Chemistry and Biochemistry 153A
Spring 2011

Final Exam

Instructions:
(Note that changes or additions to the usual instructions have been underlined.)

- You will have 3 hours to complete the exam.
- You may use a pencil (recommended) or blue or black ink pen to write your answers. Other color inks will not be graded. Your choice of writing utensil will not affect your ability to request a regrade.
- Only answers on the separate answer sheets, in the indicated space, will be graded; writing anywhere else will be ignored. Be sure to write your name and your discussion board username, if you have one, on the answer sheet.
- Do not write in the score boxes on your answer sheet; you will be docked points if you do.
- For answers with a word or sentence limit, words beyond this limit will not be read or graded.
- For short- or multi-answer questions, including irrelevant or wrong information or selections in your answer will cause you to lose points.
- Write legibly. If the grader cannot read your answer, you won't get credit.
- Items you may have on your desk:
  - non-programmable scientific calculator, *without its case or cover*
  - writing utensil(s)
  - student ID

  ALL other items must be placed into a bag, which must be zipped up or closed and pushed *completely* under your chair.
- No hats, hoods, earphones, or cellphones are allowed.
- If you continue to write after 'time' is called, your exam will be taken and docked 10 points.
- **Questions are printed on both sides, as are the two colored answer sheets. Be sure you've answered all of the questions!**

Note – although each half of the exam is worth 100 points, you may find the second (cumulative) half to be more involved, so budget your time accordingly.

Exam pages:

2-8: Questions

9-13: Help/scratch pages

Part 1 – New Material (100 points)

1. (8) Consider noncompetitive inhibition:
   a. (5) Which of the following relationships can indicate noncompetitive inhibition? Choose all that apply.

      A. $K_m^{app} > K_m$ D. $V_{max}^{app} > V_{max}$ G. $K_I > K_I{}'$

      B. $K_m^{app} < K_m$ E. $V_{max}^{app} < V_{max}$ H. $K_I < K_I{}'$

      C. $K_m^{app} = K_m$ F. $V_{max}^{app} = V_{max}$ I. $K_I = K_I{}'$

   b. (3) Noncompetitive inhibition is much less common than other types of reversible inhibition. Why? Choose the best explanation.
      A. Most inhibitors that bind in an alternate site are allosteric inhibitors.
      B. Inhibitor binding is usually influenced by the binding of substrate.
      C. Inhibitors usually have highest affinity for the free enzyme.
      D. Noncompetitive inhibition is actually a type of competitive inhibition.

2. (2) Complete the following sentence: TPP aids in the decarboxylation of _________ acids

3. (6) For each of the following parts, answer by giving *either* the name(s) of the enzyme(s) *or* the reactants and products.
   a. Which reaction(s) of *aerobic* metabolism use(s) TPP?
   b. Which reaction(s) of *anaerobic* metabolism use(s) TPP?

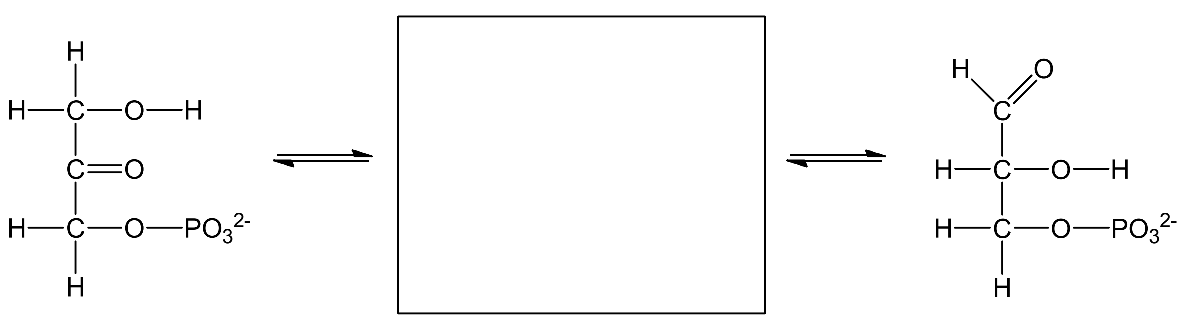
4. (5) Why do cells have multiple different redox currencies? Determine whether each of the following explanations is *correct* or *incorrect*. (Circle your choice for each.)
   a. To enhance metabolic efficiency by limiting the number of different redox reactions.
   b. To oxidize or reduce compounds of differing reduction potential.
   c. To allow transfers of different numbers of electrons.
   d. To link catabolic and anabolic pathways.
   e. To keep separate pools of reduced and oxidized currencies.

5. (12) Consider the aldol cleavage reaction catalyzed by aldolase.
   a. (2) Write a balanced equation for this reaction. (Abbreviations are OK.)
   b. (5) $\Delta G'^{\circ}$ = +22.8 kJ/mol for this reaction. However, this reaction is readily reversible in the cell, due to sub-molar concentrations of reactant(s) and product(s). If the concentrations of all reactants and products were equal, calculate what this concentration would need to be for this reaction to go in the forward direction (in human muscle cells). Show your work.
   c. (3) Other than low reactant and product concentrations, what other factor or process promotes this reaction going forward *in glycolysis*? Briefly explain in 25 words or fewer.

6. (10) Consider the enzyme glycogen phosphorylase.
   a. (2) What is the significance of this enzyme? (What is its important role?) Answer in 10 words or fewer.
   b. (3) How is this enzyme regulated in the cell? Choose all that apply.
      A. Feedback inhibition
      B. Product inhibition
      C. Substrate concentration
      D. Binding of allosteric effectors
      E. Covalent modification
   c. (5) For each of the following compounds, state whether increasing its concentration would be likely to *increase* or *decrease* the activity of glycogen phosphorylase. (The effect may be direct or indirect.)
      A. glucose
      B. glucose-1-phosphate
      C. ATP
      D. AMP
      E. NADH

7. (6) True or false? (1 point each)
   a. Specific transporters are necessary to move small metabolites across both mitochondrial membranes.
   b. Pyruvate dehydrogenase uses 5 cofactors: TPP, lipoic acid, Coenzyme Q, FAD, and NAD^+.
   c. Many vitamins are essential because of their use in enzyme cofactors.
   d. Glycolysis is an anaerobic process.
   e. In eukaryotes, fermentation occurs in mitochondria.
   f. In eukaryotes, aerobic metabolism occurs in mitochondria.

8. (7) Given the starting and ending structures, show how triosephosphate isomerase catalyzes the isomerization of DHAP. Include curved arrows to show electron movements, generic enzyme groups involved in the catalysis, and the structure of the intermediate that is formed.



9. (5) A reaction that is favorable in the forward direction (having a negative ΔG) is unfavorable in the reverse direction (having a positive ΔG of equal magnitude). The same is true for a pathway; if it is favorable in the forward direction, it is equally *un*favorable in the reverse direction. So how can glycolysis and gluconeogenesis be *simultaneously* favorable (both having negative ΔG) in a cell? Briefly explain in 35 words or fewer.

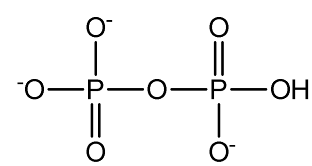
10. (3) You have been growing a culture of yeast in an *anaerobic* chamber on a glucose food source. What effect would you expect to see if oxygen is added to the chamber?
    a. Glucose consumption increases
    b. Glucose consumption decreases
    c. Glucose consumption remains the same

11. (3) Multiple choice. Carbons that enter the citric acid cycle as acetyl-CoA:
    a. Leave as CO_2 in the first round of the cycle
    b. Leave as CO_2 in the second round of the cycle
    c. Begin to leave as CO_2 in the first round of the cycle
    d. Begin to leave as CO_2 in the second round of the cycle

12. (9) Complete each of the following sentences by filling in the correct numbers:
    a. Glycolysis produces ____ ATP (or equivalent), ____ NADH, and ____ $FADH_2$ per glucose.
    b. Gluconeogenesis *uses* ____ ATP (or equivalent), ____ NADH, and ____$FADH_2$ *per glucose produced*.
    c. Each round of the citric acid cycle produces _____ ATP (or equivalent), ____ NADH, and ____ $FADH_2$.

13. (10) True or false? (2 points each)
    a. The different b-type hemes of electron transport have the same standard reduction potentials.
    b. NADH is oxidized only when oxygen is present.
    c. Electrons from $FADH_2$ only enter the electron transport chain at Complex II.
    d. Iron-sulfur clusters participate in 1- or 2-electron transfers.
    e. The reaction catalyzed by citrate synthase is anaplerotic.

14. (3) Given the structure of pyrophosphate at right (in its predominant form at pH 7), list three factors that explain why the $\Delta G'^{\circ}$ of pyrophosphate hydrolysis is large and negative.



pyrophosphate

15. (3) Given their standard reduction potentials, order the following redox centers to reflect the order in which they receive electrons in the electron transfer chain (first to receive electrons → last).

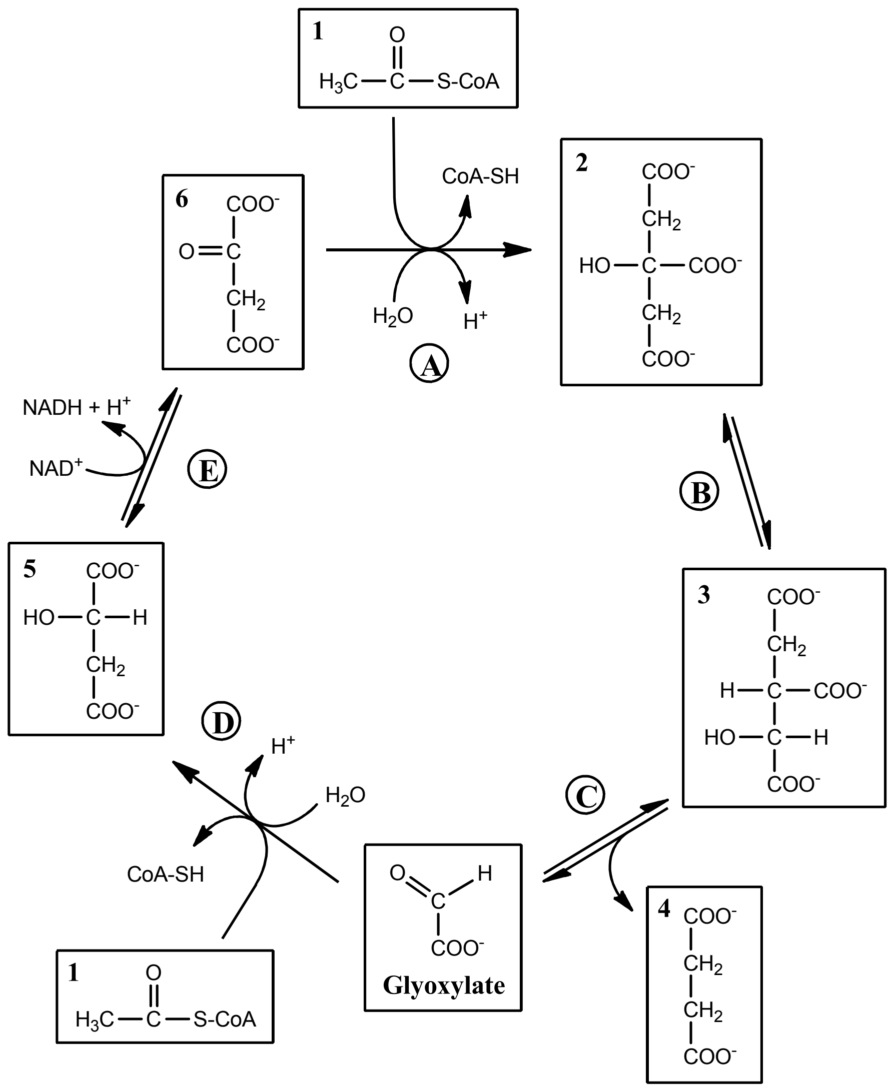
[2Fe-2S]	-0.25 V
[4Fe-4S]	-0.10 V
CoQ	+0.05 V
Cyt. a_3	+0.39 V
Cyt. c_1	+0.22 V
FMN	-0.34 V

16. (4) Why is the ability of the flavin nucleotides (FAD, FMN) to transfer 1 *or* 2 electrons important in the electron transfer chain? Briefly explain in 20 words or fewer.

17. (4) We learned that 4 protons must move from the intermembrane space into the matrix to drive the synthesis of 1ATP. How do these 4 protons contribute to ATP synthesis? Briefly explain in 25 words or fewer.

Part II – Cumulative (100 points)

The glyoxylate cycle is shown below. This pathway allows the products of fatty acid breakdown to be converted to carbon precursors for the synthesis of amino acids and sugars. (Compound **4** can be converted to amino acids or to glucose through reactions and pathways that we have studied.) While many plants, fungi, and microorganisms contain all of the enzymes of the glyoxylate cycle, vertebrates lack some of them.



18. (15) Consider the reactions of the glyoxylate cycle.

   a. (3) Most intermediates of the glyoxylate cycle are molecules we have studied. Name these compounds (1-6).

   b. (5) Name the *class* of each enzyme of the cycle (A-E).

   c. (3) Which of these reactions is/are also used in glucose catabolism (either anaerobic or aerobic)? List the enzyme(s) (A-E)

   d. (4) Which of these enzymes (A-E) would you expect to be regulatory enzymes? Why? Briefly explain in 6 words or fewer.

19. (10) Consider the net reaction of the glyoxylate cycle and how it can lead to the generation of glucose.
    a. (2) Write a balanced equation for the net reaction of the glyoxylate cycle.
    b. (3) How are the products of the glyoxylate cycle used to make glucose? Explain by listing the steps and/or pathways used in the conversion and noting the stoichiometries of the carbon compounds involved.
    c. (5) If compound **1** is labeled with ^{14}C at the methyl position, where (if at all) will this labeled carbon end up in glucose? Assume the shortest possible conversion route, and show your reasoning.

20. (9) Consider the effect of the glyoxylate cycle on the energy status and energy currencies of the cell.
    a. (3) Calculate the net use and/or production of energy currencies (ATP or ATP equivalents, NADH, $FADH_2$) that occurs as a result of the synthesis of one molecule of glucose starting from compound **1** of the glyoxylate cycle. Show your reasoning.
    b. (3) Calculate the net number of energy currencies (ATP or equivalent, NADH, $FADH_2$) that *would have been* produced if the compound **1** used to make glucose had *instead* been oxidized to CO_2.
    c. (3) From the previous 2 parts, calculate the *net loss* of ATP equivalents (that is, how many are used or not made) resulting from the production of one molecule of glucose from compound **1**.

21. (2) True or false. The glyoxylate cycle is anaplerotic for the citric acid cycle.

The glyoxylate cycle is important in plant germination. Seeds are composed largely of protein and fat, but plants also need carbohydrates for growth. The sprouting plant uses the glyoxylate cycle to help convert fatty acids into carbohydrates until it has grown big enough to carry out photosynthesis.

22. (7) Consider the fatty acids stored in seeds.
    a. (1) In what form are fatty acids stored in seeds? Name the type of molecule.
    b. (3) Draw the core structure of this type of molecule (from part **a**). You may use the generic '–R' group to indicate fatty acid tails.
    c. (3) What is the major difference between the fatty acids stored in seeds and the fatty acids stored in mammals?
        A. Seed fatty acids are more saturated.
        B. Seed fatty acids are less saturated.
        C. Seed fatty acids are longer.
        D. Seed fatty acids are shorter.
        E. Seed fatty acids are branched.

23. (4) What kinds of carbohydrates &/or glycoconjugates is the growing plant likely to need? Choose all that are likely.
    a. Cellulose
    b. Chitin
    c. Glycogen
    d. Glycoproteins
    e. Lactose
    f. Peptidoglycan
    g. Proteoglycans
    h. Sucrose

Enzyme C from many plants and microbes has been studied, including studies of kinetics and inhibition. Detailed kinetic studies of enzyme C from two plants, *Pinus pinea* and *Lupinus alba* (see photos), revealed the same overall reaction scheme, but differences in the rates of binding and catalysis. (Reviewed in Vanni et al, *Comp. Biochem. Physiol. B* 1990, **95**(3):431).

Pinus pinea: Italian stone pine

The reported reaction scheme is:

$$E + \mathbf{3} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\mathbf{3} \overset{\text{multiple steps}}{\rightleftharpoons} E\mathbf{4}G \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathbf{4} + EG \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + G$$

where E is enzyme, **3** and **4** are compounds **3** and **4**, and G is glyoxylate.

The reported kinetic values included the following:

	Pinus pinea (P)	*Lupinus alba* (L)
K_m	0.08 mM	0.03 mM
V_{max}/E_t	46 s^{-1}	10 s^{-1}
k_1	394,000 $M^{-1}s^{-1}$	725,000 $M^{-1}s^{-1}$
k_{-1}	89 s^{-1}	15 s^{-1}
k_2	60 s^{-1}	101 s^{-1}
k_{-2}	277,000 $M^{-1}s^{-1}$	192,000 $M^{-1}s^{-1}$
k_3	197 s^{-1}	12 s^{-1}
k_{-3}	986,000 $M^{-1}s^{-1}$	145,000 $M^{-1}s^{-1}$

Lupinus alba: white lupine

24. (20) For each of the following questions, answer with **P** (for the enzyme from *Pinus pinea*) or **L** (for the enzyme from *Lupinus alba*).
    a. (2) Which enzyme binds compound **3** more quickly?
    b. (2) Which enzyme has higher affinity for compound **3**?
    c. (2) Which enzyme binds glyoxylate more quickly?
    d. (4) Which enzyme has higher affinity for glyoxylate? Show your reasoning.
    e. (2) Which enzyme has the higher turnover number?
    f. (4) Which enzyme has greater catalytic efficiency? Show your reasoning.
    g. (4) Which enzyme would show greater product inhibition due to build-up of glyoxylate? Briefly explain your answer (in 30 words or fewer) and include how glyoxylate inhibits the enzyme.

25. (4) What is the relationship between enzyme C from *Pinus pinea* and enzyme C from *Lupinus alba*? They are: (Choose all that apply.)
    a. Homologs
    b. Orthologs
    c. Paralogs
    d. Isozymes
    e. Convergently related
    f. Divergently related

26. (10) The structure of enzyme C from the soil fungus *Aspergillus nidulans* has been determined by X-ray crystallography (Britton et al, *Structure* 2000, **8**:349). Although most of the structure was visible (513 out of 538 residues), some residues of the N-terminus, C-terminus, and an active-site loop were missing from the electron density. These missing regions were not predicted to form regular secondary structures.
    a. (4) One possible explanation for the missing residues is that they were removed by proteolytic cleavage. Which of the following techniques would allow you to verify that the regions missing from the electron density were *not* cleaved and are present in the crystallized enzyme? (Assume that the sequence of the enzyme is known.) Choose all that apply:
        A. Mass spectrometry
        B. Edman degradation
        C. CD spectroscopy
        D. FTIR spectroscopy
        E. NMR spectroscopy
    b. (4) Assuming no proteolytic cleavage occurred, why are regions of the enzyme not visible in the electron density? Briefly explain in 10 words or fewer.
    c. (2) Based on your knowledge of structural investigations of enzyme function, what compound (or type of compound) could you add to the crystallization experiment to cause the missing active site loop to be visible in the electron density?

27. (11) Consider the reaction catalyzed by enzyme **D** of the glyoxylate cycle.
    a. (4) How does enzyme **D** catalyze the reaction? Choose all likely catalytic mechanisms:
        A. Covalent catalysis
        B. Electrostatic catalysis
        C. General acid catalysis
        D. General base catalysis
        E. Metal ion catalysis
        F. Proximity and orientation effects
        G. Preferential binding of the transition state
    b. (4) Show the mechanism for the initial step of this reaction. Starting with the provided drawing of the substrates, add curved arrows to show the movement of electrons, and draw in relevant enzyme functional groups.
    c. (3)What reaction step promotes the overall reaction being irreversible? (5 words or fewer.)

28. (8) You want to study the kinetics of the *entire* glyoxylate cycle. But first you need to come up with an appropriate buffering system.
    a. (4) Based on your background reading, you have decided to buffer the reaction at pH 7.9. The buffers listed below are available to you. Which buffer should you use, and why? Briefly explain in 30 words or fewer.

Buffer name	pK_a	Buffer name	pK_a
Acetic acid	4.8	Sodium phosphate	7.2
Citric acid	5.4	HEPES	7.6
MES	6.2	Glycylglycine	8.2
Bis-Tris	6.5	Tris	8.3
PIPES	6.8	CHES	9.5

    b. (4) What fraction of your selected buffering compound will be protonated at pH 7.9? Show your calculation.