Recitation Section 4 Answer Key
February 14-15, 2005

Biochemistry—Energy and Glycolysis

A. Why do we care

In lecture we discussed the three properties of a living organism: metabolism, regulated growth, and replication. Today we will focus on metabolism and biosynthesis.

1. It was said in lecture that chemical reactions are the basis of life. Why do we say that?
   Being alive implies being able to change your state in response to a change in internal or environmental conditions. We discussed previously that any change in the observable characteristics of a cell begins as and is propagated by molecular interactions. Molecular interactions propagate the signal by changing the state of molecules, i.e. by reactions.

2. Why is metabolism required for life?
   A cell is subject to all laws of chemistry and physics, including the first and second law of thermodynamics. Changing the state of molecules dissipates energy along the way, so getting or making new energy is essential to life. The energy is then used for many purposes, including making the building blocks and precursors and then using them to build macromolecules that make up cells—biosynthesis.

3. Can an entity that performs no chemical reactions be considered "alive?"
   In general, no. But there are special cases of the cells that do not perform reactions right at the moment, but have the potential to perform reactions if they encounter particular conditions. Some examples of such cells are spores in nature or frozen permanents in laboratory. If they experience certain conditions, such as availability of food for spores, or defrosting and food source for frozen permanents, these cells will again perform metabolism.

4. Most reactions necessary for life are unfavorable, or do not proceed at an appreciable rate under physiological conditions. How do cells overcome this problem?
   Several mechanisms exist to allow necessary but unfavorable reactions to proceed or to speed up to levels required for cellular function. Below we explore the following mechanisms:
   - *Enzymes,*
   - *concentration gradients, and*
   - *coupling unfavorable reactions with favorable ones, including*
     - *using the common energy currency, ATP.*

B. Thermodynamics

1. What is "free energy"?
   "Free energy" (defined by Gibbs, so we use the symbol G) is the total amount of energy in a system that can be used to do work. By definition,

ΔG = *"the total free energy of products" – "the total free energy of reactants"*

2. Where is this energy stored?
   The energy is stored in the bonds of the reactant and product molecules.

We say that ΔG is a thermodynamic property, meaning that it is independent of the way that the conversion of reactants to products might proceed.

3. Based on how energy is stored in the molecules, explain why ΔG is independent of the path of the reaction.
   The energy is stored in the bonds. Regardless of how the reaction proceeds (all in one step, or in 10 steps, like glycolysis), the products will still look like the same exact molecules. Thus, the amount of energy stored in the bonds of the products will still be the same, and the difference in energy levels between reactants and products will still be the same.

4. If ΔG=0, the reaction is at equilibrium. What then is the meaning of the magnitude of ΔG?
   The magnitude of ΔG is the measure of the amount of work that can be done by a chemical reaction before it reaches equilibrium.

5. What is a favorable reaction? What would ΔG be for a thermodynamically favorable reaction?
   A favorable, or exergonic, reaction is one in which the energy state of reactants is higher than that of the products (ΔG<0).

6. What is an unfavorable reaction? What would ΔG be for a thermodynamically unfavorable reaction?
   An unfavorable, or endergonic, reaction is the one in which the energy state of the products is higher than that of the reactants (ΔG>0).

7. Not all thermodynamically favorable reactions proceed on their own. Why?
   Some reactions with negative ΔG still do not proceed at an appreciable rate. This is usually because some intermediate is in a significantly higher energy state than the reactants. The difference between the energy state of the reactants and such an intermediate is known as activation energy (E_a).

8. Catalysts overcome this problem. How do they do it?
   Catalysts (most often protein enzymes) lower the activation energy of the reaction, thus allowing the reaction to proceed. They sometimes accomplish this by physically positioning reactants in a way that brings parts of the molecules that will participate in the reaction in close contact.

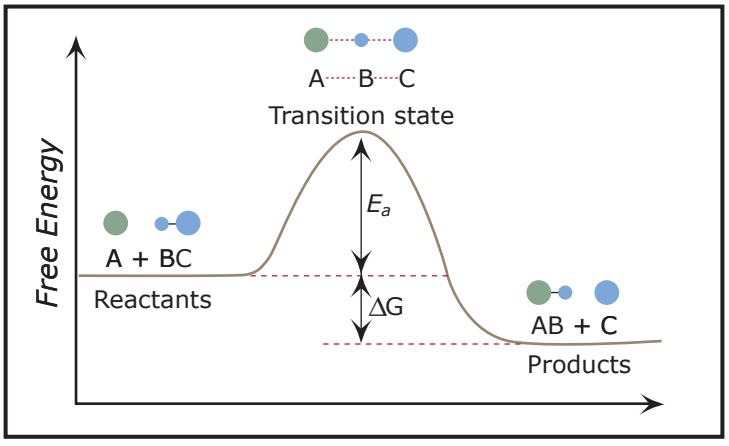

Figure by MIT OCW.

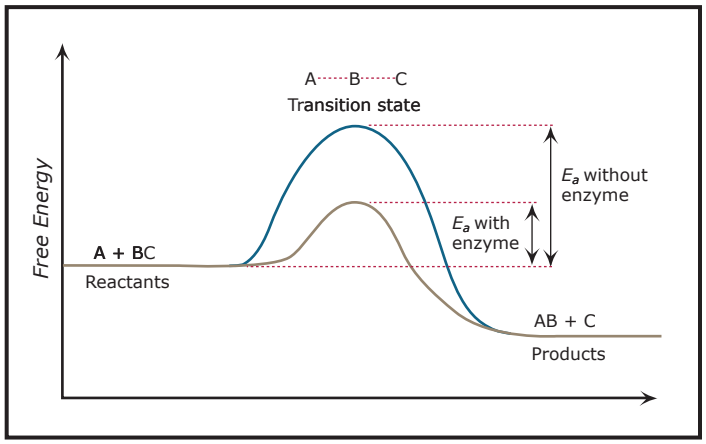

Figure by MIT OCW.

9. Is the equilibrium of the reaction affected by the action of a catalyst? Why or why not?

   The equilibrium of the reaction (relative concentrations of reactants and products) is not affected by the presence of the catalyst. This is because the equilibrium is determined by the amount of energy available to perform the work of converting reactants to products and vice versa. Since the energy is stored in the bonds, it is independent of the path the reaction takes, or the rate at which it occurs.

10. Is the rate of the reaction affected by the action of a catalyst? Why or why not?

    The rate of reaction is affected. Reactants reach the transition state due to random fluctuations in energy caused by molecular motion. If E_a is lowered, much less energy is required to reach it, so more molecules will be able to do so, and the rate of the reaction will increase.

11. Why can the direction in which a reaction proceeds be influenced by the relative concentration of reactants and products?

    If you have more molecules of a particular kind, it is more probable that some of them will reach the high energy state of the transition state.

C. Kinetics

As mentioned in lecture, effect of enzymes can be quantified. The measures commonly used, Km and Vmax are characteristic of different aspects of enzyme's actions. To illustrate these concepts, we will break into groups of four for the following experiment.

All members of the group will try to pick up from the desk and open as many eppendorf tubes as possible in a 45 second interval. One member in each group will use the hand with taped down thumb, another with the other four fingers taped together, and the last person will use a hand in a ski glove.

1. How many eppendorf tubes was each member of the team able to pick up and open in 45 seconds?

	hand	-thumb	-fingers	ski glove
low				
medium				
high				

2. What problems with enzyme functions does the demo represent?

   Problems illustrated by the demo—defective binding interactions with substrates (taped fingers), ability to catalyze the reaction (taped thumb), similar but wrong enzyme (ski glove).

Data gathered in an enzyme kinetics experiment can be represented on a graph of concentration of substrate vs. initial rate of the reaction.

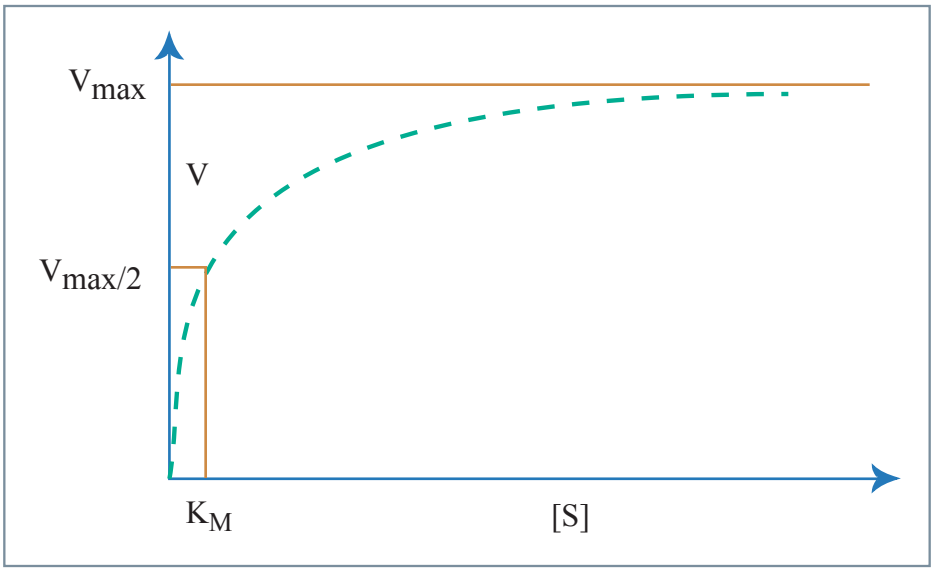

Figure by MIT OCW.

3. Why does the curve level off?

   V_{max} is the property of the enzyme at a fixed concentration. The curve levels off because the enzyme is saturated. It doesn't have to go looking for substrate—there is so much of it around, that the rate-limiting step is actually the catalysis.

K_m is the measure of how well an enzyme binds the substrate—the less substrate it takes to achieve half maximal speed, the better is the affinity of the enzyme for the substrate.

4. How would the curve below change for the mutants described in the demo?

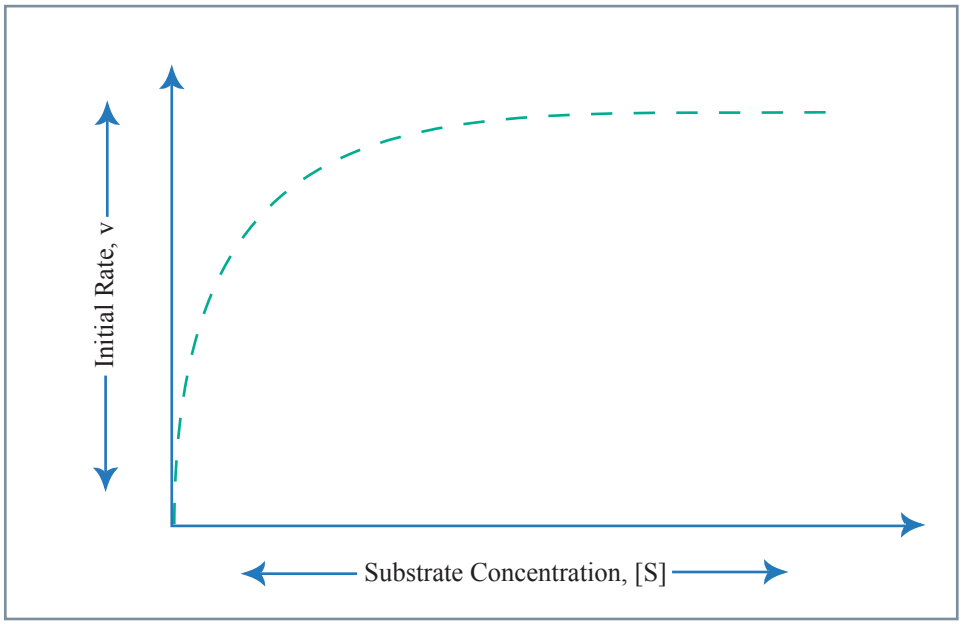

Figure by MIT OCW.

For the mutant defective in binding the substrate, K_m would be higher, but V_{max} should remain the same—if enzyme is saturated, the catalysis is the rate-limiting step.
For the mutant defective in the active site, V_{max} would decrease. K_m might actually decrease as well due to the maximal speed being lower than before, while the affinity for the substrate stays the same.
For the similar, but wrong enzyme, K_m would likely rise, while V_{max} falls—the affinity is worse, and the catalysis is slower.

D. Energy currency

Enzymes can not make thermodynamically unfavorable reactions proceed. But they do lower the activation energy of a reaction in both directions.

1. What strategy can a cell use to drive slightly thermodynamically unfavorable catalyzed reactions? How can this be achieved in a cell?

   As discussed above, skewing the concentrations in favor of reactants can drive such a reaction forward. This can be achieved by immediately siphoning off any newly formed products, thus maintaining the concentration gradient.

Sometimes the reaction is just too thermodynamically unfavorable to be driven by such tricks.

2. What is the strategy used by the cell to drive such reactions?

   In the cell, energetically unfavorable reactions are often coupled with favorable ones, such that the new overall reaction is favorable.

We say that ATP is the energy currency of the cell.

3. Why does it make sense to have energy currency?

   By producing ATP whenever possible, the cell can store the excess energy to fuel endergonic reactions whenever needed.

4. Where in ATP is the energy available to do work stored?

   In ATP energy is stored in the bonds. The most unfavorable of these bonds is the bond between the second and third phosphates.

5. What makes ATP a good candidate for the position of energy currency in the cell?

   The third phosphate can be removed, releasing a lot of energy. This phosphate can actually be transferred to one of the reactants, taking that molecule to a higher energy state, and allowing a previously non-spontaneous reaction to proceed.

ATP is also a building block of RNA.

6. Given what you know about the early history of life on Earth, why does this make sense?

   It is believed that early in evolution there were very few molecules around. The triphosphate feature of ATP made it a good candidate for energy storage, and once the pathway was developed, it became a selective liability to change it too much.
   As we discussed on the first day of class, many key pathways, such as glycolysis, are ancient, and trace their origin to the early stages of evolution of life on Earth.

E. Glycolysis

Glycolysis is an ancient pathway. It is critically important for producing ATP. It is an incremental pathway, meaning that it takes a number of steps (10) to get from the initial reactant (glucose) to the final products. Below in the energy diagram of glycolysis.

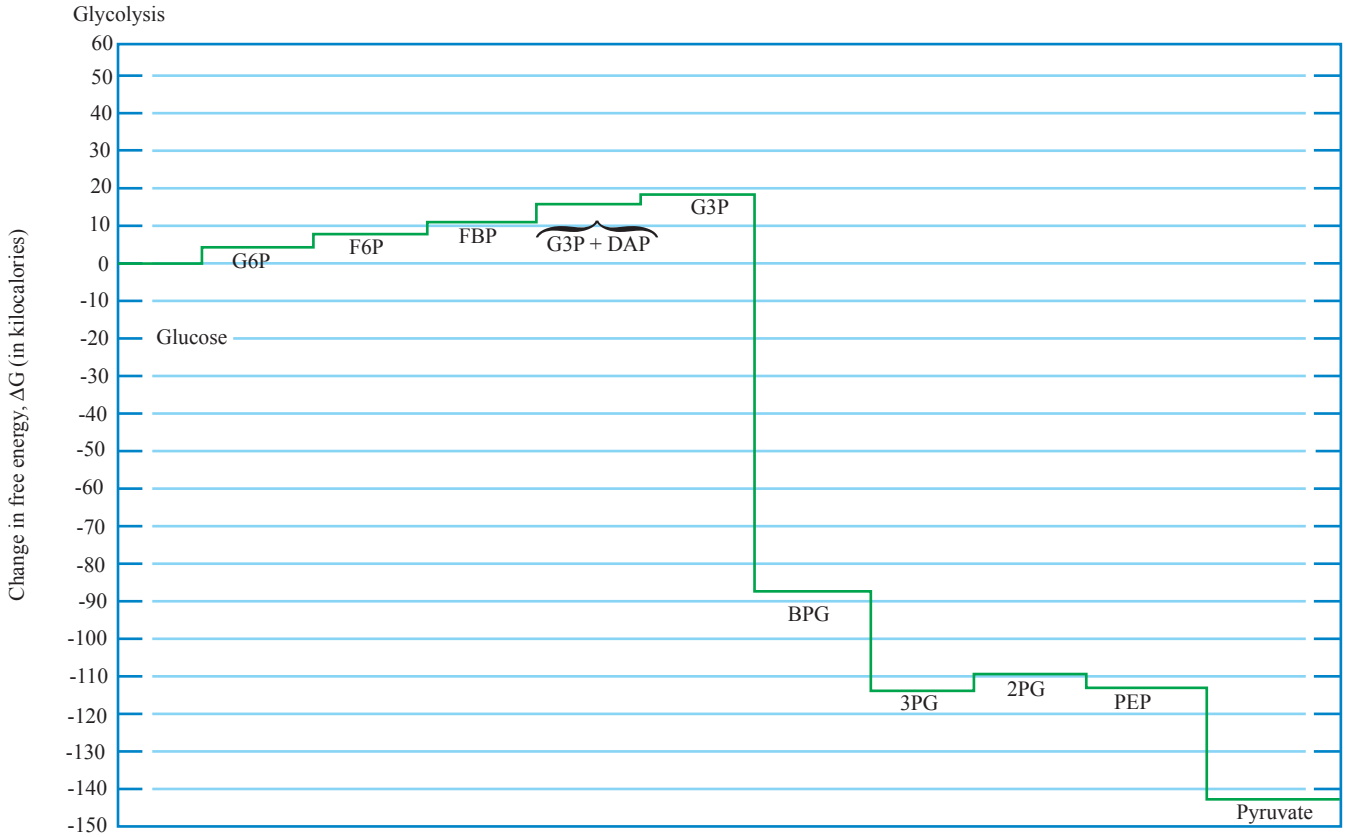

Figure by MIT OCW.

As you can see from the diagram, the overall reaction is very favorable ($\Delta G < -130$).

1. Why do the first six steps of the pathway have a positive ΔG?
   The pathway involves adding energy to reactants until the can extract energy from them.

2. Is this the most efficient way to design a pathway glucose→ pyruvate? If you were able to design it *de novo*, would you be able to come up with a better way?
   The pathway is likely not the most efficient possible. There are likely much more efficient solutions possible.

3. As we mentioned a number of times, this pathway is highly evolutionarily conserved. Why?
   Once the pathway was developed, any mutant (M) of the pathway that accidentally arises is likely to be less efficient at extracting ATP from glucose. Even if some descendant of M, M1, if it was to acquire another mutation, might end up being significantly better than the original non-mutant organism, M itself is not likely to survive or generate enough progeny to allow M1 to ever appear. Thus, evolutionary pressure works against changes to key pathways, ensuring significant conservation throughout evolution.
4. Speculate about how this pathway might have arisen.
   Maybe could get ATP from 1,3diphosphoglycerate. Developed an enzyme to get from G3P to 1,3…. Etc. Energy investment to get up to the certain level from which can get down.

5. What is the energy gain from the glycolysis pathway?
   The net energy gain from glycolysis is only 2 ATP molecules.

6. Is this enough energy to allow for the development of the diverse set of organisms populating the biosphere?
   No, the gain of 2ATP molecules from a molecule of glucose is not enough to fuel more complicated organisms that populate the biosphere today. For that, photosynthesis and respiration needed to develop. This is why it can be said that O_2 is the engine of evolution. We will look at these new ways of making ATP in the next section.