Chemistry and Biochemistry 153A
Spring 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 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 sheets; 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 colored answer sheets. Be sure you've answered all of the questions!**

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

1. (5) a. Red blood cells do not contain mitochondria. Their primary fuel source must therefore be:
   A. Fat
   B. Carbohydrate
   C. Protein

   b. Briefly explain your answer to part **a** in 35 words or fewer.

2. (5) You are studying metabolism in yeast, and you begin your experiments by feeding the yeast 50 mM glucose labeled at carbon 4. Among the labeled compounds, you find dihydroxyacetonephosphate (DHAP).
   a. Briefly explain why DHAP is labeled (in 30 words or fewer).
   b. Which carbon of DHAP is labeled (if the phosphorylated carbon is #3)?

3. (2) True or False? Isocitrate dehydrogenase catalyzes an oxidative decarboxylation with the help of NAD^+ and TPP.

4. (2) True or False? Succinate dehydrogenase does not use NAD^+ as an oxidant because the reduction potential of NAD^+ is too low.

5. (2) True or False? Phosphofructokinase is an allosteric enzyme.

6. (2) True or False? Coenzyme A is a 1- or 2-electron carrier.

7. (2) True or False? Glycogen phosphorylase is regulated by both covalent modification and effector binding.

8. (20) For each of the following enzymes (1-6), select all characteristics (A-I) that match:

   1) Glyceraldehyde-3-phosphate dehydrogenase
   2) Pyruvate dehydrogenase complex
   3) Succinyl-CoA synthetase
   4) Pyruvate kinase
   5) Phosphoglycerate mutase
   6) Succinate dehydrogenase

   A. Catalyzes the replacement of a thioester with a phosphoryl attachment (formation of a mixed anhydride).
   B. Catalyzes a substrate-level phosphorylation.
   C. (The enzyme) is or becomes phosphorylated
   D. (The enzyme) becomes reduced
   E. Binds a cosubstrate
   F. Uses metal ion(s) in catalysis
   G. Is a regulatory enzyme
   H. Forms a Shiff base
   I. Uses electrostatic catalysis

9. (4) The inhibition of ATCase by CTP is an example of (choose all that apply):
    a. Feedback inhibition
    b. Product inhibition
    c. Competitive inhibition
    d. Uncompetitive inhibition
    e. Mixed inhibition
    f. Allosteric inhibition
    g. Homotropic effects
    h. Heterotropic effects

10. (29) As we discussed in class, the enzyme creatine kinase catalyzes the transfer of a phosphoryl group from ATP to creatine, yielding ADP and creatine phosphate.
    a. (3) The $\Delta G'^\circ$ for the hydrolysis of creatine phosphate is -43.1 kJ/mol, and the $\Delta G'^\circ$ for the hydrolysis of ATP is -30.5 kJ/mol. Which compound, creatine phosphate or ATP, has the higher phosphoryl transfer potential?
    b. (3) Calculate the $\Delta G'^\circ$ for the creatine kinase reaction. Show your reasoning.
    c. (3) In class we discussed that $\Delta G \approx 0$ for this rection in the cell. Briefly explain why this is true (in 10 words or fewer).
    d. (5) Briefly explain why $\Delta G \approx 0$ is important in the creatine kinase reaction being able to buffer ATP and ADP levels. (50 words or fewer.)
    e. (4) Calculate the ratio of [creatine phosphate]/[creatine] in human muscle if [ATP]/[ADP] = 10.
    f. (3) It is common for athletes to take creatine (or creatine phosphate) as a supplement. Its consumption can lead to improvements in short duration, high intensity (anaerobic) exercise, but not longer duration (aerobic) exercise. Why would aerobic exercise not be improved by supplementation with creatine? Briefly explain in 25 words or fewer.
    g. (3) Would you expect creatine kinase to be a regulatory enzyme? Explain why or why not in 8 words or fewer.
    h. (5) Humans have four isozymes of creatine kinase. How (in general) can it benefit an organism to have isozymes? Choose all that apply.
        A. Different isozymes may catalyze the same type of reaction with different substrates.
        B. Different isozymes may be regulated differently.
        C. Different isozymes may function in different tissues.
        D. Different isozymes may catalyze different reactions using the same substrate.
        E. Different isozymes may have different affitinites for their substrate(s).
        F. Different isozymes may modify different parts of the same substrate.
        G. Different isozymes may function in different pathways.

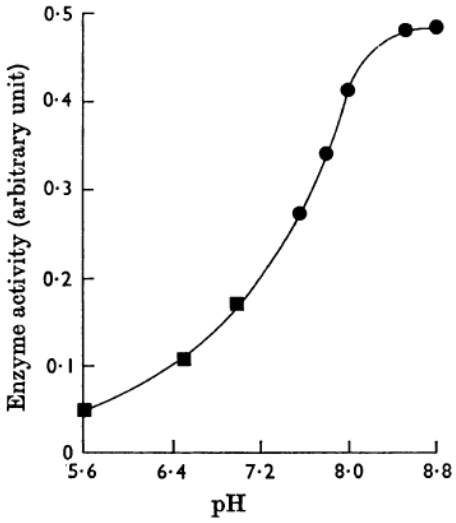
11. (22) The kinetics of citrate synthase have been well studied. The following excerpts and figures come from a study of citrate synthase published in 1969 (Shepard and Garland, *Biochem. J.* **114**, 597). From the abstract:

    1. Citrate synthase (EC 4.1.3.7) was purified 750-fold from rat liver. 2. Measurements of the Michaelis constants for the substrates of citrate synthase gave values of 16 μM for acetyl-CoA and 2 μM for oxaloacetate.

    a. (4) For which substrate does citrate synthase have a higher binding affinity? Why is this relevant in the cell? Briefly explain in 15 words or fewer.

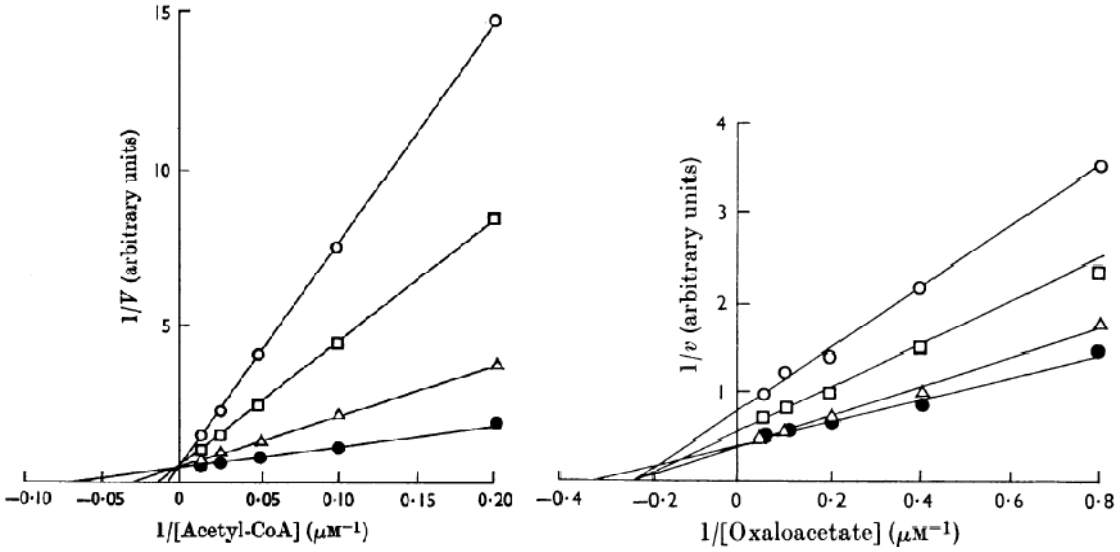
    The activity of citrate synthase was measured as a function of pH, yielding the plot on the right:

    b. (3) Why is it beneficial for citrate synthase to be maximally active at the pH shown in the curve, as opposed to physiologic pH? Briefly explain in 20 words or fewer. *(Hint: consider its environment.)*



The adenylates ATP, ADP, and AMP were found to be inhibitors of citrate synthase, as shown in the following plots. The data points correspond to:

● No adenylate nucleotide
○ 4 mM ATP
□ 4 mM ADP
△ 4 mM AMP



c. (4) Based on the data shown in the above plots, complete the following sentence using the choices below: In binding citrate synthase, ATP is a ___(i)____ inhibitor with respect to acetyl-CoA and a ___(ii)____ inhibitor with respect to oxaloacetate.

   A. Competitive
   B. Uncompetitive
   C. Mixed
   D. Pure noncompetitive
   E. Allosteric

d. (5) The authors report that the K_m^{app} for acetyl-CoA in the presence of 4 mM ATP is 143 μM. Calculate the dissociation constant for ATP binding to citrate synthase.

e. (4) Where does ATP bind to citrate synthase? Give a structural explanation for how this is possible (in 10 words or fewer).

f. (2) Based on the left plot above, which of the adenylates has the highest affinity for binding citrate synthase?

12. (5) Which of the following are ways that protons cross the mitochondrial inner membrane? Choose all correct answers.
    a. Bound to membrane-diffusible molecules
    b. Through proton jumping
    c. Diffusion
    d. Osmosis
    e. Through transport enzymes, with other molecules

Extra Credit: ATP Synthase (10 points)

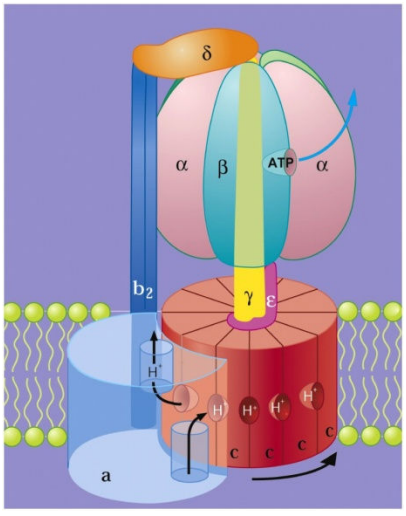
A. What UCLA professor won the Nobel Prize for his work on ATP synthase? Give his first and last names.

B. Which subunits make up the rotor?

C. Can the synthase function without the b subunits? Briefly explain why or why not in 30 words or fewer.

D. Why does it take ~4 protons moving across the membrane to synthesize 1 ATP? Briefly explain (in 25 words or fewer).

E. What happens if the proton gradient is reversed?



Part II (140 points) – Cumulative

The theme is: **BABY FOOD! ;)**

Mother's milk is a baby's first and most important food. Milk provides nutrients (proteins, fats, carbohydrates, vitamins & minerals), hydration, and immunological protection (via antibodies and immune cells) to the baby.

The composition of milk varies widely depending on the species of mammal, reflecting the different needs of different species of young.

Composition of Milk from Different Mammalian Species (per 100 g fresh milk).
(From the University of Guelph Dairy Science and Technology site)

	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (kcal)
Cow	3.2	3.7	4.6	66
Human	1.1	4.2	7.0	72
Water Buffalo	4.1	9.0	4.8	118
Goat	2.9	3.8	4.7	67
Donkey	1.9	0.6	6.1	38
Elephant	4.0	5.0	5.3	85
Monkey, rhesus	1.6	4.0	7.0	73
Mouse	9.0	13.1	3.0	171
Whale	10.9	42.3	1.3	443
Seal	10.2	49.4	0.1	502

The major class of fats in milk is triglycerides (triacylglycerols). The triglycerides in milk are composed of a mixture of short-chain (ex: 4:0, 6:0, 8:0) and long-chain (ex: myristic, palmitic, stearic) fatty acids, whose melting points vary widely. In cow's milk, the mixed composition of triglycerides results in an overall melting point of 37°C, such that the fats are liquid at the cow's body temperature.

13. (2) Why does the milk of whales and seals have a much higher fat content than the milk of other mammals?

14. (3) Why is there a rough correlation between fat content and energy for milk from the various animals? (For example, seal milk has the highest fat content and also the highest energy.)

15. (5) In the milk of an animal with *lower* body temperature, would you expect the proportion of short-chain fatty acids to be *greater* or *smaller* than cow's (assuming the milkfat is likewise liquid)? Briefly explain why in 35 words or fewer.

Casein is the major protein component of milk. It is an unusual protein, containing little defined secondary or tertiary structure. Instead, it loosely aggregates to form casein micelles.

16. (4) Casein has many proline residues. How does this high proline content contribute to the formation of a loose, undefined structure? (20 words or fewer.)

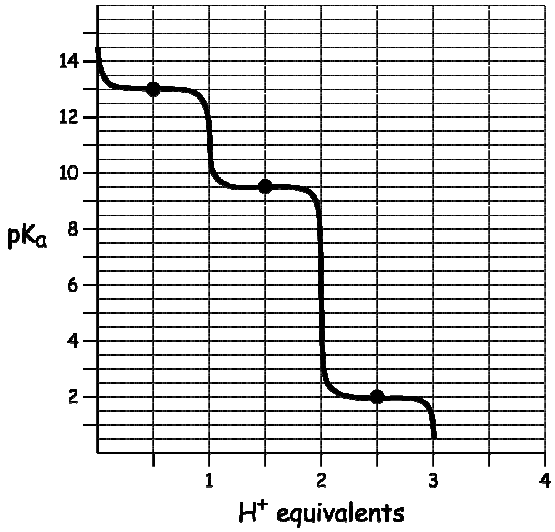
17. (4) Casein also has a large proportion of hydrophobic residues. How is this important in forming micelles? (25 words or fewer.)

18. (6) The pI of casein is 4.6.
    a. Based on the pI, which of the following amino acids is most likely to be abundant in casein?
        A. Glu
        B. Gln
        C. Pro
        D. Lys
        E. Leu
    b. The pH of milk is 6.7. What is the net charge of casein in milk?
        A. Positive
        B. Zero
        C. Negative
        D. Cannot be determined without more information

A Ser-Thr dipeptide is contained within the sequence of human casein.

19. (7) Starting with the provided atoms, draw the structure of Ser-Thr. Circle the atoms whose positions define the peptide's ϕ dihedral angle. Put asterisks (*) next to the atoms whose positions define the peptide's ψ dihedral angle.

20. (9) Does the titration curve at right accurately represent the titration of the Ser-Thr dipeptide? If not, list each error (one per line).



21. (29) Lactose is the major carbohydrate of milk. Its catabolism begins with hydrolysis of its glycosidic bond, then the monosaccharides are broken down via glycolysis. (Galactose is converted to glucose-6-phosphate through a series of reactions, one of which uses ATP and produces ADP.)

    a. (5) List the energy currencies produced as a result of the aerobic breakdown of lactose. Show your reasoning.

    b. (4) How many ATPs can be made from these energy currencies? Show your work.

    In the breakdown of proteins, different amino acids are catabolized to different intermediates of glycolysis &/or the citric acid cycle. For the dipeptide Ser-Thr, ser is converted to pyruvate in one step, and thr to pyruvate and acetyl-CoA in several steps:

    $$\text{Ser} \rightarrow \text{pyruvate} + NH_3$$
    $$\text{Thr} + NAD^+ + \text{CoA} \rightarrow \rightarrow \rightarrow \rightarrow \text{pyruvate} + \text{acetyl-CoA} + \text{NADH}$$

    c. (2) Name the class of enzyme that catalyzes the conversion of serine to pyruvate.

    d. (4) List the energy currencies produced as a result of the aerobic breakdown of Ser-Thr. Show your reasoning.

    e. (2) How many ATPs can be made from these energy currencies? Show your work.

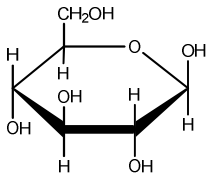
    f. (4) Given the molecular masses of the two nutrient compounds, lactose = 342 g/mol; Ser-Thr = 206 g/mol, which is the more efficient energy source? Show your reasoning.

    g. (4) Suggest two different compounds that could regulate the breakdown of Ser-Thr (to pyruvate and acetyl-CoA): a likely product inhibitor, and a likely feedback inhibitor.

    h. (4) Of the two inhibitors you listed for part f, choose one, and briefly explain how its levels reflect the energy status of the cell (25 words or fewer).

22. (4) Although we usually see it in its furanose form, fructose can also adopt a pyranose structure. Which of the following structures correctly depicts α-D-fructopyranose?

a



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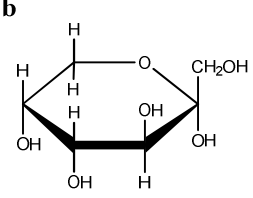

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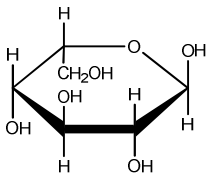

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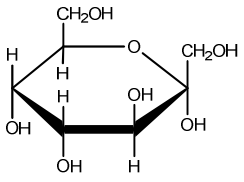

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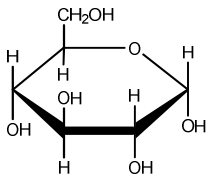
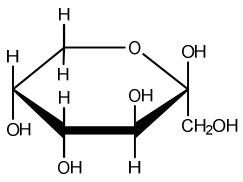


f



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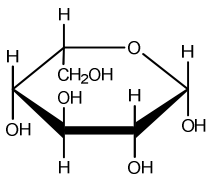
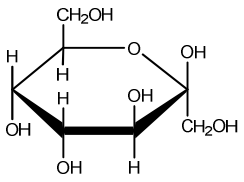


h



23. (20) Although minor components in terms of concentration, there are several important digestive enzymes in milk, including lipases, peptidases, and glycosidases. These help the baby digest the milk.

    a. (3) For some moms, their fresh, refrigerated milk will gradually acquire a strong, unpleasant, *soapy* taste. Which of the above digestive enzymes is responsible for this change?

    b. (2) What is the reaction product that has the soapy taste?

    c. (4) Freezing the milk slows the reaction, but does not stop it. Briefly explain why (20 words or fewer).

    d. (4) Warming the milk speeds up the reaction. Briefly explain why (20 words or fewer).

    e. (4) Scalding the milk (heating to the point of steaming) stops the reaction. Briefly explain why (in 20 words or fewer).

    f. (3) When fresh milk is added to baby cereal (like rice cereal or oatmeal), the initially thick mixture becomes runnier over time. Which of the above digestive enzymes is responsible for this change?

24. (8) Some breastfed babies display allergies to cow's milk; when Mom drinks the milk, baby breaks out in a rash.

    a. Sometimes people think this type of reaction is due to lactose intolerance. Why this is not the case?

    b. What type of compound (ex: carbohydrate, protein, fat, vitamin, …) is the most likely allergen and why? Briefly explain in 35 words or fewer.

25. (10) Yogurt is a favorite food of babies, despite its sour flavor. Yogurt is produced through the fermentation of milk by two types of bacteria: *Lactobacillus bulgaricus* and *Streptococcus thermophilus.*

    a. What type of fermentation occurs in making yogurt? (*Hint: consider yogurt's sour taste.*)

    b. Complete the sentence. The products of fermentation are _____ the substrates.
        A. More oxidized than
        B. More reduced than
        C. Of equivalent oxidation state as

    c. If lactose were labeled at carbon 1 of galactose, where would the label end up in the fermentation product(s)? Draw the product(s) and circle the labeled carbon(s).

26. (25) Some parents like making their own purees to make sure their baby eats the freshest veggies. But certain home-made baby foods should be avoided with young babies. Vegetables like beets, carrots and spinach are naturally high in nitrates, which can oxidize the iron in hemoglobin, decreasing hemoglobin's effectiveness, and causing a condition called 'methemoglobinemia' (or blue baby syndrome). Nitrate-contaminated well water can also cause methemoglobinemia.

    The oxidation of heme by nitrate involves the following redox half-reactions:

    $NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$ $\quad \mathscr{E}'^\circ = 0.42$ V

    $Hb\text{-}Fe^{3+} + e^- \rightarrow Hb\text{-}Fe^{2+}$ $\quad \mathscr{E}'^\circ = 0.12$ V

    a. (2) Write the net reaction for the oxidation of one heme iron by nitrate.

    b. (4) Calculate the $\Delta\mathscr{E}'^\circ$ for this reaction.

    c. (4) Calculate the $\Delta G'^\circ$ for this reaction.

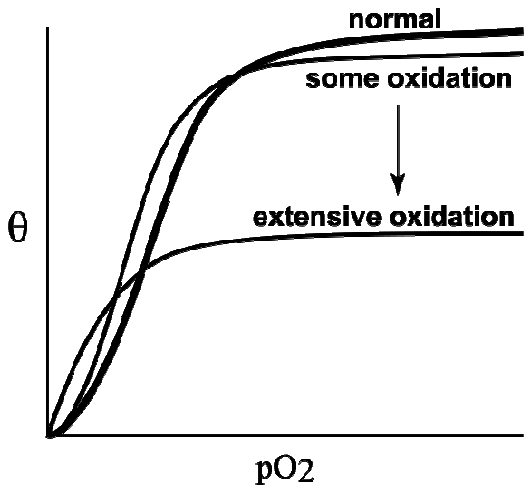
    d. (2) True or false? This reaction is spontaneous under standard conditions.

    e. (4) In studies of the *kinetics* of O_2 binding by hemoglobin, the T- and R-states bind oxygen equally quickly (same k_A). What can you infer about the rates of O_2 dissociation from the two states? In other words, does O_2 dissociate from a T-state Hb *more quickly than*, *less quickly than*, or *equally quickly as* from an R-state Hb? Briefly explain your answer in 30 words or fewer.

    The oxidation of heme iron in some of the subunits of hemoglobin (forming 'methemoglobin') changes hemoglobin's oxygen binding curve as shown:

    f. (6) Based on this curve, explain how heme oxidation affects hemoglobin's ability to bind and transport oxygen (40 words or fewer).

    g. (3) Name an effector can induce a similar curve shift.



27. (2) True or False? Allosteric enzymes usually show Michaelis-Menten kinetics.

28. (2) True or False? Phosphoglycerate mutase can produce 2,3-BPG, but cannot catalyze its production.