Due Wednesday April 26th – in class

1. (8 pts, 10 min) View the Jmol page on PFK. There you will view the structure of PFK-1 from a bacteria (E. coli). The image shows PFK bound to either AMP, ADP, or ATP (i.e. AXP) and to phosphorylated fructose (either F-6-P or F-1-6-P, FXP). Although the displayed protein is a homodimer, only one set of bound ligands will be shown. The following navigation guides will be useful:

- Click on the "AXP Highlight" to turn on/off a yellow surface on the AXP residue(s). Note that there are two bound AXPs
- Click on the "FXP Highlight" box to turn on/off a green surface on the FXP residue.

i) Which AXP (AXP-1 or AXP-2) is bound in the active site? Which is found in the allosteric site? Briefly justify your answer with reference to the proximity to the other substrate (2 pts).

ii) Does this structure show the enzyme complexed with reactants or products? Justify your answer with reference to the structure (2 pts).

iii) Use the data from the dry lab to determine whether PFK is activated or inhibited by the AXP that is bound in the allosteric site (2 pts).

iv) Does the regulation you discovered in iii make physiological sense? Briefly justify your answer (2 pts).

2. (9 pts, 20 min) The curves to the right show the effect of fructose-2,6-bisphosphate (5 μM) on the activity of fructose-1,6-bisphosphatase. The data that was used to generate this plot is given in the table below:

<table>
<thead>
<tr>
<th>[F-1,6-Phosphate] (μM)</th>
<th>uM Product/sec +F-2,6P</th>
<th>uM Product/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>5.5</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>7.3</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
<td>8.8</td>
</tr>
<tr>
<td>50</td>
<td>13.6</td>
<td>10.0</td>
</tr>
<tr>
<td>100</td>
<td>14.3</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Please answer the following questions (most of these are straightforward and are designed to draw your attention to differences in F16P and F26P).

i) Draw the substrate and product of the reaction catalyzed by fructose-1,6-bisphosphatase. Include any cofactors/co-substrates that may be involved in the reaction (e.g. ATP, NADH, etc.) (1 pt)

ii) In which metabolic pathway does fructose-1,6 bisphosphatase operate (1 pt)?

iii) What is the structural difference between F-1,6-Phosphate and F-2,6-Phosphate (1 pt)?

iv) Would you characterize F-2,6-P as which of the following (1 pt):
   - Competitive inhibitor of F-1,6-bisphosphatase
   - Allosteric activator of F-1,6-bisphosphatase
   - Allosteric inhibitor of F-1,6-bisphosphatase

   Briefly justify your answer on the basis of the experimental data (2 pts).

v) Based on your answer to part d, draw a simple, cartoon-like diagram of fructose-1,6-bisphosphatase. In your diagram should indicate the binding sites for F-1,6-P and F-2,6-P and indicate the active site region on the enzyme (3 pts).

3. (10 pts, 20 min) Your liver has been actively synthesizing proteins all morning; consequently, its ATP levels are lowered and AMP/ADP levels are high. While crossing the street on your way to your favorite class, a fast moving car comes directly towards you.

i) What hormone is released by your central nervous system, once you notice the car? (2 pts)

ii) What will happen to the phosphorylation state of proteins due to release of this hormone? (2 pts)

iii) How will the presence of this hormone affect the levels of F2,6-P in the cell? (Will it rise or fall?) (2 pts)
iv) Under these conditions, will the liver produce glucose from glycogen, perform gluconeogenesis or both? Briefly justify your answer with reference to how these pathways are regulated – consider both hormonal control, energy sensing, and the availability of ATP for biosynthesis. (4 pts)

4. (5 pts, 10 min) The concentration of chloride in seawater is 0.6 M. An ocean dwelling bacteria uses chloride transport to generate ATP. What is the minimum number of chloride ions that would have to be moved through its ATP synthase to generate ATP? You can assume the intracellular concentration of chloride is 0.1 M and that the voltage difference across its membrane is 0.06 V, inside positive, and the temperature is 300K.

5. (6 pts, 10 min) The reaction catalyzed by Malate dehydrogenase in the TCA cycle has a standard free energy change \( (\Delta G^\circ) \) of +29 kJ/mol, but a Gibbs free energy of 0 kJ/mol during the normal operation of the TCA cycle. Explain how the Gibbs free energy is reduced to zero for this reaction; is this accomplished by either direct or indirect coupling? (2 pts)

6. (5 pts, 5 min) A reaction that is identical in chemistry to that of pyruvate dehydrogenase occurs in the TCA cycle. Identify the name of the enzyme that catalyzes this reaction and show that the chemical changes are identical to those catalyzed by pyruvate dehydrogenase.

7. (5 pts, 5 min) Transaminases are enzymes that reversibly convert \( \alpha \)-keto acids to \( \alpha \)-amino acids by the replacement of a ketone group by an amide group, thus providing a way to both synthesize and degrade amino acids. For example, pyruvate can be converted to alanine by a transaminase, as illustrated in the reaction shown to the right. Two other amino acids can be synthesized directly from intermediates in the TCA cycle by virtue of the transaminase reaction.
   i) Draw the substrate and product of the transaminase reaction for both reactions and give the name of the resultant amino acid.
   ii) Based on your answer to this question, do you consider the TCA cycle to be catabolic, anabolic, or both?

8. (5 pts, 10 min). The diagram to the right shows actual data for the recovery of muscle glycogen after exercise for an individual on either high-carbohydrate diet (solid line) or for an individual on a diet that consists of fat and protein (dashed line). The recovery period starts at T=0 after an exercise period of 2 hours (from T = -2 to 0 hours). Explain, using your knowledge of the metabolic pathways discussed in this course, the reason for the slow (almost non-existent) recovery of muscle glycogen for the individual on the fat and protein diet.

9. (10 pts, 10 min). Both glucose and hexanoic acid contain 6 carbons and will be converted to CO\(_2\) in metabolic pathways.
   i) Determine the number of ATP molecules that are produced from glucose and hexanoic acid. Hint, determine the number of ATP and acetyl-CoAs produced and then determine the ATP content of one acetyl-CoA. You may assume NADH=3 ATP, FADH\(_2\)=2 ATP (4 pts).
   ii) Balance the hypothetical redox reaction of hexanoic acid to glucose, is this an oxidation or a reduction (4 pts).
   iii) Based on your answers to parts i) and ii) what is the relationship between the oxidation state of carbon and the amount of energy produced when that carbon is converted to CO\(_2\) (2 pts).

10. (8 pts, 10 min) Ethanol can be produced from glucose by yeast (and other organisms), a process referred to as fermentation. Glucose can be readily obtained from sugar cane syrup, and with more expense, from corn.
   i) If you wanted to maximize the production of ethanol – would you grow the yeast cells in the presence of a high concentration of oxygen or low? Why? (2 pts)
   ii) How efficient is the process of ethanol production, i.e. how much of the original energy content in glucose is retained in the ethanol? You can estimate the energy remaining in ethanol by first determining how many ATP molecules can be produced from one glucose molecule (see question 9), and then subtracting the net number of ATPs that were generated going from glucose to pyruvate (6 pts).