

## BIOENERGETICS

As we understand them, cells are incredibly well-designed, self-sufficient microscopic machines. They must therefore be ruled by the same principles that govern the function of all physicochemical systems. Specifically, they must handle energy in the same way as all man-made machines.

These rules, or *laws*, were studied and well-established by scientists analyzing simple steam machines and were formally developed as a branch of physical science known as thermodynamics.

Fortunately for us, the three laws of thermodynamics are quite simple to understand and can be formulated with very little mathematics. Even more fortunate for our purposes, we only need two of the three laws.

### The First Law of Thermodynamics

The *first law of thermodynamics* says that the energy cannot be created or destroyed, though it can be converted into various forms, including potential energy, kinetic energy, chemical energy, electrical energy, and heat. This *conservation of energy law* is very familiar to all of us; it is not exclusive to the field of thermodynamics. We all know that potential energy can be transformed into kinetic energy as a solid body falls down and that a piece of wood will burn, thereby converting chemical energy in heat.

The way this law is expressed is simple: In any process, the energy of the universe remains the same. The same applies to any isolated system that does not exchange matter or energy with the environment. This can be expressed as follows:

$$\text{ENERGY}_{\text{at the end of a process}} = \text{ENERGY}_{\text{at the beginning of the process}} \quad (1a)$$

or

$$\text{ENERGY}_{\text{at the end}} - \text{ENERGY}_{\text{at the beginning}} = 0 \quad (1b)$$

or

$$\Delta E_U = 0 \quad \text{where } E = \text{energy and } U \text{ is an isolated system (the universe, for example)} \quad (1c)$$

The Greek letter “delta,”  $\Delta$ , symbolizes the difference between the final value and the initial value of a property of a system as a result of a process. In this case, it indicates that the change in energy

### The Second Law of Thermodynamics

The second law is equally simple to formulate. It says that the *entropy* of the universe increases or remains the same in any spontaneous process. The same applies to any isolated system that does not exchange matter or energy with the environment.

$$\text{ENTROPY}_{\text{at the end}} \geq \text{ENTROPY}_{\text{at the beginning}} \quad (2a)$$

or

$$\text{ENTROPY}_{\text{at the end}} - \text{ENTROPY}_{\text{at the beginning}} \geq 0 \quad \text{or} \quad \Delta S_{\text{U}} \geq 0 \quad \text{where } S = \text{entropy} \quad (2b)$$

This law is also simple and easy to formulate, but is not as familiar to us as the first law, primarily because it involves the term *entropy*. However, we will see that entropy can be thought as a measure of the probability of a state. Then, the law says something very intuitive: the universe moves to more likely conditions.

Example. Suppose we have a deck of 52 cards. If taken one card at a time, the cards in the deck can be ordered in a great number of ways –  $8 \cdot 10^{67}$  ways, in fact! From this large set of arrangements, we would consider only a few as “ordered” (for example, all the suites separated, and increasing from ace to king). All of the other arrangements – that is, the immense majority of them– correspond to “disordered” arrangements. Intuitively, we would say that the “disordered” state is more likely; that is, it has higher probability than the “ordered” state. The second law says that if we take an “ordered” set of cards and throw it away, when we recover the 52 cards, they will be “disordered”, which is exactly what any of us would had predicted. Note, though, that the second law does not exclude the possibility that a system can go from disorder to order, but then the system must not be isolated and the sum of the change of entropy of the system plus that of the environment must be positive. After all, a set of cards can go from a disordered to an ordered arrangement, but only if somebody is willing to order the deck in a specific way.

We can agree, then, that disordered states are more probable than ordered states. Having your closet ordered requires a given arrangement of your clothes. Any other arrangement is likely to be labeled “disordered” by your mother. The second law explains why, if you not employ energy in keeping it ordered, it will evolve to a disordered state.

### **The Paradox of Life**

Living organisms are systems characterized by an extremely high degree of order. If you take all the molecules corresponding to a cell and mix them together, it would be very unlikely that you would obtain a living cell. Only a very few highly ordered arrangements of these molecules will render a cell; hence, according to thermodynamics, life is a very unlikely state. However anybody knows how difficult is to keep things sterile; our culture media get contaminated if we are not very careful handling them. And our food spoils even in the refrigerator. Yet there is life in the deep sea, in the deserts, and mountains. Life seems to blossom everywhere on our planet, even though living organisms are highly ordered and hence thermodynamically improbably. In the next several sections, we will try to solve this paradox.

### **Molecular Thermodynamics in a Box**

To understand how cells handle energy, we need to have an intuitive idea of the molecular basis of the laws of thermodynamics. The formal way to connect molecules and thermodynamics is called statistical mechanics; however, we will try to maintain an intuitive understanding of these concepts. For this, we need a simple macroscopic system that we can easily visualize and that behaves as a set of molecules exchanging energy.

Imagine a system containing small balls that are in constant movement because a stream of air is blowing from below (Figure 1). In the four boxes from left to right, the stream of air is blowing with increasing speed.

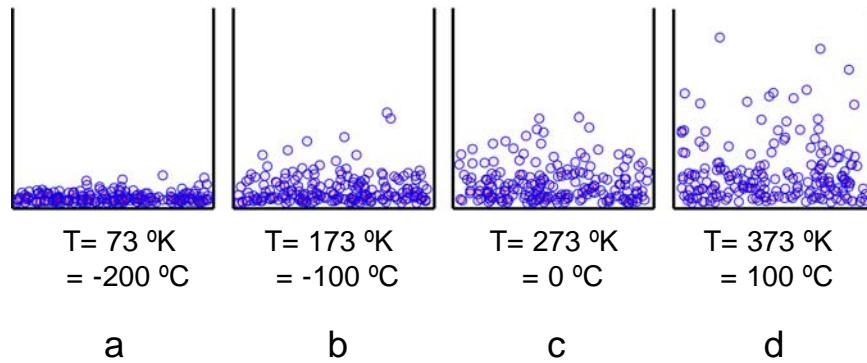


Figure 1

Think of the balls in each of the four boxes as the equivalent of molecules in this simulation and imagine them dancing with increasing energy as the “wind” coming from below becomes stronger. The wind in this system is equivalent to the *absolute temperature* in thermodynamics (expressed in Kelvin degrees, °K, which is equal to °C + 273). The higher the temperature, the more kinetic energy the molecules in a system will have. Notice that there are always more balls in the lower levels of the box (minimum energy levels). As the temperature (i.e., the air stream in our analogy) increases, more balls will be found higher in the box (at higher energy levels); however the lower levels are always the more populated.

These balls (i.e., molecules) are populating different energy levels. As the temperature increases, higher energy levels are reached. When the temperature is zero Kelvin degrees, all the balls stay at the bottom of the box, which is the lowest possible energy level. The number of molecules at different energy levels follows what is called a “Boltzmann distribution”.

### Energy and Entropy in the Box

Imagine the same box but now divided in two sectors (Figure 2a). Note, however, that the dashed line between the sectors is *not* a barrier to the balls; it is simply a means of distinguishing between the two sectors. As before, the molecules are dancing around as they are moved by the air. In this box, the two sectors have the same bottom area and the number of molecules in each part of the box is, on average, the same.

In the second box (Figure 2b), the sector at the left is smaller (smaller bottom area), and the

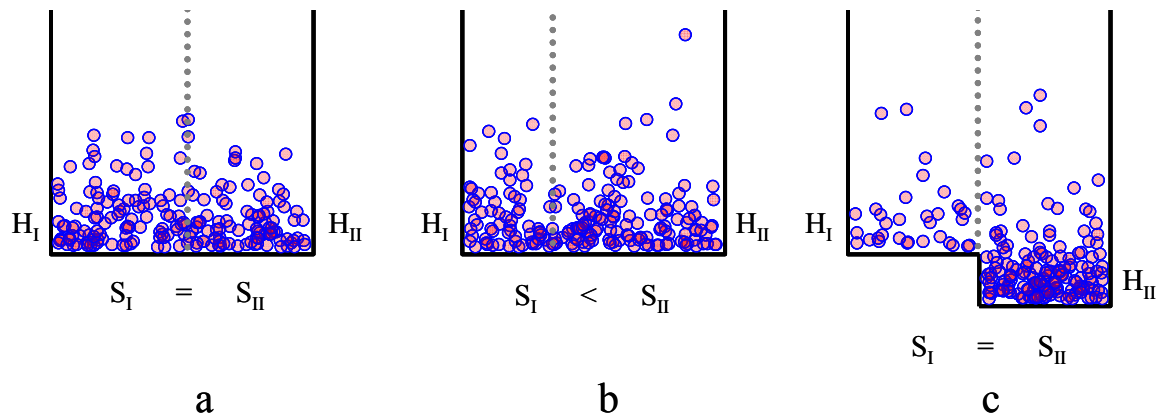


Figure 2

number of molecules in this part of the box is smaller. Why? An even distribution of balls in different areas of the box is much more probable than any other arrangement where balls accumulate in special regions. Assuming an even distribution, the smaller sector will have fewer molecules. (This may seem a very trivial conclusion, but you must realize that you are visually grabbing the difference in entropy of both sectors.) In the third box (Figure 2c), the area is the same, but the floor of the sector at the right is at a lower level (lower energy). Now if the molecules move freely between the two sectors, it is intuitive that the left sector will have fewer molecules. Movement to the left is “uphill”; only the balls at the right that have jumped higher than the difference in the floor levels between the two sectors can move to the left sector. In contrast, movement from left to right is “downhill” and does not require overcoming a difference in energy.

In these boxes, entropy is represented by the floor area of the sector (large areas means large S values) and the energy in the different levels of the floor. A low floor level means a low *enthalpy* and a high floor level means a high enthalpy. This quantity (identified by the letter H) is the preferred expression for energy in most biological systems.

By looking at the molecules dancing in the box, we can say that the molecules will have a tendency to move toward sectors with larger areas and lower floor levels. Thermodynamics formally establishes this by saying that all systems move toward conditions with lower *free energy*, represented by the letter G.

$$\Delta G = \Delta H - T \cdot \Delta S \tag{3}$$

Where        G = free energy  
                   H = enthalpy  
                   S = entropy  
                   T = absolute temperature in K°

It is an important principle of thermodynamics that *all spontaneous reactions and processes proceed in the direction of a free energy decrease.*

### Free Energy and Work

Next, we need to realize that not only the sign but also the magnitude of  $\Delta G$  has a meaning (Figure 3).

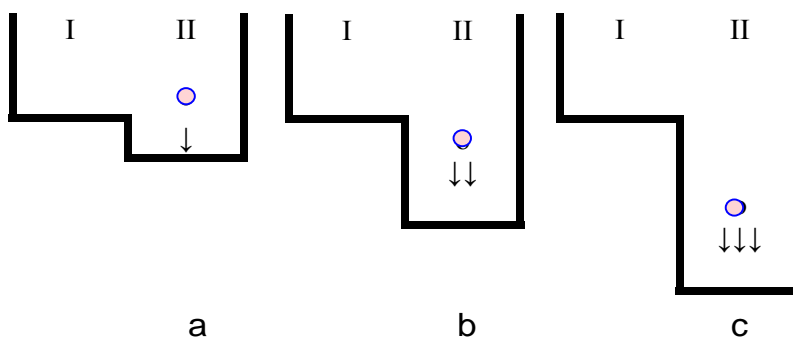


Figure 3

If you pay attention to a ball falling from a sector with a high floor level to a sector with a low floor level, you will see that it is accelerated as it falls. The larger the difference in floor level, the faster will be the speed of the ball when it hits the floor or another ball in

the low-energy compartment. The speed of the ball represents *kinetic energy*, which can, at least

in principle, be used to generate work. For example, if the ball were to hit a wheel, the wheel can spin and if the wheel is appropriately harnessed, it can be used to generate electricity or move a weight. In the absence of a wheel – i.e., with no means of using the kinetic energy of the ball – it will bounce onto the walls and other balls until the energy is lost as heat. In summary,  $\Delta G$  indicates how much work can be extracted from a reaction or process. The term free energy, refers to how much energy is available (i.e., is “free”) to do useful work.

### Building Boxes That Represent Real Processes

To illustrate the usefulness of our box models, let’s start with a simple process:



Water is a more disordered condition than ice because water molecules can move freely around in the liquid whereas they form stable bounds in the solid. At the same time, all the tethers among molecules make ice a state with lower energy (you need to add energy to the ice to break the bounds formed among water molecules). Hence, we build a box with larger area for the liquid and a lower floor level for the solid (Figure 4)

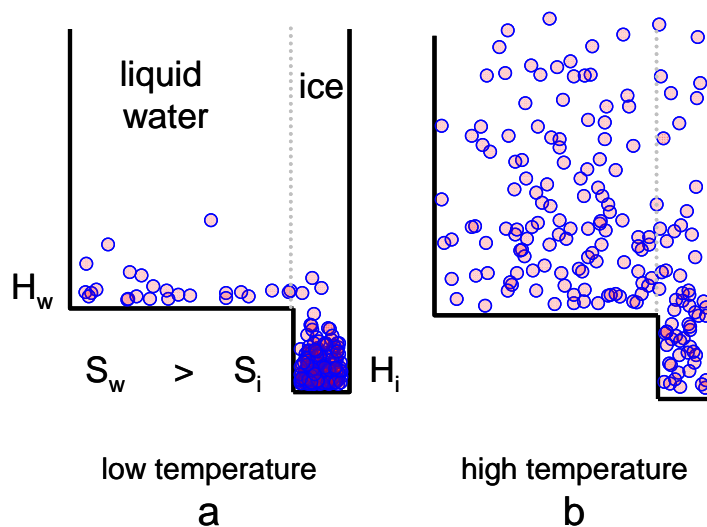


Figure 4

Suppose you decrease the temperature (i.e., less air is coming from below). Where will the molecules accumulate? Next, suppose you increase the temperature; now where will the molecules go? At low temperatures, the balls will fall down into the ice sector; the air does not have enough strength to move them back to the liquid condition. At high temperature the balls will move all around the box and most of them will be in the larger liquid compartment. Thus, the box correctly predicts the freezing of water at low temperature and the melting of ice when the temperature increases.

So, if we build the boxes correctly, we can visualize many processes intuitively, as the examples in Figure 5 illustrate.

## Examples

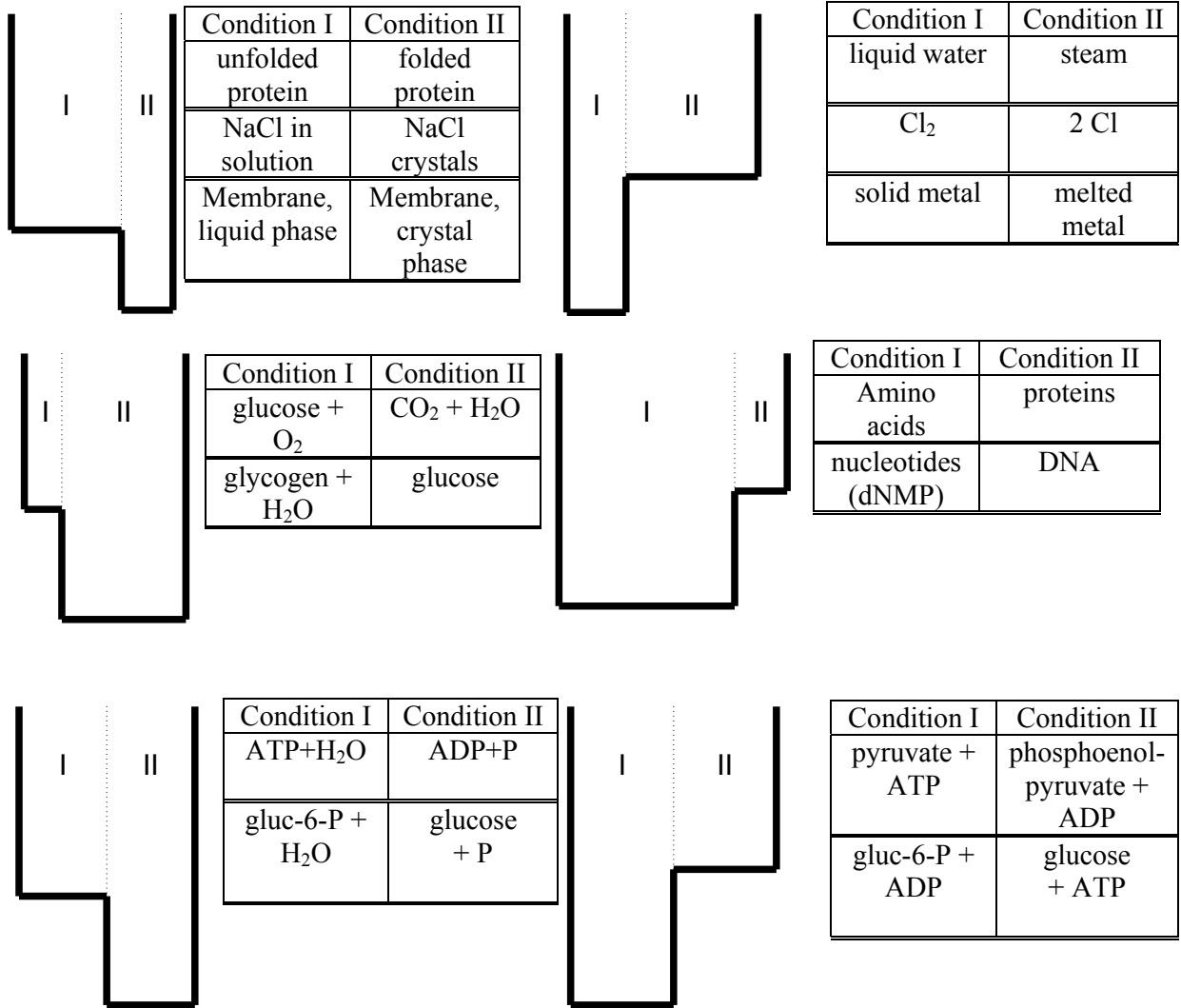


Figure 5

All H and S values are only qualitatively assigned. However, the boxes are useful because they enable us to predict intuitively which condition is more favorable in each case and what the effect of temperature is in the process.

## The Meaning of $\Delta G$ and $\Delta G^\circ$

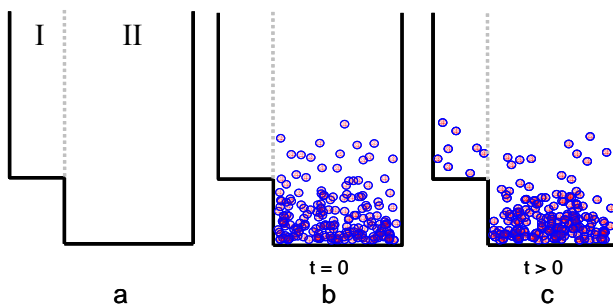


Figure 6

We met  $\Delta G$  in equation 3, where it was defined as *free energy*. Now we're ready to meet  $\Delta G^\circ$ , which is called the *standard free energy*.

In Figure 6, it is clear that there is a tendency for the molecules to move toward the II condition (lower H, higher S). However, suppose that we introduce all the balls in the II sector and then turn on the air stream (Figure

6b). We can readily understand that some balls will jump to sector I (Figure 6c). When all the balls are in one condition, there will be a tendency to populate the other.

In conclusion, the “shape” of the box tells us to which sector molecules will move when both states have the same amount of molecules. In this particular box, if both sectors have the same number of balls initially, many more molecules will be transferred from I to II than from II to I. This is called the standard free energy, symbolized by  $\Delta G^\circ$ . The real  $\Delta G$  will tell us to what sector the molecules will move depends on i)  $\Delta G^\circ$  (the shape of box) and ii) the number of molecules in each state.

We can now write an equation for the calculation of  $\Delta G$ , assuming that  $\Delta G^\circ$  is known, along with the values for R (the gas constant) and T (the absolute temperature in  $^\circ\text{K}$ ) and the concentrations of both the product(s) and reactant(s).

$$\Delta G = \Delta G^\circ + RT \ln [\text{products}]/[\text{reactants}] \quad (5)$$

where [products] and [reactants] are the concentrations of products and reactants, respectively, at a given point of a reaction. For example, for the reaction  $\text{I} \rightarrow \text{II}$ , [product] = concentration of II and [reactant] = concentration of I

Notice that if [product] is zero (i.e., only reactants are present) then  $\Delta G = -\infty$  (remember,  $\ln(0) = -\infty$ ) and the reaction will proceed to the right to generate products. In contrast, if only products are present, then  $\Delta G = +\infty$  and the reaction will proceed to the left.

### **Equilibrium and the Equilibrium Constant**

To summarize, if all the molecules are present on one side, a certain number will move to the other side. But notice that at a given point, the number of molecules moving from one to the other side is the same than the number moving back. At that point, the system has reached a condition where there is no further tendency to move from either side to the other. In other words, the system is at *equilibrium*. At this point,  $\Delta G$  will be zero.

If we look at equation 5 at equilibrium

$$\Delta G^\circ + RT \ln [\text{product}]/[\text{reactant}] = 0 \quad \text{then} \quad (6a)$$

$$\Delta G^\circ = - RT \ln K_{\text{eq}}, \quad (6b)$$

where  $K_{\text{eq}} = [\text{product}]/[\text{reactant}]$  at equilibrium,  $K_{\text{eq}}$  is called the *equilibrium constant*

It is intuitive that the number of molecules in each sector at the equilibrium (hence, the  $K_{\text{eq}}$ ) depends on the box shape. Equation 6b shows that there is a direct relationship between the equilibrium constant and  $\Delta G^\circ$ . Hence, the box shape determines  $\Delta G^\circ$  and  $K_{\text{eq}}$ ; whereas,  $\Delta G$  depends on the box shape and on the number of molecules in the sectors.

### **Activation Energy and Reaction Rate**

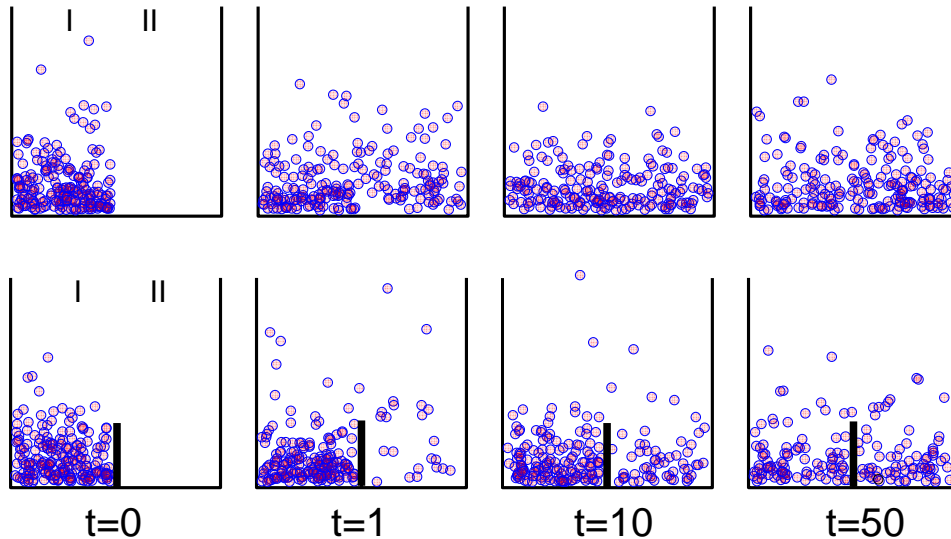


Figure 7

The next two concepts we need to understand are *activation energy* and *reaction rate*. Suppose we have the boxes shown in Figure 7. The top and bottom boxes are identical except for a small wall that separate sector I from sector II in bottom boxes. All the balls are in the left compartment when the experiment begins ( $t=0$ ). The air is turned on and the molecules start to move. In this experiment, you measure the number of molecules that are jumping from I to II per time unit (per second, for example). It is easy to see that, because of the barrier between the compartments, the initial rate of ball transfer between I to II is slower in the bottom box. Also, it will take more time for the bottom box to reach a point where the balls jumping back equilibrate with the balls jumping forward – that is, where it reaches equilibrium. Notice though, that the  $K_{eq}$  will be the same for both boxes (in this case  $K_{eq}=1$ ; the number of balls in sectors I and II is the same because they have the same area and floor level).

The interesting concepts here are:

1. The higher the wall, the longer the time required to reach equilibrium. The energy required to jump to the other side is called the *activation energy* and is represented by the height of the wall.
2. The activation energy does not change  $\Delta G^\circ$ ; hence, it does not affect  $K_{eq}$ . Notice that neither the area nor the floor level of the compartments is affected by the separation between the compartments.

Notice that up to now, we have not talked about time. Thermodynamics establishes whether or not a process or reaction will occur (depending of the  $\Delta G$  of the transformation) but it does not indicate how much time will be required for the process to occur.

However, time plays a central role for living organisms and the box representation of processes is useful to address some kinetic considerations. For example, it is easy to visualize that the activation energy will delay any process but will not change the equilibrium conditions that only depend on the shape of the box. It is also evident that temperature will speed up any reaction. For example, in the bottom box, the time to reach the equilibrium will be shorter if we increase the air stream. Notice, however, that temperature can also affect the equilibrium constant.

In summary, the rate of a reaction can be increased by i) decreasing the activation energy of the reaction or ii) increasing the temperature



## Bioenergetics

All the concepts that we have been working with using the box representation are valid for any system. We will focus now on some topics that are especially relevant for “living” machines, such as cells, with Figure 8 as our guide.

Figure 8a shows a typical reaction that occurs in almost all cells: the synthesis of glucose-6-phosphate (G6P).

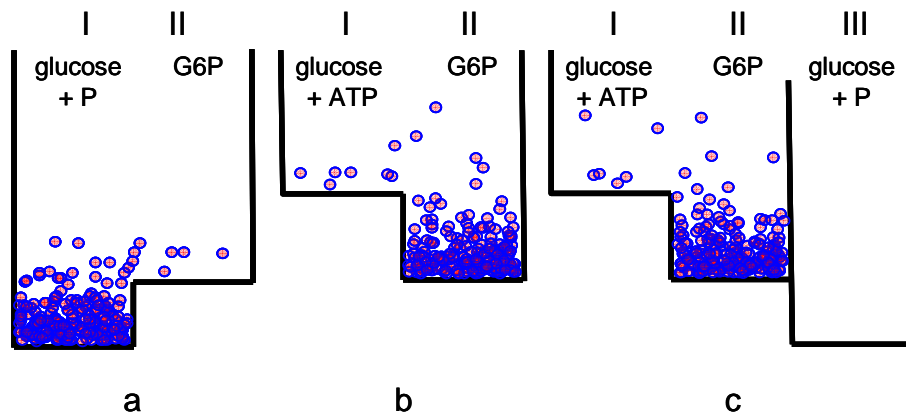
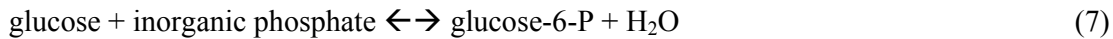


Figure 8

This is a reaction with a positive  $\Delta G^\circ$ . When the box is filled with the same amount of molecules in both compartments, it will proceed to the left, hydrolyzing glucose-6-P to form glucose and inorganic phosphate. When equilibrium is reached, there will be very little glucose-6-P left (Figure 8a).

To solve this problem, *i.e.*, to drive the synthesis of glucose-6-phosphate, the cell uses a high-energy molecule in replace of phosphate (Figure 8b):



Now the reactants have more energy than the products, and the box representation clearly shows that if we begin with ATP and glucose, the balls will accumulate in the glucose-6-P compartment.

In summary, to produce glucose-6-P, the cell requires:

- 1- ATP in the medium as a high-energy molecule capable of delivering phosphate.
- 2- A means of accelerating reaction 8. This is exactly what enzymes do. They can very efficiently decrease the activation energy of specific reactions, thereby accelerating the rate many orders of magnitude. Most cells have a specific enzyme that catalyses reaction 8 – *i.e.*, decreases the height of the wall between compartment I and II in Figure 8c. Most commonly, the enzyme is either hexokinase or glucokinase.
- 3- A means of preventing glucose-6-P hydrolysis. Most reactions have large activation energies and therefore do not proceed at measurable rates unless specific enzymes are present. Cells in general do not have an enzyme capable of accelerating the hydrolysis of glucose-6-P. Therefore, this reaction, although very favorable from the  $\Delta G^\circ$  point of view, occurs only very slowly, resulting in the accumulation of glucose-6-P. Notice the high

activation energy separating compartments II and III in Figure 8c. Liver cells are an exception: They do have an enzyme that decreases this barrier and are therefore able to release glucose from glucose-6-P to maintain sufficient amount of glucose in blood. (A third compartment was introduced in Figure 8c; balls move in this box as they do in the two-compartment boxes encountered previously.)

In conclusion, part of the answer to the apparent paradox of living organisms is that cells have enzymes that function in a variety of reaction pathways by lowering the activation energies of specific reactions, thereby guiding the flux of molecules in the desired direction. In addition, they can couple reactions with energetically favorable processes (*i.e.*, reactions with large negative  $\Delta G^\circ$  values), such as hydrolysis of ATP or movement of ions downhill electrochemical gradients; thereby driving metabolic pathways capable of producing high-energy end products.

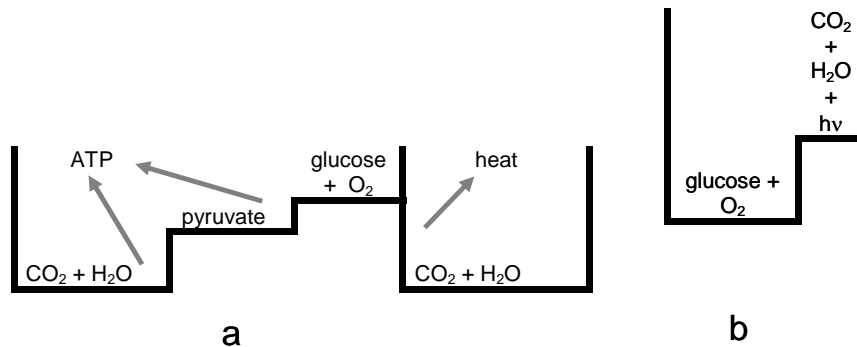


Figure 9

In the cell, energy is obtained by transforming chemical or light energy using complex sets of enzymes. For example, oxidation of glucose to  $\text{CO}_2$  is a very favorable reaction with a very large  $\Delta G^\circ$  (Figure 9a). However, the reaction has a large activation energy; you can store glucose for years at room temperature in contact with air, which has 21% oxygen, and oxidation will not proceed to any measurable extent. If you increase the temperature, the molecules will of course be able to overcome the activation energy and glucose will burn to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . However, cells must maintain a constant temperature and cannot simply burn energy supplies. Instead, the glycolytic pathway in the cytosol in combination with the TCA (tricarboxylic acid) cycle and the electron transport system in the mitochondrion produces the same end product, using a large percentage of the energy of the reaction to generate ATP (Figure 9a). Plant cells can drive molecules using a still more astonishing pathway: taking energy from sunlight, they manage to activate processes that synthesize glucose from  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , liberating oxygen in the process (Figure 9b).

### Bioenergetics: How to Handle the Entropy Problem

Figure 10a represents the process of DNA synthesis reaction:



This is an energetically unfavorable reaction with a positive  $\Delta G^\circ$  (Figure 10a). As we have seen previously, a way to solve this is to use high-energy molecules as reagents and this is what cells do. By using high energy nucleotides, the DNA synthesis becomes energetically favorable (Figure 10b).

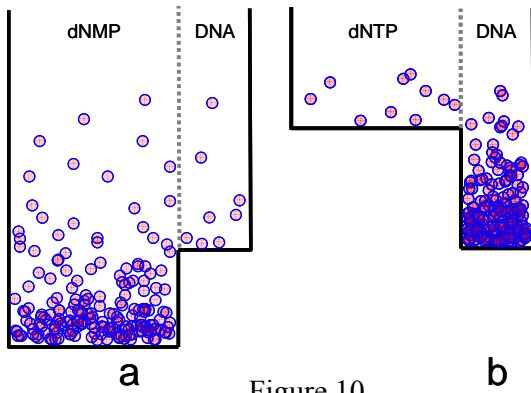


Figure 10

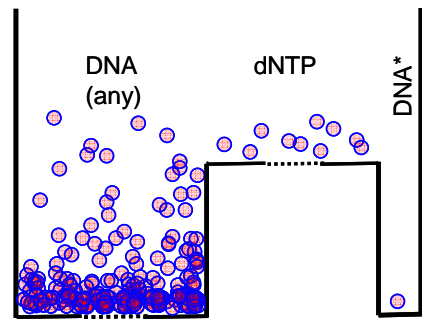


Figure 11

However, the cell must synthesize a very specific DNA, a unique sequence of nucleotides. The total number of different combinations for a small piece of DNA having 600 nucleotides is  $4^{600} = 10^{360}$ . For the entire DNA of a human cell, it would be  $4^{3000000000}$ . So, in fact, the representation for the synthesis of DNA should be a box as shown in Figure 11. The left sector represents the  $10^{360}-1$  incorrect combinations and the one on the right the single correct combination (DNA\*). Notice that the area on the left should be  $10^{360}$  times larger than the one at the right, something that is hard to imagine and impossible to plot (the punctuated lines on the left and middle sectors represent these huge entropic differences). So, although the energetic problem of synthesizing DNA can be solved by using dNTPs, the huge entropic problem is still there.

### Maxwell's demon and DNA synthesis

To address this problem we need to imagine the following experiment in our box representation, as shown in Figure 12.

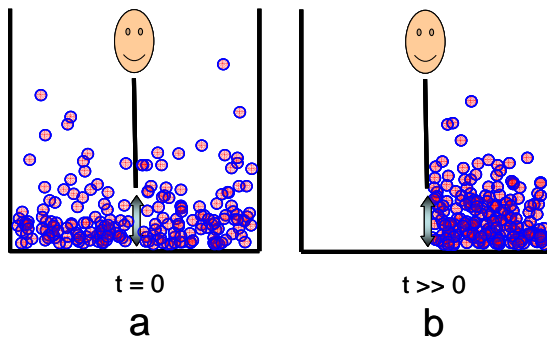


Figure 12

We start with a box with two identical sectors and balls dancing all around. At a given time, a very large activation barrier is built between the two compartments ( $t = 0$ ). At this point, the two sectors should have the same amount of molecules. Now, a Maxwell's demon is included in the system (this is a thought experiment introduced by Maxwell in 1867). The demon is able to open a hole in the wall when a ball is coming from the left and to keep the hole closed when a ball comes from the right. After a while, the right sector will have more balls than the left one. At the end, all molecules will be in the right

sector ( $t \gg 0$ ). This process seems to violate the second law of thermodynamics. We started with a disordered state (molecules equally distributed between the two compartments) and ended in a state where all molecules are in a single compartment. This paradox is solved by assuming that the demon has *information*: It could distinguish molecules coming from the left from molecules coming from the right. But what about the apparent violation of the second law? The answer is that information is not free and the energy required to obtain and use the information preserves the validity of this law. With this demon in the system, the more probable outcome is that, at the end of the experiment, all molecules are in the right compartment. In brief, the system

has moved to the more likely condition in the presence of a demon – with the necessary information, of course.

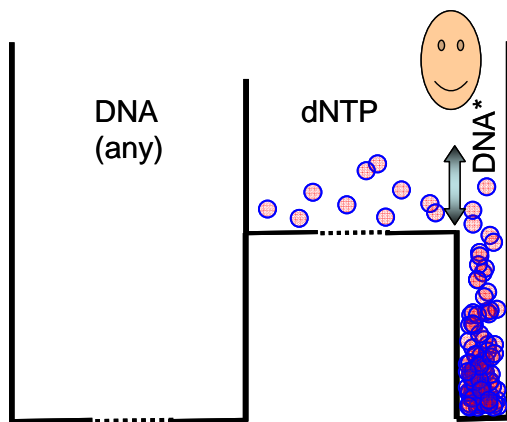


Figure 13

Let's go back to the box representing the almost impossible synthesis of a unique DNA molecule (Figure 13). The cell needs a demon with the right information to perform this Herculean task and the cell actually has this demon. Can you guess who the demon is? Exactly! It is the enzyme called DNA polymerase. The addition of a nucleotide to the growing strand has high activation energy; hence, it will not occur unless an enzyme can decrease this barrier. The DNA polymerase will only catalyze the incorporation of a new nucleotide if it matches with the template strand, which contains the information used by the demon. So from the  $10^{360}$  possible combinations, the polymerase will only decrease the activation energy for the synthesis of the

complementary strand. The synthesis of all other possible chains is prevented by the large activation energy.

In summary, all living organisms require both ENERGY and INFORMATION. Energy comes most commonly from food and solar radiation, and is accumulated inside the cell as high-energy molecules such as ATP and as electrochemical gradients across membranes, such as the  $\text{Na}^+$  and  $\text{H}^+$  gradients maintained in the plasma membrane and the inner mitochondrial membrane, respectively.

Where does the information come from? It comes from another living organism, generally the parents or the preexisting mother cell. The requirement of preexisting information justifies two basic and connected principles in biology.

- 1- Spontaneous generation of life does not occur (no information is available)
- 2- All cells comes from a preexisting cell (which carries the information)

In conclusion, in the presence of energy (for example, a nutritive culture medium) and information (for example, an healthy bacterium) life is not improbable, it is inevitable. Molecular demons carrying the necessary information force the output of what a priori would be very unlike events.