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Biochemistry I, Spring Term 2000 - Third Exam:

This exam has a total of 100 points and is divided into two sections. You must do ALL of the questions.

There are a total of 9 pages in this exam, *excluding* the formula page. Please check that you have all the pages and write your name on every page before you begin. Use the space provided to answer the questions.

NOTE: It may not be necessary to completely fill the space provided! Clear and succinct answers are appreciated by the graders!

Grade:

Part A:	___________
B1	___________
B2	___________
B3	___________
B4	___________
B5	___________
B6	___________
B7	___________
B8	___________
B9	___________
TOTAL	___________

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Section A (28 pts): (2 pts/question). Circle the letter corresponding to the **best** answer.

1. Phospholipids are molecules that contain:

   a) large amounts of polysaccharides.
   b) cholesterol.
   c) hydrophilic heads and hydrophobic tails.
   d) Both b) and c) are correct.

2. Micelles of fatty acids in water are organized such that the acyl (hydrocarbon) groups are directed toward the interior because

   a) of the van der Waals forces between the tails.
   b) of the free energy gained by exclusion of water from the interior.
   c) hydrogen bonding between water and the polar head groups.
   d) none of the above.

3. Most integral membrane proteins have the following characteristics:

   a) Composed of α-helices and/or β-sheets
   b) Most of the polar groups are on the outside of the protein, in contact with the membrane.
   c) Many unsatisfied hydrogen bonds.
   d) Contain FAD and other redox active centers.

4. Which of the following is an example of a storage polysaccharide made by animals?

   a) cellulose.
   b) glycogen.
   c) amylopectin.
   d) starch.

5. Cellulose, a β(1->4)-linked glucose polysaccharide differs from starch in that starch is

   a) a β(1->6)-linked manose polysaccharide.
   b) an α(1->6)-linked glucose polysaccharide.
   c) an α(1->4)-linked glucose polysaccharide.
   d) an α(1->4)-linked mannose polysaccharide.

6. In a eukaryotic cell, the enzymes of glycolysis are located in the ______ and the enzymes of the TCA cycle are located in the _____:

   a) plasma membrane; mitochondra
   b) inner mitochondrial membrane; cytosol
   c) cytosol; mitochondrial matrix.
   d) cytosol; inner mitochondrial membrane

7. Energy for the synthesis of ATP by eukaryotic organisms during oxidative phosphorylation is obtained directly from

   a) High energy phosphate compounds.
   b) A proton gradient across the inner mitochondrial membrane.
   c) A proton gradient across the outer mitochondrial membrane.
   d) A proton gradient across the cell membrane.

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8. Kinases are enzymes that
   a) produce Coenzyme Q from $FADH_2$
   b) transfer a phosphate group from ATP to another compound.
   c) do not play a role in the regulation of glycogen.
   d) require NAD as a cofactor.

9. The oxidation of one acetyl-CoA by the TCA (Krebs, citric acid) cycle results in the synthesis of about ___ ATPs.

   a) 6
   b) 9
   c) 12
   d) 15

10. Pyruvate, the end product of glycolysis, is converted to which of the following in humans under anaerobic (i.e. no oxygen) conditions?

   a) ethene
   b) ethanol
   c) lactic acid.
   d) acetic acid.

11. The conversion of NADH to NAD^+, involves (hint, what does Leo the Lion do?):

   a) a gain of one electron by NAD^+.
   b) a loss of two electrons from NADH.
   c) no change in the number of electrons in NADH.
   d) a gain of two electrons by NAD^+.

12. The proton gradient and the membrane potential

   a) are sufficient, separately, to make ATP from ADP + Pi.
   b) are both required to make ATP.
   c) cancel one another when uncouplers are present.
   d) reinforce one another when uncouplers are present.

13. Each cycle of fatty acid oxidation (β-oxidation) produces:

   a) 1 FAD, 1 NADH, and 1 acetyl-CoA.
   b) 1 $FADH_2$, 1 NADH, and 1 acetyl-CoA.
   c) 1 $FADH_2$, 1 NADH, and 2 CO_2 molecules.
   d) 1 $FADH_2$, 1 NAD^+, and 1 acetyl-CoA.

14. Which of the following is not a common mechanism for altering the flux of metabolites through the rate-determining step of a pathway?

   a) Covalent modification (i.e. phosphorylation) of the enzyme.
   b) Diffusional coupling between adjacent active sites.
   c) Allosteric control of the enzyme activity.
   d) Inverse control of opposing reactions.

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Section B:

B1(14 pts): The following is a table of proteins and a brief summary of their properties. Devise a purification scheme that will result in a purified preparation of hexose kinase in its native (i.e. active) form. You may use as many affinity columns as you like, however beware that some may work better than others, depending on the properties of the protein. Although space is allotted for three steps, you may be able to accomplish the purification in fewer steps.

For each of the steps in your scheme, briefly discuss the physical basis for the separation

Protein	Solubility in 3M Ammonium Sulfate (gm/L)	Isoelectric Point (pI)	Molecular Weight
Glycogen phosphorylase	0	6.0	150,000
Phosphofructose kinase	0	6.0	150,000
Hexose kinase	10	7.0	80,000
glyceraldehyde 3-P dehydrogenase	10	7.0	80,000

Step #	Separation Technique	Basis of Separation	Protein(s) retained for next step	Protein(s) discarded by this step
1				
2				
3				

B2 (10 pts) : The standard free energy of hydrolysis of a number of phosphate esters is given on the formula page. On the basis of these free energies, answer the following questions:

i) Can Glucose-1-P be used to efficiently phosphoryate ADP to make ATP under standard conditions (i.e. if you mixed 1M of Glucose-1-P with 1 M of ADP, do you expect a large amount of ATP to be formed)? Justify your answer using a thermodynamic argument(6 pts).

ii) *Assuming that the intracellular concentration of ADP and ATP are 1mM, calculate the ratio of [glucose] to [glucose-1-P] such that the reaction described in part i becomes spontaneous.(4 pts)

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B3 (6 pts): *The membrane potential across the mitochondrial membrane is approximately -150 mV (inside negative). The oxidation of each NADH pumps about 8 protons across this membrane. This generates a pH difference of 1 pH unit and stores about 160 kJ/mol of free energy (i.e. 20 kJ/mol per proton). Now, assume that the energy from NADH oxidation was used to pump phosphate ions across the membrane instead, giving an identical concentration gradient. Calculate the free energy stored by the phosphate gradient. Assume that the membrane potential is unchanged (i.e. remains at -150 mV) and the phosphate is entirely in the *doubly* ionized form (i.e. $HPO_4^{=}$).

B4 (4 pts): The catalytic mechanism of lysozyme requires that Asp53 is ionized and that Glu35 is not. A mutant lysozyme was made such that Asp53 was replaced with Asn. Would this enzyme still be active? Why? Do you expect the activity to be the same, decrease, or increase? Justify your answer.

B5 (4 pts) Answer **one** of the following two questions:

i) What are the similarities and differences between product inhibition and feedback inhibition.

OR

ii) What are the similarities and differences between substrate level phosphorylation and oxidative phosphorylation.

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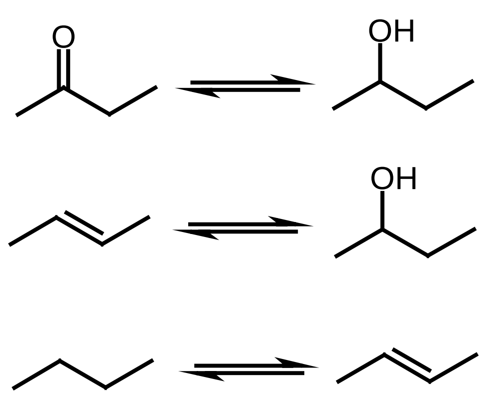
B6 (10 pts): Choose **one** of the following compounds: NADH, NAD^+, ATP, or AMP.

i) Which compound did you choose?(0 pts)

ii) Do high levels of this compound indicate high or low energy reserves in the cell. Why?(3 pts)

iii) Briefly discuss how the compound you chose regulates the complete oxidation of glucose (i.e. the pathways of glycolysis, TCA cycle, electron transport, oxidative phosphorylation). Where possible give at least one specific example of an enzyme and describe how it is regulated by the compound of your choice.(7 pts).

B7 (2 pts) Three biochemical reactions are shown below. Circle the one that is **NOT** an example of a redox reaction.



B8 (6 pts): The following is a list of enzymes that catalyze spontaneous, or energetically downhill, reactions that we have encountered in glycogen metabolism, glycolysis, regulation of glycolysis, or fatty acid metabolism:

a) Glycogen phosporylase
b) Fructose 1,6 bisphosphatase
c) Fructose 2,6 bisphosphatase
d) Thiolysis (last step in fatty acid oxidation, producing Acetyl-CoA)

For **one** of the above, answer the following:

i) Indicate your choice.

ii) What is the reverse reaction:

iii) What is the energy source, or method, used to drive the reverse reaction:

iv) Briefly discuss how the forward and reverse reactions are coordinately regulated. If they are not regulated, say so!

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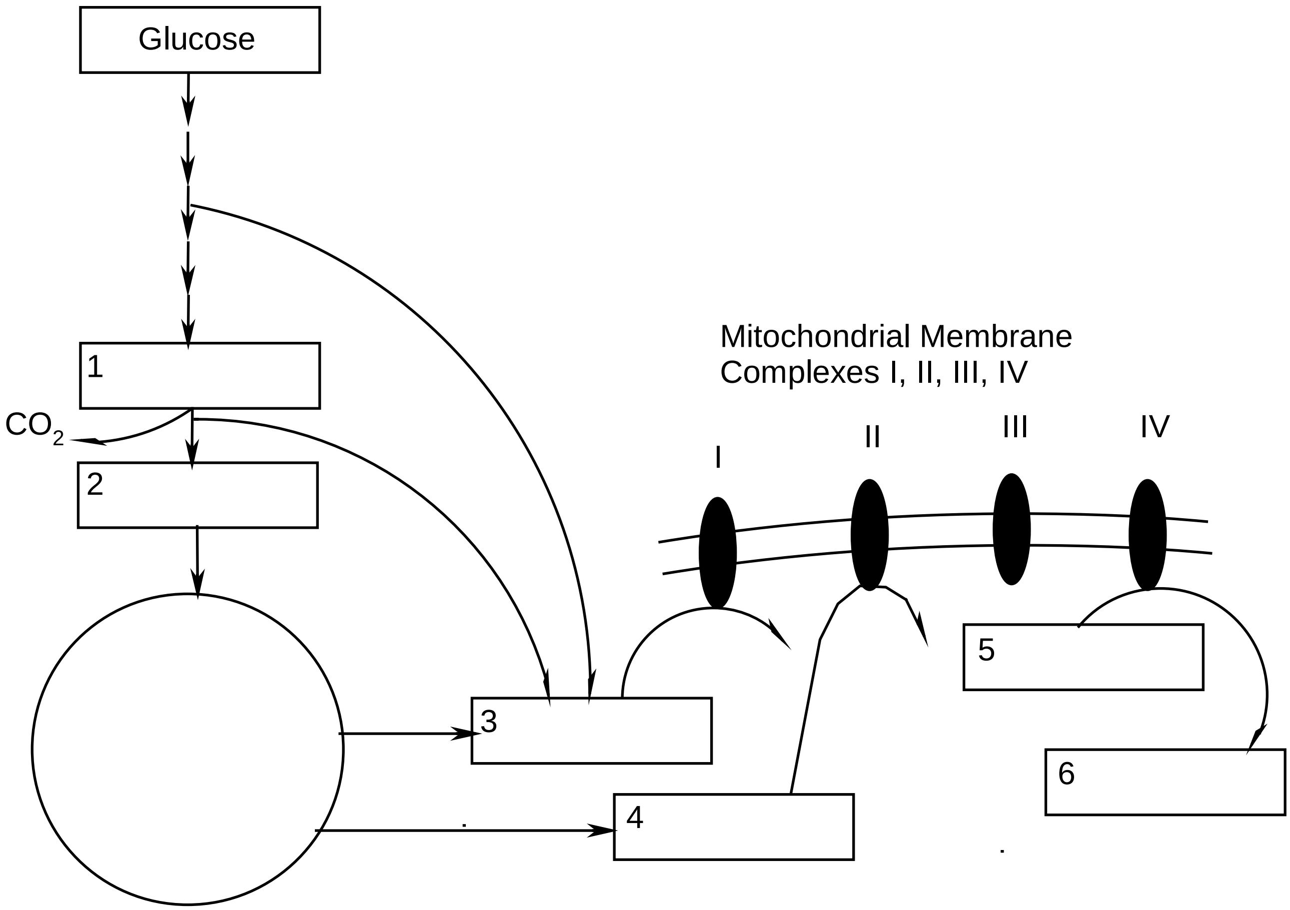
B9: (16 pts) The following diagram is an overview of glucose oxidation under **aerobic** conditions. Note that this diagram is incomplete; for example, CO_2 generation from the TCA (Krebs) cycle is not shown.



i) Fill in the **six(6)** empty squares with the name of the appropriate molecule. (9 pts).

ii) Indicate, by circling and labeling the appropriate sections of the above diagram, which segment corresponds to glycolysis and electron transport. Please circle the segments in a neat fashion so that there is no ambiguity when grading! (2 pts).

iii) Briefly describe the molecular mechanism by which the proton gradient is coupled to ATP generation (ie. describe how the F_1F_oATPase converts the proton gradient to chemical energy) (3 pts)

iv) Add the appropriate step(s) to account for anerobic metabolism in yeast. What is the main purpose of this pathway (besides generating alcohol)?(2 pts) (The chemical formula for pyruvate is $CH_3COCOOH$).