# Johns Hopkins Engineering

## Molecular Biology

## Transport Across Membranes: Overcoming the Permeability Barrier

## Part 2

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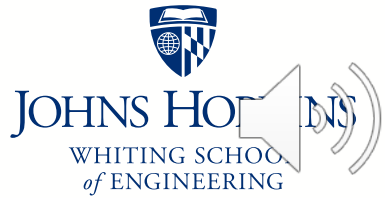
**Module 6 / Lecture 2**



# Second law of thermodynamics

**Second law of thermodynamics:** “Chemical reactions and physical processes always proceed in the direction of decreasing free energy.”

Diffusion through membranes is no exception: free energy is **minimized** as molecules flow down their concentration gradient and as ions flow down their electrochemical gradient.

In most membrane transport processes, the free energy change depends on the concentration or electrochemical gradient alone, but factors such as heat, pressure, and entropy also contribute to the total free energy and may in some cases need to be taken into consideration as well.

Therefore, ***diffusion always proceeds from regions of higher to lower free energy.***

At thermodynamic equilibrium, no further net movement occurs because the free energy of the system is at a **minimum**.

# Simple diffusion - limited to small, nonpolar molecules

Simple diffusion across a membrane is only possible for solutes that can pass readily through the hydrophobic interior of the lipid bilayer. To determine what governs membrane permeability and how permeable a membrane is to different solutes, scientists frequently use membrane models.

Lipid bilayers represent the primary permeability barrier of a membrane. Small molecules can pass through the barrier by simple diffusion, but sodium and potassium ions can hardly pass at all.

Based on subsequent experiments by many investigators using a variety of lipid bilayer systems and thousands of different solutes, we can predict with considerable confidence how readily a solute will diffuse across a lipid bilayer.

From **Table 8-1 (next slide)**, the three main factors affecting diffusion of solutes are **size**, **polarity**, and **charge**.

# Comparison simple diffusion, facilitated diffusion and active transport

**Table 8-1** Comparison of Simple Diffusion, Facilitated Diffusion, and Active Transport

| Properties | | Simple Diffusion | Facilitated Diffusion | Active Transport |
|---|---|---|---|---|
| **Solutes transported** | **Examples** | | | |
| Small nonpolar | Oxygen | Yes | No | No |
| Large nonpolar | Fatty acids | No | Yes | No |
| Small polar | Water, glycerol | Yes | Yes | No |
| Large polar | Glucose | No | Yes | Yes |
| Ions | $Na^+$, $K^+$, $Ca^{2+}$ | No | Yes | Yes |
| **Thermodynamic properties** | | | | |
| Direction relative to electrochemical gradient | | Down | Down | Up |
| Effect on entropy | | Increased | Increased | Decreased |
| Metabolic energy required | | No | No | Yes |
| Intrinsic directionality | | No | No | Yes |
| **Kinetic properties** | | | | |
| Membrane protein mediated | | No | Yes | Yes |
| Saturation kinetics | | No | Yes | Yes |
| Competitive inhibition | | No | Yes | Yes |





Table 8-1

# Solute size

Lipid bilayers are more permeable to smaller molecules than to larger molecules.

The smallest molecules relevant to cell function are **water, oxygen, and carbon dioxide**. Membranes are quite permeable to these molecules; no specialized transport processes are required to move them into and out of cells. Even these small molecules do not move across membranes freely, however.

Water molecules can diffuse across a bilayer **10,000 times slower** than they move when allowed to diffuse freely in the absence of a membrane!

The best way to think about **passive movement** of small molecules across membranes is that their **diffusion is strongly hindered by the presence of a lipid bilayer,** but that they occasionally permeate the bilayer and diffuse randomly to the other side, where they leave the membrane again.

The size rule holds for molecules up to about the size of **glucose**.

Ethanol and glycerol are able to diffuse across membranes at reasonable rates, but **glucose** cannot.

Most cells need **specialized proteins** in their plasma membranes to facilitate the entry of glucose and other large nutrient molecules.

# Solute polarity

Lipid bilayers are relatively permeable to nonpolar molecules and less permeable to polar molecules. This is because nonpolar molecules dissolve more readily in the hydrophobic phase of the lipid bilayer and can therefore cross the membrane much more rapidly than polar molecules of similar size.

**Figure 8-5** illustrates the relationship between the hydrophobicity of a solute as measured by its relative solubility in a nonpolar solvent (olive oil) and its rate of diffusion across a membrane.

In general, **the more** hydrophobic, or nonpolar, a substance is, the more readily and rapidly it can move across a membrane.

# Relationship between membrane permeability versus solubility in olive oil / water

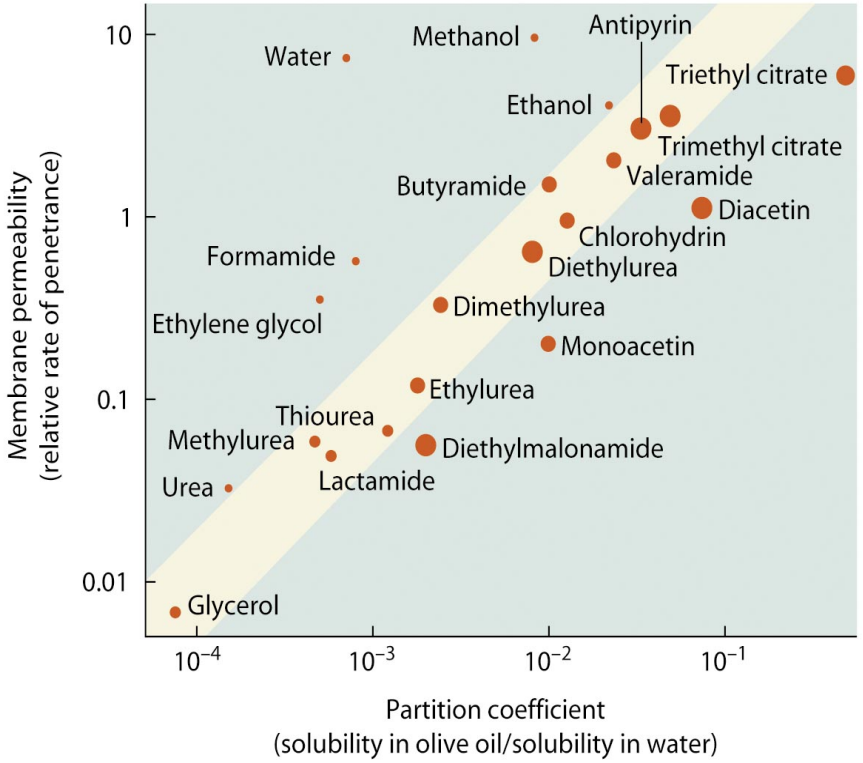





**Figure 8-5**

# Ion permeability

Lipid bilayers are very impermeable to ions because a great deal of energy (about 40 kcal/mol) is required to move ions from an aqueous environment into a nonpolar environment.

The impermeability of membranes to ions is very important to cell activity, because every cell must maintain an ion gradient across its plasma membrane in order to function.

In most cases, this is a gradient either of sodium ions (animal cells) or protons (most other cells).

**Mitochondria** and **chloroplasts** depend on proton gradients for their function. On the other hand, membranes must also allow ions to cross the barrier in a controlled manner.

**The proteins that facilitate ion transport provide hydrophilic channels that shield the ions from the hydrophobic interior of the membrane.**

# Rate of simple diffusion is directly proportional to concentration gradient

Consider the **thermodynamic** and **kinetic** properties of the process (**Table 8-2 / next slide**).

**Thermodynamically** simple diffusion is always an exergonic process, requiring no input of metabolic energy. Individual molecules diffuse randomly in both directions, but net flux is always in the direction of minimum free energy which in the case of uncharged molecules means down the concentration gradient.

**Kinetically** a key feature of simple diffusion is that the net rate of transport for a specific substance is directly proportional to the concentration difference for that substance across the membrane over a broad concentration range.

# Factors governing rate of diffusion across lipid bilayers

**Table 8-2** Factors Governing the Rate of Diffusion Across Lipid Bilayers

| Factor | Examples | | Permeability Ratio* |
|---|---|---|---|
| | More Permeable | Less Permeable | |
| 1. Size: bilayer more permeable to smaller molecules | $H_2O$ (Water) | $H_2N—CO—NH_2$ (Urea) | $10^2$:1 |
| 2. Polarity: bilayer more permeable to nonpolar molecules | $CH_3—CH_2—CH_2—OH$ (Propanol) | $HO—CH_2—CHOH—CH_2—OH$ (Glycerol) | $10^3$:1 |
| 3. Charge: bilayer highly impermeable to ions | $O_2$ (Oxygen) | $OH^-$ (Hydroxide ion) | $10^9$:1 |

*Ratio of diffusion rate for the more permeable solute to the less permeable solute.



Table 8-2

# Simple diffusion vs. Michaelis-Menten kinetics

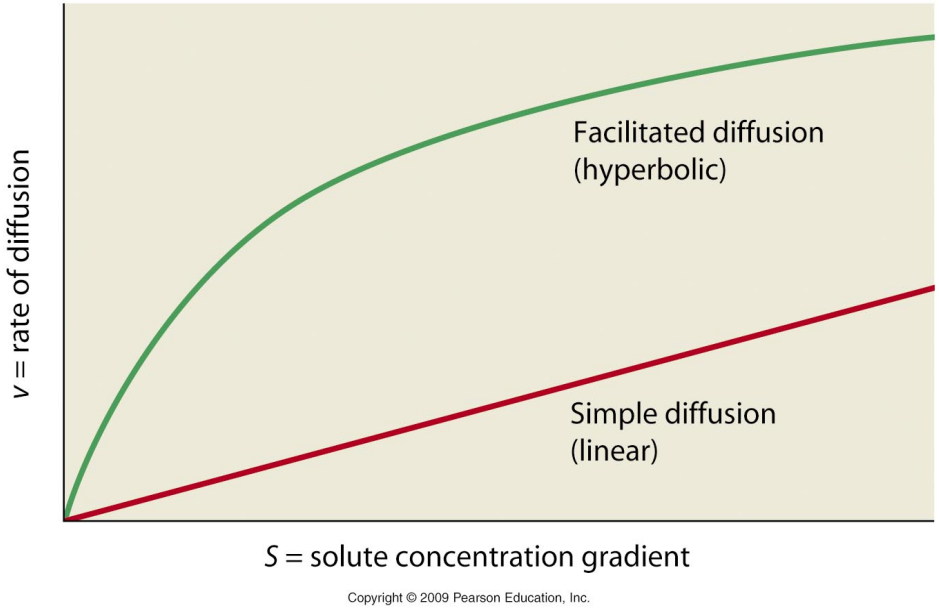
**Simple diffusion** is characterized by a **linear relationship** between the inward flux of the solute across the membrane and the concentration gradient of the solute, with no evidence of saturation at high concentrations.

**Simple diffusion** differs in this respect from facilitated diffusion, which is subject to saturation and generally follows **Michaelis - Menten kinetics (see next slide)**.

Simple diffusion can therefore be distinguished from facilitated diffusion by its kinetic properties, as indicated in **Table 8-2**.

**Simple diffusion** is relevant only to molecules such as ethanol and $O_2$ that are small enough and/or nonpolar enough to cross membranes at a reasonable rate without the aid of transport proteins.

# Comparison of the kinetics of simple diffusion and facilitated diffusion





For simple diffusion across a membrane, the relationship between ***v*** (the rate of diffusion), and [S], the solute concentration gradient, is linear over a broad concentration range (red line).

For facilitated diffusion, the relationship is hyperbolic, in accord with Michaelis - Menten kinetics (green line).

For simplicity, the initial solute concentration is assumed to be on one side of the membrane and zero on the other side.

**Figure 8-6**

# Facilitated diffusion: protein-mediated movement down the gradient

Most substances are **too large** or **too polar** to cross membranes at reasonable rates by simple diffusion.

Such solutes can move into and out of cells and organelles at appreciable rates only with the assistance of **transport proteins** that mediate the movement of solute molecules across the membrane.

If such a process is **exergonic**, it is called **facilitated diffusion, or passive transport,** because the solute still diffuses down the concentration or electrochemical gradient, with no input of energy needed.

The role of **transport proteins** is to facilitate the diffusion of a polar or charged solute across the barrier.

Example of facilitated diffusion: movement of **glucose** across the plasma membrane of an erythrocyte (or almost any other cell in your body, for that matter).

Concentration of **glucose** is higher in blood than in erythrocytes (red blood cells), so transport of glucose across the plasma membrane is passive.

**Glucose** is too large and too polar to diffuse across the membrane unaided; a **transport protein** is required to facilitate its inward movement (**Figure 8-2b / next slide**).

# Facilitated diffusion mediated by carrier protein

**(b) Facilitated diffusion mediated by carrier proteins.** The movement of glucose across the plasma membrane is facilitated by a specific glucose transporter called GLUT1. An anion exchange protein facilitates the reciprocal transport of chloride ($Cl^-$) and bicarbonate ($HCO_3^-$).

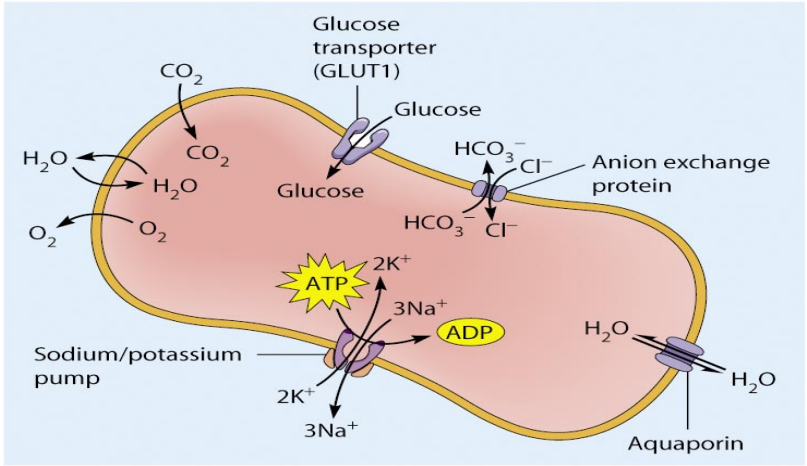







Figure 8-2b

# Carrier and channel proteins facilitate transport by different mechanisms

**Channel proteins** form hydrophilic ***channels*** through the membrane that allow the passage of solutes without any change in the conformation of the protein.

Some of these channels are relatively large and nonspecific, such as the ***pores*** found in the outer membranes of bacteria, mitochondria, and chloroplasts.

Pores are formed by **transmembrane proteins** called ***porins*** and allow selected hydrophilic solutes with molecular weights up to about 600 Da to diffuse across the membrane.

Most channels are small and highly selective. These smaller channels are involved in transport of ions rather than molecules, and are referred to as ***ion channels***.

The movement of solutes through ion channels is more rapid than transport by carrier proteins because complex conformational changes are not necessary.

# Carrier proteins are analogous to enzymes in their specificity and kinetics

**Carrier proteins** are sometimes called **permeases**.

Like an enzyme catalyzed reaction, the process of facilitated diffusion always involves an initial binding of the solute to a specific site on a protein surface, the subsequent release of product, and a reduction in the activation energy of the reaction due to the involvement of the protein catalyst.

## Specificity of Carrier Proteins

Another property that carrier proteins share with enzymes is ***specificity***. Like enzymes, transport proteins are highly specific, often for a single compound or a small group of closely related compounds and sometimes even for a specific stereoisomer.

An example is the carrier protein that facilitates the movement of glucose into erythrocytes (see **Figure 8-2b**). This protein recognizes only glucose and a few closely related monosaccharides, such as galactose and mannose.

# Carrier proteins transport either one or two solutes

Although carrier proteins resemble each other in their kinetics and theft presumed mechanism of action, they also differ in significant ways.

One difference concerns the number of salutes transported and the direction in which they move.

When a carrier protein transports a single solute across the membrane, the process is called **uniport** (**Figure 8-7a**).

The glucose carrier protein in **Figure 8-8** is a **uniporter**.

When two solutes are transported simultaneously and their transport is coupled such that transport of either stops if the other is absent, the process is referred to as **co-transport or** *coupled* transport (**Figure 8-7b**).

In **co-transport**, process is called ***symport*** if the two solutes are moved in the same direction or ***antiport*** if the two solutes are moved in opposite directions.

# Uniport and coupled transport

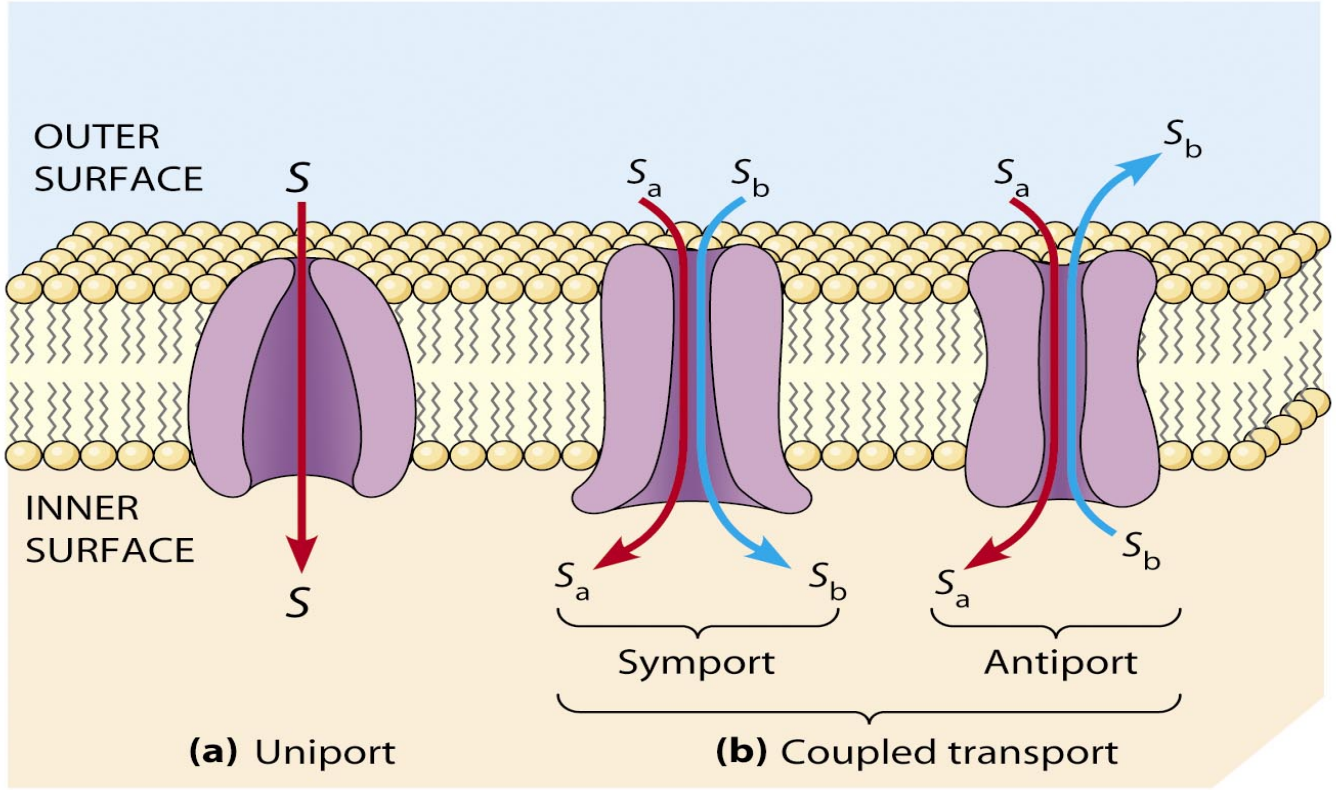





Figure 8-7

# The glucose transporter:  a uniport carrier

Movement of glucose into an erythrocyte (blood cell) is an example of facilitated transport across the plasma membrane mediated by a uniport carrier protein (**Figure 8-2b**).

The concentration of glucose in the blood plasma is usually in the range of 65-90 mg/100 mL, or about 3.6 - 5.0 mM.

The erythrocyte (or almost any other cell in contact with the blood) is capable of glucose **uptake by facilitated diffusion** because of its low intracellular glucose concentration and the presence in its plasma membrane of a glucose carrier protein, or ***glucose transporter*** **(GluT).**

The GluT of erythrocytes is called G1uT1 to distinguish it from related GluTs in other mammalian tissues.

GluT 1 allows glucose to enter the cell about **50,000 times as fast** as it would enter by free diffusion through a lipid bilayer.

# The alternating conformation model for facilitated diffusion of glucose by the glucose transporter giut1

GluT1, the glucose transporter present in the erythrocyte plasma membrane, is a transmenbrane protein that provides a hydrophilic channel for n-glucose molecules and is capable of alternating between two conformations, called T and T2.

The transport process is shown in **Figure 8-8** in three steps, arranged around the periphery of a cell.

(1) With GluTl in its *T1* conformation, a molecule of n-glucose collides with and binds to the binding site on the protein.

(2) Binding of glucose causes the transporter to shift to its alternate (T2) conformation, with the binding site now open to the inside of the cell.

(3) As glucose is released from the binding site to the inside, the GluTl protein reverts to its original CT1) conformation, ready for a further transport cycle.

**Figur 8-8**

# Overview of GLUT1 glucose transporter

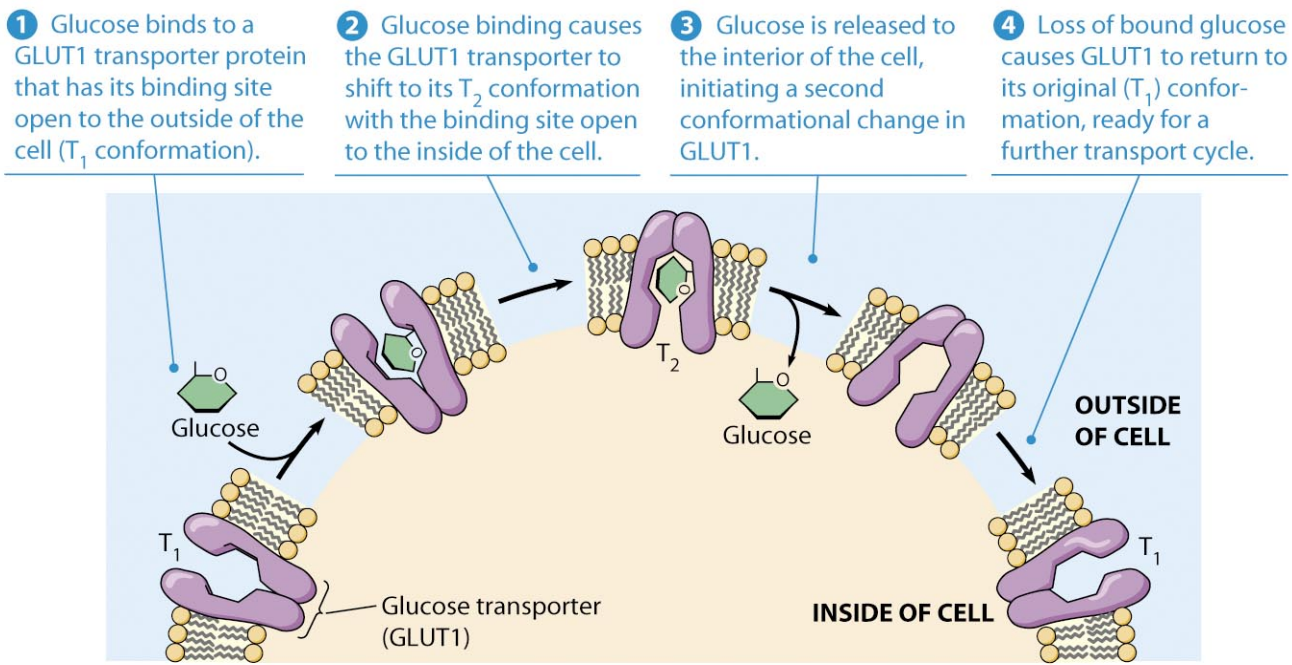







Figure 8-8

# Active transport: protein-mediated movement up the gradient

**Facilitated diffusion** is an important mechanism for speeding up the movement of substances across cellular membranes but it only accounts for the transport of molecules *toward equilibrium, which means down* a concentration or electrochemical gradient.

What happens when a substance needs to be transported *against* a gradient?

**Active transport is** a process that differs from facilitated diffusion in one crucial aspect: active transport **always moves solutes away from thermodynamic equilibrium** (that is, up a concentration or electrochemical gradient), and therefore it always requires the input of energy.

Active transport is thermodynamically unfavorable (i.e., **endergonic**) and occurs only when coupled to an exergonic process.

As a result, the membrane proteins involved in active transport must provide not only for translocation of the desired solute molecules across the membrane but also for coupling of that translocation to an energy yielding reaction.

# Active transport: protein-mediated movement up the gradient

Active transport performs **three major functions** in cells and organelles.

(1) **it makes possible the uptake of essential nutrients** from the environment or surrounding fluid, even when their concentrations in the environment are much lower than inside the cell.

(2) **it allows various substances, such as secretory products and waste materials**, **to be removed from the cell or organelle**, even when the concentration outside is greater than that inside.

(3) **it enables the cell to maintain constant, nonequilibrium intracellular concentrations of specific** inorganic ions, notably $K+$, $Na+$, $Ca^{2+}$, $H^{+}$.

The ability to maintain a constant internal environment far removed from equilibrium is the **most important** aspect of active transport.

# Pumps

The membrane proteins involved in active transport are often called ***pumps***.

Membrane pumps selectively transport specific components molecules or ions from one fluid mass to another.

An important distinction between active and passive transport concerns the direction of transport.

**Passive transport** is inherently ***nondirectional*** with respect to the membrane; solute can move in either direction, depending entirely on the prevailing concentration or electrochemical gradient.

**Active transport** has **directionality.**

An active transport system that transports a solute across a membrane in one direction will not transport that solute actively in the other direction.

Active transport is therefore said to be a ***unidirectional*** or a ***vectorial*** process.

# Comparison of direct and indirect active transport

**(a) Direct, or primary, active transport** involves a transport system coupled to an exergonic chemical reaction, most commonly the hydrolysis of ATP.

ATP drives the outward transport of protons, thereby establishing an electrochemical proton gradient across the membrane.

**(b) Indirect, or secondary, active transport** involves a transport system that is driven by the co-transport of cations-protons, in this case down the electrochemical gradient.

The exergonic inward movement of protons provides the energy to move the transported solute, [S], against its concentration or electrochemical gradient.

**See Figure 8-9**

# Direct active transport

**(a) Direct active transport** involves a transport system coupled to an exergonic chemical reaction, most commonly the hydrolysis of ATP. As shown here, ATP hydrolysis drives the outward transport of protons, thereby establishing an electro-chemical potential for protons across the membrane.

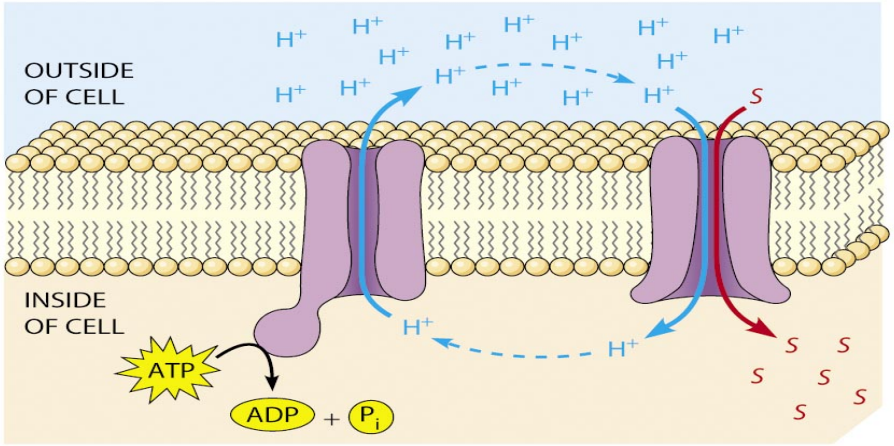







Figure 8-9a

# Indirect active transport

**(b) Indirect active transport** involves the coupled transport of a solute *S* and ions—protons, in this case. The exergonic inward movement of protons provides the energy to move the transported solute, *S*, against its concentration gradient or electrochemical potential.

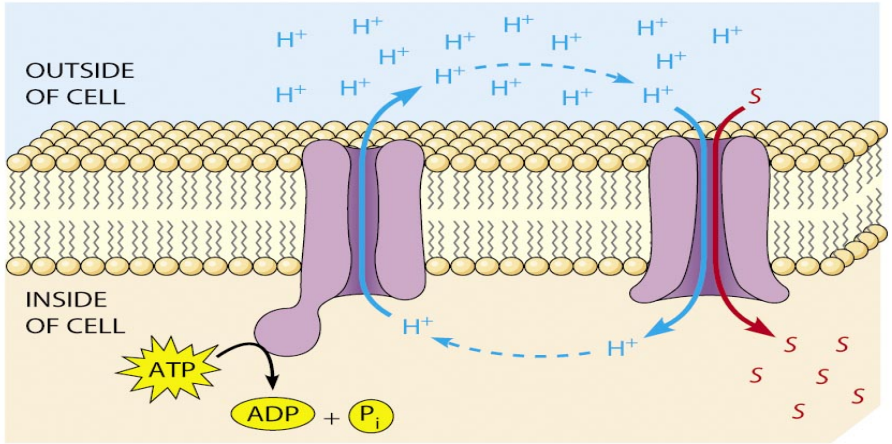







Figure 8-9b

# Direct and indirect active transport

**(a) Direct active transport** involves a transport system coupled to an exergonic chemical reaction, most commonly the hydrolysis of ATP. As shown here, ATP hydrolysis drives the outward transport of protons, thereby establishing an electro-chemical potential for protons across the membrane.

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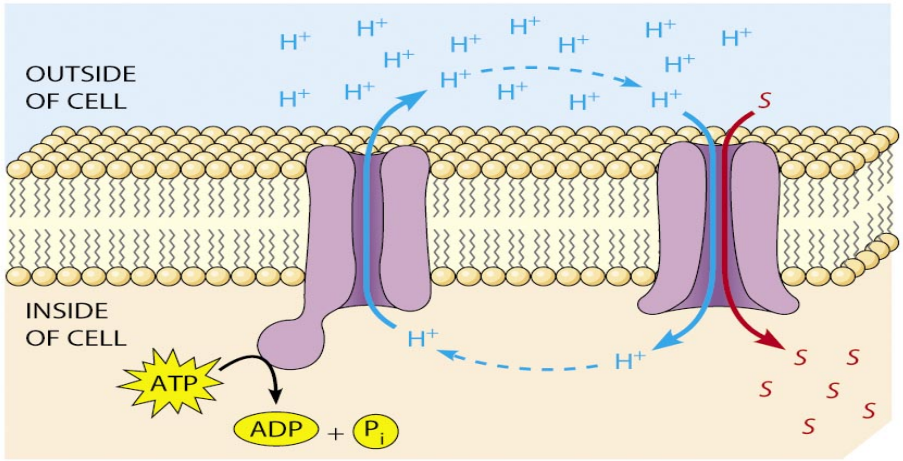






Figure 8-9

# Direct active transport
# The $Na^+/K^+$ pump maintains electrochemical ion gradients

The $Na^+//K^+$ pump uses **ATP** as its energy source and is therefore an example of a transport ATPase.

The $Na^+//K^+$ pump is present in the plasma membrane of virtually all animal cells, but it has been studied in the greatest detail in red blood cells.

Like other active transport systems, this pump has inherent directional: potassium ions are always pumped inward and sodium ions are pumped only outward.

Sodium and potassium ions activate the ATPase only on the side of the membrane from which they are transported sodium ions from the inside, potassium ions from the outside.

# Sodium potassium pump

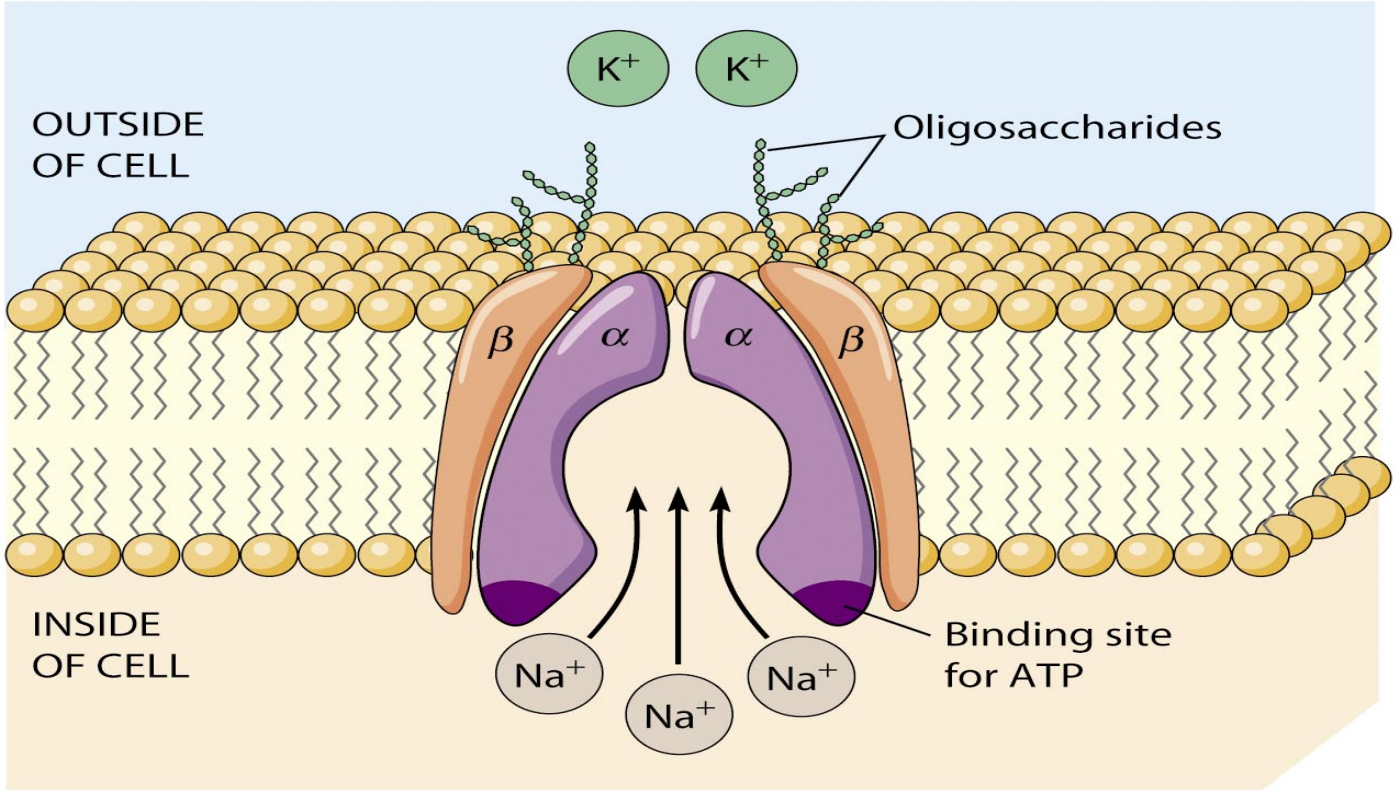




**Figure 8-10**

# The $Na^+/K^+$ pump

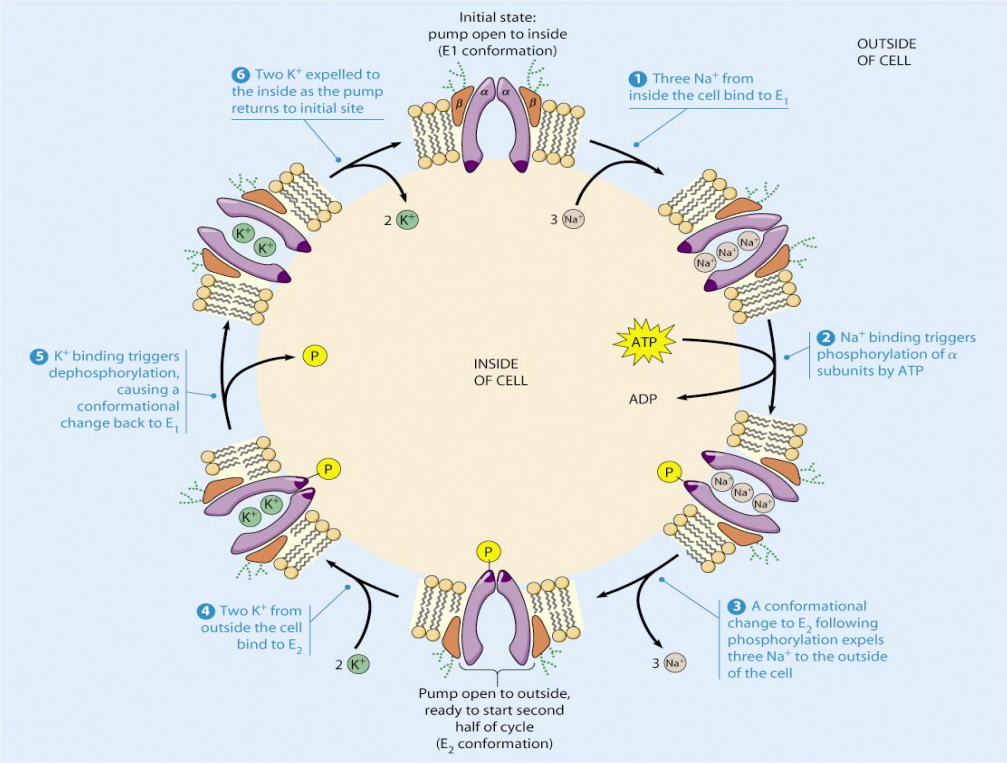






Figure 8-12