted diffusion.

A wide variety of active transporter proteins have been discovered. These proteins all use energy from one of several possible sources to catalyze the motion of molecules against electrochemical gradients.

Many active transporters involve ATP, including the  $F_0F_1$  ATPase, which is responsible for the oxidative phosphorylation process that results in the majority of ATP synthesis. The  $Na^+K^+$ -ATPase is an example of the large P-type ATPase family of proteins (the P-type refers to the fact that the pump has a phosphoprotein intermediate); the  $Na^+K^+$ -ATPase is a critical protein that creates the membrane potential and the sodium gradient.

Electrochemical gradients can also be used as an energy source for active transport. The sodium gradient can be used to drive the transport of a variety of molecules via specific antiports and symports. Proton gradients are also widely used for transport and related processes.

In addition to ion gradients and ATP hydrolysis, the energy obtained from reduction of oxygen to water is used to generate the proton gradient required for ATP synthesis. Finally, the energy in light can be used to drive active transporters.