ENERGY TRANSFORMATIONS, LINKED PROCESSES AND PROTEINS*

Summary: This set of notes starts with a quick overview of thermodynamics and then proceeds to understanding energy transformation that occur when different processes are linked together. Understanding coupled processes, especially in terms of energy, is key to the understanding of physiology. One the cell and molecular level, proteins are responsible for linking favorable and unfavorable thermodynamic processes and so we will also consider them.

I. AN INTRODUCTION TO ENERGETICS

A. ENERGETICS: the study of how energy transformations take place and how these affect the organism.

A short review will be in order before we begin. You should be familiar with this material; if not, review it.

B. THERMODYNAMICS:

1. **First law**: matter/energy is neither created nor destroyed in any process, it simple changes form. (The law of conservation of matter/energy). Thus, we know that:

a. For mass: the total amount of material that enters an organism must equal that which leaves or which remains a part of the organism.

b. Likewise, for energy: the total energy obtained by food or via some physical means (e.g. sunlight, heat) must either remain stored in the organism or be lost to the environment.

2. **Second Law:** in any **SPONTANEOUS PROCESS** the entropy of the **UNIVERSE** must increase. By entropy, of course, we mean disorder. There are a number of ways to think about this process and it often becomes quite complex.

-- Being brief, we can say that entropy generating processes result in:

a. **greater numbers of particles** and/or

b. the **lowering of the potential energy of a system**. This frequently is the result of the energy being spread over a greater volume of space than previously, that is, leaving the system it was previously contained in.

In either case, the value of the entropy (S) of the system is expressed in units of energy per temperature, e.g., $\frac{J}{^oK}$.

C. EQUILIBRIUM

D. ENERGY, WORK AND EQUILIBRIUM

4. Potential energy, as you are quite aware, comes in many forms besides gravitational potential energy. Many of these have uses in biological systems. The forms that we will be most interested in include diffusive, chemical and electrical. Before we start, we should note a general assumption:

3a. Work = Free Energy

where we will assume that unless we say otherwise, energy and work can be inter-converted completely. Thus, if it takes X joules to create a certain concentration, electrical, chemical disequilibrium, then we assume that X joules are stored as a result of that creation. Now, as we just saw with the teeter-totter example there will also be cases where the transformation occurs at less than the 100% efficiency implied by 3a.
Also note that the general form of all of the energy/work equations is:

3b. ΔG = # units involved * a measure of energy (work) per unit

Hopefully this will be a bit clearer in a moment.

! **Reminder**: when a system can perform work on its surroundings it is convention to indicate this with a **negative ΔG**; positive ΔG values mean than any change requires that work be performed on the system.

(a) Let's start with diffusive free energy (joules):

$$\Delta G_D = -nRT \ln \frac{[\text{side 1}]}{[\text{side 2}]}$$

4a.

here ΔG_D is the free energy available when a system goes from a given concentration gradient or ratio, written as $(\frac{[\text{side 1}]}{[\text{side 2}]})$, to a situation where there is no concentration difference[1] , ***R*** is the gas constant (**R= 8.31 X 10 $^{-3}$ $\frac{\text{kJ}}{\text{(mol }^{\text{o}}\text{K)}}$**), **T** is the absolute temperature (kelvins), and **n** is the number of mols that. Thus, $RT\ln\frac{[\text{side 1}]}{[\text{side 2}]}$ gives the amount of energy released per mol of material that actually diffuses down a concentration gradient (or the energy stored per mol that needs to be moved up a gradient to create the ratio $\frac{[\text{side 1}]}{[\text{side 2}]}$ and **n** is the total number of mols that actually diffuse. Notice that equation #4 gives total energy, not energy per mol, which is the other common way of considering free energy changes (just leave out n if that is what you want).

Another form of equation 4a is one based on $\log_{10}$; it is useful since we are more used to thinking and working in a decimal system than a system of base **e**.

$$\Delta G_D = -2.3 * nRT \log \frac{[\text{side 1}]}{[\text{side 2}]}$$

4b.

(b) **Chemical Potential Energy**: Chemical work can be thought of as nothing more than the re-arrangement of chemical bonds. There is a potential energy change associated with the re-

[1] On a macro scale, of course.

arrangement and the total amount of the energy change will therefore be proportional to the total number of bonds re-arranged. This number will be determined by:

- a measure of difference between the starting ratio of products and reactants (called the reaction quotient (or sometimes, the mass-action ratio) symbolized by ***q*** or $\boldsymbol{Q_r}$) to their ratio when the reaction reaches equilibrium (given by the equilibrium constant $\boldsymbol{K_{eq}}$). This is given as $\boldsymbol{K_{eq}}$ **/** ***q***. Thus, the greater the disparity between these two measures (the more the initial system is displaced from equilibrium) the greater the number of bonds changed per mol of reactant that was originally present.
- Therefore, the number of mols of reactants present also matters

Here is the equation -- notice that it is set up exactly like the diffusion equation:

5.
$$\Delta G_C = -nRT\ln\left(\frac{K_{eq}}{q}\right) = -2.3 * nRT\log\left(\frac{K_{eq}}{q}\right)$$

! The reaction quotient (mass action ratio), ***q***, is defined mathematically the same way as the equilibrium constant, K_{eq}. Thus for the reaction we all learned in chemistry classes:

$$K_{eq} = \frac{[C]^c * [D]^d}{[A]^a * [B]^b}$$

(where A and B are reactant and C and D are product species), the mass action ratio is given as:

$$q = \frac{[C]^c * [D]^d}{[A]^a * [B]^b}$$

(continue on next page)

which looks just like the equilibrium constant.
So what is the difference? K_{eq} refers to the ratios of products to reactants at equilibrium, a situation of zero potential energy. On the other hand, ***q*** refers to any mix (ratio) or products to reactants -- *q* would usually not equal K_{eq} .

? With respect to equilibrium, if *q* is greater than K_{eq}, has the reaction reached equilibrium? If not, is it displaced towards the products or reactants of the forward reaction?

- For the reaction A <--> B which has a value of K_{eq} = 1.0, satisfy yourself that if the mass action ration is greater or less than 1.0 that **free energy will either be available or will need to be placed in the system to reach that value of *q***.
- When will the system contain no free energy? (See eq. #4 above)
- Do the same thing for a reaction with a K_{eq} of 10. (What is the difference between a systems with a K_{eq} of 1 vs. 10?)
- **Make a rough plot of the absolute value of free energy (ΔG) vs. the log of K_{eq} /*q*. What fact does a plot such as this emphasize?**

(c) **Electrical potential energy**: we have considered this one before. Energy in electricity is related to the number of separated charges and the electrical potential that exists between them. Thus:

6. $$\Delta G_E = -nz\mathfrak{F}E = -nz\mathfrak{F}\Delta E$$

where **n** is the number of particles moved, **z** is the charge on each particle, $\mathfrak{F}$ is Faraday's constant gives the number of charges in electrical instead of chemical units as the number of coulombs in one mol of charged particles, and **E** is the electrical potential through which the particles are moved.

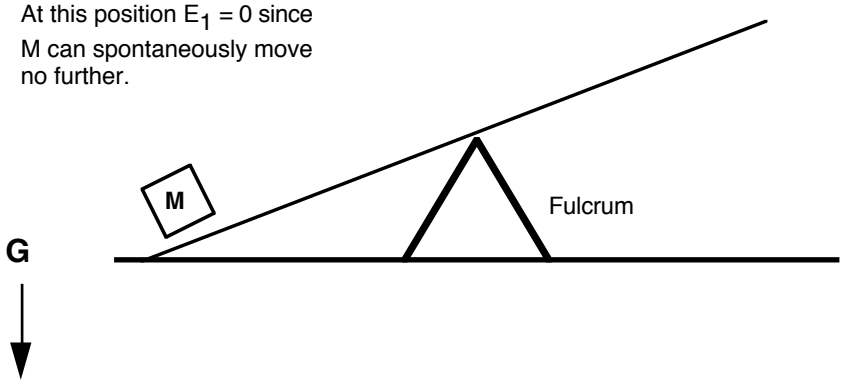
Anyone of these processes may in theory be used to drive any other. The only requirements are that one system must contain more potential energy than the other and that a suitable coupling agent exists. These agents will most commonly take the form of chemical intermediates (products of one reaction that are reactants in the next) or proteins, for example, enzymes or membrane transport proteins. However, we can (and will) also think of organs or our entire body as the coupling agents.

E. COUPLED PROCESSES: As far as biological systems go the more interesting part of the 2nd law relates to the fact that a process can be spontaneous if the entropy of the universe as a whole increases. This brings us to the important concept of **coupled reactions** or more generally, **coupled processes**:

a. A process that is thermodynamically unfavorable can be driven by one that is favorable.

b. A **coupling agent** is required to link the favorable and unfavorable process into an overall process. This agent can be just about anything, all that is required for its operation is that both processes (the spontaneous and non-spontaneous) utilize it, not necessarily at the same time.

Here's a simple example -- a lever is a coupling device. Suppose that an object with mass of M kg is on one arm of a first class lever[2] with mechanical advantage of one.:



[2] Don't panic -- there is no need to go back and review levers.

The object will not spontaneously lift itself into the air; this would require it to increase in potential energy and is forbidden by the second law. In fact, it would like to fall towards the center of the earth, this is a spontaneous process

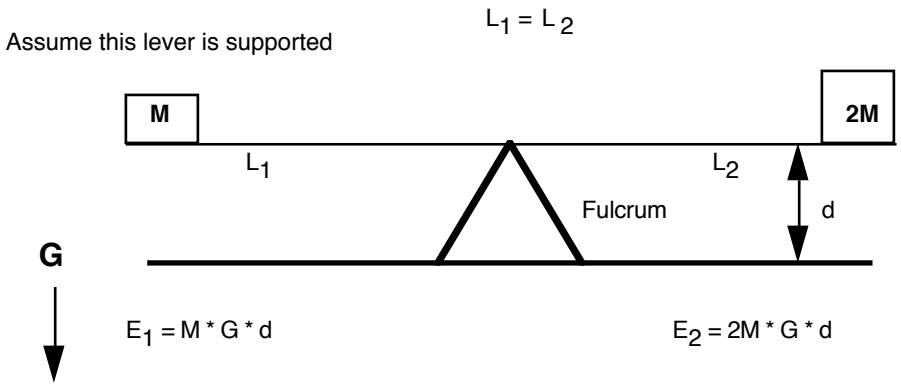
The potential energy (free energy) contained within this object is, of course, the product of its mass, the acceleration due to gravity, and the distance that it can fall; $E_1 = F * D_1$. Since F is due to mass M and the acceleration due to gravity G, then the potential energy of this system is:

1. $E_1 = M * G * D_1$

Suppose that we have a second object with mass 2M kg. The potential energy of this object will then be $E_2 = 2M * G * D_2$

Notice that $E_2 > E_1$. Now, let's assume that both masses are at the same height so that $D_1 = D_2 = D$. It is possible to simply allow the second mass to fall distance D and release its potential energy. In so doing, it would not affect the first mass. However, since the potential energy (free energy) of mass#2 is greater than mass#1, *it is also possible to use some of this energy to do work on mass #1*. This will only be possible if there is some mechanism to **couple** the release of potential energy by the movement of mass#2 with a gain in potential energy by mass#1. One example of such a coupling device is a simple lever.:

The Principle of Coupling Illustrated by a Lever



Now, suppose that both of the arms of this lever are of equal length. If we stop supporting the arms, movement will occur and useful work will be done on Mass M (#1). The energy for this work comes from gravitational pull on Mass 2 M (#2). Notice, that without the lever, this transfer of energy from a spontaneous process (the movement of mass #2 towards the earth) to a non-spontaneous process (the movement of mass#2 away from the earth) would not be possible.

? How much has the potential energy of masses #1 and #2 **changed**? Assume that movement was 1 m and use (-) to indicate a decrease in E and (+) to indicate an increase.
How much has the potential energy of the **entire system changed**?
What does this change have to do with the requirements of the second law?

? How efficient was this system at doing useful work? The only measure of efficiency that we will use is that of mechanical efficiency -- for this system, it is defined as:

eq. 2. $$\text{Efficiency} = \frac{\text{Conserved Energy (useful work)}}{\text{Expended Energy (work done)}} * 100$$

Identify the quantities for the numerator and denominator for our system and calculate its efficiency.
What happened to the energy that wasn't conserved by raising the light arm?
Define equilibrium in terms of energy. Adjust the lever system we have been discussing so that it is at equilibrium. Explain. How much useful work can this system do?

C. COUPLING AND METABOLISM: We will treat metabolism (broadly -- the sum of all chemical reaction in the body) in detail later in the course. But some notions relating to metabolism will be useful now, so let's look

1. Metabolism is usually viewed as the sum of two processes: CATABOLISM and ANABOLISM (+ movement).

a. **Catabolism** -- reactions that break down complex compounds often with the purpose of energy release with the goal of temporarily conserving some of the released energy in compounds such as ATP.

b. **Anabolism and Movement**: reactions that use energy to join relatively simple compounds into more complex ones (ex: protein synthesis, DNA synthesis, etc) or which produce motion.

Catabolic processes are thermodynamically favorable, anabolic and movement processes are not. So how do we ever get any increase and complexity and/or movement. The answer is of course, its magic! Just kidding. The answer is **coupling**. It is the only way it is possible for us to synthesize complex molecules and grow.

For example, the addition of a glucose molecule onto a molecule of glycogen, a process that results in a larger and more complex glycogen molecule is not thermodynamically favorable:

7. glucose + glycogen (n) + energy -----> glycogen (n+1)

1. This is obviously not a spontaneous reaction -- the potential energy of the system increases and the number of particles decreases with the synthesis of glycogen. K_{eq} is considerably less than 1.
Obviously this is an example of a class of reactions that result in greater complexity: they are collectively referred to as **anabolic reactions**.

A moment ago we referred to **CATABOLIC** reactions very broadly. . A good example is the hydrolysis of Pi from any nucleotide tri- or di-phosphate (such as ATP, GTP, UTP or ADP, to name a few)

8. ATP ----> ADP + Pi + energy **or** UTP ------> UDP + Pi + energy

NOTE: You will often see the statement that the "bond energy" for this reaction is ~-30.54 kJ/mol (-7.3 kcal/mol) It is important that you understand what this means.

... Chemical work refers to the number of bonds that are broken or formed. The more such formation, the more total energy change that will occur. The energy change involved in the formation of each bond (of a certain type) is constant, but the more a reaction is displaced from equilibrium, the more total reactions that will occur in achieving equilibrium. Thus, the further a reaction system is displaced from equilibrium, the more reactions will occur in moving towards equilibrium and the more the total change in potential energy. Therefore, the "bond energy" changes with the concentrations of products and reactants (see equation #4 above). Obviously the quantity that changes is not the energy per bond but the total number of bonds that are formed or broken since q changes as product and reactant concentrations change.
... The potential (free) energy change for the breakdown of ATP mentioned above (-30.54 KJ/mol) is for standard conditions. This is defined as when all the products and reactants are all in 1 molal concentrations, pH = 7 and temperature = 298 kelvins. The potential energy of any such a system is termed the standard free energy, $\Delta G^{0'}$ (delta G zero prime). Note that the gas constant, *R*, is 8.31 J **mol^{-1}** * °K

Problem: assume K_{eq} = 230,125 mols for ATP <----> ADP + P_i , further assume that the actual cellular concentrations are:

[ATP] = 3 mM, [ADP] = 1mM, [P_i] = 10 mM (ignore the concentration of water)

Find the potential energy of the cellular ATP system at 25^{o} C. ***Hint: be sure to put concentrations in mols (molal here) and be careful with units – final answer needs to be in kJ.***

Ans.: ~ -44.7 kJ/mol. Explain why this makes sense.

2. The hydrolysis of ATP is obviously a very thermodynamically favorable reaction. Notice that it results in a decrease in potential energy and an increase in the total number of particles; both processes indicative of increases in entropy. This is reflected in the large, negative ΔG (see box above).

3. By **COUPLING** the two reactions shown above (#s 6 and 7), the favorable reaction (hydrolysis of ATP) can be use to drive the unfavorable reaction:

9. a. glucose + ATP ------> glucose-6-phosphate + heat
   b. glucose-6-phosphate --> glucose -1- phosphate
   c. glucose -1 - phosphate + UTP --> UDP-glucose + 2 Pi + heat
   d. UDP-glucose + glycogen (N) ---> glycogen (N+1) + UDP + heat

For later this semester -- notice that 2 ~P bonds are used to attach one glucose to glycogen. Notice once again that chemical work is being done -- molecules are being rearranged.

In this case the coupling agents are enzymes that perform each of the two reactions; the separate reactions are coupled by common products and reactants.

4. An important note on terminology: the overall reaction shown above (using UTP to synthesize glycogen is referred to as ANABOLIC even though it has a catabolic component (the hydrolysis of UTP)). Likewise, glycolysis results in the synthesis of ATP but it also degrades more complex substances in doing so and is therefore referred to as CATABOLIC. Thus, in naming a

pathway anabolic or catabolic, the trend is to look and see what happens to the complexity of rather long-lived compounds (such as glycogen or glucose) and ignore what happens to intermediates such as ATP or NADH.
Note that as with the lever, a physical agent is required to tie the anabolic (unfavorable) and catabolic (favorable) process together. In this case, the physical agents (as will be typical at the molecular level) are enzymes. Reactions 9a and 9c (last page) are the coupling reactions.

C. EQUILIBRIUM -vs.- STEADY-STATE

1. **EQUILIBRIUM:** Generally, all of your training in chemistry and physics has dealt with equilibrium systems. These can be characterized as:

a. closed systems where under favorable conditions a net (macroscopic or forward) reaction will proceed to the point predicted by thermodynamics.

b. From there on no further change will be noted since forward and reverse reactions will be equal. We classically write such reactions as:

9. aA + bB <-----> cC + dD

and define the equilibrium situation in terms of the concentrations of the various chemical species:

10. $$K_{eq} = \frac{[C]^c * [D]^d}{[A]^a * [B]^b}$$

c. **Equilibrium is often an adequate concept for the analysis of <u>individual</u> biological reactions.** Reactions are often either at equilibrium or very close to it.

d. However, equilibrium is not a suitable way to view the organism as a whole. The presence of:

1. large numbers of potential energy (PE) rich, structurally complex molecules, -- and --

2. continual, measurable reaction rates

both imply strongly that organisms are not at equilibrium. This should be obvious since the 2nd law predicts that the spontaneous breakdown of complex, high PE materials will be favored.

e. **Thus, for any organism to maintain a complex structure, it will require a constant input of energy and materials just to maintain itself.**

1. Closed systems at equilibrium by definition do not add new materials. For a biological system, we can equate a prevalence of reactions at equilibrium with death.

2. We know that the only way to continue a reaction indefinitely is to add new materials to the system and remove the wastes. This is precisely what living organisms do.

D. REMINDER -- STEADY-STATE: in its simplest sense, a steady-state implies that the system is maintained at some constant distance from equilibrium by the constant addition of reactants, removal or products or (usually) both.

1. Due to the flux of materials and energy into, through and out of a steady-state system, its fundamental differences with an equilibrium system are:

a. it is OPEN instead of closed

b. It constantly does work (a system at equilibrium does none). The amount of work that can be done is a function of the distance the system is displaced from equilibrium. The mathematical descriptions of this work have been discussed previously (see equations 4a and b and the problems and discussion related to them)

2. With living systems, the position of the steady-state is usually always changing and so it is best to think of organisms as having a **DYNAMIC STEADY-STATE.**

E. KINETICS: The second law can be used to predict whether or not a reaction can occur as written or whether it needs to be coupled to some other reaction. However, it says nothing about the rate of reaction. This falls under the province of **KINETICS**, we will not consider it any further here except to state that the kinetics of biological reactions are primarily controlled by three factors:

1. **CATALYSTS** in the form of enzymes (usually proteins; very rarely RNA). These are the most important single factor in determining reaction rate. Nearly all biologically important reactions are catalyzed by enzymes and are tightly controlled by them. The ability to synthesize various enzymes and the timing of synthesis is, of course, under genetic control.

The most important things to remember about enzymes in addition are that the lack of a particular enzyme will result in the inability of an organism to perform a certain task.

2. **Temperature** -- Up to a point, increases in temperature provides additional activation energy and increases the frequency of collisions between participants in a reaction. More will be said about this when we discuss temperature in a few days.

3. Direct, chemical addition of **activation energy** as in the example of glycogen synthesis above where the addition of energy contained in the outer high-energy phosphate bond of UTP to glucose resulted in a change in its electronic structure and made it more reactive.

? Using the equations and data given above (see the earlier box with the ATP free energy problem), **calculate the largest concentration difference a living cell could produce for a non-charged solute species using the ATP energy system. (Remember to use the actual energy value of the ATP system, not the standard free energy value). Express your answer simply in terms of the number of times the particle is more concentrated on one side of the membrane compared to the other.**

Can cells really concentrate materials to this amount? If not, explain any discrepancies between your calculation and typical concentration factors of 10 to 1000X. Why would there be a difference?

How would you make the calculation for a charged species?

Note: ignore the **n** term in the diffusive work equation; we will assume that for each particle transported, one ATP is used.

Could an ATP-powered system transport particles in either direction (i.e. in or out of the cell). Explain.

What will happen to the cell's ability to produce a concentration gradient if the cell's ability to produce ATP from ADP and Pi is lessened?

Quite aside from whether or not a cell would actually concentrate a substance such as glucose to the extent that it is theoretically possible, address this question:

In some cells there are very large stores of carbohydrate -- most of the glucose is in a non-soluble starch form, **glycogen**. According to eq. #8 (these notes) all that is required **to make glycogen is the use of one ~P per glucose added** to the glycogen.

? What does adding glucose to glycogen do the thermodynamic requirements of putting a certain amount of glucose into a cell -- assume in transport (independent of glycogen synthesis) that one ATP is required per glucose transported in (-- this is roughly correct although the actual process is indirect)

Be careful -- think about the costs carefully the following ways:

- cost of getting a certain amount of glucose into the cell if the intracellular concentration is kept low as it would be if glycogen is made
- cost of getting the same amount of glucose into the cell is intracellular concentration of dissolved glucose is high
- costs of making glycogen
- other advantages to putting glucose into glycogen.

Do your best -- this is a tough problem but will be very useful to us in understanding some of the things organisms are really doing in contrast to what we might initially want to think they are doing!!