Chemistry and Biochemistry 153A
Winter 2010

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 answer sheet, 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 is the answer sheet. Be sure you've answered all of the questions!**

Part I (100 points) – Kinetics of Enzyme Inhibition through ATP Synthase

1. (5) Which compound has the higher phosphoryl-transfer potential? 1,3-bisphosphoglycerate (1,3-BPG) or fructose-1,6-bisphosphate (FBP)? Briefly explain the evidence for your answer (in 30 words or fewer).

2. (4) Why are energy currencies useful to the cell? Choose all correct answers.
   a. They provide a link between catabolic and anabolic processes in the cell.
   b. They act as a storehouse for free energy that can be used to drive endergonic reactions.
   c. They act as a storehouse for free energy that can be used to drive exergonic reactions.
   d. They react rapidly, enhancing metabolic reaction rates.

3. (12) True or False? (3 points each)
   a. Several glycolytic enzymes require metal ion cofactors.
   b. In fermentation (starting from glucose), there is no net oxidation of carbon.
   c. Anaplerotic reactions use citric acid cycle intermediates to build other molecules.
   d. Acetyl-CoA can be used as a substrate in the net synthesis (generation) of citric acid cycle intermediates.

4. (7) Regarding competitive inhibition:
   a. What is the relationship between K_m and K_m^{app}?
      A. $K_m < K_m^{app}$
      B. $K_m = K_m^{app}$
      C. $K_m > K_m^{app}$
   b. Why is this relationship observed? In other words, briefly explain how the action of a competitive inhibitor leads to this relationship between K_m and K_m^{app} (30 words or fewer).
   c. True or false? Transition state analogs act as competitive inhibitors.

5. (12) Given the mitochondrial framework on your answer sheet, show how electrons are passed *from succinate to oxygen* through the electron transport chain. In your drawing, include:
   - All diffusible substrates and products of the various electron transfer reactions (with labels)
   - All relevant protein complexes (with labels)
   - The number, direction, and location of proton transfers (assuming you begin with one molecule of succinate)
   - The positive (+) and negative (-) sides of the membrane

   Be sure to distinguish between electron movements and proton movements (label your arrows).

6. (10) In class, we discussed 3 catabolic enzymes that undergo an induced fit on binding their substrates (the 'pacman' enzymes).

   a. Briefly define 'induced fit' (30 words or fewer).

   b. Select the three enzymes displaying induced fit from the list provided:

A. Hexokinase	G. α-Ketoglutarate dehydrogenase
B. Glyceraldehyde-3-phosphate dehydrogenase	H. Succinyl-CoA synthetase
	I. Phosphoglycerate kinase
C. Pyruvate kinase	J. Phosphofructokinase
D. Citrate synthase	K. Aldolase
E. Pyruvate dehydrogenase	L. Enolase
F. Isocitrate dehydrogenase	M. Succinate dehydrogenase

   c. Why is induced fit binding important in these three enzymes? Provide a brief explanation that applies to all three (15 words or fewer).

7. (19) In the glycolytic reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glyceraldehyde-3-phosphate (GAP) is converted to 1,3-bisphosphoglycerate (1,3-BPG).

   $GAP + P_i + NAD^+ \rightarrow 1,3\text{-BPG} + NADH + 2H^+ \qquad \Delta G'^\circ = +6.7$ kJ/mol

   a. In the above reaction, GAP is the (choose all that apply):
      A. Reductant
      B. Oxidant
      C. Hydride donor
      D. Hydride acceptor

   Normally, the direct addition of a phosphate group to a metabolite is a highly unfavorable process. However, under standard conditions, this reaction is only slightly disfavored, because it is coupled to the favorable redox reaction:

   $$GAP + H_2O + NAD^+ \rightarrow 3\text{-PG} + NADH + 2H^+$$

   b. Calculate the $\Delta G'^\circ$ for this favorable redox reaction given that:

   $$1,3\text{-BPG} + H_2O \rightarrow 3\text{-PG} + P_i \qquad \Delta G'^\circ = -49.4 \text{ kJ/mol}$$

   (Hint: the GAPDH reaction is the sum of the phosphorylation and redox reacions)

   c. Calculate the $\Delta E'^\circ$ for this favorable redox reaction. Show your work.

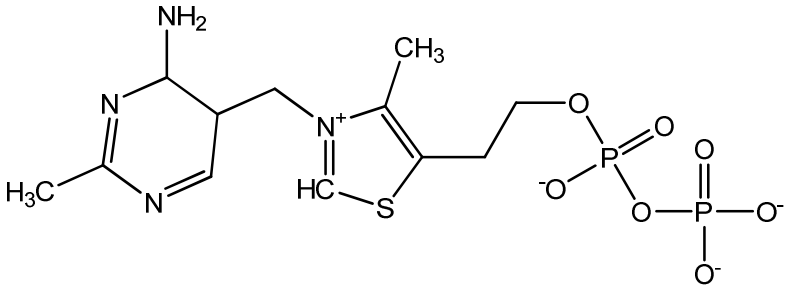
   d. Given that the standard reduction potential, E'°, of the NAD^+/NADH pair is -0.315 V, calculate the E'° for the GAP/3-PG. Show your work.

   e. Write the balanced reduction half reaction for the GAP/3-PG redox pair.

   f. Why is the conversion of GAP to 1,3-BPG favorable in the cell? Briefly explain in 25 words or fewer.

8. (4) Pyruvate dehydrogenase complex is regulated by (choose all that apply):
   a. Covalent modification
   b. Feedback inhibition
   c. Allosteric modulators
   d. Product inhibition
   e. Competitive inhibition

9. (15) Regarding the compound thiamine pyrophosphate (TPP), whose structure is shown:



   a. TPP is a (choose all correct answers):
      A. Cofactor
      B. Coenzyme
      C. Cosubstrate
      D. Prosthetic group
      E. Redox center

   b. TPP is used by an enzyme of the citric acid cycle. Which enzyme is it?
      A. Citrate synthase
      B. Aconitase
      C. Isocitrate dehydrogenase
      D. α-Ketoglutarate dehydrogenase
      E. Succinyl-CoA synthetase
      F. Succinate dehydrogenase
      G. Fumarase
      H. Malate dehydrogenase

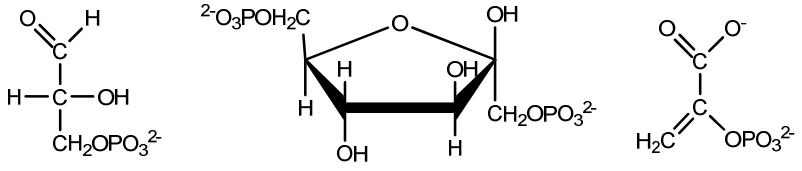
   c. In the box provided, draw the *curved arrow mechanism* for the first step of this (citric acid cycle) decarboxylation. A portion of the substrate structure is included. Complete the structure of the substrate and draw in the relevant portion of TPP.

   d. True or false? TPP has an acidic carbon.

   e. True or false? TPP helps stabilize a carbanion intermediate.

10. (12) You are studying yeast metabolism, and you begin by feeding the yeast radio-labeled glucose, with ^{14}C at position 3.

   a. Circle where you would expect this label to appear in the following compounds (be sure to put your answers on the answer sheet):



   b. When the yeast are grown anaerobically, you measure the rapid appearance of the label in one byproduct. What is this compound?

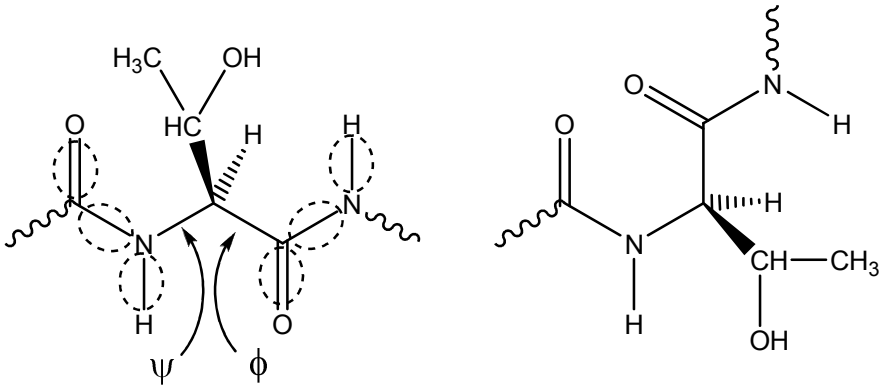
   c. Do you expect a different result when the yeast are grown aerobically? If so, what would be the difference?

Part II (140 points) – Cumulative

11. (10) As a teaching assistant for a biochemistry course, you are expected to grade exams. The question that you have been assigned to grade asks is two parts. In the first part, students must complete a portion of a polypeptide chain (left) with:
    - A sidechain for L-tyrosine
    - Circles on bonds about which there is no rotation
    - Arrows indicating the bonds about which ϕ and ψ conformational angles are measured.

    In the second part (right), students are asked to draw the same peptide segment with ϕ=180° and ψ=0°.

    One student gives the following answers:



    What is wrong with these drawings? Briefly list up to 6 different errors, one per line, (there may actually be more or fewer), that you see in the students' answers. (Include whether the error is in the left structure, right structure, or both)

12. (8) There is evidence that the complexes of electron transport associate into large, 'supramolecular' complexes. For example, electron micrographs suggest that one copy of complex III and two copies of complex IV associate.

    a. What is/are the benefit(s) of forming this type of association? Choose all that apply:
        A. The number of protons pumped per electron can increase.
        B. The rate of proton pumping can be enhanced.
        C. The reduction potentials of the redox centers can be modified.
        D. The P/O ratios for NADH and $FADH_2$ can be increased.
        E. The rate of electron transfer can be enhanced.

    b. What is the predominant type of interaction driving the formation of these supramolecular complexes?
        A. H-bonds
        B. van der Waals contacts
        C. Salt bridges
        D. The hydrophobic effect
        E. Covalent bonding

13. (10) Regarding chemical reactions:
    a. We learned that the thermodynamics (favorability) of a reaction are independent of its kinetics (rate). Briefly explain why these are independent of each other (30 words or fewer).
    b. We also learned that the measured ΔG values of cellular reactions can be used to determine which reactions of a pathway are rate-limiting. Briefly explain how reaction rates influence the ΔG in this situation (25 words or fewer).

14. (30) Your labmate is studying the kinetics of the enzyme jacobase with its substrate chipotlate, and has come to you for help in analyzing the results. When performing reactions with 0.01 μM jacobase, he measured the K_m and V_{max} and found that they equal 0.5 mM and 4 mM/s, respectively.
    a. Draw a double-reciprocal (Lineweaver-Burke) plot for these data; be sure to label the axes with names, units, and values.
    b. True or false? Jacobase has a high affinity for its substrate chipotlate.
    c. What is the catalytic efficiency of jacobase with chipotlate as its substrate? Show your work.
    d. Based on your answer to part c, is the following statement true or false? Jacobase is a catalytically perfect enzyme. (You must answer part c to get credit for this answer.)

    Your labmate also measured jacobase's kinetics in the presence of an inhibitor
    e. He tells you that $K_m^{app} < K_m$ and $V_{max}^{app} < V_{max}$. Based only on this information, can you tell what kind of inhibitor this is? If so, what kind? If not, why not, and what additional information would you need?
    f. He wants to know about the affinities of this system. Help him by writing an equation that relates measurable quantities (such as [jacobase], [inhibitor], [chipotlate], K_m, K_m^{app}, V_{max}, V_{max}^{app}) to the K'_I.
    g. K'_I is a measure of the affinity between:
        A. The inhibitor and jacobase
        B. The inhibitor and chipotlate
        C. The inhibitor and the jacobase-chipotlate complex
        D. Chipotlate and jacobase
        E. None of the above
    h. What are the units of K'_I?

15. (4) The p50 for myoglobin is _____(a)_____ the p50 for hemoglobin, indicating that myoglobin's affinity for O_2 is _____(b)_____ that of hemoglobin.
    A. higher than
    B. lower than
    C. equal to

16. (6) Given what we learned about enzyme classification and naming, why is 'ATP synthase' a misleading name for this enzyme? What should it be called instead?

17. (27) Lactate dehydrogenase (LDH) is a homotetrameric enzyme found in a variety of organisms, from bacteria to animals. Some bacterial LDHs show allostery (positive cooperativity with respect to pyruvate, and positive modulation by fructose-1,6-bisphosphate, or FBP), while others don't. According to a publication by Uchikoba, et al, 2001, "The quaternary structure of [non-allosteric LDH] was similar to the active conformation of allosteric LDHs."

    a. Write a balanced equation for the reaction catalyzed by LDH.

    b. Based on the quote from the publication, what is the likely relationship between the $K_{0.5}$ of the allosteric LDH and the K_m of the non-allosteric LDH?

        A. $K_m < K_{0.5}$ B. $K_m > K_{0.5}$ C. $K_m = K_{0.5}$

    c. Draw the expected V_o versus [pyruvate] curves for:

        A. Non-allosteric LDH
        B. Allosteric LDH
        C. Allosteric LDH + FBP

        Be sure to label each curve with the appropriate letter, and label the axes.

    d. Which of the following catalytic mechanisms are important in the function of LDH?

        A. Covalent catalysis
        B. Electrostatic catalysis
        C. Metal ion catalysis
        D. Proximity and orientation effects
        E. Preferential binding of the transition state
        F. Acid/base catalysis

    You are interested in studying the kinetics of the LDH reaction using lactate as a substrate. Your first step is to pick a buffer that will keep the reaction as close to pH 7.4 as is possible. The following buffers are available to you:

Buffer name	pK_a
Citric acid	5.4
Bis-Tris	6.5
PIPES	6.8
Sodium phosphate	7.2
HEPES	7.6
Tris	8.3
CHES	9.5

    e. Which buffer will you use? Briefly explain your selection in 25 words or fewer.

18. (6) Three molecules of CO_2 are produced from pyruvate after one round of the citric acid cycle. Although O_2 is not involved these oxidations, the absence of O_2 quickly brings the reactions of the citric acid cycle to a halt. Briefly explain this phenomenon in 40 words or fewer.

The Philippines is a tropical island nation with hot and humid weather most of the year.

19. (10) Traditional Philippine cuisine varies depending on the region, but sour flavors are common throughout the islands. This sourness is achieved through the use of ingredients like vinegar (acetic acid) or citrus (ascorbic acid).

    a. Sour flavors help protect food against spoiling (that is, against microbial growth), which is important in a hot place where not everyone owns a refrigerator. Briefly explain (in terms of the effect on macromolecules) how these flavors are able to provide antimicrobial activity. (20 words or fewer.)

    b. The acetic acid in vinegar, when ingested, is converted to acetyl-CoA (with the concurrent hydrolysis of 1 ATP per reaction), then oxidized through the citric acid cycle. What is the *net* production of ATP (or ATP equivalents) per metabolized molecule of acetic acid? Show your work.

20. (16) Soy sauce is another common ingredient in Philippine cooking. It is made by fermenting a mixture of mashed soybeans (mostly protein) and flour (mostly starch), followed by brining (soaking the fermented mixture in salt water). The brining liquid becomes the soy sauce.

    a. To what class of macromolecules does starch belong?
    b. What are the structural features of the polymers in starch? Choose all that apply:

        A. Linear
        B. Branched
        C. Helical
        D. Extended
        E. Rigid
        F. Hydrated
        G. Anhydrous
        H. Fibrous

    c. What are the monomer units of starch? Be as specific as possible.
    d. Name the linkages that connect these monomers in starch. Be as specific as possible.
    e. Briefly state the most likely purpose of adding flour to the soybeans. (10 words or fewer.)

21. (13) Geckos are a common sight in the Philippines, crawling on walls and ceilings.

    a. Studies of geckos' uncanny ability to stick to all sorts of surfaces have revealed that only van der Waals contacts are formed between geckos' toes and the surfaces they crawl on. How could such a weak interaction counteract the force of gravity on this relatively large organism? Briefly explain in 15 words or fewer.

    b. Given the climate of the Philippines, the fatty acids of geckos' membrane lipids are likely to be _____ those of Artic (cold-climate) walruses. (Choose all that apply):

        A. longer than
        B. shorter than
        C. of equal length as
        D. more saturated than
        E. less saturated than
        F. of equal saturation as

    c. Briefly explain your answers to part b (in 25 words or fewer).