

MEMBRANES:

Structure, Physical and Chemical Properties, and Function

Biological membranes are the basis for many important properties of the cell, not the least of which is to physically define the cell boundary, and in eukaryotes, the boundaries of each intracellular organelle. However, they are not completely impermeable boundaries, and through embedded proteins, the membrane serves as the gatekeeper for the passage of specific molecules into (e.g. nutrients) and out of (e.g. waste) the cell. Other embedded proteins can identify the cell to other cells, and participate in numerous interactions with the environment or other cells. Finally, the membrane, or more precisely, the chemical gradients across the membrane, is an important energy source for the cell.

Membrane Structure and Composition

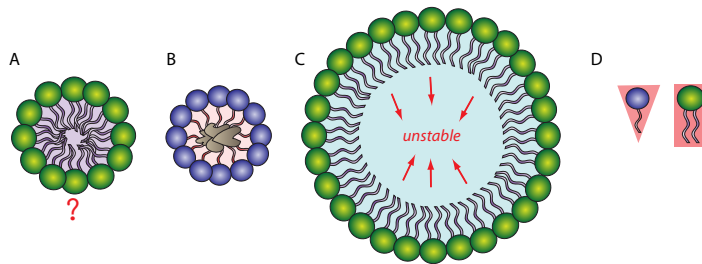
Since most cells live in an aqueous environment and the contents of the cell are also mostly aqueous, it stands to reason that a membrane that separates one side from the other must be *hydrophobic* to form an effective barrier against accidental leakage of materials or water. In the earlier chapter on the basic biomolecules, cellular membranes were partially defined as being composed primarily of phospholipids: molecules consisting of a phosphorylated polar head group attached to a glycerol backbone that has two long hydrocarbon tails. The composition of the hydrocarbons can vary in length and degree of saturation, and there is also variation in the head groups. It is also important to remember that although we concentrate on the phospholipids as the primary components of the membrane, there are other significant parts: other lipids, including cholesterol, integral and peripheral membrane proteins, and glycosylated lipids and proteins.

Because the phospholipids are amphipathic, meaning they have a hydrophilic head and hydrophobic tail, the simplest conformation for a small group of phospholipids in aqueous solution might be expected to be a micelle (fig. 1A), but is this actually the case? Mixtures of hydrophobic molecules and water are thermodynamically unstable, so this structure would protect the hydrophobic fatty acyl tails from the aqueous environment with which the head groups interact. Micelles can form with other amphipathic lipids, the most recognizable being detergents such as SDS (sodium dodecyl sulfate, also called

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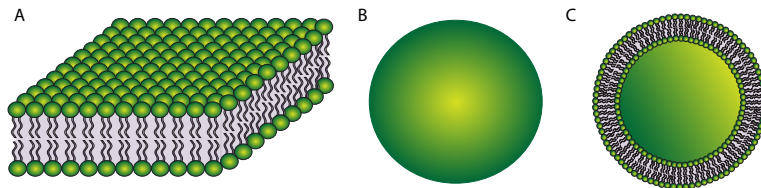
sodium lauryl sulfate), used in common household products such as shampoos. The detergents act by surrounding hydrophobic dirt (1B) and holding it in solution within the micelle to be rinsed away with the water. At smaller sizes, the micelle is fairly stable; however, when there are a large number of phospholipids, the space inside the micelle becomes larger and can trap water in direct contact with the hydrophobic tails (1C). This renders the micelle unstable, so a large single layer of phospholipid is unlikely to serve stably as a biological membrane. Micelles form easily with SDS and other single-tailed lipids because their overall shape (van der Waals envelope) is conical (1D), which lends itself to fitting tight curvatures. However, phospholipids are more cylindrical, and it is harder to fit them into a tight spherical micelle. If they do form micelles, they tend to be larger, and likely to collapse.

Figure 1.



On the other hand, a phospholipid bilayer (figure 2A) could form a fatty acyl sandwich in which the polar head groups face outward to interact with an aqueous environment, and the fatty acids are sequestered in between. However, this does not resolve the problem on the edges of the sandwich. Sometimes, a collapsed micelle can form a closed bilayer in which the edges appear to be sealed, but due to the shape of the phospholipids, there is poor contact between acyl chains. Such a tight bend is unstable and the edge phospholipids are likely to break apart from one another. So, the solution to the ideal phospholipid structure in an aqueous environment is a spherical phospholipid bilayer (2B and cutaway in 2C) : no edges mean no exposed hydrophobicity.

Figure 2.

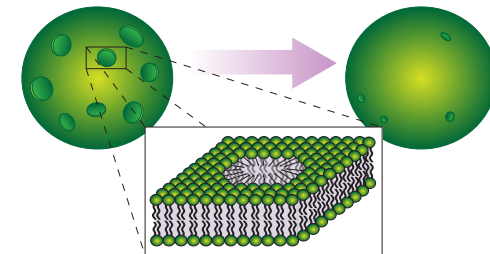


Just as overly large micelles will be unstable, there is also a minimal concentration of lipid needed for a micelle to form. This is known as the critical micelle concentration (cmc) and is a property of each particular lipid. Below the cmc, there are not enough amphipathic lipids to mutually shield their hydrophobic tails from the water, and the more likely position of the lipids is on the surface of the aqueous solution, hydrophilic heads in contact, with the hydrophobic tails in the air.

Liposomes are artificial spherical bilayers (Fig.2) that are used both in research for study of both membrane lipids and integral membrane proteins. They are also used to deliver drugs and other macromolecules into cells for either research of therapeutic purposes. A common method for getting DNA, a large molecule that is not normally transported into cells, into a cell is called lipofection. This technique involves creating liposomes within a solution containing the DNA of interest. This traps the DNA into the liposome, which can then be applied to cells. The liposomes then fuse with the plasma membrane and deliver the DNA into the cell.

Another technique for introducing foreign DNA into a cell is electroporation, which illustrates the self-sealing property of the phospholipid bilayer (figure 3). The cell (usually thousands of cells, actually) is placed in a DNA solution and subjected to an electric current. This transiently pulls on the cell membrane in all directions, causing many small holes to open up. DNA then moves into the cell, and the holes spontaneously heal. Of course, the process is not perfect, and due to variations in the cells and in the electric field, some cells will be unable to reseal the holes, or for other reasons may not survive the process.

Figure 3.



The stability of the spherical phospholipid bilayer does not imply that it is static in its physical properties. In most physiologically relevant conditions, the membrane is cohesive, but fluid. It can mold its surface to the contours of whatever it is resting on, and the same thermodynamic and hydrophobic properties that make it so stable also allow it to seal up minor tears spontaneously. Maintaining a working range of fluidity is important to the cell: if the membrane is too rigid, then it may be unable to move or undergo necessary processes such as endocytosis, in which a cell takes up large extracellular molecules by enveloping them with the cell membrane and pinching it off in a vesicle; while if it becomes too fluid, it may lose integrity and fall apart.

There are three major factors that govern the fluidity of the membrane: (1) degree of saturation of the fatty acyl chains, (2) the temperature, and (3) the concentration of cholesterol. Fully saturated fatty acyl chains can rotate freely around any bond and therefore can pack together very tightly, thus decreasing membrane fluidity. As more unsaturated fatty acyl chains are introduced into the membrane, the more space there is between some of the fatty acyl tails, and there is an increase in fluidity. Similarly at higher temperatures, even saturated fatty acyl chains, with their increased energy, move more and create more space between the chains, also increasing fluidity. Finally, cholesterol, as a small planar lipid molecule, can intercalate between the fatty acyl tails. Interestingly, in normal physiological temperatures, the effect of cholesterol is dependent on its concentration. At normal concentrations, the cholesterol restricts acyl tail movement and decreases fluidity. However at very low concentrations, cholesterol has the opposite effect, separating the hydrophobic tails and slightly increasing fluidity, especially in the innermost regions of the membrane. **[In case you were wondering, human medical problems with cholesterol are unrelated to the cholesterol in the cell membranes, and refers to cholesterol (bound to lipoprotein carriers) in the bloodstream. That cholesterol can build up in the blood vessels, decreasing the internal diameter and along with it, blood flow. When this happens to the heart, which uses a lot of oxygen, the oxygen deprivation can cause necrosis and a heart attack.]**

In addition to the three factors noted above, phospholipid composition can also alter membrane fluidity: shorter acyl chains lead to greater fluidity, while longer chains, with more surface area for interaction, generate membranes with higher viscosity. The phospholipid composition of biological membranes is dynamic and can vary widely. The table below shows the differences in the ratios of major phospholipid species phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and sphingomyelin (SM) in plasma membranes from two different cell types. As you might expect based on their differing functions, the ratios of plasma membrane lipids of a myelinating Schwann cell are very different from the lipids in the plasma membrane of a red blood cell.

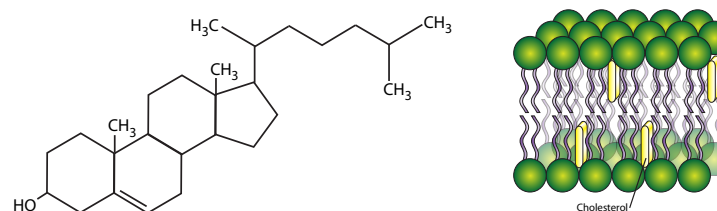


Figure 4. Cholesterol is a small planar molecule that can fit in between the fatty acyl tails of phospholipids in the membrane.

The effect of cholesterol is actually a little more complicated than explained to the left. Cholesterol is not only planar but rigid, and it intercalates close between acyl tails near the head region. Because cholesterol is somewhat shorter than many acyl tails, while the parts of the hydrocarbon chains nearer the head groups are stabilized and restricted in movement, the cholesterol actually acts as a spacer for the other (methyl) end of each chain, so in the center domain of the hydrophobic core of the bilayer, there is actually increased fluidity around cholesterol. Cholesterol concentration varies greatly among organelles of the same cell, and can even be dynamically regulated in response to temperature changes. Membrane samples taken from fish living in warmer temperatures contain more cholesterol than samples from the same species acclimatized to a lower temperature.

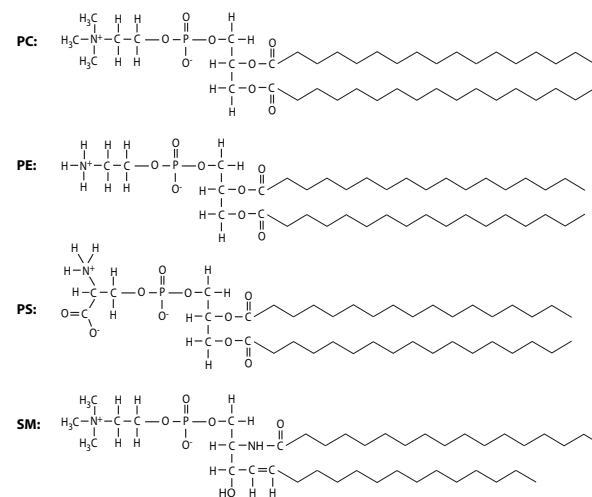


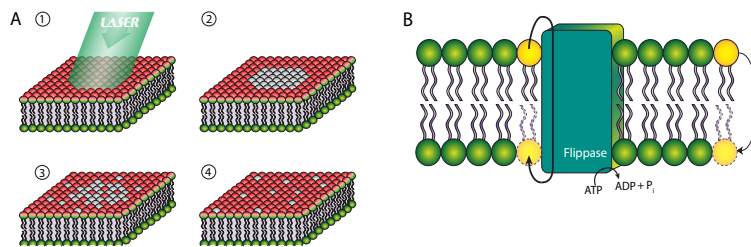
Figure 5. The molecular structures of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and sphingomyelin (SM). The acyl chains of all four molecules are of variable length except the 13-C chain of SM.

<i>Lipid</i>	<i>Schwann Cell PM</i>	<i>Erythrocyte PM</i>
Phosphatidylcholine	44%	19%
Phosphatidylethanolamine	14%	18%
Phosphatidylserine	3%	8%
Sphingomyelin	29%	17%

Even within a single cell, the composition of the plasma membrane differs from that of intracellular organelles. There is even heterogeneity within a membrane itself - the lipids are not simply distributed randomly in the membrane. Research over the last two decades have identified lipid “rafts” that appear to be specific for embedding particular proteins. Since they are unanchored lipids, the rafts can move laterally within the membrane just like most individual lipid molecules. Finally, there are different ratios of the lipids between the two layers of the bilayer. The cytoplasmic face of every membrane will have different associations and functions than the non-cytoplasmic face, so why should we expect the lipid composition to be the same?

Although some phospholipids are directly linked to proteins and the cytoskeleton, most are not, and are therefore free to move within the plane of its layer of the bilayer. In the experiment shown in figure 6 below, the cell surface has been labeled with a covalently bound dye that fluoresces red when excited by green light. When the dye molecules are exposed to high intensity light for an extended period, they no longer fluoresce, a phenomenon known as photobleaching. Photobleaching is a permanent effect, and therefore it can be inferred that if a photobleached spot fluoresces again, then other, non-bleached, phospholipids must have moved into the spot. This experiment clearly proves the lateral mobility of phospholipids in a membrane.

Figure 6. (A) photobleaching experiments can demonstrate lateral mobility of lipids in a bilayer. But, transverse mobility is severely limited and is usually facilitated by a flippase enzyme (B).



However, this is not the case with transverse mobility from one face of the membrane to the other. Due to the highly unfavorable energetics of pushing a polar head group through the lipid tail layers, phospholipids very rarely move from one layer to the other within a membrane (fig. 6B, far right yellow phospholipid). Such movement is greatly facilitated by a flippase enzyme, which hydrolyzes a molecule of ATP for the energy to push a phospholipid from one face of the bilayer to the other.

Though lipid rafts were considered a possibility in the fluid-mosaic membrane model proposed by Singer and Nicholson in 1972, only in the last two decades has the idea been researched seriously, but there have been technical difficulties with visualizing a small domain of lipids within a virtual ocean of lipids. In broad terms, the rafts are considered small areas of ordered lipids within a larger undirected membrane. Lipid rafts most often form in association with specific membrane proteins while excluding others. Some of the proteins are peripheral membrane proteins such as src or the GPI-linked adhesion molecule Thy-1, while others are transmembrane proteins such as the T-cell receptor. Usually the included proteins have signaling-related functions, and one model proposes that these proteins may direct the organization of selected lipids around them, rather than the other way around.

For over a century since Meyer (1899) and Overton (1901) first suggested it, the prevailing theory for the mechanism of gaseous general anesthesia (e.g. ethyl ether, halothane, nitrous oxide, cyclopropane) has been that they partition into and interact with the lipids of the plasma membranes of neurons and by altering the physical membrane properties accomplish the anesthesia. However, in 1985, Franks and Lieb published a report in Nature that, for the first time, showed that an enzyme could be directly affected by a gaseous anesthetic. Since that report, the evidence has been building against the old model of altering membrane lipid properties, and now the current model of direct gas-protein interaction is assumed.

In a pure phospholipid bilayer, membrane proteins as well as lipids have lateral mobility, but in living cells, the proteins are generally constrained either by preferential association with certain kinds of lipids, by direct attachment to cytoskeletal elements, by indirect attachment to the cytoskeleton, or by being “fenced in” by cytoskeletal gridwork directly underlying the cell membrane. Since 1972, the Singer-Nicholson “fluid mosaic” model of membrane structure has been accepted as a general model for biological membranes. It proposes that integral membrane proteins as well as membrane lipids have lateral freedom of movement. This has since been refined with the recognition of lipid rafts and clustered membrane protein patches, but is still viable as a basic model.

Membrane Permeability

A pure phospholipid bilayer, whatever the lipid composition, is a semi-permeable membrane that is generally repellent to large molecules and to ions. Small polar molecules can sometimes pass easily (e.g. ethanol), but more often pass at low rates if at all (e.g. water). However, small nonpolar molecules are able to pass through the membrane with relative ease. The reasons should be self-evident: larger molecules simply cannot fit between the lipid molecules to make their way through. Small molecules

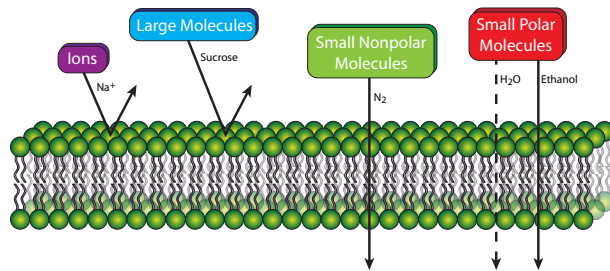


Figure 7. A pure phospholipid bilayer is inherently semi-permeable.

that can fit must be hydrophobic, otherwise the fatty acyl core of the membrane will repel them and block them from proceeding. Higher concentrations of cholesterol, by filling in gaps between phospholipid tails, decreases permeability even for small molecules that can normally pass through the membrane easily. Cells need far more than small nonpolar molecules for their material and energy requirements. Fortunately for life on earth, the membranes of living cells are not purely phospholipids, and as we will see, proteins embedded in the phospholipid bilayer can form conveyances for the transport of many different molecules in and out of the membrane.

In fact, the observation of saturation kinetics in glucose transport in erythrocyte membranes was the first indication of protein-mediated transport (the GLUT1 glucose transporter). Another telling observation was the finding that glucose permeability through erythrocyte membranes is a million times greater than that through an artificial lipid bilayer. The concentration of glucose in the blood is relatively high compared to that inside of most cells, so this is mediated transport, but passive transport since it is going down the concentration gradient. To facilitate the process by preventing a buildup of glucose concentration in the cell, the first step of glucose metabolism is phosphorylation to convert it into a different molecule, glucose-6-phosphate. Thus the concentration of glucose stays very low, and it flows readily from the bloodstream into the cell.

There are some obvious differences between transport of molecules directly through the lipid bilayer (nonmediated transport) and transport using a protein facilitator embedded in the membrane (mediated transport). *Nonmediated transport* is governed by diffusion: the solute moves from areas of high concentration to areas of low concentration, thereby eliminating the gradient. As long as a solute (A) can get through the membrane, its flux (J) is determined solely by concentration difference and the permeability (P) of the membrane: $J_A = P_A ([A]_{out} - [A]_{in})$ and the relationship between the flux across the membrane and the concentration differential is linear.

This is not the case in *mediated transport*. As the name implies, a protein intermediary is required, and alarm bells should be going off in your head saying, “there’s a limit,” to the number of available transport proteins at any given time. Therefore, just as we saw with enzyme kinetics in chapter 3, the flux of solutes going through a transporter is not linearly related to the concentration differential across the membrane, though there is still a concentration effect. Instead, the relationship is logarithmic, reaching a saturation plateau once all the available transport proteins are in use. At that point, increasing the concentration of the solute will not increase its flux across the membrane. Thus for simple unidirectional mediated transport of a solute (B), the flux (J) can be expressed as a value of the affinity of the transporter for the solute (K_M) and the concentration of the solute:

$$J_B = (J_{max}[B])/(K_M + [B])$$

Membrane permeability allows for the possibility of concentration gradients across membranes, which in turn have potential energy associated with the concentration differential across the membrane. This turns out to be a phenomenally important source of cellular energy, and is the basis for aerobic synthesis of ATP by oxidative phosphorylation (chapter 5). However, to have a meaningful discussion of how concentration differences across semipermeable membranes store energy, we should review some basic concepts first.

If a point source (e.g. a “glob”) of a solute (e.g. honey) is placed into a solvent (e.g. tea), it starts to dissolve, and as it does so, the concentration of solute near the point source will start out much higher than the concentration towards the periphery of the container (e.g. teacup). Over time, the solute then diffuses from the point source outward in all available directions, and eventually the concentration of solute is equal at any point in teacup-space. This behavior is governed by the Second Law of Thermodynamics. The solute is initially concentrated, which means that its constituent molecules are relatively organized. By the second law, these molecules will tend toward chaos, moving away from the constraints of the initial point toward an area with lower concentrations of the solute.

In case you have already forgotten and are too lazy to flip back to chapter 3 to check, this is the law that states that the entropy of the universe (or any closed system) tends toward maximum.

Now, imagine a temporary wall around the point source. The natural tendency is for the solutes to spread out, so by preventing that movement, you have bottled up some potential energy. Of course, this is only potential energy if there is some chance that the solutes can eventually go through the barrier (e.g. the wall has windows that can be opened). If the solutes have absolutely zero chance of passing through, then there is no potential energy because there is no potential to get out and about. Recalling the Energy chapter in which the second law was introduced, the chemical potential energy of a solute is $G=RT \ln[A]+G'$, so the *chemical potential* difference across a membrane is then $\Delta G= RT \ln([A_i]/[A_o])$.

Now imagine this as something like a hydroelectric dam, where there is a great deal of pressure building up behind the dam, which can be utilized when some of the water is allowed through, powering turbines that generate electricity. In the biological case, there is concentration pressure building up both inside and outside the cell because the natural thermodynamic tendency is to bring the inside and outside concentrations of each solute to equilibrium. When this pressure is released by allowing the ions or other molecules to flow across the membrane, energy is released, and may be captured and used. The most direct example of this the proton-gradient-driven ATP synthase in the inner mitochondrial membrane (see Chapter 5, Metabolism), which contains a direct molecular equivalent to the turning of a water wheel with the flow of water. For another example, if we look at $[Na^+]$ in an animal cell, the extracellular concentration is much higher than that intracellularly. When a Na^+ channel is opened, the Na^+ ions rush inward to try to equalize the concentration of Na^+ inside and outside of the cell. Equilibrium is not actually reached in a living cell because Na^+ channels are tightly regulated and only open for short periods of time.

In cells, concentration gradients of ions are great energy sources because the lipid part of the membrane is strongly repellent to ions, preventing them from passing through, but the membrane is embedded with channels and transporters that can allow the ions through if and when they are open. Because ions have both concentration differentials and charge differentials across the membrane, the *electrochemical potential* difference across the membrane is represented by a modification of the chemical potential difference equation with a term that takes that electrical charge into account:

$$\Delta G=RT \ln([A_i]/[A_o]) + ZF\Delta\Psi$$

Z is the charge of the ion (e.g. +1 for Na^+ , -1 for Cl^- , +2 for Ca^{++}), F is the Faraday constant (9.6485×10^5 C/mol), and $\Delta\Psi$ is the membrane potential. In an average animal cell, the membrane potential is approximately -70mV. The number is negative to show that the inside of the cell is negative with respect to the outside. Thus, again considering Na^+ , not only is there a chemical gradient of more Na^+ ions outside the cell than inside, there

Potassium leak channels are structurally as well as functionally different from other potassium channels. Where most K^+ channels have one pore domain, the leak channels have two. While leak channels, by definition are not voltage gated, nor appreciably activated or inactivated, this is not true of all members of the tandem pore domain family of potassium channels. Interestingly, some (e.g. TASK-1) are mechanoreceptors, opening in response to membrane stretch, and others act as thermoreceptors, with heat-sensitive activation (e.g. TREK-1).

is also a charge gradient of more positive charges outside the cell to inside, so both forces contribute to the energy of Na⁺ flow into the cell. The equilibrium potential of one ion (e.g. Na⁺) across a membrane is determined by the Nernst equation:

$$E_m = (RT/zF) \ln ([Na^+]_{out}/[Na^+]_{in})$$

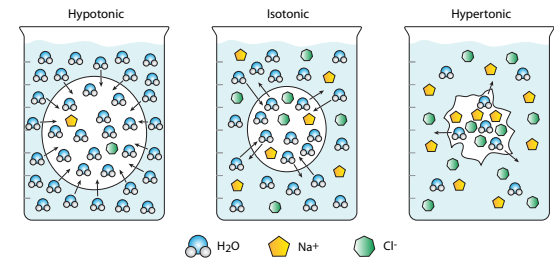
which is extended in the Goldman equation (also Goldman-Hodgkin-Katz equation) that calculates membrane potential based on multiple ion gradients. For most animal cells, a good approximation of the overall membrane potential can be calculated using the three major gradients: Na⁺, K⁺, and Cl⁻. There are, of course, other ion gradients, but their contributions are normally much smaller than these three.

$$V_m = (RT/F) \ln \frac{P_{Na^+}[Na^+]_{out} + P_{K^+}[K^+]_{out} + P_{Cl^-}[Cl^-]_{out}}{P_{Na^+}[Na^+]_{in} + P_{K^+}[K^+]_{in} + P_{Cl^-}[Cl^-]_{in}}$$

The membrane potential is relatively stable in non-excitable cells, but in neurons and muscle cells, the membrane potential is quite dynamic, so the membrane potential in a non-excited state is referred to in these cells as the resting potential. The membrane (resting) potential in most animal cells is around -70mV. This is due in large part to the presence of K⁺ leak channels. These channels leak K⁺ from the cell down the concentration gradient until the chemical potential difference of K⁺ is at equilibrium with the membrane potential. In other words, the gradient pushing K⁺ out will eventually be stopped by an equal force from the gradient pushing positive ions (including K⁺) back in. There are also Na⁺ and Cl⁻ leak channels, but there are far fewer and they contribute far less to the resting potential than K⁺.

Although we most commonly think of water as the solvent in which “interesting” molecules (e.g. ions) diffuse, its concentration and movement across membranes has important biological consequences. *Osmosis* is a term that specifically refers to the diffusion of water across a membrane. In this case, water is considered a solute rather than a solvent, so that if a water-permeant liposome, embedded with aquaporin channels to allow the passage of water, is placed in a very salty saline solution, the cell will shrink because there is a lower ratio of water to dissolved salts outside of the cell than inside. This is a *hypertonic* solution relative to the cell. Therefore the water flows from the cell (higher water concentration) out into the saline (with lower water concentration). Conversely, a cell placed in distilled and deionized water will swell and potentially burst because the water rushes from the highest possible concentration (pure water) to a cytoplasm with lower water concentration (because dissolved in it are various ions and other molecules). This is an example of a *hypotonic* solution. An *isotonic* solution will have the same concentration of water inside and outside of the cell.

Figure 8. Osmosis. An artificial “cell” that is permeable to water but not to Na⁺, K⁺, or Cl⁻ is placed in a aqueous solutions of varying salinity.



Membrane Transport Proteins

Membrane proteins come in two basic types: integral membrane proteins (sometimes called intrinsic), which are directly inserted within the phospholipid bilayer, and peripheral membrane proteins (sometimes called extrinsic), which are located very close or even in contact with one face of the membrane, but do not extend into the hydrophobic core of the bilayer. Integral membrane proteins may extend completely through the membrane contacting both the extracellular environment and the cytoplasm, or they may only insert partially into the membrane (on either side) and contact only the cytoplasm or extracellular environment. There are no known proteins that are completely buried within the membrane core.

Integral membrane proteins (fig. 9) are held tightly in place by hydrophobic forces, and purification of them from the lipids requires membrane-disrupting agents such as organic solvents (e.g. methanol) or detergents (e.g. SDS, Triton X-100). Due to the nature of the bilayer, the portion of integral membrane proteins that lie within the hydrophobic core of the membrane are usually very hydrophobic in character, or have outward-facing hydrophobic residues to interact with the membrane core. These transmembrane domains usually take one of the two forms depicted in figures 8 and 14: alpha helices - either individually or in a set with other alpha helices, or barrel-shaped insertions in which the barrel walls are constructed of beta-pleated sheets. The hydrophobic insertions are bounded by a short series of polar or charged residues that interact with the aqueous environment and polar head groups to prevent the hydrophobic portion of the protein from sliding out of place. Furthermore, proteins can have multiple membrane-spanning domains.

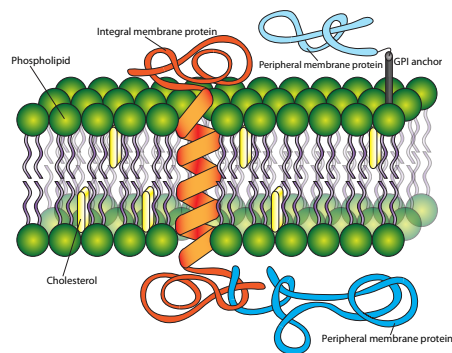


Figure 9. Integral (orange) and peripheral (blue) membrane proteins embedded in a phospholipid bilayer.

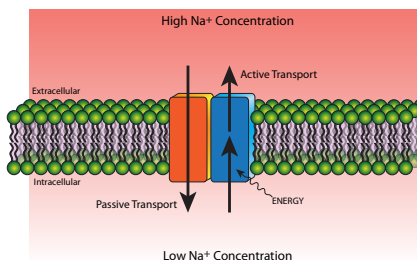
Peripheral membrane proteins (also shown in figure 9) are less predictable in their structure, but may be attached to the membrane either by interaction with integral

membrane proteins or by covalently attached lipids. The most common such modifications to peripheral membrane proteins are fatty acylation, prenylation, and linkage to glycosylphosphatidylinositol (GPI) anchors. Fatty acylation is most often a myristoylation (a 14:0 acyl chain) and palmitoylation (a 16:0 chain) of the protein. A protein may be acylated with more than one chain, although one or two acyl groups is most common. These fatty acyl chains stably insert into the core of the phospholipid bilayer. While myristoylated proteins are found in a variety of compartments, almost all palmitoylated proteins are located on the cytoplasmic face of the plasma membrane. Prenylated proteins, on the other hand, are primarily found attached to intracellular membranes. Prenylation is the covalent attachment of isoprenoids to the protein - most commonly isoprene (a C5 hydrocarbon), farnesyl (C15), or geranylgeranyl (C20) groups (fig. 10). GPI anchors (fig. 11) are found exclusively on proteins on the outer surface of the cell, but there does not appear to be any other commonality in their structures or functions.

Of course, not all membrane proteins, or even all transmembrane proteins, are transporters, and the many other functions of membrane proteins - as receptors, adhesion molecules, signaling molecules, and structural molecules - will be discussed in subsequent chapters. The focus here is on the role of membrane proteins in facilitating transport of molecules across the cell membrane.

Transport across the membrane may be either passive, requiring no external source of energy as solute travels from high to low concentration, or active, requiring energy expenditure as solute travels from low to high concentration (fig. 12). Passive transport can also

Figure 12. For Na⁺ ions and animal cells, passive transport is inward, sending Na⁺ from the high concentration outside the cell to the low concentration inside. Active transport requires energy such as ATP hydrolysis to push a Na⁺ ion from the low concentration inside the cell to the higher concentration outside.



be divided into nonmediated transport, in which the movement of solutes is determined solely by diffusion, and the solute does not require a transport protein, and mediated passive transport (aka facilitated diffusion) in which a transport protein is required to help a solute go from high to low concentration. Even though this may sometimes involve a change in conformation, no external energy is required for this process. Nonmediated passive transport applies only to membrane-soluble small nonpolar molecules, and the kinetics of the movement is ruled by diffusion, thickness of the membrane, and the electrochemical membrane potential. Active transport is always a mediated transport process.

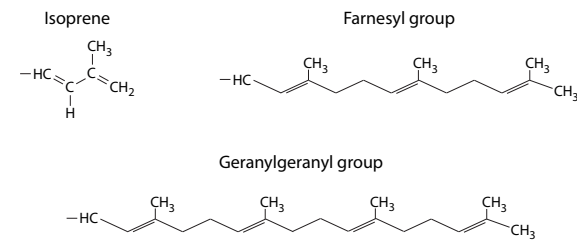


Figure 10. Prenylation

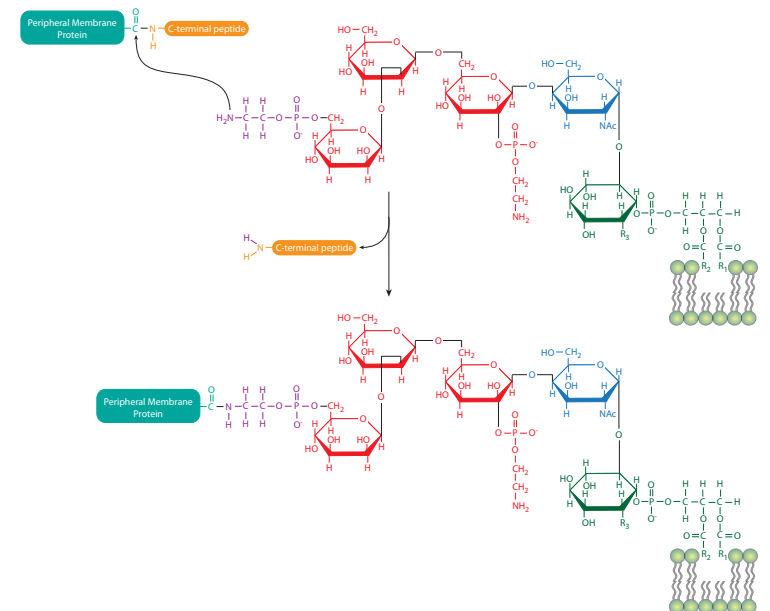


Figure 11. GPI-linked proteins are connected by the C-terminal carboxyl group to phosphoethanolamine, which is linked to a core tetrasaccharide of three mannose residues and one N-acetylglucosamine, the latter of which is bound by glycosidic linkage to a phosphatidylinositol.

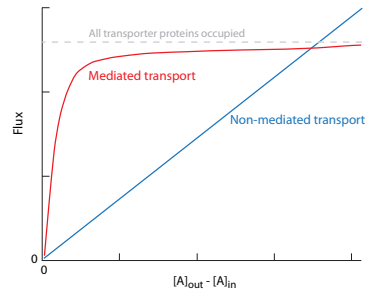


Figure 13. Non-mediated and Mediated transport: flux vs concentration.

Comparing the solute flux vs initial concentration in figure 13, we see that there is a linear relationship for nonmediated transport, while mediated passive transport (and for that matter, active transport) shows a saturation effect due to the limiting factor of the number of available proteins to allow the solute through. Once there is enough solute to constantly occupy all transporters or channels, maximal flux will be reached, and increases in concentration cannot overcome this limit. This holds true regardless of the type of transporter protein involved, even though some are more intimately involved in the transport than others.

Channels are essentially hands-off transport systems that, as the name implies, provides a passage from one side of the cell to another. Though channels may be gated - able to open and close in response to changes in membrane potential or ligand binding, for example - they allow solutes through at a high rate without tightly binding them and without changes in conformation. The solute can only move through channels from high to low concentration. The potassium channel depicted below (fig. 14A) is an example: there is a selectivity filter (14B) of aligned carbonyl oxygens that transiently

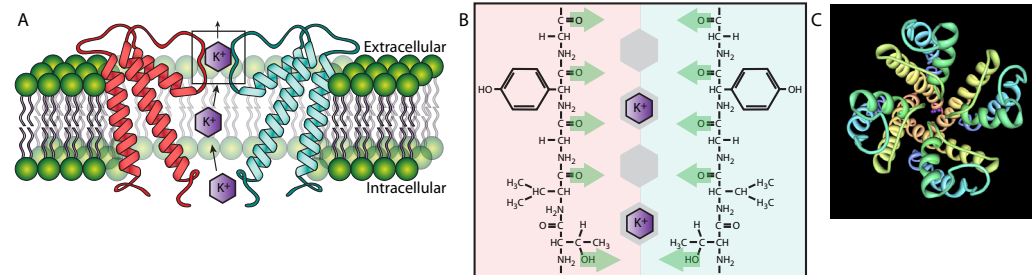


Figure 14. (A) Half of the tetrameric K⁺ channel showing two subunits. (B) Detail of the selectivity filter boxed in A. (C) Top-down image generated from data from the RCSB Protein Data Bank.

positions the K⁺ ions for rapid passage through the channel, but it does not bind the K⁺ for any significant period, nor does the channel undergo any conformational changes as a result of the interaction. Smaller Na⁺ ions could (and on rare occasion do) make it

In addition to protein transporters, there are other ways to facilitate the movement of ions through membranes. Ionophores are small organic molecules, often (but not exclusively) made by bacteria, that help ions move through membranes. Many ionophores are antibiotics that act by causing the membranes to become leaky to particular ions, altering the electrochemical potential of the membrane and the chemical composition inside the cell. Ionophores are exclusively passive-transport mechanism, and fall into two types.

The first type of ionophore is a small mostly-hydrophobic carrier almost completely embedded in the membrane, that binds to and envelopes a specific ion, shielding it from the lipid, and then moves it through the cell membrane. The most studied carrier-type ionophore is valinomycin, which binds to K⁺. Valinomycin is a 12-residue cyclic depsipeptide (contains amide and ester bonds) with alternating D- and L- amino acids. The carbonyl groups all face inward to interact with the ion, while the hydrophobic side chains face outward to the lipid of the membrane. Carrier ionophores are not necessarily peptides: the industrial chemical 2,4-dinitrophenol is an H⁺ carrier and important environmental waste concern, and nystatin, an antifungal used to treat *Candida albicans* infections in humans, is a K⁺ carrier.

The second type of carrier forms channels in the target membrane, but again, is not a protein. Gramicidin is a prototypical example, an anti-gram-positive antibacterial (except for the source of gramicidins, the gram-positive *Bacillus brevis*) and ionophore channel for monovalent cations such as Na⁺, K⁺, and H⁺. It is impermeable to anions, and can be blocked by the divalent cation Ca²⁺. Like valinomycin, gramicidin A is also a made of alternating D- and L- amino acids, all of which are hydrophobic (L-Val/Ile-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp). Gramicidin A dimerizes in the membrane to form a compressed β -sheet structure known as a β -helix. The dimerization forms N-terminal to N-terminal, placing the Trp residues towards the outer edges of the membrane, with the polar NH groups towards the extracellular and cytoplasmic surfaces, anchoring the pore in place.

through the K^+ channel, but because they are too small to be properly positioned by the K^+ filter, they usually pop back out. It should be noted that this channel is a tetramer (14C) and the cutaway diagram in (14A) only shows half of the channel for clarity.

While most proteins called “channels” are formed by multiple alpha-helices, the porins are formed by a cylindrical beta sheet. In both cases, solutes can only move down the concentration gradient from high to low, and in both cases, the solutes do not make significant contact with the pore or channel. The interior of the pore is usually hydrophilic due to alternating hydrophilic/hydrophobic residues along the beta ribbon, which places the hydrophobic side chains on the outside, interacting with the membrane core.

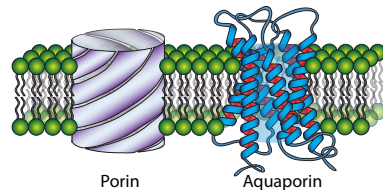


Figure 15.

Porins are primarily found in gram-negative bacteria, some gram-positive bacteria, and in the mitochondria and chloroplasts of eukaryotes. They are not generally found in the plasma membrane of eukaryotes. Also, despite the similarity in name, they are structurally unrelated to aquaporins, which are channels that facilitate the diffusion of water in and out of cells.

Transport proteins work very differently from channels or pores. Instead of allowing a relatively fast flow of solutes through the membrane, transport proteins move solutes across the membrane in discrete quanta by binding to the solute on one side of the membrane, changing conformation so as to bring the solute to the other side of the membrane, and then releasing the solute. These transport proteins may work with individual solute molecules like the glucose transporters, or they may move multiple solutes. The glucose transporters are *passive transport* proteins, so they only move glucose from higher to lower concentrations, and do not require an external energy source. The four isoforms are very similar structurally but differ in their tissue distribution within the animal: for example, GLUT2 is found primarily in pancreatic β cells, while GLUT4 is found mostly in muscle and fat cells.

On the other hand, the classic example of an *active transport* protein, the Na^+/K^+ ATPase, also known as the Na^+/K^+ antiport, utilizes the energy from ATP hydrolysis to power the conformational changes needed to move both Na^+ and K^+ ions against the gradient. Referring to the figure 16, in its resting state, the Na^+/K^+ ATPase is open to the cytoplasm and can bind three Na^+ ions (1). Once the three Na^+ have bound, the

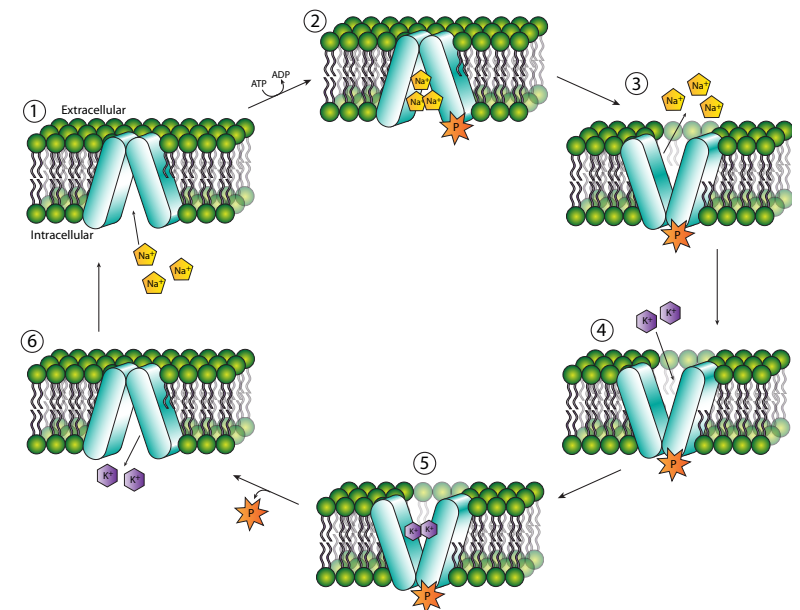


Figure 16. Active Transport by Na^+/K^+ ATPase. This enzyme pushes three Na^+ ions out of the cell and two K^+ ions into the cell, going against the gradient in both directions and using energy from ATP hydrolysis. [Note: some texts diagram this enzyme activity with separate binding sites for Na^+ and K^+ , but recent crystallographic evidence shows that there is only one ion binding site that changes conformation and specificity.]

transporter can catalyze the hydrolysis of an ATP molecule, removing a phosphate group and transferring it onto the ATPase itself (2). This triggers a conformational change that opens the protein to the extracellular space and also changes the ion binding site so that Na^+ no longer binds with high affinity and drops off (3). However, the ion binding site specificity is also altered in this conformational change, and these new sites have a high affinity for K^+ ions (4). Once the two K^+ bind, the attached phosphate group is released (5) and another conformational shift puts the transporter protein back into its original conformation, altering the K^+ binding sites to allow release of the K^+ into the cytoplasm (6), and revealing Na^+ affinity once again.

The Na^+/K^+ ATPase is a member of the P-type family of ATPases. They are named because of the autophosphorylation that occurs when ATP is hydrolyzed to drive the transport. Other prominent members of this family of ATPases are the Ca^{++} -ATPase that pumps Ca^{++} out of the cytoplasm into organelles or out of the cell, and the H^+/K^+ ATPase, though there are also P-type H^+ pumps in fungal and plant plasma membranes, and in bacteria.

Unlike Na^+ or K^+ , the Ca^{++} gradient is not very important with respect to the electrochemical membrane potential or the use of its energy. However, tight regulation of Ca^{++} is important in a different way: it is used as an intracellular signal. To optimize the effectiveness of Ca^{++} as a signal, its cytoplasmic levels are kept extremely low, with Ca^{++} pumps pushing the ion into the ER (SR in muscles), Golgi, and out of the cell. These pumps are themselves regulated by Ca^{++} levels through the protein calmodulin. At low Ca^{++} levels, the pump is inactive, and an inhibitory domain of the pump itself prevents its activity. However, as Ca^{++} levels rise, the ions bind to calmodulin, and the Ca^{++} -calmodulin complex can bind to the inhibitory region of the Ca^{++} pump, relieving the inhibition and allowing the excess Ca^{++} to be pumped out of the cytoplasm.

There are three other families of ATPases: the F-type ATPases are proton pumps in bacteria and mitochondria and chloroplasts that can also function to form ATP by running “backwards” with protons flowing through them down the concentration gradient. They will be discussed in the next chapter (Metabolism). Also, there are V-type ATPases that regulate pH in acidic vesicles and plant vacuoles, and finally, there are anion-transporting ATPases.

Hydrolysis of ATP, while a common source of energy for many biological processes, is not the only source of energy for transport. The active transport of one solute against its gradient can be coupled with the energy from passive transport of another solute down its gradient. Two examples are shown in figure 17: even though one is a *symport* (both solutes crossing the membrane in the same physical direction) and one is an

Cardiac glycosides (also cardiac steroids) inhibit the Na^+/K^+ ATPase by binding to the extracellular side of the enzyme. These drugs, including digitalis (extracted from the purple foxglove plant) and ouabain (extracted from ouabio tree) are commonly prescribed cardiac medications that increase the intensity of heart contractions. The inhibition of Na^+/K^+ ATPase causes a rise in $[\text{Na}^+]_{\text{in}}$, which then activates cardiac $\text{Na}^+/\text{Ca}^{++}$ antiports, pumping excess sodium out and Ca^{++} in. The increased $[\text{Ca}^{++}]_{\text{cytoplasm}}$ is taken up by the sarcoplasmic reticulum (see chap. XX), leading to extra Ca^{++} when it is released to trigger muscle contraction, causing stronger contractions.

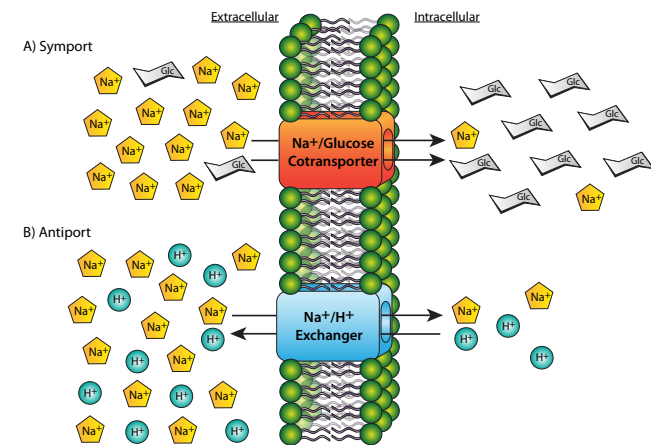


Figure 17. Symport and Antiport. The terms refer only to direction of solutes in or out of cell, not to energetics. In this symport, the energy release from passive transport of Na^+ into the cell is used to actively transport glucose in also. In the antiport example, Na^+ transport is again used, this time to provide energy for active transport of H^+ out of the cell.

Acetylcholine receptors (AChR), which are found in some neurons and on the muscle cells at neuromuscular junctions, are ligand-gated ion channels. When the neurotransmitter (acetylcholine) or an agonist such as nicotine (for nicotinic type receptors) or muscarine (for muscarinic type receptors) binds to the receptor, it opens a channel that allows the flow of small cations, primarily Na^+ and K^+ , in opposite directions, of course. The Na^+ rush is much stronger and leads to the initial depolarization of the membrane that either initiates an action potential in a neuron, or in muscle, initiates contraction.

antiport (the two solutes cross the membrane in opposite physical directions), they both have one solute traveling down its gradient, and one solute traveling up against its concentration gradient. As it happens, we have used Na^+ movement as the driving force behind both of these examples. In fact, the Na^+ gradient across the membrane is an extremely important source of energy for most animal cells. However this is not universal for all cells, or even all eukaryotic cells. In most plant cells and unicellular organisms, the H^+ (proton) gradient plays the role that Na^+ does in animals.

The Action Potential in Neurons

The transport of solutes in and out of cells is critical to life. However, in neurons, the movement of ions has another crucial function in metazoan animals: production of action potentials used for neurotransmission. This specialization allows for extremely rapid transmission of information across long distances. An example my mentor would use when teaching basic neuroscience to schoolchildren was a bipolar neuron that extends from the toe to the brain. For information from the toe to be useful, it must reach the brain for processing very quickly. This signal may have to travel several meters in a giraffe, far more in whales. No chemical signal can move that quickly, and despite the popular simplification and dramatic depictions on television, electricity does not flow through neurons as though they were copper wires.

The reality does involve rapid changes to membrane potential. Since that is measured in volts and is a difference in electrical charge across the membrane, it is sometimes thought of colloquially as electrical transmission, but the mechanism is completely different from electrons flowing through a wire. Instead, there is a moving change in membrane potential due to sequential opening of ion channels along the length of the axon (the long thin part of the nerve cell). Generally, the signal starts with an

A number of potent toxins have been used in the study of the AchR, including histrionicotoxin, an arrow poison extracted from a South American tree frog, *Dendrobates histrionicus*, curare (d-tubocurarine) isolated from certain plants, and α -bungarotoxin, which is isolated from the Taiwanese snake, the banded krait. All three act by competitive inhibition, blocking the opening of the AchR channel.

Acetylcholine spontaneously dissociates from AchR in about a millisecond, closing the channel. It could then be reactivated by the acetylcholine, but this is tightly regulated by the enzyme acetylcholinesterase (AChE), which is a GPI-anchored protein on the membrane of the target cell, and prevents overstimulation by rapid degradation of the acetylcholine. The military nerve gas sarin, which gained notoriety in a 1995 attack on the Tokyo subway system, works by inactivating AChE, thus causing overstimulation of AchR.

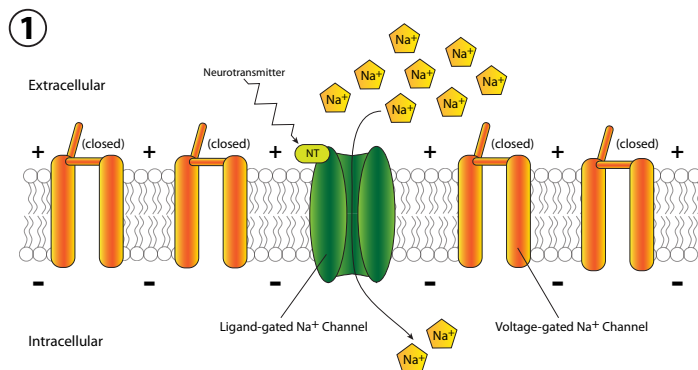


Figure 18.

excitatory chemical signal, or neurotransmitter (fig. 18), that binds to a receptor on the neuron. This receptor may be linked to an ion channel or it may itself be a ligand-gated ion channel. In either case, the channel opens and Na^+ comes into the cell.

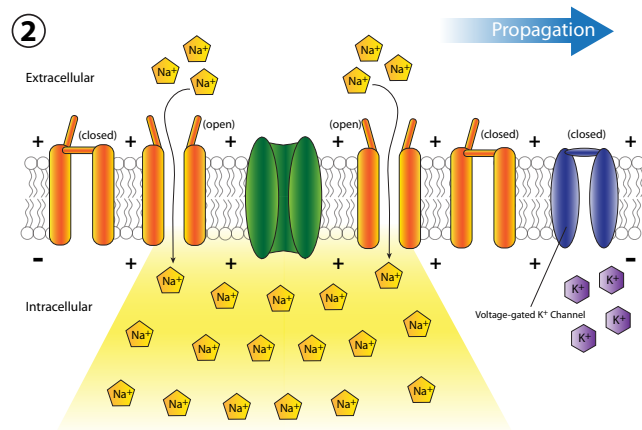


Figure 19.

The sudden influx of positive charges transiently and locally depolarizes the membrane (more + charges along the inside of the membrane than outside). The depolarization of the membrane near the channel causes all nearby voltage-gated Na^+ channels to transiently open (fig. 19), thus allowing a new rush of Na^+ ions into the cell that can then depolarize another small section of membrane. Although technically, channels open up on either side of the receptor, normally, the receptor is located on the main cell body, or soma, while most of the ion channels are lined up along the axon. Therefore, there is a de facto directionality to the propagation of the depolarization.

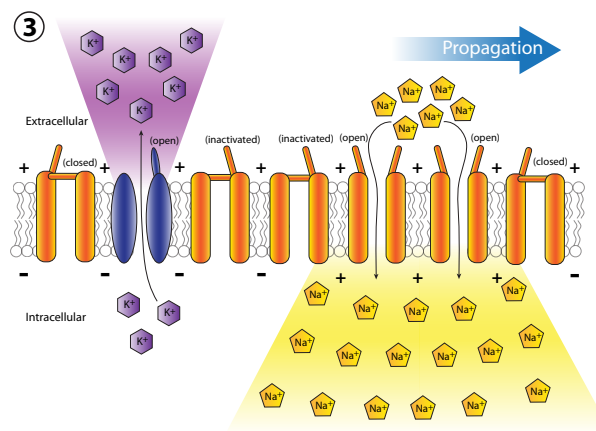


Figure 20.

Since neuronal transmission of information is critical to many systems, it is not surprising that a variety of organisms have evolved toxins that can paralyze or kill an attacker or prey. Interestingly, the primary target of the toxins is the voltage-gated Na^+ channel, and not the K^+ channel.

The toxin most widely used in research, is tetrodotoxin (TTX), which is derived from the skin and certain organs of the puffer fish (*Fugu rubripes*). This fish is considered a delicacy (primarily in Japan), and even when prepared by expert and specially-trained chefs, the small amount of residual TTX in the flesh causes a tingling numbing of the tongue as neural excitation is blocked. Interestingly (but unrelated to ion channels) fugu has a highly compressed genome - where most other eukaryotes have large swaths of non-coding regions, fugu has little, and its genes are very close together. In fact, it has almost as many predicted genes as the human genome in only 1/8th the DNA!

Saxitoxin (STX) is another poison that acts on the voltage-gated Na^+ channel. It is produced by the plankton that cause "red tide" (dinoflagellates of the genus *Alexandrium*, *Gymnodium*, and *Pyrodinium*) and is concentrated by filter-feeders such as mussels, oysters, and other shellfish. Interestingly, STX was once investigated by the US military as a nerve agent for chemical warfare, under the designation TZ. Both STX and TTX bind to the external surface of the closed voltage-gated Na^+ channel near the mouth of the pore, preventing opening, and thereby blocking Na^+ flow.

Batrachotoxin, on the other hand, binds to voltage-gated Na^+ channels when they are open rather than closed, so there is a massive continuous influx of Na^+ . A product of another South American tree frog, *Phylllobates aurotaenia*, this toxin is used as a hunting poison by aboriginal tribes in the area, and is the most potent venom currently known. With a lethal dose of less than 2 $\mu\text{g}/\text{kg}$, batrachotoxin is roughly ten times more deadly than TTX. However, to put this in perspective, botulinum toxin, used pharmacologically as Botox[®] and the cause of botulism, is well over a hundred times more potent (though acting through a completely different mechanism - prevention of neurotransmitter release).

More voltage gated Na^+ channels are opened up quickly after their neighboring channels have opened and depolarized their section of the membrane, leading to a long chain reaction of voltage-gated depolarizations all the way down the axon (fig. 20). Once the signal is propagating down the axon, there are two questions that should come into your head. First, neurons are not disposable cells, so how are all these opened channels reset and the membrane re-polarized for the next action potential? And second, why wouldn't the depolarization wave become bidirectional since each little depolarization area spreads out in both directions from the opened Na^+ gate?

In fact, the answers to both questions are closely related, and has to do with the gating mechanism of voltage-gated ion channels (fig. 21). Voltage-gated ion channels have two gates: one that opens in response to an increase in membrane potential, and one that closes the channel after a short period of time. This means that there are three potential states for most voltage-gated ion channels: closed, open, and inactivated.

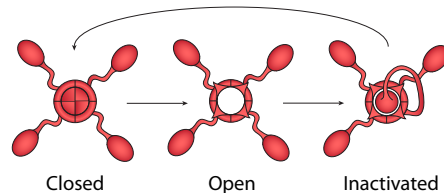


Figure 21. Three states of a voltage-gated K^+ channel.

The inactivated state occurs when the second gate (more like a plug, really) closes, because this is voltage-insensitive. The plug eventually comes out of the pore and the voltage-sensitive gate is set back in place to the closed state. Voltage-sensitive K^+ channels are tetramers, as shown here, and each subunit carries a potential inactivating domain. The choice of which domain appears to be random. Voltage-sensitive Na^+ channels, on the other hand, also have four transmembrane regions, but they are all part of a single protein, and the channel only has one inactivation gate/domain. However, the three-state gating mechanism is still the same.

Back to the action potential: each new depolarization opens the next adjacent set of voltage gated Na^+ channels, and so on. In a neuronal axon, which is where action potentials occur, the movement of the depolarizations happens very quickly and unidirectionally. It happens quickly because the axon is a very long thin projection of the cell, so the volume is small and therefore the influx of Na^+ can quickly depolarize a long section of membrane. It happens unidirectionally because the previously opened voltage-sensitive Na^+ channels go into a refractory inactivated state that cannot be reopened immediately.

Thus when the adjacent wave of depolarization hits, the previously opened channels do not open again, and the opening of more voltage-gated channels continues unidirectionally away from the start.

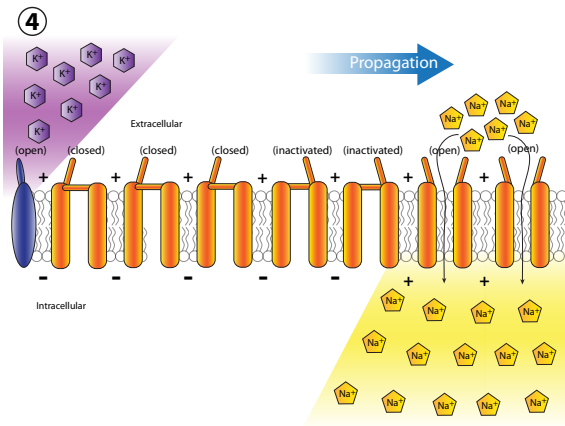


Figure 22.

But wait, there's more! Just because the Na^+ channels are locked closed doesn't mean that the membrane goes back to normal. What repolarizes the membrane behind the action potential? The voltage-gated potassium channels, which are interspersed among the Na^+ channels (note purple ions and channels in figs. 20, 22). They open more slowly than the sodium channels (fig. 23) and thus reach peak flux shortly after the peak flux of Na^+ . Thus, the recovery phase, or repolarization back towards the resting potential, is helped by both cessation of the inward Na^+ movement and continued outward K^+ movement.

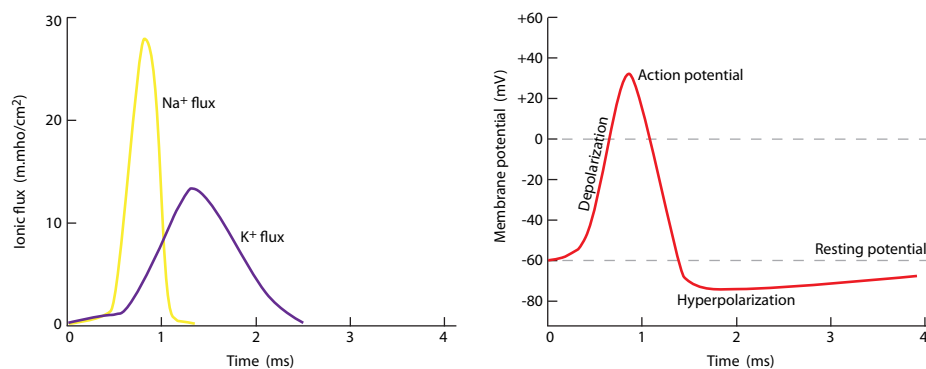


Figure 23. The contributions of Na^+ flux (depolarizing) and K^+ flux (repolarizing) to the neuronal action potential. Remember that the Na^+ movement is into the cell, while K^+ is moving out.

Since all action potentials behave the same way from the standpoint of changes in membrane potential, the difference between stronger and weaker nerve signals is in the frequency/rate of action potential firing, not in magnitude of the ion flux.