Solutions to 7.014 Problem Set 2

Question 1

The following experimental data were collected during a study of the catalytic activity of an intestinal peptidase capable of hydrolyzing its substrate, the dipeptide glycylglycine:

<table>
<thead>
<tr>
<th>[substrate] mM</th>
<th>Initial reaction velocity* mmol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>15</td>
</tr>
<tr>
<td>1.5</td>
<td>21</td>
</tr>
<tr>
<td>2.0</td>
<td>24</td>
</tr>
<tr>
<td>3.0</td>
<td>28</td>
</tr>
<tr>
<td>4.0</td>
<td>33</td>
</tr>
<tr>
<td>8.0</td>
<td>40</td>
</tr>
<tr>
<td>16</td>
<td>49</td>
</tr>
<tr>
<td>32</td>
<td>50</td>
</tr>
</tbody>
</table>

*Initial velocity is the velocity early in the reaction, before the product has built up enough to slow the reaction rate.

a) Plot initial velocity vs [substrate]
Question 1, continued

b) Using the graph drawn in part (a), estimate $K_M$ and $V_{\text{max}}$ for the peptidase enzyme. From this data, you can see that the velocity is leveling off at around 50 mmol/min, so this is $V_{\text{max}}$. $K_M$ is defined as the substrate concentration at which the reaction velocity is half maximal. Half of $V_{\text{max}}$ is approximately 25 mmol/min, a rate which is reached at approximately 2 mM of substrate, so $K_M$ is approximately 2 mM.

c) How would doubling the peptidase concentration affect $K_M$?

Enzyme concentration has no effect on $K_M$.

d) How would doubling the peptidase concentration affect $V_{\text{max}}$?

Increasing [E] will cause an increase in $V_{\text{max}}$.

e) How would doubling the peptidase concentration affect the equilibrium constant ($K_{\text{eq}}$) for the catalyzed reaction?

Enzyme concentration does not affect the equilibrium constant because enzymes cannot change $\Delta G$ or $K_{\text{eq}}$.

f) How would doubling the peptidase concentration affect the initial rate of the reaction?

There is not a single correct answer to this as it depends upon what the starting [substrate] is. Below 2 mM [substrate], V is proportional to [E], so doubling [E] will double the initial reaction. At high [substrate], doubling the [E] will have no effect.

g) If you look at the graph you have drawn in part (a), it is linear at low [S] and it reaches a plateau at high [S]. Why does the rate of an enzyme-catalyzed reaction plateau at high [S]?

At high [S], the enzyme becomes completely occupied processing the substrate; eventually (as [S] gets high) all the enzyme molecules are ‘busy’ so the rate cannot increase.

Question 2

The enzyme Phosphofructokinase (PFK) catalyzes the conversion of fructose 6-phosphate (F6P) to fructose 1, 6-bisphosphate (FBP), in step 3 of glycolysis.

\[
\text{fructose 6-phosphate + ATP} \underset{\text{phosphofructokinase}}{\rightleftharpoons} \text{fructose 1,6-bisphosphate + ADP}
\]

\[\Delta G^o = -3.4 \text{ kcal/mol}\]

a) What two functions does ATP serve in this coupled reaction?

The hydrolysis of ATP provides the energy to drive this coupled reaction. ATP also serves to donate a phosphate to fructose 1,6-bisphosphate from fructose 6-phosphate.
b) Draw and label an energy diagram for this reaction. Include the relative energy levels of the substrates and the products, the activation energy and the $\Delta G^\circ$ for the reaction.

![Energy Diagram](image)

$\Delta G^\circ = -3.4 \text{ kcals/mole}$

In erythrocytes, the following intracellular concentrations of metabolites are found:

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose 6-phosphate</td>
<td>.014 mM</td>
</tr>
<tr>
<td>Fructose 1,6-bisphosphate</td>
<td>.028 mM</td>
</tr>
<tr>
<td>AMP</td>
<td>1 mM</td>
</tr>
<tr>
<td>ADP</td>
<td>0.2 mM</td>
</tr>
<tr>
<td>ATP</td>
<td>2 mM</td>
</tr>
<tr>
<td>Pi</td>
<td>1 mM</td>
</tr>
</tbody>
</table>

c) What is the free energy change of the phosphofructokinase reaction under these cellular conditions (37 °C)? Show your work. Is the reaction spontaneous under these conditions?

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{products}]}{[\text{reactants}]}$$

$$\Delta G = -3.4 \text{ k}cals/\text{mole} + 0.61 \text{ k}cals/\text{mole} \ln \frac{[\text{FBP}][\text{ADP}]}{[\text{F6P}][\text{ATP}]}$$

$$\Delta G = -3.4 \text{ k}cals/\text{mole} + 0.61 \frac{\ln (0.2)}{\ln}$$

$$\Delta G = -4.38 \text{ Spontaneous}$$
Question 2, continued

Phosphofructokinase (PFK) is the main regulatory enzyme of glycolysis.

d) What is the specific signal or molecule(s) to which PFK responds?
PFK responds to the decreased ATP to ADP ratio (increased levels of ADP).

e) Why does this mechanism of regulation make sense?
Normally, a low ATP to ADP ratio indicates that cells need more energy currency available ready to use. By speeding up glycolysis, more ATP can be formed from ADP to raise the ATP to ADP ratio.

f) In certain tumor cells, an enzyme called ATPase becomes abnormally active, resulting in increased hydrolysis of ATP to ADP. What would be the effect on the overall rate of glycolysis in these cells?
The activity of the glycolytic enzymes would increase, increasing the overall rate of glycolysis.

Question 3

The energy released by ATP hydrolysis can be used to drive unfavorable (endergonic) reactions in the cell.

<table>
<thead>
<tr>
<th>ATP hydrolysis Reaction</th>
<th>Under Standard Conditions</th>
<th>Under Cellular Conditions (approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP ( \rightarrow ) ADP + P(_\text{i})</td>
<td>( \Delta G_0 = -7.5 \text{ kcal/mol} )</td>
<td>( \Delta G = -12 \text{ kcal/mol} )</td>
</tr>
<tr>
<td>ATP ( \rightarrow ) AMP + PP(_\text{i})</td>
<td>( \Delta G_0 = -7.7 \text{ kcal/mol} )</td>
<td>( \Delta G = -12.2 \text{ kcal/mol} )</td>
</tr>
</tbody>
</table>

For example, fireflies use the energy of hydrolysis of ATP to AMP + PP\(_\text{i}\) (pyrophosphate – the two terminal phosphate groups of ATP) to drive the reaction that produces light. See below:

Overall reaction is:

\[
\text{rxn 1: } \text{luc} + O_2 \quad \rightarrow \quad \text{ox-luc} + CO_2 + \text{light} \\
+ \text{rxn 2: } \text{ATP} \quad \rightarrow \quad \text{AMP} + PP_i \\
\text{overall rxn: } \text{luc} + O_2 + \text{ATP} \quad \rightarrow \quad \text{ox-luc} + CO_2 + \text{light} + \text{AMP} + PP_i
\]
In fact, the reaction proceeds by a two-step process. In step 1, ATP reacts with luciferin to produce AMP-luciferin + PP$_i$. In the second step AMP-luciferin reacts with O$_2$ to give + AMP + CO$_2$. This is similar to the glutamine synthetase reaction described in lecture where ATP is used to “activate” glutamic acid and then the activated glutamic acid reacts to form glutamine.

Given the above $\Delta G$ values, and the fact that light production occurs under cellular conditions, what, if anything, can you say about the values of the following $\Delta G$’s?

Note that, from the information given, you cannot give exact values, only limits or ranges. For example, “$\Delta G_{\text{rxnX}}$ under cellular conditions $\leq$ 27 kcal/mol”, “$\Delta G_{\text{rxnY}}$ under standard conditions $>$ 0 kcal/mol”, or “not enough information given”.

a) $\Delta G_{\text{overall rxn}}$ under cellular conditions?
Since the reaction proceeds under cellular conditions, and therefore it is spontaneous, the $\Delta G$ must be between 0 and -12.2 kcal/mol.

b) $\Delta G_{\text{overall rxn}}$ under standard conditions?
Without knowing the cellular conditions (concentrations of all products & reactants), you cannot tell anything about the $\Delta G$ for this reaction under standard conditions.

c) $\Delta G_{\text{rxn1}}$ under cellular conditions?
Since the $\Delta G$ for the overall reaction must be < 0 and we know that $\Delta G_{\text{rxn2}} = -12.2$ kcal/mol, $\Delta G_{\text{rxn1}}$ must be < + 12.2 kcal/mol.

Question 4

You notice a slimy patch of goo eating away at your carpet. But wait, your carpet is made of 1950’s nylon! How can this be? You expect the slimy patch is a colony of rare Archaebacteria, and set out to determine how these bacteria utilize nylon as an energy source. You culture large quantities of the bacteria and painstakingly purify a protein with the ability to degrade nylon. You name the newly discovered enzyme Leggase. Nylon is a polymer made up of many repeating subunits (like polysaccharides). It looks like this:

The hatched lines at the ends indicate that this same unit is repeated many times in both directions. The arrow points to the bond that is cleaved by the Leggase enzyme.

a) If we look at the degradation of nylon in the absence of enzyme the rate at which this bond is cleaved is about 1 per year. In the presence of the enzyme, the rate of cleavage is about 500 bonds/second. What is the increase in the rate of the reaction?

The increase in the rate of the reaction is equal to the rate of the reaction with enzyme divided by the rate of the reaction without enzyme. So we get $\left\{(500 \text{ molecules/sec.})(3600 \text{ sec./hr.})(24 \text{ hr./day})(365 \text{ days/year})\right\} / (1 \text{molecule/year}) = 1.58 \times 10^{10}$ fold increase in reaction rate with the enzyme Leggase.
Question 4, continued

b) You make a solution that is 0.1M nylon polymers. You add Leggase and allow the reaction to reach equilibrium at 25°C. You determine the concentration of nylon polymers at equilibrium is 0.0001M. In this reaction, assume that 1 molecule of nylon polymer is cleaved into two molecules of cleaved nylon.

i) What is the equilibrium constant for the reaction?  
For this reaction $K_{eq} = \frac{[\text{cleaved nylon}]^2}{[\text{nylon}]}$  
We know that at equilibrium $[\text{nylon}] = 0.0001M$, so $[\text{cleaved nylon}] = 0.1M - 0.0001M = 0.0999M$.  
$K_{eq} = \frac{(0.0999M)^2}{(0.0001M)} = 99.8M$

ii) What is the change in free energy ($\Delta G$) in kcal/mol? Is this an exergonic or endergonic reaction?  
Because the reaction is at equilibrium, $\Delta G = 0$.  
If, however, we had asked for $\Delta G_o$,$\Delta G_o = -RT\ln K_{eq} = -(0.00198 \text{ kcal/M K}) (298K) \ln (99.8M) = -2.72 \text{ kcal/mol}$.

c) You realize that this species could be crucial to waste management, but uncontrolled, this bacterium could change life as we know it! You want to be able to control this bacterium, and discover that a dipeptide, Gly-Gly is an inhibitor of Leggase.

i) Draw the dipeptide Gly-Gly below.

![Gly-Gly dipeptide](image)

ii) Explain, why this dipeptide would inhibit Leggase?  
The site of the nylon molecule at which Leggase cleaves looks like a peptide bond. The glycine dipeptide has a peptide bond, and the CH$_2$'s of the glycines mimic the CH$_2$'s in nylon that are next to the bond that is cleaved. Since the dipeptide is structurally similar to nylon, it could bind to the active site of Leggase.

d) If nylon66 polymers were aligned in a crystal array, what force or bond would dominate the structure? (http://aml.arizona.edu/classes/mse222/1998/nylon66/mse222.htm)  
Hydrogen bonds between the NH and the CO groups on adjacent polymers.