

## MEMBRANE FUNCTION, Part 3

### Active Transport<sup>1</sup>

#### **Active Transport:**

If the cell must expend energy to transport material across the membrane, the movement is called **active transport**. Energy expenditure is required when a substance is moved up a concentration gradient or when a concentration gradient is created where one did not exist previously. As we saw earlier, such increases in order are improbable events and require energy for their creation and maintenance. In one sense they appear to violate the Second Law of Thermodynamics in that with respect with the transported materials more order is created. However, the creation of this order is linked to the creation of comparatively larger amounts of disorder elsewhere. Or put another way, **a non-spontaneous process (the concentration of particles) is driven by a highly spontaneous process** (usually the breakdown of some compound accompanied by a large release in energy). In order for one of these processes to drive the other, some sort of **coupling agent** is required. In the case of active transport, that coupling agent is the transporter protein.

Let's make this a bit more concrete. A good example is the **Na<sup>+</sup> / K<sup>+</sup> pump** (also known as the **Na<sup>+</sup> / K<sup>+</sup> ATPase**). **It translocates both Na<sup>+</sup> and K<sup>+</sup> in unfavorable directions -- it pumps Na<sup>+</sup> out of a cell to where it is more concentrated and at the same time it moves K<sup>+</sup> into the cell where it is also more concentrated.**

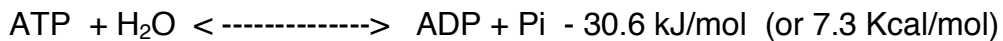
Part of this exchange of ions makes the process easier -- note that if one + ion is exchanged for another there is no change in the electrical charge across the cell membrane. Its worth noting, however, that **in most versions of the Na<sup>+</sup> /K<sup>+</sup> ATPase that the exchange rate is 3 : 2 and therefore the charge is affected.**

As is obvious from the name, the process is driven by the breakdown of ATP. Recall that this compound is a ribonucleotide triphosphate and that we have said earlier that its outer two bonds have an unusually high standard transfer potential. This means that, if we start with a mixture of 1 M ATP, 1 M ADP, 1 M Pi and water that by the time equilibrium is reached, nearly all of the ATP will be converted to ADP and Pi. In other words, a lot of bonds will have been rearranged and therefore a great deal of work was done (equivalent to saying that the system released a lot of energy in re-arranging all of those bonds).

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We also learned that the reason that so much work will be done is related to the fact that the outer two phosphate groups contain a number of negative charges and they strongly repel each other. This is what makes the bond relatively likely to break and not reform. We also learned in the metabolism section that in order to synthesize ATP from ADP and "**inorganic**" phosphate (**P<sub>i</sub>** or **HPO<sub>4</sub><sup>2-</sup>**) we must supply enough energy to overcome this repulsion. If we consider the amount of energy associated with breaking all of these bonds when we start from standard 1 molal concentrations, then:



Without going into great detail, focus on the amount of energy released under standard conditions, -7.3 kcal/mol. This is a very large amount of energy and essentially means that it is very difficult and therefore very unlikely that this reaction can be reversed. In fact we will see that the reaction is normally only reversed in very specialized cell processes that have very large amounts of energy available for the reversal. **Thus, when ATP is broken down by a transport protein such as the Na<sup>+</sup>/K<sup>+</sup> ATPase, it will not immediately reform to ATP.** This is important to our story.

**ATP AND ENERGY:** You almost certainly know from high school biology that ATP is an energy source. What you probably don't know is what it does -- what does it mean to be an energy source? It certainly does not act like gasoline or steam and heat up or expand to do work. Instead, it simply provides a step in a process that is irreversible and thereby forces a process to move in one direction. Let's see what this means. We will consider our Na<sup>+</sup> / K<sup>+</sup> pump.

- Recall that like all proteins, the pump has a certain shape or conformation. In this case it is a quaternary protein.
- Recall also that many proteins are very sensitive to environmental changes in that such changes can induce conformational changes. Such changes are also called **allosteric changes**.

OK, here we go (**see Campbell, p136 (8<sup>th</sup> Ed) fig 7-16** -- what I give is a bit more detailed.

- Our pump is a transmembrane protein. On its inner face is a place for Na<sup>+</sup> to bind .
- On the inside, in a place different from that for Na<sup>+</sup> is a binding place for ATP.
- If no Na<sup>+</sup> binds to the pump protein, it assumes a shape that also gives it very low affinity for both ATP and for K<sup>+</sup> . Thus, even when ATP

and  $K^+$  are present (which they usually are) they will have trouble binding.

- If internal  $Na^+$  gets high enough, it starts binding to the  $Na^+$  sites on the pump.

Why “high enough concentration” – the reason is that the pump has an affinity, defined like  $K_M$  for  $Na^+$  and  $K^+$ .

- By the time three  $Na^+$  ions have bound, the protein has undergone a conformational change. After all,  $Na^+$  are a charged particles and it should be easy to imagine that they would cause the protein to change its shape. The result of this shape change is to increase the affinity of the ATP binding site.
- The addition of ATP, a large, very charged molecule, once again induces another conformational change in the pump protein. This conformational change makes the protein a good catalyst and it breaks ATP into ADP and  $P_i$ .
- The result of the reaction is yet another conformational change. This causes the release of ADP while the  $P_i$  remains attached to the pump.
- The loss of the ADP once again triggers a significant conformational change -- after all, a very charged, large molecule has partially left the pump (when the ADP is released). This change causes the protein to translocate the  $Na^+$  to the outside.
- As the translocation is completed, the affinity of the protein for  $Na^+$  decreases
- Guess what happens next -- the release of the  $Na^+$  increases the affinity of the molecule for  $K^+$  and so two  $K^+$  from the outside bind.
- Once this happens, another conformational change occurs and the phosphate is lost.
- This results in another conformational change that translocates the  $K^+$  into the cell. When it is released, the final conformational change occurs that gets us back to the original shape. The cycle is now ready to start over.

A couple of crucial **final comments**:

1. Every step (each transition from one shape to another) except for:
  - the translocation (with a large positive  $\Delta G$ ) and
  - the hydrolysis of ATP (a large negative  $\Delta G$ ) is easily reversible.

In other words, most steps can go one way almost as easily as the other. One step, the translocation, considered in isolation, is relatively likely to go – but in the opposite direction than is useful to the cell. Thus, the translocation is more likely to bring in  $Na^+$  and kick out  $K^+$ .

2. **The free energy of the hydrolysis of ATP is even greater than the translocation.** Since the ATP system is so far from equilibrium and

since there is an enzyme that catalyzes its breakdown that is part of the pump (and therefore coupled to the shape changes of the pump), **the entire process must move in one direction -- expel Na<sup>+</sup> and bring in K<sup>+</sup>.** If this step was not included, the "pump" protein would move Na<sup>+</sup> in and K<sup>+</sup> out.

**Let's see what you understand beyond a mechanical description of the operation of the pump:**

Assume that the concentration of K<sup>+</sup> was the same inside and outside and the concentration of Na<sup>+</sup> was also the same inside and outside.

- (i) What is  $\Delta G$  for the translocation of these ions across the membrane in opposite directions (answer at bottom of page).  
(ii) if  $\Delta G$  for the hydrolysis of ATP = 0 kJ, which way would the pump go?
- For the same situation, assume  $\Delta G$  for the hydrolysis of ATP was -42 kJ/mol.  
(i) Which way would the pump operate?  
(ii) Which is the only essentially irreversible step (see the steps given above) when the pump first starts to operate?
- Now suppose the concentration gradients are established (more Na<sup>+</sup> out than inside and more K<sup>+</sup> inside than outside) and the free energy associated with moving ions against this gradient is  $\Delta G = + 20\text{kJ/mol}$ .  
(i) Which way will the cycle operate and why?  
(ii) As more ions are pumped, what happens to the  $\Delta G$  value (qualitatively)?  
(iii) Assuming that the  $\Delta G$  for the ATP system remains at -42kJ/mol – what will be the total  $\Delta G$  of the K<sup>+</sup> and Na<sup>+</sup> gradients when pumping stops?
- If we lowered the ATP/ADP ratio such that the  $\Delta G$  for the hydrolysis of ATP became zero, and if we kept the concentration gradients so that K<sup>+</sup> was more concentrated inside, Na<sup>+</sup> more concentrated outside and the  $\Delta G$  for their movement "uphill" (Na<sup>+</sup> out and K<sup>+</sup> in) was  $\Delta G = +20$  kJ/mol, what would happen?

**Answers**

- (i)  $\Delta G = 0$  – there are no concentration gradients.  
(ii) Neither way – it would be as likely to move ions in either direction. The system is at equilibrium.
- (i) It would pump Na<sup>+</sup> out and K<sup>+</sup> in since the hydrolysis of ATP would force the pump cycle to move in that direction.  
(ii) The hydrolysis of ATP – since that step is irreversible and since it is coupled to the other steps in the pump, it will force the overall cycle to move in the direction of pumping K<sup>+</sup> in and Na<sup>+</sup> out.
- (i) The overall (coupled)  $\Delta G$  is  $-42+20 = -22\text{kJ/mol}$  – pumping will continue.  
(ii) It becomes more positive (since the gradients are getting larger and movement up gradients is becoming more improbable).  
(iii) +42 kJ/mol
- K<sup>+</sup> and Na<sup>+</sup> would move in the directions for diffusion and ATP would be synthesized from ADP and Pi – the "pump" would run backwards using the ion gradients as fuel. Notice that this is analogous to what ATP synthase of the mitochondria does!