MEMBRANE TRANSPORT

Suggested reading

Stryer, 4th Edition
Chapter 12.
Overview

In this class we will consider the following:

1. What is membrane transport?
2. Why do cells need transport systems?
3. What are channels and carriers?
4. An example of a channel.
5. An example of a carrier.
**What is Membrane Transport?**

Membrane transport is defined as the movement of molecules across cell membranes.

There are two classes of membrane transport.

- Rapid, stereoselective, saturable, protein mediated transport.
- Slow, non-specific diffusion of molecules across the cell membrane.

This class will focus on protein-mediated mechanisms.
**Cells often show very high permeability to specific molecules.**

e.g. human erythrocytes are 100,000 times more permeable to D-glucose than they are to L-glucose.

Metabolically depleted human erythrocytes are 1,000 fold more permeable to potassium (at. wt. = 39.09) than they are to sodium (at. wt. = 22.99) ions.

*This tells us that specific, stereoselective systems intervene to mediate transport of D-glucose and K!!*
Passive versus Active Transport

For some cells exposed to certain solutes, the equilibrium intracellular concentration of solute is identical to that outside the cell. 

**e.g. erythrocytes & D–Glucose**

Because equilibrium $[\text{D-glucose}]_i = [\text{D-glucose}]_o$, the red cell glucose transport system is described as "passive" — the distribution of sugar across the cell membrane is the same as that produced by simple passive diffusion.

---

Time course of 3-O-methylglucose (3OMG) uptake by a single squid giant axon expressed as $[3\text{OMG}]_i/[3\text{OMG}]_o$. 3OMG is a nonmetabolizable sugar.

The dotted line indicates the water space of the axon.
For different cells or solutes, the equilibrium, intracellular concentration of solute is not identical to that outside the cell.

e.g. epithelial cells of small intestine & D–Glucose.

Because

\[ [\text{D-glucose}]_i = 20 \ [\text{D-glucose}]_o \]

the epithelial cell glucose transport system is described as “ACTIVE” – the distribution of sugar across the cell membrane is NOT that produced by simple passive diffusion.
When charged species are examined (e.g. Na⁺) we must consider the effect of the membrane potential (V) on transmembrane solute distributions.

Most cells are characterized by a membrane potential difference (V) of −70 mV (inside negative with respect to the outside).

Now examine the concentrations of cations and anions in serum and cytosol:

<table>
<thead>
<tr>
<th>Species</th>
<th>[Extracellular] [mM]</th>
<th>[Intracellular] [mM]</th>
<th>Equilibrium potential [mV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>150</td>
<td>15</td>
<td>+61.5</td>
</tr>
<tr>
<td>K⁺</td>
<td>5.5</td>
<td>150</td>
<td>-88.3</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>1.5</td>
<td>0.0002</td>
<td>+119.2</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>125</td>
<td>9</td>
<td>-70.3</td>
</tr>
</tbody>
</table>

\[\text{K}^+, \text{Na}^+, \text{Cl}^-, \text{K}^+\]
Consider Cl⁻. The Cl⁻ concentration gradient is directed into the cell. Thus Cl⁻ tends to diffuse along the concentration gradient into the cell. The interior, however, is negative with respect to the outside and Cl⁻ ions are pushed out along the electrical gradient. An equilibrium is achieved when Cl⁻ influx = Cl⁻ efflux. The membrane potential at which this equilibrium exists is the equilibrium potential. Its magnitude is calculated from the Nernst equation as follows:

\[
\frac{RT}{FZ_{Cl}} \ln \left( \frac{[Cl^\circ]}{[Cl_i]} \right) = -70.3 \text{mV}
\]

where R is the gas constant (1.987 cal/deg/mol)
T is absolute temperature (37°C = 310°K)
F is the faraday (23060 cal/volt/mol)
\(Z_{Cl}\) is the valence of Cl (-1)
Because $E_{\text{Cl}} = V$ (membrane potential), no forces other than those represented by the chemical and electrical gradients (the electrochemical gradient) need be invoked to explain the distribution of Cl\(^-\) across the cell membrane.

Because $E_{\text{Na}}$, $E_{\text{K}}$ and $E_{\text{Ca}} \neq V$, this suggests that other processes intervene to exclude Na and Ca and to accumulate K. These are transport processes and must be ACTIVE.
Control of transport function

The permeability of cells to specific molecules can be rapidly modified by a change in physiologic conditions.

For example, application of acetylcholine to the neuromuscular junction increases the permeability of the muscle membrane to sodium, potassium and calcium ions very rapidly (within 1 msec).

Electrical stimulation of nerve and muscle also results in the very rapid (1 msec) and reversible increases in sodium and potassium permeability in the cell membrane.

Application of insulin to muscle cells and fat cells results in the rapid (30 sec to 5 min) and reversible increase in cellular permeability to D-glucose.
Selective transport is protein-mediated

This increased membrane permeability to specific molecules suggests that an enzyme or class of enzymes intervenes to accelerate the rate at which molecules are distributed across the cell membrane.

These enzymes are known as membrane transporters.

Some transporters are classic enzymes – they accelerate the rate at which a molecule achieves its equilibrium distribution across the cell membrane by providing (literally) an alternative reaction pathway. These are the PASSIVE transporters.

Some active transporters exploit high energy intermediates (ATP-hydrolysis) to catalyze rapid net solute movement against a concentration gradient (uphill).

Yet other active transporters exploit Na+ and K+ gradients to drive a molecule against an chemical gradient.

As with other enzymes, membrane transporters display saturation kinetics and competitive or non-competitive inhibition by relatively low concentrations of specific inhibitors.
EXAM-TYPE QUESTION

Human fibroblast cells were maintained in culture and were exposed to 5 mM 3OMG (a nonmetabolizable but transported neutral sugar). Following 5 hr incubation at 37 °C, intracellular 3OMG achieved steady state and the ratio of intracellular [3OMG] : extracellular [3OMG] was calculated. The 3OMG transport system is passive when:

a) Intracellular [3OMG] > extracellular [3OMG]
b) Intracellular [3OMG] < extracellular [3OMG]
c) Intracellular [3OMG] = extracellular [3OMG]
d) Intracellular [Na] < extracellular [Na]
e) The transporter does not hydrolyze ATP.
Why Do Cells Need Membrane Transporters?

The lipid bilayer is an effective barrier to the movement of small hydrophilic molecules. Two factors govern the rate at which molecules can diffuse across the lipid bilayer.
(1) The membrane solubility of the molecule

Most molecules are hydrophilic and are thus poorly soluble in hydrophobic membrane bilayers.

Basal permeability coefficients $P$ for the human red blood cell plotted against their hexadecane–water partition coefficients on double logarithmic axes. The straight line is the least squares line of unit slope.
(2) The size of the molecule

Most molecules are too large to diffuse easily within the membrane bilayer (the average void volume in a bilayer is the size of a methylene group).

\[ \text{MOLECULAR WEIGHT} \]

Comparison of the size dependence of the resistance to diffusion (units of \(10^6 \text{ sec cm}^{-2}\)) in water (lower curve) and within the human red cell membrane (upper curve). (We define resistance as the reciprocal of the diffusion coefficient.) Diffusion data as in Fig. 2.3, except for the value for glucose diffusing in water at 25°C, which was taken from Longsworth (1953). [The data point for \(n\)-hexanol \((M_r = 102)\) could not be plotted, since its diffusional resistance within the red cell membrane was too high \((370 \times 10^6 \text{ sec cm}^{-2})\).] Only the red cell data points (●) are numbered (see below). The data points for diffusion in water (○) refer, in order of increasing molecular weight, to \(\text{H}_2\), \(\text{N}_2\), \(\text{O}_2\), \(\text{CO}_2\), urea, glycerol and glucose.

The compounds are: 2, ethanediol; 3, ethanol; 4, glycerol; 6, methanol; 7, propanol; 9, urea; 10, water.
In order for cell to selectively regulate its metabolite content it must use transporter molecules which accelerate the rate of entry or export of these species into or out of the cell. Selective expression of specific transporters allows the cell to retain or import molecules that are important for survival and to export molecules that are incompatible with cellular survival.
EXAM-TYPE QUESTION

Protein-independent, transmembrane solute diffusion:

a) shows saturation kinetics
b) is stereoselective
c) is independent of solute molecular size
d) increases with increasing molecular size
e) increases with increasing lipid solubility.
There are two classes of protein-mediated transport systems:
1) channels
2) carriers

Channels and Carriers
The channels form membrane-spanning pores that allow molecules to diffuse down the electrochemical gradient into or out of the cell.

Some channels are gated. They are opened or closed by binding of a ligand or by altered membrane potential.
The carriers are an altogether different class of transport mechanism. The carriers appear to present either an import or an export site to the transported molecule but not both sites simultaneously.
EXAM-TYPE QUESTION

One of the following is incorrect:

a) channels and carriers are comprised of membrane spanning proteins

b) Channels form membrane spanning pores.

c) Some channels are gated by ligands

d) Carriers alternate between 2 states –one presenting an import site, the second presenting an export site.

e) Carriers simultaneously present import and export sites to substrate.
The acetylcholine receptor of the neuromuscular junction. The figure below shows a schematic of a synapse between two neurons. Nerve impulses are communicated across most synapses by small diffusible molecules called neurotransmitters.

The presynaptic membrane of the synapse is separated from the postsynaptic membrane by a gap (about 50 nm wide) called the synaptic cleft. The interior of the presynaptic axon terminus is filled with synaptic vesicles which contain about 10,000 acetylcholine molecules (in a cholinergic synapse).

When a nerve impulse arrives at the presynaptic terminal, this leads to the synchronized export of the contents of about 300 vesicles. This raises the acetylcholine concentration in the cleft from 10 nM to about 500 µM in less than a millisecond.

\[
H_3C-C-O-CH_2-CH_2-N-(CH_3)_3^+
\]

Acetylcholine
The binding of acetylcholine to the postsynaptic membrane changes the ionic permeabilities of this membrane markedly. The conductance of both sodium and potassium increases greatly within 100 μsec leading to a large inward current of sodium and a smaller outward current of potassium. The inward sodium current depolarizes the postsynaptic membrane and triggers an “action potential”.

Acetylcholine depolarizes the postsynaptic membrane by increasing the conductance of Na⁺ and K⁺.
Acetylcholine binding to the postsynaptic membrane opens a single kind of cation channel which is almost equally permeable to sodium and potassium. The influx of sodium is much larger than the influx of potassium because the gradient for sodium flow is greater than that for potassium. This change in ion permeability is mediated by the nicotinic acetylcholine receptor which we will refer to here simply as the acetylcholine receptor.

{Acetylcholine also activates a different kind of acetylcholine receptor in the heart - the muscarinic acetylcholine receptor which has a different function to slow pacemaker activity in the heart.}

The acetylcholine receptor is known as a ligand gated channel. When acetylcholine binds, the channel is opened. Let us look at this channel in some detail.
The Acetylcholine Receptor is a Multisubunit Complex

Background

The electric organ of *torpedo* - an electric fish- is a good source of acetylcholine receptor because its electroplax are very rich in cholinergic postsynaptic membranes.

Another biological source that has been very useful in studying the acetylcholine receptor is the family of snake neurotoxins. α-bungarotoxin and cobra toxin block neuromuscular transmission. These small basic proteins bind specifically and very tightly to acetylcholine receptors and thus can be used as molecular tags of the receptor.
Structure

The acetylcholine receptor (AChR) of the electric organ has been solubilized by adding non-ionic detergents to a postsynaptic membrane preparation and then purified by affinity chromatography using a column bearing covalently attached to cobra toxin. The receptor is then displaced by addition of free cobra toxin resulting in the release of a 268 kilodalton receptor comprised of four kinds of subunits. These subunits are $\alpha$, $\beta$, $\gamma$ and $\delta$ subunits. The stoichiometry is $\alpha_2, \beta, \gamma, \delta$.

Each of the $\alpha$-chains contains a single binding site for acetylcholine. The cloning and sequencing of the cDNAs encoding for these subunits, each about 50-58 kDa shows that they have similar sequence. It now seems likely that the genes for $\alpha$, $\beta$, $\gamma$ and $\delta$ subunits arose by duplication and divergence of a common ancestral gene.
The structure has a 5-fold symmetry which is consistent with the similarity of its five constituent subunits. The receptor is cylindrical with a mean diameter of about 65 Å. All five rod-shaped subunits span the membrane. The receptor protrudes about 60 Å on the synaptic side of the membrane and about 20 Å on the cytosolic side. The two acetylcholine binding sites are at the synaptic end of the two α subunits.
Figure 12-5
Schematic diagram of the closed form of the acetylcholine receptor channel. The narrowest part of the pore is occluded by side chains coming from five helices. [Courtesy of Dr. Nigel Unwin.]
Single Channel analysis by Patchclamp Conductance Measurements

Single ion channels can be studied using the patchclamp technique.

Using this method, a clean glass pipette with a 1 µm diameter tip is pressed against an intact cell to form a seal. Suction leads to the formation of a very tight seal so that the resistance between the inside of the pipette and the bathing solution is many gigaohms. A gigaohm seal, which is $10^9$ ohms, ensures that an electric current flowing through the pipette is identical to the current flowing across the membrane covered by the pipette. This gigaseal makes possible high resolution current measurements while a voltage is applied across the membrane. With a little luck, each seal may contain only a single channel in the membrane. This makes it possible to follow the flow of ions through a single channel and the transitions between states of a channel. Moreover, these changes can be monitored with a time resolution of microseconds.
The activity of a single acetylcholine receptor is graphically displayed in patchclamp recordings of postsynaptic membranes of skeletal muscle. The addition of acetylcholine is followed by transient openings of the channel.

The current, or $i$, flowing through an open channel is 4 picoamperes when the membrane potential $V$ is -100 millivolts (mV).
An ampere represents the flow of $6.24 \times 10^{18}$ charges per second. Hence,

$$4 \times 10^{-12} \times 6.24 \times 10^{18} = 2.5 \times 10^7$$

sodium ions per second flow through an open channel. The conductance, or $g$, of the channels is equal to

$$g = \frac{i}{V - E_R}$$

where $E_R$ is the reversal potential at which there is no net flux. $g$ is expressed in units of siemens (the reciprocal of an ohm), $i$ in amperes and $V$ in volts. If $E_R$ equals 0, a current of 4 pA at a potential of 100 mV corresponds to conductance of 40 pS.
**Xenopus Oocytes Express Microinjected Messenger RNAs Encoding Subunits of the Acetylcholine Receptor**

The availability of cDNAs for subunits of the acetylcholine receptor has opened new and exciting ways of exploring how this channel functions. mRNA is transcribed in vitro from the cDNAs, microinjected into *xenopus* oocytes which lack their own acetylcholine receptors. Polypeptides translated from these injected mRNAs are targeted to the plasma membrane of the oocyte. Patchclamp measurements then reveal whether functional acetylcholine receptors are formed.

![Diagram](image)

*Xenopus oocytes can be used to express mRNAs derived from cDNAs. The steps in expression are in vitro transcription of the cDNA, microinjection of the resulting mRNA, translation of the mRNA by the oocyte, and targeting of the channel to the plasma membrane. Microinjection of the mRNAs for the four kinds of subunits of the acetylcholine receptor results in the formation of functional channels, as evidenced by patch-clamping studies of membrane conductance.*

Fully active channels are formed only when all four kinds of subunits are expressed. Partially active channels are assembled if either the $\gamma$ or $\delta$ chain is missing.
The Binding of Two Acetylcholine Molecules Transiently Opens a Cation Selective Pore

Measurements of the dependence of channel opening on the concentration of acetylcholine results in a Hill coefficient of 1.97. Hence, at least two molecules of acetylcholine must bind to the receptor to open the channel.

Patchclamp studies suggest that the acetylcholine (A) binds to the closed state, (C) of the receptor to form the AC and A\(_2\)C states. The A\(_2\)C state then undergoes a transition to the open state A\(_2\)O.

Multiple conformational states of the acetylcholine receptor. The abbreviations used are A, acetylcholine; C, closed state of the receptor; O, open state; I, inactive state. The binding of two molecules of acetylcholine rapidly opens the channel. If the concentration of acetylcholine stays high, the channel spontaneously closes (desensitizes) by making a transition to an inactive state.
Acetylcholine binding under physiologic conditions occurs in less than 100 µsec. The rate constant for acetylcholine association with either site of the receptor is in the order of $10^8$ per molar per second - close to the diffusion controlled limit.

Likewise, the intramolecular transition from the $A_2C$ state to the open $A_2O$ state is very rapid - 30 µsec. Thus, the channel opens swiftly in response to a sudden increase in the acetylcholine concentration in the synaptic cleft.

Under physiological conditions, the channel remains open for only about 1 msec because acetylcholine in the cleft is hydrolyzed rapidly to acetate and choline by acetylcholinesterase. This enzyme is anchored to the postsynaptic membrane by a covalently attached glycolipid group.
Acetylcholinesterase has a very high turnover number, 25,000 per second and achieves kinetic perfection because

\[
\frac{k_{\text{cat}}}{K_m} = 2 \times 10^8 \text{ M}^{-1} \text{s}^{-1}
\]

The catalytic power of acetylcholinesterase enables synapses to transmit action potentials at high frequency.

Organic fluorophosphates like diisopropylphosphofluoridate (DIPF) inhibit acetylcholinesterase by forming very stable covalent phosphoryl enzyme complexes. The phosphoryl group becomes bonded to the active site serine, as in serine proteases that have reacted with PIPF. Many organic phosphate compounds have been synthesized for use as agricultural insecticides or as nerve gases for chemical warfare.

The structural basis for channel opening is not understood. The open form of the channel has not been imaged thus far because it is so short lived. If the concentration of acetylcholine remains high, the channel spontaneously closes in less than a second through a process called desensitization.
Cation Selectivity

Monovalent or divalent cations but not anions readily flow through the open form of the acetylcholine channel. How is cation selectivity determined? The amino acid sequence of the pore forming helices are informative.

Three rings of negatively charged residues are present. One ring is located within the transmembrane region of the pore and the other two flank the entrances to this narrow segment. Anions, such as chloride, cannot enter the pore because they are repelled electrostatically by the rings.

Studies of the permeability of a series of organic cations differing in size (e.g. alkylammonium ions) suggest that the narrowest part of the pore has a diameter of 6.5 Å.
Other examples of Channels

1) Voltage gated Na channel
2) Voltage gated K channel
3) Ca activated K channel
4) ATP-regulated K channels
5) Glutamate receptor
EXAM-TYPE QUESTION

One of the following is correct. The acteylcholine receptor:

a) Is an example of a voltage gated channel
b) Is a membrane carrier
c) Is a ligand-gated carrier
d) Closes by a process called desensitization
e) Transports acetylcholine into the cell.
**Medical Relevance**

An autoimmune disease called *myesthenia gravis* develops when individuals develop self-antibodies against the AChR. This leads to weakened synaptic transmission and neuromuscular control. A classic early-stage symptom is twitching or drooping eyelids.

Tetradotoxin (TTX) is a potent poison isolated from the puffer (fugu) fish. Indeed it is the action of TTX that makes fugu such a dangerous delicacy. TTX blocks the voltage-gated Na channels that play a vital role in depolarizing neurons and conduction of action potentials. Saxitoxin (STX) is produced by marine dinoflagellates and acts in the same way. Shellfish (clams and mussels) feeding on dinoflagellates during a red tide, are poisonous. A contaminated mussel may contain enough STX to kill 50 humans.

A common feature of TTX and STX is the presence of a guanido group. This positively charged group on the surface interacts with negatively charged carboxylates at the mouth of the channel on the extracellular side of the membrane. TTX and STX block the flow of sodium by plugging the entrance of the pore. Radiolabeled STX was used as a molecular tag to purify the NA-channel.
A CARRIER MECHANISM

ATP Hydrolysis Drives the Transport of Sodium and Potassium Ions Across the Plasma Membrane

Most cells have a high concentration of K and a low concentration of Na relative to the external medium. These transmembrane ionic gradients are generated by a specific transport system that is called the Na,K pump because the movement of these ions is linked.

The active transport of Na and K is of great physiologic importance. Indeed, more than a third of the ATP consumed by a resting animal is used to pump these ions. The Na K gradient in animal cells controls cell volume, renders nerve and muscle cells electrically excitable and drives the active transport of sugars an amino acids.
In 1957 Jens Skou discovered an enzyme that hydrolyzes ATP only if Na and K are present in addition to Mg, which is required by all ATPases. This enzyme was named the Na\(^+\)K\(^+\)ATPase.

The Na\(^+\)-K\(^+\) ATPase, an integral part of the Na\(^+\)-K\(^+\) pump, hydrolyzes ATP only if both Na\(^+\) and K\(^+\) are present, in addition to Mg\(^{2+}\).
The reaction scheme was viewed as

\[
\text{ATP + H}_2\text{O} \xrightarrow{\text{Na}^+, \text{K}^+, \text{Mg}^{2+}} \text{ADP} + \text{P}_i + \text{H}^+
\]

It was proposed that the Na\(\text{+K}^+\)ATPase is an integral part of the Na K pump and that the splitting of ATP provides the energy needed for the active transport of these cations. This is important because in order to move Na against an electrochemical gradient, the cell requires an input of energy. Similarly, for the transporter to move K from the outside of the cell to the inside of the cell against an electrochemical gradient, an input of energy is needed.

This hypothesis has been supported by the finding that the level of ATPase’s activity is correlated with the level of pump activity. Also, variations in Na and K levels have parallel effects on the ATPase activity in transport. Finally both the Na\(\text{+K}^+\)ATPase and the NaK pump activities are specifically inhibited by cardiotonic steroids.
The isolation of the Na⁺K⁺ATPase and its reconstitution into artificial lipid bilayers confirms that the ATPase and the pumping activity are mediated by the same molecular complex, the NaK pump. The results of these various studies have confirmed the identity of the Na⁺K⁺ATPase and the NaK pump and have revealed new insight into a new class of transport mechanisms called carriers.

The NaK pump consists of two types of subunits: α and β subunits that are associated in the membrane as an α₂β₂ tetramer. Hydrophobicity analyses and membrane localization studies suggest that the α chain contains at least eight transmembrane helices.

Much of the α chain plus its ATPase activity is located on the cytosolic side of the membrane. The small portion of the α chain on the extracellular side contains the binding site for cardiotonic steroid inhibitors. The β chain which contains a single membrane helix does not appear to be essential for ATPase or transport function.
What is the catalytic mechanism of Na and K pumping? An important clue is the discovery that the ATPase is phosphorylated by ATP in the presence of Na and Mg. The site of phosphorylation is the side chain of a specific aspartate residue. This β-aspartylphosphoryl intermediate or EP is then hydrolyzed if K is present. Phosphorylation does not require K whereas dephosphorylation does not require Na or Mg.

The reaction scheme can thus now be viewed as:

\[
E + ATP \overset{Na^+, Mg^{2+}}{\rightleftharpoons} E-P + ADP \\
E-P + H_2O \overset{K^+}{\rightarrow} E + P_i
\]
Na-dependent phosphorylation and K-dependent dephosphorylation are not the only critical reactions. Because the pump serves to extrude Na and to import K, it is reasonable to conclude that Na binds at the cytosolic surface of the transporter and dissociates at the exoplasmic surface of the ATPase while K binds at the exofacial surface and is released at the endofacial surface of the transporter.

Kinetic evidence also suggests that the pump interconverts between two states: e1 and e2. Na binds to the e1 or intracellular state while potassium binds to the e2 or extracellular state of the carrier. The catalytic cycle may now be represented by:

![Enzymatic cycle of the Na\(^+\)-K\(^+\) ATPase.](image)
The binding cavity alternately faces the inside and outside of the cell in each transport cycle

Insufficient data exist to form a detailed structural model for NaK pumping. However, the following model was proposed by Jardetzky and by Glynn.

1) The pump must contain a cavity large enough to permit entry of a small molecule.

2) The pump must be able to assume 2 conformations such that the cavity is open to the inside in one form and to the outside in the other form.

3) The affinity for the transported species must be different in the 2 conformations.
Schematic diagram of a proposed mechanism for the Na⁺-K⁺ pump. The upper sequence of reactions depicts the extrusion of three Na⁺ ions, whereas the lower reactions show the entry of two K⁺ ions. The $E_1$ (light) and $E_2$ (dark) forms are shown here as having very different conformations. The actual conformational differences may be quite small.
The structural basis of this conformational change is unknown.

The NaKATPase is sometimes referred to as an E1.E2.ATPase.

{Other examples of E1.E2.ATPases include the CaATPase and the protonATPase}

NOTE:

All carrier-mediated transport systems undergo this E1-E2 conformational change. Some of these systems use ATP to drive molecules against a concentration gradient. Other transporters simply use this E1-E2 conformational change to move solutes down a concentration gradient.
Several other findings have been obtained:

1) N-ethylmaleimide inhibits the pump and traps Na\textsuperscript{22} in an occluded (nonreleasable) state.

2) Intracellular vanadate inhibits the pump and traps Rb\textsuperscript{86} (a substitute for K) in an occluded (nonreleasable) state.

3) In the absence of K\textsubscript{o} and K\textsubscript{i} and in the presence of high Na\textsubscript{o} and low Na\textsubscript{i}, the pump synthesizes ATP from ADP and Pi. When the Na gradient is reversed in the absence of K, ATP is hydrolyzed. Under both conditions, the pump catalyzes Na/Na exchange.

4) In the absence of Na but in the presence of Pi, the pump catalyzes K/K exchange.

5) E2.P dephosphorylation is inhibited by cardiotonic steroids
These allow further refinement of the model:
Medical Relevance

The cardiotonic steroids obtained (originally) from the foxglove *Digitalis purpurea* have been used as cardiac stimulants for centuries.

Their application at low doses results in increased force of contraction of heart muscle. It is, therefore, a drug of choice in treating congestive heart failure. It is thought that inhibition of the Na,KATPase results in diminished transmembrane Na gradients. This in turn produces less Ca efflux via the Na/Ca exchange transporter (an antiporter), raising sarcoplasmic Ca levels and thereby enhancing contraction of the contractile apparatus.

Inhibition of Na reabsorption by the renal tubule results in diuresis (water loss). This can be achieved by application of NaKATPase inhibitors which block active Na transport out of the epithelial cell into the interstitium or by application of substances such as furosemide which inhibit Na and Cl symport from the renal tubule lumen into the epithelial cells of the tubule.
EXAM-TYPE QUESTION

One of the following is incorrect: The Na,KATPase:

a) Is not voltage-gated channel
b) Couples ATP hydrolysis to Na extrusion and K import
c) Is not a ligand-gated channel
d) Breaks thermodynamic principles by transporting Na against an electrochemical gradient.
e) Is inhibited by cardiotonic steroids.
**Passive**

**Uniport**  
(simple carrier)  
Bidirectional fluxes  
Net flux from high [S] to low [S]  
"relaxation" operational

**Antiport**  
(exchange only carrier)  
Bidirectional fluxes  
Net flux from high [S] to low [S]  
"relaxation" absent

**Examples**

- Sugar transport in most cells
- Nucleoside transport
- Amino acid transport
- Anion exchange in most cells
- Na/Ca exchange
- ADP/ATP exchange in mitochondria
**Active**

**Symport**
(co-transport)
Net uphill transport of solute driven by net downhill flow of cation.

**Uniport**
(ATPase)
relaxation operational

**Antiport**
(ATPase)
"relaxation" absent or very slow

**Examples**
sugar and amino acid transport in absorptive epithelia

Neurotransmitter transport in synaptic clefts

H-ATPase
Ca-ATPase

Na,K-ATPase