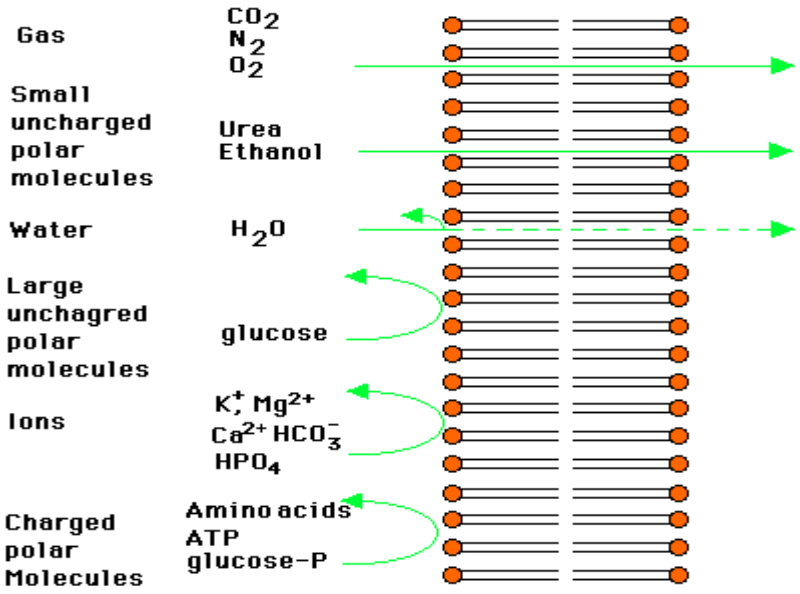
Chapter 2
Transport Systems

The plasma membrane is a selectively permeable barrier between the cell and the extracellular environment. It permeability properties ensure that essential molecules such as glucose, amino acids, and lipids readily enter the cell, metabolic intermediates remain in the cell, and waste compounds leave the cell. In short, the selective permeability of the plasma membrane allows the cell to maintain a constant internal environment. Similarly, organelles within the cell often have a different internal environment from that of the surrounding cytosol, organelles membranes maintain this difference. Good example within the animal-cell lysosome or the plant -cell vacuole- the organelles involve in digestive and degradative processes. The concentration of protons (H^+) is 100-1000 times that of the cytosol; this gradient is maintained by proteins in the organelle membrane.

An artificial membrane composed of pure phospholipid or of phospholipid and cholesterol is permeable to gases, such as 0_2 and CO_2. and small, relatively hydrophobic molecules, such as ethanol. Such molecules can cross cellular membranes unaided by transport proteins.

No metabolic energy is expended because movement is found a high to a low concentration of the molecules, down it chemical concentration gradient. As noted positive S value (increase in entropy) and a negative G.

In contrast, a pure phospholipid membrane is only slightly permeable to water and is essentially impermeable to most water-soluble molecules, such as hydrogen, sodium, calcium, and potassium. Proteins are required to transport such molecules and ions across all cellular membranes because different cell types require different mixtures of these low-molecular-weight compounds, the plasma membrane of each cell type contains a specific set of transport proteins that allow only certain ions or molecule to cross, as does the membrane surrounding each type of subcellular organelle.



The above illustration represents three major types of membrane transport proteins. One type couples ATP hydrolysis with energetically unfavorable uphill) movement of ions. The other two types of membrane transport proteins are not ATPases,

and their ability to transport ions or molecules is not dependent on ATP hydrolysis. Some of these proteins catalyze only energetically favorable (downhill) movement of substances, whereas others couple the downhill movement of one substance to the uphill movement of a different substance.

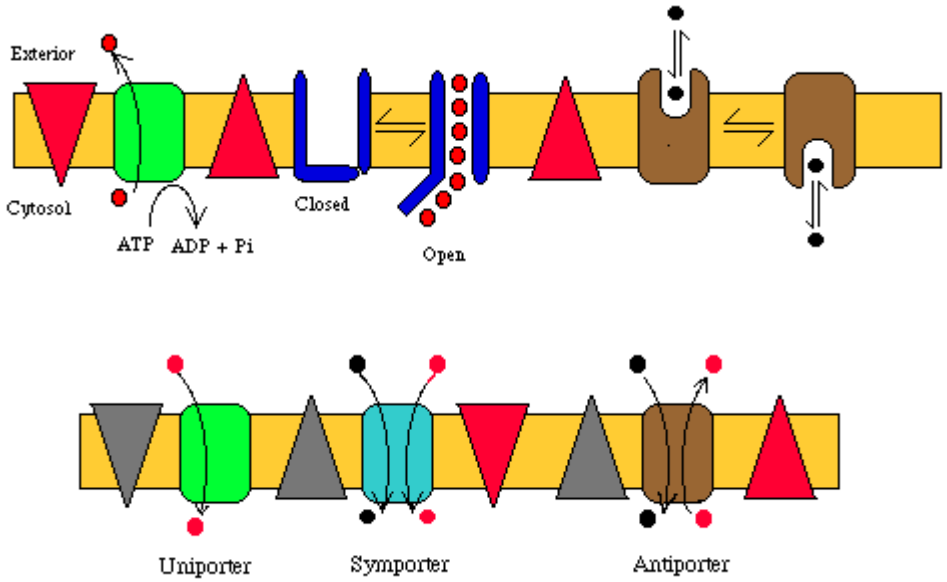
ATP-powered pumps are ATPases that use the energy of ATP hydrolysis to move ions across a membrane against a chemical concentration gradient or electric potential. This type of ion movement, referred to as active transport.

Channels Proteins

Channel proteins transport water or specific types of ions down their concentration (or electric potential) gradients, energetically favorable reactions. They form a protein-lined passageway across the membrane through which multiple water molecules or ions move simultaneously, single file at a very rapid rate (10^8 per second. For example, the plasma membrane of all animal cells contain potassium, and only this ion, to move across the membrane down its concentration gradient. We will show how movement of K^-, through these always-open channels generates an electric potential across the plasma membrane. Many other type of channels proteins are usually closed, and open only in response to specific signals. Because these types of ion channels play a fundamental role in the functioning of nerve cells.

The third class of membrane-s transport called transporters, move a wide variety of ions and molecules across membranes. Transporters, in contrast to channel proteins, bind only one or a few substrate molecules at a time; after binding substrate molecules, the transporter undergoes a conformational change such that the bound substrate molecules, and only these molecules, are transported across the membrane. Because movement of each substrate molecule (or small number of molecules) requires a conformational change in the transporter, transporters move only about 10^2-10^4 molecule per second, a lower rate than that associated with channel proteins.

Three types of transporters have been identified. Uniporters transport one molecule at a time down a concentration gradient. This type of transporter, for example, moves glucose or amino acids across the plasma membrane into mammalian cells. In contrast, antiporters and symporters catalyze movement of one type of ion or molecule against its concentration gradient coupled to movement of a different ion or molecule down its concentration gradient. Like ATP pumps, they are often referred to as active transporters but, unlike pumps, no hydrolysis of ATP is involved.



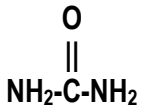
Diffusion of Small Molecules Across Pure Phospholipid Bilayers

In the transport process called diffusion, a small molecule in aqueous solution dissolves into the phospholipid bilayer, crosses it, and then dissolves into the aqueous solution on the opposite side. No proteins are required and the diffusion rate of a substance across the bilayer is proportional to its concentration gradient across the layer and to its hydrophobicity.

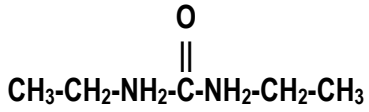
The first step in passive diffusion is movement of a molecule from the aqueous solution into the hydrophobic interior of the phospholipid bilayer. The hydrophobicity of a substance is measured by it partition coefficient K, the equilibrium constant for its partition between oil and water. Since the composition of the interior of the phospholipid bilayer resembles that of oil. the partition coefficient of a substance moving across a bilayer equals the ratio of its concentration just inside the hydrophobic core of the bilayer C^m to its concentration in the aqueous solution C^{aq}:

$$K=C^m/C^{aq}$$

The partition coefficient is a measure of the relative affinity of a substance for lipid versus water. For example, urea



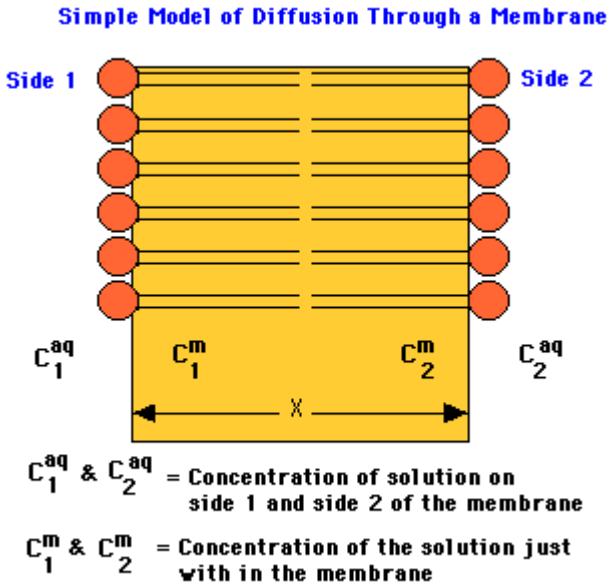
has a K = 0.0002, whereas diethyurea (with two ethyl groups)



has a K =0.01. Diethylurea, which is more hydrophobic than urea, will diffuse into phospholipid bilayer membranes about 50 time faster than urea.

0.01/0.0002 = 50 Faster

Once a molecule moves into the hydrophobic interior of a bilayer, it diffuses across it; finally, the molecule moves from the bilayer into the aqueous medium on the other side of the membrane. Because the hydrophobic core of a typical cell membrane is 100-1000 times more viscous than water, the diffusion rate of a all substances across phospholipid membrane is very much slower than the diffusion rate of the same molecule in water. Thus, movement across the hydrophobic portion a membrane is the rate-limiting step in diffusion.



Quantitative Treatment of Diffusion

A membrane of surface area A and thickness x separates two solution of concentration C_1^{aq} and C_2^{aq}, where $C_1^{aq} > C_2^{aq}$ (see the above figure). In this case, the diffusion rate is given by a modification of Fick's law, which states that the diffusion rate across the membrane dn/dt (in mol/s) is directly proportional to the permeability coefficient P, to the difference in solution concentrations C_1^{aq}-C_2^{aq}, and to the area A, or

$$dn/dt = PA(C_1^{aq} - C_2^{aq})$$

P, and thus the rate of passive diffusion, is proportional to the partition coefficient K and to the diffusion coefficient within the membrane D and is inversely proportional to membrane thickness x. To see the important point that the value of P for any substance is proportional to its partition coefficient K we can write the equation for flow of material within the membrane (which must equal flux of material across the membrane) as

$$dn/dt = (D/x)A(C_1^m - C_2^m)$$

where C_1^m and C_2^m are the concentrations just inside the hydrophobic region of the membrane. Since K equals C_1^m/C_1^{aq} and , equivalently, C_2^m/C_2^{aq}, the equation becomes

$$dn/dt = K(D/x)A(C_1^{aq} - C_2^{aq})$$

Comparing this to equation **$dn/dt = PA(C_1^{aq} - C_2^{aq})$, we see that $P = KD/x$.**

Thus, the rate of diffusion of any substance through the membrane will be proportional to its particular permeability coefficient P. Since D and x are the same for most substances, the rate of diffusion of any substance is thus proportional to its partition coefficient K. Quantitatively, the rate of passive diffusion of a water-soluble molecule is proportional to its hydrophobicity.

Fick's law does not apply to charged molecules. The diffusion of charged molecules across a membrane permeable to the ion is determined not only by the concentration gradient but also by any electric potential gradient that might exist across the membrane.

Uniporter-Catalyzed Transport

Very few molecules enter or leave cells, or cross organelle membranes, unaided by proteins. Even transport of molecules, such as water and urea, that can diffuse across pure phospholipid bilayers is frequently accelerated by transport proteins. Thus we need to understand the properties of the different kinds of membrane-transport proteins and their many roles in cell and organism physiology. Many studies on the function of membranes transport protein involve extraction and purification a specific protein and its reincorporation into pure phospholipids bilayer membranes, such as liposomes. With the help of liposomes functional properties of the various membrane proteins can be examined. Liposomes containing a single type of transport protein can be used to investigate properties of the transport process. The integral proteins of an erythrocyte membrane are solubilized by a nonionic detergent, such as octyglucoside. The glucose transport protein, a uniporter, can be purified and then incorporated into liposomes made of pure phospholipids.

Uniporters catalyze movement of one molecule at a time down a concentration gradient. Similar to enzymes, uniporters accelerate a reaction that is already thermodynamically favored, and the movement of a substance across membrane down its concentration gradient will have a negative □G. This type of movement is sometimes referred to as facilitated transport or facilitated diffusion.

Three properties of uniporter-catalyzed movement:

- The rate of uniport transport is far higher than predicted by Fick's equation describing passive diffusion.
- Uniport transport is specific.
- Uniport transport occurs via a limited number of transporter proteins, rather than throughout phospholipid bilayer. Consequently, there is a maximum transport rate, V_{max}, that is achieved only when the concentration gradient across the membrane is very large.

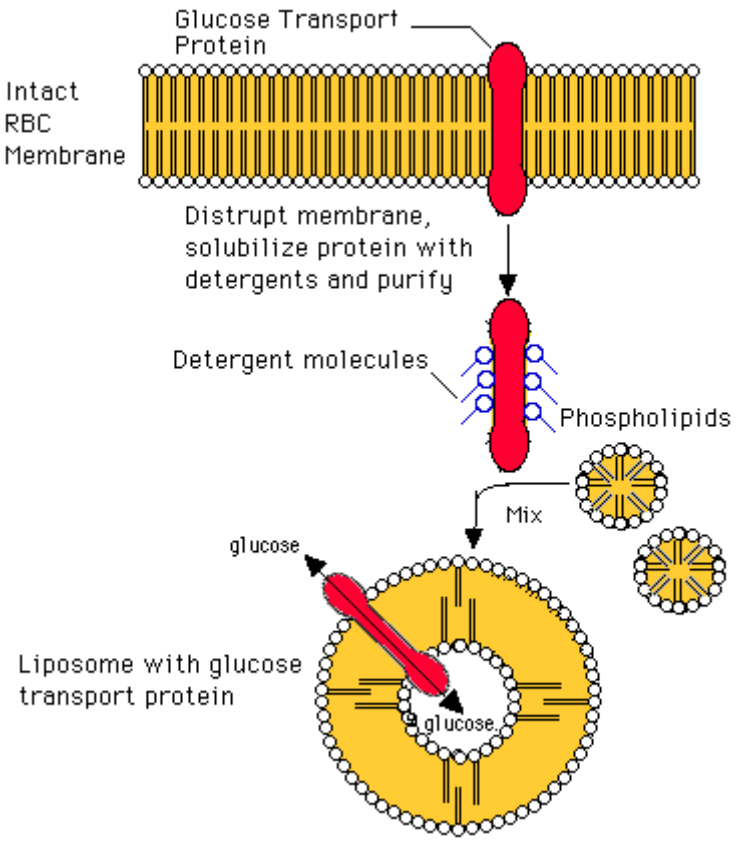
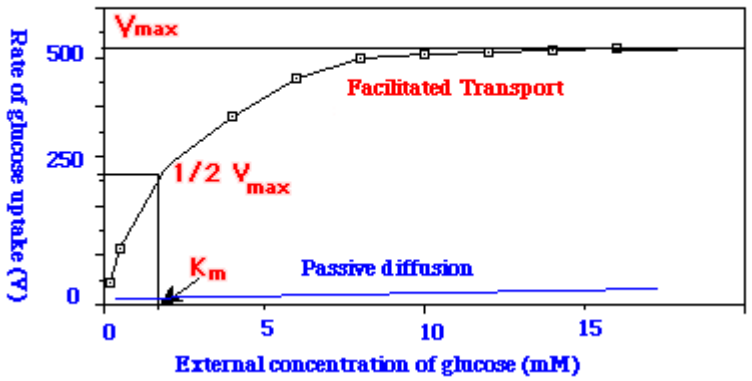


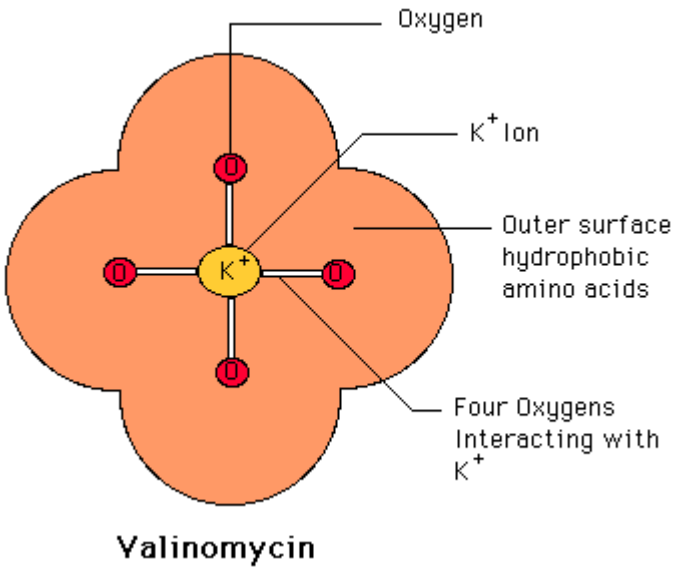
Figure 1 shows the rate of glucose uptake by erythrocytes at different external glucose concentrations. The initial intracellular glucose concentration is zero, so V_{max} is achieved at high external glucose concentrations. Since the concentration of glucose is usually higher in the extracellular medium than in the cell, the plasma membrane glucose transporters usually catalyze net movement of glucose in one direction from the medium into the cell. However, if the concentration gradient is reversed, glucose transporter, like all uniporters, is equally able to catalyze the movement in the reverse direction form the cell into the medium. This occurs when cells are being starved and liver cell synthesize glucose which move into the blood.

Fig. 1: Rate equation



Two General models have been proposed.

According to the carrier model, the transporter protein binds the molecules to moved at one face, moves through the membrane, and releases the molecule at the other face. Carrier models do explain the properties of certain small peptide antibiotic that accelerate the movement of certain ion across membranes. This is not the case for large proteins; it would require too much energy. The antibiotic valinomycin increases the transport of K^+ ions across biological membranes by forming a sphere around the each K^+ ion. The hydrophobic amino acid side chains of the antibiotic lie on the outer surface, and six or eight oxygen atoms on the inside coordinately bind to the K^+. The hydrophobic exterior makes the K^+ carrier complex soluble in the lipid interior of the membranes and thus facilitates movement of the ion through the interior of the membrane. Most investigators now believe that membrane transporters undergo conformational changes that permit bound ions or molecules to pass through the membrane.



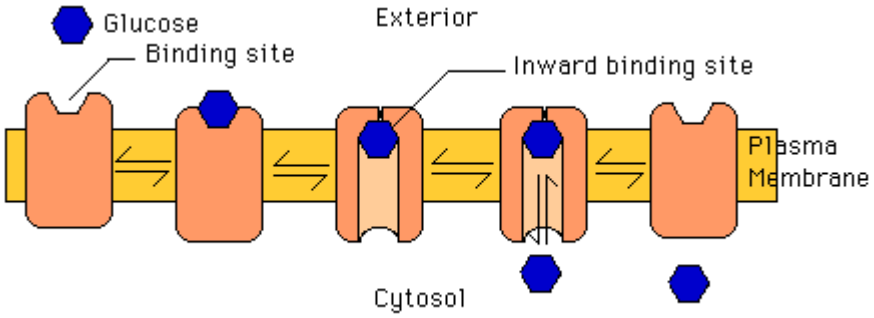
Valinomycin

Conformational States of the Glucose Transporter

The glucose transporter alternates between two conformational states:

- Glucose-binding site faces the outside of the membrane.
- In the other glucose face the inside of the cell.

Unidirectional transport of glucose from the exterior inwards occurs when the transporter, with glucose bound to the outward facing site, undergoes a conformational change such that the outward-facing site is inactivated and the bound glucose move through the protein and becomes attached to newly-formed inward-facing site. The glucose is released into the cell interior, the transporter undergoes the reverse conformational change, inactivating the inward-facing glucose binding site and recreating the outward-facing glucose binding site.



Kinetics of Glucose Transport.

As noted previously, a plot of the entry rate of glucose into erythrocytes versus external glucose concentration is not linear; it is a curve that levels off at V_{max} at high external glucose concentrations. The kinetics of the unidirectional transport of glucose and other small molecule from the outside of a cell inward, catalyzed by a uniporter, can be described by same type of equation used to describe a simple enzymatically catalyzed chemical reaction. Let assume that a substance S (glucose) is present initially only on the outside of the membrane.

$$K_m \; V_{max}$$

S_{out} + Transporter <--------> S.transporter complex <--------> S_{in} +Transporter

where K_m is the substance-transporter binding constant and V_{max} is the maximum transport rate of S into the cell. If C is the concentration of S_{out} (initially, the concentration of S_{in} =0), then, by exactly the same derivation used for the Michaelis-Memten equation we can write

$$v = V_{max}/(1 + K_m/C)$$

Where v is the transport rate of the species into the cell; V_{max} is the rate of transport if all molecules of the transporter contain a bound S, which occurs at high S concentrations; and K_m is the substrate concentration at which half-maximal transport occurs across the membrane. The lower the value of K_m, the more tightly the substrate binds to the transporter, and the greater the transport rate. For a RBC transporter, the K_m for glucose transport is 1.5 millimolar (mM); at this concentration roughly half of the transporters with outward-facing binding sites would have a bound glucose. Blood glucose is normally 5 mM or 0.9g/L. At this concentration, the RBC glucose transporter is functioning at 77% of maximum rate V_{max} as can be seen from figure 1.

Specificity

The data in Table 1 show that the erythrocytes glucose transporter is highly specific. For example the K_m for the nonbiological L-isomer of glucose is >3000. D-glucose is readily transported into the RBC and L-glucose does not enter at a measurable rate.

After glucose is transported into the erythrocyte, it is rapidly phosphorylated, forming glucose 6-phosphate which cannot leave the cell. The glucose concentration gradient across the membrane is maintained, as is the rate of glucose is used by the cell.

Table 1: Specificity of Glucose transporter on Erythrocyte

Substrate	K_m(mM)*	Substrate	K_m(mM)*
D-glucose	1.5	D-Mannose	20
L-Glucose	>3,000	D-Galactose	30

*concentration in millimolar required for half-maximal rate of transport

Ion Channels and Membrane Electric Potential

Plasma membranes usually contain several uniporters that enable molecules such as amino acids, nucleosides, and sugars to enter or leave the cell down their chemical concentration gradients.

The movement of ions across the plasma membrane and organelle membranes also is mediated by transport proteins: symporter and certain antiporter cotransport ions together with specific small molecules, whereas ion channels, ion pumps and some antiporters transport only ions. In all cases, the rate and extent of ion transport across membranes is influenced not only by the ion concentrations on the two sides of the membrane but also by an electric potential that exist across the membrane thus, we turn now to the origin of the electric potential across the membrane , and its relationship to ions channels within the membrane.