Transport Across Membranes: Energetics and Pumps/Channels

Transport involves Energy transformations:

Transport is vectoral - has directionality:

Stryer Biochemistry Chapter 13

Background for Chapter 18

Dr. Ray

Types of Transport of Metabolites (Energetics)

Transport of a metabolite, ion, or polar molecule across a membrane is driven by its chemical potential difference (based on having different concentrations of the same substance on the two sides of the membrane).

- Transporters are membrane proteins that facilitate the passage of molecules across a membrane, such as the cell membrane.
- Generally, passage of a polar molecule across a membrane involves:
  1. Binding of molecule
  2. Conformation change of protein
  3. Release of molecule
- Transport proteins embedded in membranes display substrate specificity (like enzymes) allowing passage of some molecules but not other very similar ones.

Transporters are ligand-selective proteins (like enzymes) with dissociation constants $K_D \sim 10^{-7}$ (low $K_D$ ~ tightly bound ligands).

→ Cellular Transport : Passive Transport
1. Arrange the following in the order of decreasing permeability (ability to traverse through the membrane).

A) Urea
B) Tryptophan
C) H₂O
D) Na⁺
E) glucose

**Answer:**

Most permeable

Least permeable

Bacterial cell membranes (in prokaryotes):
(A) one membrane  (B) two membranes
Permeability coefficients of small molecules span a wide range, and are correlated with their solubility in a nonpolar solvent relative to that in water.

A small molecule might traverse a lipid bilayer in the following way:
1. it sheds its solvation shell of water
2. it becomes dissolved in the hydrocarbon core of the membrane
3. it diffuses through the hydrocarbon core to the other side
4. it becomes resolvated by water

For ions such as Na\(^+\), replacement of the coordination shell of polar water molecules by nonpolar interactions with the membrane interior, is highly unfavored energetically → so ions traverse across membranes very slowly
Types of Transport of Metabolites (Energetics)

Transport of a metabolite, ion, or polar molecule across a membrane is driven by its chemical potential difference (based on having different concentrations of the same substance on the two sides of the membrane).

Second Law of Thermodynamics: molecules spontaneously move from a region of higher concentration to one of lower concentration, thus DOWN their concentration gradient, without the input of energy.

Three are three types of transport:

1. Nonmediated transport
2. Passive-mediated transport (CHANNELS, facilitated diffusion)
3. Active transport (PUMPS)

1. Nonmediated transport - nonpolar, hydrophobic compounds can diffuse on their own through the nonpolar interior of membranes.

- Involves simple diffusion of lipophilic molecules DOWN their concentration gradient from high to low concentration (ex: steroid hormones)

→ Cellular Transport : Diffusion
Three are three types of transport:

1. **Nonmediated transport**
   - for hydrophobic molecules (LIPOPHILIC)
   - do NOT require assistance of a transport protein

2. **Passive-mediated transport** (CHANNELS, facilitated diffusion)
   - for polar / charged molecules
   - moves molecules from regions of higher concentration to regions of lower concentration
   - no energy cost

3. **Active transport** (PUMPS)
   - for polar / charged molecules
   - creates a concentration gradient (moves molecules from low to high concentration)
   - needs an input of energy such as hydrolysis of ATP


--> Cellular Transport : Active Transport
Energetics of Passive-Mediated Transport

2. **Passive transport proteins (CHANNELS)** allow **ions and polar molecules** (solute) to cross a membrane in the direction of DECREASING concentration (high → low):

In “Facilitated Diffusion” either channels form or proteins alternate between two conformational states. Types are:

1. carrier ionophores
2. porins (channel forming ionophores)
3. other transport proteins have (2-conformations)


--> Cellular Transport : Passive Transport
Energetics of Transport

- **Passive transport is an exergonic process**
- **uses an existing gradient, so metabolite moves from an area of high concentration to an area of low concentration**
  \[ \rightarrow \text{end up with SAME concentration on both sides (NO gradient)} \]
- **This is a spontaneous direction of flow, powered by an increase in entropy:**

\[ \Delta S \text{ is positive} \]

\[ \Delta G = \Delta H - T \Delta S \]

\[ \text{so } \Delta G \text{ is negative} \]

- More ordered state (gradient exists)
  - Higher energy state

- More disordered state (no gradient)
  - Lower energy state

**Can STORE ENERGY in a gradient!**

**Movement of metabolite to region of low concentration RELEASES energy**
Energetics of Active Transport

3. **Active transport** proteins need an input of energy, since polar/charged molecules are moving across a membrane in a direction of INCREASING concentration:
   
   moving ______________ their concentration gradient

   solute moves from area of low conc → high conc

- **Active transport is energy requiring process that is driven by another source of free energy:**

  (A) **Primary transporter** – *uses energy released by ATP hydrolysis*

  *Example:* Ion transporters like (Na\(^+\)-K\(^+\))-ATPase, couple the vectoral (directional) transport of ions with ATP hydrolysis.

  (B) **Secondary transporter (co-transporter)** – *uses existing gradient of one molecule (or ion) to drive active transport of another molecule (to CREATE a gradient for the 2nd molecule).*

    - Symport
    - Antiport

  (C) **Other: Electron Transport Chain** _gets energy from electron flow_

Endergonic (active) transport must be coupled to an exergonic process so that collectively the two reactions have an overall negative ΔG.
**Types of Transport**

- **Uniport** - transport of a SINGLE solute across a membrane, in **one direction** (A), can be passive or active.

**SECONDARY TRANSPORTERS**  
**(COTRANSPORTERS)** – are active:

couple the energetically uphill transport of one species (B) to the downhill transport of another species (A):

Thus (A) moves high \( \rightarrow \) low (releases energy) and (B) moves low \( \rightarrow \) high (creating gradient for B, so costs energy)

1) **Symport** – *simultaneous* cotransport of two solutes across a membrane in the **SAME** direction

2) **Antiport** – *simultaneous* cotransport of two solutes across a membrane in **OPPOSITE** directions
Energetics of Active Transport

• Active transport is an energy requiring process that is driven by another source of free energy:

**Pump action** – one way to pump molecules across a membrane is to have two conformational states, each with a binding site accessible to a different side of a membrane.

**Secondary transporter (cotransporter)** – uses energetically downhill flow of molecules or ions from an existing gradient (rather than ATP hydrolysis) to drive active transport of another molecule, thus CREATING a gradient for the 2nd molecule.

![Image of conformational states](image)

![Image of pump action](image)

**TABLE 11-4** Cotransport Systems Driven by Gradients of Na⁺ or H⁺

<table>
<thead>
<tr>
<th>Organism / tissue/cell type</th>
<th>Transported solute (moving against its gradient)</th>
<th>Cotransported solute (moving down its gradient)</th>
<th>Type of transport</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Lactose</td>
<td>H⁺</td>
<td>Symport</td>
</tr>
<tr>
<td></td>
<td>Proline</td>
<td>H⁺</td>
<td>Symport</td>
</tr>
<tr>
<td></td>
<td>Dicarboxylic acids</td>
<td>H⁺</td>
<td>Symport</td>
</tr>
<tr>
<td>Intestine, kidney</td>
<td>Glucose</td>
<td>Na⁺</td>
<td>Symport</td>
</tr>
<tr>
<td>(vertebrates)</td>
<td>Amino acids</td>
<td>Na⁺</td>
<td>Symport</td>
</tr>
<tr>
<td>Vertebrate cells</td>
<td>Ca²⁺</td>
<td>Na⁺</td>
<td>Antiport</td>
</tr>
<tr>
<td>(many types)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Higher plants</td>
<td>K⁺</td>
<td>H⁺</td>
<td>Antiport</td>
</tr>
<tr>
<td>Fungi (Neurospora)</td>
<td>K⁺</td>
<td>H⁺</td>
<td>Antiport</td>
</tr>
</tbody>
</table>

Table 11-4

Lehninger Principles of Biochemistry, Fifth Edition
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Free Energy can be Stored in Concentration Gradients

- An unequal distribution of molecules or ions is an energy rich condition because free energy ($\Delta G$) is minimized when all concentrations are equal.

- Active transport creates concentration gradients (unequal distribution of molecules), whose formation requires an input of energy.

- Ion gradients are an important biochemical mode of energy storage!

- Chapter 18: Fuel catabolism results in the thermodynamically downhill flow of electrons down the electron-transport chain, which creates a proton gradient across the inner mitochondrial membrane.

  $\rightarrow$ $H^+$ pumps _______ driven by _____________ Electron Transport

- During oxidative phosphorylation the energy stored in this ion gradient is converted into chemical energy, in the form of ATP.

  $\rightarrow$ ATP synthesis driven by ________________ $H^+$ Diffusion

Active transport and energy conversion in IMM

OP (ATP synthesis) = 

Use ________________ 

$\frac{1}{2}O_2 + H_2O \rightarrow ADP + P_i \rightarrow ATP$
Passive Mediated Transporters: Ionophores

**Ionophore action** – increase permeability of membrane to selected IONS by facilitating their diffusion across a membrane

(a) **Carrier ionophores** bind to and surround ion and transport it by diffusion through the lipid bilayer

(b) **Channel forming ionophores (porins)** span the membrane with a solvent-filled channel or pore through which selected ions can diffuse

- Ions are moving from _______ $\rightarrow$ _______ concentration, so _______ energy cost. Ionophore allows passage of charged species through ________________ core of membrane.
Carrier Ionophores: Valinomycin

Valinomycin (carrier ionophore) has a **high rate of transport, moving upto $10^4$ K$^+$ ions across a membrane per second**. After delivering one K$^+$ ion, the uncomplexed ionophore rapidly returns to original side of the membrane to pick up another K$^+$ ion.

- X-ray crystal structure of Valinomycin complexed to K$^+$ ion via six coordinate-covalent bonds: cyclic peptide, contains L & D-amino acids
- **What is the structural consequence of having D-amino acids?**

Allows different backbone conformations that are not present in proteins with only L-amino acids.

Healthy cells actively maintain certain ion gradients. **Antibiotic Ionophores** function by discharging and dissipating these conc. gradients. They passively permit ions to diffuse from high to low concentrations, thus **dissipating** useful gradients.

- Many antibiotics are ionophores of bacterial origin (secreted by one bacterium to kill neighboring types of bacteria in their environment).
Porins: Channel Forming Ionophores

- Porins are channel-forming proteins in the outer membrane of gram-negative bacteria, mitochondria and chloroplasts, that allow entry of small polar solute molecules.

- Porins contain transmembrane channels, formed from a 16 to 18 stranded antiparallel beta-barrel, lined with polar residues on inside surface of barrel and nonpolar ones on the outside, facing the membrane.

- Surprisingly, most membranes are permeable to water - it is polar but neutral. Its small size and 55 molar (high) concentration allows easy diffusion of $H_2O$ across a membrane.

- Yet aquaporins are needed in some tissues (such as lens of eye).

(a) Transmembrane antiparallel $\beta$-barrel  
(b) trimer of identical subunits  
(c) hydrophobic band encircles exterior of protein

$\rightarrow$ $\beta$-barrel channel

*E. coli* OmpF porin
2. Ascribe the characteristics given below for transport of ions or polar molecules to either:
   (a) active transport (pumps)
   (b) transport through channels

   (1) flux (rate of flow of molecules between two sides of a membrane) \( \sim 10^7 \text{ s}^{-1} \) ________ \( (10^7 \text{ molecules moved / sec}) \)
   (2) flux \( 3 \times 10^1 \) to \( 2 \times 10^3 \text{ s}^{-1} \) ________ \( (\text{often involves protein conformational change}) \)
   (3) ions can flow from either side of the membrane  
   Direction of flow, depends on ________________________________
   (4) flux occurs only in a specific direction  
   vectoral transport

3. An uncharged polar molecule is transported from side 1 to side 2 of a membrane. Explain each answer.
   (a) If its concentration is \( 10^{-3} \text{ M} \) on side 1, and \( 10^{-6} \text{ M} \) on side 2, will the transport be active or passive?

   (b) If its concentration is \( 10^{-4} \text{ M} \) on side 1, and \( 10^{-1} \text{ M} \) on side 2, will the transport be active or passive?
1. The rate of transport of two molecules, indole and glucose across a cell membrane is shown in the figure. What are the differences between the transport mechanisms of the two molecules?

(A) Observe initial increase in rate of transport as [Glucose] increases, until rate plateaus (levels off), indicating the transporter is saturated (plot is similar to Michaelis Menten Kinetics)
→ Glucose is very _________ (so _________ a transporter to cross membrane)

(B) Rate of Indole transport increases linearly with its concentration
→ Indole probably diffuses freely through the membrane (it is lipophilic)
→ so there is no need for a transporter
Passive Metabolite Transport: Glucose Transporter – 2 conformational states

Model for glucose transport in muscle cells by *Glucose Permease*:

- Protein alternates between **two mutually exclusive conformations**, that alternately exposes polar ligand binding site to different sides of the membrane.

  - **The conformational transition is triggered by binding of the ligand** on one side of the membrane.
  - This occurs instead of forming an open, aqueous channel, as in porins.

**Passive transport:**
- glucose flows **DOWN** its concentration gradient (high → low) so there is **NO energy cost**
- this is a **Facilitated Uniport**

http://www.wiley.com/college/fob/quiz/quiz10/10-35.html

- Some other cells (intestinal) transport glucose using active transport (symporter)
Active Transport: (Na\(^+\)-K\(^+\))ATPase Pump

Membrane bound ATPases:
- Uses energy released by ATP hydrolysis to translocate cations across membranes to FORM a gradient

PUMP = transports ions AGAINST their concentration gradient: Low \(\rightarrow\) High

(Na\(^+\)-K\(^+\))ATPase structure:
- \((\alpha\beta)_2\) tetramer
- Creates a charge across the plasma membrane of eukaryotes: (low internal Na\(^+\) concentration)
- The ATP hydrolysis site, which provides the energy to drive this thermodynamically unfavorable gradient, is located on the internal surface of the plasma membrane.

Cellular Transport \(\rightarrow\) Active Transport (use of Na\(^+\)-K\(^+\) ATPase in ligand gated ion channels)
Active Transport (Na⁺-K⁺)ATPase Pump: Primary Transporter (Energy comes from ATP hydrolysis)

Membrane bound ATPases – translocate cations across membrane

The (Na⁺-K⁺)ATPase (pump), simultaneously:

- pumps 3 Na⁺ ions OUT of the cell and
- pumps 2 K⁺ ions INTO the cell (antiport)
- while hydrolyzing (on cell interior):
  - one molecule of ATP → ADP + Pᵢ

This creates a charge separation across membrane: more positive (+) on the outside than the inside (less positive, more negative)

In animal cells, Na⁺-K⁺ controls:
1. Cell volume
2. Allows neurons and muscle cells to be electrically excitable
3. Drives active transport of sugars/amino acids

Example: Result of pumping out Na⁺ ions, creating a low internal Na⁺ concentration, prevents osmotic bursting of cell with water inflow → water flows out towards high concentrations to dilute Na⁺ ions
(Na\(^+-\)K\(^+\))ATPase Pump

More than one-third of the ATP consumed by a resting animal is used to pump Na\(^+\) and K\(^+\) ions

- Na\(^+\)-K\(^+\)ATPase is a ___primary___ transporter which ___uses___ ATP hydrolysis to create gradients.

- (Na\(^+\)-K\(^+\))ATPase pump has steriod binding sites on outer surface for regulation and carbohydrates for recognition (glycosylated receptor)

- Digitoxigenin is a cardiotonic steroid that is a potent inhibitor of the Na\(^+\)-K\(^+\) pump (K\(_I\)~10 nM)

→ SKIP: details of P-type ATPase mechanism for Ca\(^{2+}\)ATPase and gastric H\(^+\)-K\(^+\) ATPase (in Stryer text)

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter6/animations.html#

→ Sodium-Potassium Exchange Pump → Cotransport (next slide)
Function of (Na\(^+-\)K\(^+\))ATPase Pump

- **Membrane bound Na\(^+-\)K\(^+\) ATPase is a primary transporter.** It translocates cations across the membrane by converting the free energy of phosphoryl transfer (ATP hydrolysis) into the free energy of a sodium ion gradient (forms a gradient).

- **The Na\(^+\) ion gradient is then USED to PUMP materials into the cell, through the action of a secondary transporter (such as the Na\(^+\)-glucose symporter).**

Free Energy can be stored in Na\(^+\) ion concentration gradients!

Two different transporters are working together: ___________________

\( \text{\textbf{KNOW concept, SKIP calculations}} \)
Active transport using an energy source other than ATP hydrolysis. A **secondary** transporter uses existing gradient of one molecule or ion, to drive active transport of another molecule.

- This **symporter** uses the $H^+$ gradient across *E. coli* membrane (generated by the oxidation of fuel molecules) to drive (PUMP) the uptake of lactose and other sugars against a concentration gradient (low outside to high inside).

- $H^+$ and lactose bind to sites facing outside of cell. Permease with both sites full **everts**, releasing both ligands. Another **eversion** places empty sites on outside.

**Lactose Permease:**

$$\Delta G \text{ } - \text{ for } H^+$$

$$\Delta G \text{ } + \text{ for lactose}$$
In E.Coli, ET chain occurs across the plasma membrane.

1. During fuel catabolism is the creation of the $H^+$ gradient across the inner mitochondrial membrane, by the electron-transport chain, involve a $1^\circ$ or $2^\circ$ transporter, or neither? 

_______, the energy source for active transport (creating a gradient) results from the _____________________________, as they are transferred from a carrier with lower affinity for electrons, to a carrier with higher affinity for electrons (finally to $O_2$).

$\rightarrow$ $H^+$ pumps (Active) driven by Exergonic Electron Transport
$\rightarrow$ ATP synthesis driven by Exergonic (Passive) $H^+$ Diffusion
Compartments in Eukaryotic Cells Control Metabolism

The plasma membrane of each type of cell and different organelle membranes with a cell contain unique transporters.

Regulation of glucose uptake by GLUT transporters:

Mitochondrial transporters regulate flow of specific charged metabolites across IMM:

Receptor Mediated Endocytosis:

Cholesterol transport

Transferrin Receptor Cycle regulates Iron transport
Cholesterol Transport

Model of low-density lipoprotein particle (LDL), which transports cholesterol in body fluids:

- Apoproteins are **hydrophilic**
  - Solubilize hydrophobic lipids in core
  - Contain cell targeting signals

How does cholesterol enter cells?

**Endocytosis** brings material into cells

**Exocytosis** takes material out of cells

- (1) **BUDDING**
  - LDL binding → Internalization → Lysosomal hydrolysis

- (2) **FUSION**
  - Cholesterol esters are **hydrophobic**
  - Cholesterol esters
  - Unesterified cholesterol
  - Phospholipid
  - Cholesteryl ester
  - Apoprotein B-100

- **Cholesterol Transport**
  - (2) Fusion
  - (1) Budding

- **Model of low-density lipoprotein particle (LDL)**
  - Transport cholesterol in body fluids:
    - Cholesterol esters
    - Unesterified cholesterol
    - Phospholipid
    - Cholesteryl ester
    - Apoprotein B-100

**Apoproteins**
- Hydrophillic
- Solubilize hydrophobic lipids in core
- Contain cell targeting signals

**Cholesterol esters**
- Hydrophobic
- Solves cholesterol in body fluids
Eukaryotes: Functions of Membrane Enclosed Organelles and Vesicles - Secretory Pathways

Budding and fusion are used to move molecules around within a cell, to the outside of a cell (secretion by antibodies), or from outside into the cell (ingestion of antigens by macrophage cells)

- Following protein synthesis (translation) at the ribosomes (step 1), many secreted and transmembrane proteins move directly into the lumen (interior) of the endoplasmic reticulum to undergo post-translational processing. Nascent proteins are targeted to different compartments by signal peptides.

1. Why can membrane budding/fusion occur?

- In the ER proteins are modified, glycosylated, and disulfide bonds are formed.

- Step 2 - vesicles “budding off” the endoplasmic reticulum (ER) membrane

- Step 3 - “fusion” of vesicles with plasma membrane to allow delivery to cell surface (to form receptor proteins)
Regulation of Glucose Uptake by GLUT Transporters

- A cell can only perform biochemical reactions on compounds which it has taken up from its environment. Glucose enters a cell through a homologous set of transporters: GLUT1 to GLUT5.

<table>
<thead>
<tr>
<th>Name</th>
<th>Tissue location</th>
<th>$K_m$</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT1</td>
<td>All mammalian tissues</td>
<td>1 mM</td>
<td>Basal glucose uptake</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Liver and pancreatic β cells</td>
<td>15–20 mM</td>
<td>In the pancreas, plays a role in the regulation of insulin In the liver, removes excess glucose from the blood</td>
</tr>
<tr>
<td>GLUT3</td>
<td>All mammalian tissues</td>
<td>1 mM</td>
<td>Basal glucose uptake</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Muscle and fat cells</td>
<td>5 mM</td>
<td>Amount in muscle plasma membrane increases with endurance training</td>
</tr>
<tr>
<td>GLUT5</td>
<td>Small intestine</td>
<td>—</td>
<td>Primarily a fructose transporter</td>
</tr>
</tbody>
</table>

Table 16.4 Family of glucose transporters

![Diagram of glucose metabolism](image-url)

Active pathways:
1. Glycogen breakdown, Chapter 21
2. Gluconeogenesis, Chapter 16
3. Glycolysis, Chapter 16
4. Citric acid cycle, Chapter 17
5. Oxidative phosphorylation, Chapter 18

Figure 21.14 Biochemistry, Sixth Edition
© 2007 W.H. Freeman and Company
Porins: Channel Forming Ionophores

• Structure of aquaporin viewed from the side and from the top, shows that hydrophilic residues line the central water channel.

1. How do ionophores distinguish between similar ions, allowing passage of one ion but not another?

- A K\(^+\) ion entering the K\(^+\) channel travels partly through the channel still solvated with water (blue). Here the pore diameter narrows to 3Å (yellow).

- Ions larger than the pore size at the narrowest point in the channel are rejected (ex: Rb\(^+\) and Cs\(^+\))

- K\(^+\) ions must shed their water shell and interact directly with C=O groups (red) in the selectivity filter of the channel.

- Smaller ions like Na\(^+\) cannot interact favorable with pore functional groups, forming a less stable complex, and are unable to shed their water shell.

**Table 13.1** Properties of alkali cations

<table>
<thead>
<tr>
<th>Ion</th>
<th>Ionic radius (Å)</th>
<th>Hydration free energy in kJ mol(^{-1}) (calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li(^+)</td>
<td>0.60</td>
<td>-410 (98)</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>0.95</td>
<td>-301 (72)</td>
</tr>
<tr>
<td>K(^+)</td>
<td>1.33</td>
<td>-230 (55)</td>
</tr>
<tr>
<td>Rb(^+)</td>
<td>1.48</td>
<td>-213 (51)</td>
</tr>
<tr>
<td>Cs(^+)</td>
<td>1.69</td>
<td>-197 (47)</td>
</tr>
</tbody>
</table>
Maintaining the Body's Chemistry: Dialysis in the Kidneys
Membranes and Proteins: Dialysis and Proton Gradients

Active and Passive Transport of ions and metabolites out of Nephron Tubules through their surface membrane into the surrounding capillaries

Authors: Rachel Casiday and Regina Frey
Department of Chemistry, Washington University, St. Louis, MO 63130

http://www.chemistry.wustl.edu/~edudev/LabTutorials/Dialysis/Kidneys.html