CHEM 330 **Exam #3** **Dr. Brandt**
Fall 2012-13

Read each question carefully. Please answer the questions asked.

Rules:

1. **You may use any inanimate source you like. *Note, however, that neglecting to cite your sources appropriately is unethical and is potentially an instance of Academic Misconduct*.**
2. **You may work in groups of up to three.**
3. **You may discuss the questions with your partner(s) or with your instructor (don't count on answers, but you might get hints).**
4. **You may not discuss (neither verbally, via email, nor using any other method, including mental telepathy) the exam questions with anyone not currently enrolled in the class.**
5. **Please submit your text in *typed* form (equations/drawings may be *neatly* drawn by hand in ink). (You may submit one set of answers per group).**
6. **Due Date: Friday, November 9, 2012 before 5 PM.**
7. **Good luck, and have a good Thanksgiving!**

. . . the result of not treating cave bears with respect.

1. The hexokinase reaction is biologically irreversible. However, liver cells convert glucose-6-phosphate to glucose. Are these two statements contradictory? Why or why not?
2. The lactate dehydrogenase reaction has a $\Delta G'^{\circ}$ of –25.2 kJ/mol. In erythrocytes, the reaction **pyruvate + NADH → lactate + NAD** has a ΔG of –14.8 kJ/mol. In liver cells and heart cells, the reaction usually exhibits a small positive ΔG. How is this possible?
3. The most common pathway for liver metabolism of ethanol requires the use of alcohol dehydrogenase.
   a. Ethanol cannot (in humans) be converted to pyruvate. How will the presence of ethanol affect gluconeogenesis in a healthy liver? Please justify your answer.
   b. How does a healthy liver respond to glucagon to maintain plasma glucose levels? How does the presence of ethanol perturb this effect?
4. Protein H is known to bind compound O. An experimental setup contains two chambers separated by a membrane permeable to compound O but not to protein H: Chamber A has a volume of 1 liter and Chamber B has a volume of 10 mL.
   a. In studying protein H, you obtain the data in the spreadsheet on the course website. What are the binding parameters (B_{max}, K_d or $K_{0.5}$, and n) for protein H? Is protein H cooperative?
   b. You add compound O to both chambers of the experimental setup with a final concentration of 150 μM. You then dissolve 1.5 g of protein H in chamber B without changing the volume of the chamber. Please determine the moles of compound O present in each chamber following equilibration.
   c. What is the effect of protein H on the distribution of compound O? Please justify your answer.
   d. Is there an analog to this system in humans? Is so, what is it? Please justify your answer.
5. You are lost in the woods in a snowstorm, and to your great relief, find a cabin. The owner of the cabin tells you that, as you knocked on the door, he drank 80 ml methanol (by mistake). Assume that methanol and ethanol have densities of 0.79 g/ml, and that the alcohols distribute themselves rapidly over a total volume of 45 liters of body fluids (45 liters is your estimate of the fluid volume of your host). A quick check of the biochemistry textbook you always carry with you reveals that the K_m of alcohol dehydrogenase for ethanol is 1 mM and for methanol the K_m is 10 mM, and reminds you that alcohol dehydrogenase converts ethanol into acetaldehyde (which can then be converted to acetyl-CoA). Alcohol dehydrogenase also converts methanol into formaldehyde, which is quite toxic to humans. Drinking 80 ml of methanol will permanently harm your host, unless you intervene.
   a. How would ingesting ethanol prevent damage from formaldehyde?
   b. Why does this work? Is there anything else that you would need to know to be sure that ethanol ingestion would be effective?
   c. You happen to have a 750 ml bottle of single malt scotch (40% ethanol). Assume that (based on these numbers) 5% of the rate of methanol conversion to formaldehyde that would occur without treatment would probably avoid permanent damage. Do you have enough scotch to save your host? How much, if any of the scotch will be left over for you? (Please justify your answer.)

6. You synthesize six different glucose molecules, each with a single (radioactive) ^{14}C label in one of the possible positions. You prepare test tubes containing the glycolytic enzymes, pyruvate dehydrogenase, and the TCA cycle enzymes ***excluding*** malate dehydrogenase in an aqueous buffer with oxaloacetate and appropriate coenzymes.

a. Given the enzymes you used, which positions in glucose will result in release of ^{14}C-labeled carbon dioxide? Please justify your answer.

b. What is the effect of leaving out malate dehydrogenase?

c. Will adding pyruvate carboxylase to your test tubes allow release of ^{14}C-labeled carbon dioxide from additional positions? Please justify your answer.

7. In Organism P (see the next question), the ATP synthase enzyme complex can synthesize ATP using a proton gradient, and can use ATP hydrolysis to generate a proton gradient. For the bacterium you measure, $pH_{inside} = 8.5$, $pH_{outside} = 7.2$, and membrane potential = –75 mV.

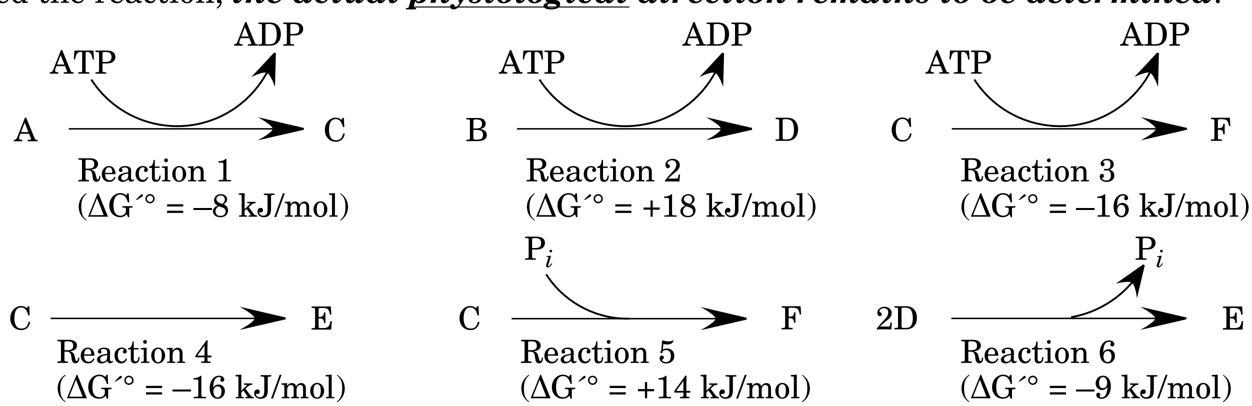
a. Assuming that the bacterium is a sphere with a diameter of 2.2 μm, how many "free protons" are present in the cell?

b. Assuming [ATP] = 4 mM, [ADP] = 0.4 mM, and [P_i] = 4 mM, and the cellular temperature is 25°C, what is the minimum number of protons that must enter the cell to yield enough energy to synthesize an ATP from an ADP. (Please show your calculations).

c. Assume that the stoichiometry for ATP synthase is three protons per ATP. Under these conditions, is the ATP synthase synthesizing or hydrolyzing ATP? Please justify your answer.

8. As the result of a storm while on a three-hour boat tour, you have found yourself on an uncharted island with several other people. The (additional) bad news is that you and your fellow castaways have become infected with a previously unknown, lethally pathogenic organism, P. Fortunately, however, you have discovered scientific instrumentation and a biochemistry laboratory on the island, and you begin to study the organism.

You find that organism P has a biochemical pathway that is not present in humans. The pathway appears to contain six compounds, which you, in a stunning burst of imagination, label A, B, C, D, E, and F. Further analysis reveals that organism P has six enzymes that catalyze reactions involving these compounds. The reactions are shown below, with the corresponding ΔG´° values. The reactions are all shown in the direction in which you first tried the reaction; ***the actual <u>physiological</u> direction remains to be determined***.



Of the compounds listed, you find that compound A is present at 5 mM in the "growth medium" (actually, a sample of human blood obtained from another castaway) you are using; allowing the organism P cells to grow results in depletion of compound A. If compound A is not present, the organism will not grow, nor can A be replaced with any of the other compounds. Typical concentrations of the various metabolites in organism P after some period of growth in the presence of a constant 5 mM of compound A are given in the table below.

Compound	**Concentration (mM)**	**Compound**	**Concentration (mM)**
ATP	4	C	2
ADP	0.4	D	0.5
Phosphate (P_i)	4	E	2.7
A	5	F	0.02
B	4		

You perform experiments in which you measure velocities of some of the enzymatic reactions at different concentrations of some of the compounds. These data are available in spreadsheet form on the course website.

a. Kinetic data for enzyme 4 is given in a spreadsheet on the course website. Please analyze the data. Is this enzyme a normal Michaelis-Menten enzyme? Please determine the enzyme parameters (*i.e.*, K_m, V_{max}, and any other values that may be of interest). Is enzyme 4 part of the pathway? (Hint: concentrations matter!) Please justify your answers.
b. Kinetic data for enzyme 3 is given in a spreadsheet on the course website. Please qualitatively analyze the data. Is this enzyme a normal Michaelis-Menten enzyme? Is enzyme 3 part of the pathway? (Hint: concentrations matter!) Please justify your answers.
c. Please use the available information to work out the overall pathway, and briefly justify your answer.
d. Is the overall pathway thermodynamically favorable? Is the overall pathway kinetically favorable? (How can you tell?)
e. What is (are) the role(s) of ATP in this pathway? Does this pathway generate or utilize net amounts of ATP? Please justify your answers.
f. Using information and examples from pathways you have studied, explain why six enzymes are probably necessary for this pathway.
g. Which enzymes are likely to have high catalytic rates in comparison to the others? Which enzymes may be slow relative to the others? Why do you think so?
h. Which of the enzymes appear to be regulated? Which compounds are involved? What benefit to the organism does this regulation confer? Please justify your answers.
i. Compound A is present at about 5 mM in human blood. Can you, by developing an inhibitor of one of the enzymes, save the lives of the castaways (including yourself)? If so, please (***briefly***!) explain how you might rapidly produce an inhibitor drug that might work, and please justify why inhibiting this enzyme might be more effective than one of the others. (Hints: your treatment should involve no more than one drug, because you will not be able to develop more before everyone dies; removing compound A from your circulation is not a viable option, and any inhibitor too similar to compound A might also be lethal to the humans.)
j. Extra credit: propose a mathematical model for enzyme 3 that accounts for its observed reaction kinetics.