Final Exam

*St. Patty’s Day is almost here, so why not have a test on BEER?!!*

**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!
Part 1 – New Material

1. (10) True or False? The following questions are worth 1 point each:
   a. Human cells can perform alcoholic fermentation.
   b. Fermentation yields more energy than oxidation.
   c. Pyruvate is an α-keto acid.
   d. The reduced form of \( \text{NAD}^+ \) is \( \text{NADH}_2 \).
   e. Coenzyme A is an electron carrier.
   f. Biotin helps catalyze carboxylation reactions.
   g. The oxidation of glucose to \( \text{CO}_2 \) and \( \text{H}_2\text{O} \) is exergonic.
   h. Citrate has three carboxylate groups.
   i. Coenzyme Q is found in the cytosol.
   j. \( \text{FADH}_2 \) produced in the citric acid cycle diffuses to complex II of the electron transport chain, where it releases its electrons.

2. (3) Why aren’t hexokinase and glucose-6-phosphatase both fully active under the same cellular conditions? (Choose the best answer.)
   a. The cell would explode.
   b. ATP would be wasted.
   c. Heat would be generated.
   d. Glucose levels would fluctuate too much.
   e. Fermentation wouldn’t be possible.

3. (12) Given the following hypothetical metabolic pathway:

   \[
   \begin{align*}
   &\text{A} \quad \begin{array}{c} 1 \end{array} \quad \text{B} \quad \begin{array}{c} 2 \end{array} \quad \text{C} \quad \begin{array}{c} 3 \end{array} \quad \text{D} \quad \begin{array}{c} 4 \end{array} \quad \text{E} \quad \begin{array}{c} 5 \end{array} \quad \text{F}
   \end{align*}
   \]

   a. (2) Which is/are the slow step(s) (among 1-5)?
   b. (2) Name an intermediate (among A-F) that could act as a product inhibitor of enzyme 2.
   c. (2) Name an intermediate (among A-F) that could act as a feedback inhibitor of enzyme 2.
   d. (2) Name an intermediate (among A-F) that could act as an activator of enzyme 2.
   e. (4) Briefly explain how regulated steps of a pathway are experimentally identified (35 words or fewer).

4. (5) Imagine a game in which you need to get a classmate to say ‘ATP synthase,’ but you can’t just ask them to say it. List 5 key words or short phrases you would use to let your classmate know what biochemical term you are thinking of. (Each word should somehow relate to the structure, function, or features of ATP synthase. Do not list ‘ATP’ or ‘synthase’ in your answer.) As an example, if I wanted someone to say ‘spider,’ I might use the following key words: eight, legs, arachnid, tarantula, web.
5. (9) True or False? The following questions are worth 3 points each:
   a. GTP and ATP have similar phosphoryl transfer potentials.
   b. Decarboxylations are reversible in the cell.
   c. Lactate and glucose have equal oxidation states.

6. (3) In the respiratory chain, many types of redox centers are used multiple times, with each center having a different standard reduction potential. Examples include the iron-sulfur clusters and hemes:

How can the same type of redox center have multiple different standard reduction potentials? Briefly explain in 15 words or fewer.

7. (4) Given the table above, why are heme groups found in Complexes III and IV, while iron-sulfur clusters are found mainly in Complexes I and II? Choose all that apply:
   a. Electrons move from compounds of lower to higher reduction potential.
   b. To allow for releases of free energy in each step of the electron transport chain
   c. Electrons move from Complex I to Complex II to Complex III to Complex IV
   d. Heme can bind O\textsubscript{2} for reduction
   e. The centers (heme vs. Fe-S) transfer different numbers of electrons
When we drink beer, the alcohol is absorbed into our blood stream and is catabolized mainly in the liver. In the major pathway for ethanol catabolism, ethanol is converted to acetaldehyde, then to acetate, as shown in the figure below.

![Diagram showing ethanol catabolism pathway]

8. (20) In the first step of ethanol catabolism, ethanol is converted to acetaldehyde through the action of cytosolic alcohol dehydrogenase (ADH):

\[ \text{ethanol} + \text{NAD}^+ \rightarrow \text{acetaldehyde} + \text{NADH} + \text{H}^+ \]

a. (2) Circle the correct choice for each blank:

In the reaction catalyzed by ADH, ethanol is the (1: reductant / oxidant) because it becomes (2: reduced / oxidized).

b. (3) Below are the reduction half reactions and standard reduction potentials for the participants of the ADH reaction. Using these values, calculate the change in standard reduction potential for the ADH reaction (as written above). Show your work.

\[
\begin{align*}
\text{acetaldehyde} + 2\text{H}^+ + 2e^- &\rightarrow \text{ethanol} \quad \varepsilon^{\circ} = -0.197 \text{ V} \\
\text{NAD}^+ + \text{H}^+ + 2e^- &\rightarrow \text{NADH} \quad \varepsilon^{\circ} = -0.315 \text{ V}
\end{align*}
\]

c. (2) True or False? The ADH reaction as written above is favored under standard conditions.

d. (3) Calculate the \(\Delta G^\circ\) for this reaction. Show your work.

e. (5) Given that, in the cytosol, the ratio of \([\text{NAD}^+]:[\text{NADH}] \approx 700:1\), what must be true of the ratio of \([\text{ethanol}]:[\text{acetaldehyde}]\) in order for this reaction to proceed as written? Show your work.

f. (2) What would you expect the relationship between \(\Delta G^\circ\) and \(\Delta G^{\circ\circ}\) to be for this reaction?

A. \(\Delta G^\circ < \Delta G^{\circ\circ}\)
B. \(\Delta G^\circ \approx \Delta G^{\circ\circ}\)
C. \(\Delta G^\circ > \Delta G^{\circ\circ}\)

g. (3) Briefly explain your answer to part f (in 20 words or fewer).

9. (12) In the second step of ethanol catabolism, acetaldehyde is converted to acetate through the action of aldehyde dehydrogenase (ALDH):

\[ \text{acetaldehyde} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{acetate} + \text{NADH} + 2\text{H}^+ \]

a. (4) Which molecules or intermediates (and parts thereof) in this reaction act as nucleophiles? Which act as electrophiles?

b. (2) Complete the second sentence. There are two versions of ALDH in the liver, one in the cytosol, and one in the mitochondria. These two enzymes are ________________.

c. (6) Which enzyme of glucose catabolism catalyzes a similar type of oxidation-reduction reaction? How are the two reactions similar? How do they differ? Briefly list your answers using 20 total words or fewer.
10. (10) Acetate, the product of ALDH, can leave the liver and be metabolized by other cells. This catabolism of acetate begins with the conversion of acetate to acetyl-CoA at the expense of ATP:

\[ \text{acetate} + \text{CoA} + \text{ATP} \rightarrow \text{acetyl-CoA} + \text{ADP} + \text{P}_i \]

a. (2) What class and subclass of enzyme catalyze this reaction?
b. (2) Which enzyme of glucose catabolism catalyzes a similar type of reaction?
c. (6) Propose a 2-3 step reaction scheme for how acetyl-CoA formation could be coupled with ATP hydrolysis. Base your answer on the coupling mechanism employed by the enzyme of part b above. List each step, and name which molecule would act as the nucleophile (nuc) and electrophile (elec).

For example, if describing the first step of the PDH complex rxn, you’d list:
Decarboxylation of pyruvate, nuc – TPP, elec – pyruvate

11. (12) Ethanol cannot be used by humans in the net synthesis of glucose.

a. (3) Briefly explain why not (20 words or fewer.) Hint: consider the nature of the citric acid cycle as a hub of metabolism.
b. (5) How can $^{14}$C-labeled ethanol that is catabolized in the liver give rise to labeled glucose? Briefly explain in 25 words or fewer.
c. (4) If ethanol is labeled at the methylene carbon (that is, the one bonded to oxygen), where would this label end up in glucose? Circle all potential points of labeling in the provided structure.
Part II – Cumulative

12. (15) Consider the complete, oxidative catabolism of ethanol to CO₂, starting with the steps described on the previous page.

   a. (4) What is the net yield of energy and redox currencies per molecule of ethanol. Show your reasoning.

   b. (3) What is the net yield of ATP per molecule of ethanol? Assume that electrons from cytosolic NADH are passed to Complex I. Show your work.

   c. (3) Given that the molecular weight of glucose is 180, versus 46 g/mol for ethanol, which is the better energy source? (Which yields more ATP per gram?) Show your reasoning.

   d. (3) Consider the structures of glucose and ethanol. What aspect of their structure explains your finding in part c? (8 words or fewer).

   e. (2) True or false? The oxidative catabolism of ethanol results in some substrate-level phosphorylation.

13. (22) Many inhibitors of alcohol dehydrogenase (ADH) have been studied. One publication examined the following set (right) of related molecules (Anderson et al, BBA 1965, 99:46-55):

   Studies of the inhibition kinetics of the compounds resulted in two groups of behavior. The smaller compounds, with n < 6, produced plots like the two shown below:

   ![Plot 1](image1)
   ![Plot 2](image2)

   In each plot, line 1 represents the enzyme in the absence of inhibitor. Lines 2 and 3 represent increasing concentrations of inhibitor.

   a. (1) Why type of plot is shown above?

   b. (4) How are the values of individual points on the plots measured? Briefly explain in 30 words or fewer.

   c. (2) What type of inhibition is shown in the plot where NAD⁺ is the substrate?

   d. (2) What type of inhibition is shown in the plot where ethanol is the substrate?
e. (3) Which form(s) of the enzyme does the inhibitor bind most tightly? (Choose all that apply.)
   A. Free enzyme
   B. Enzyme-NAD⁺ complex
   C. Enzyme-ethanol complex
   D. Enzyme-NAD⁺-ethanol complex

f. (3) Based on the kinetic plots and on the structure of the inhibitors, where (on the enzyme) would you predict that these inhibitors bind?

The kinetic data using NAD⁺ as the substrate were used to calculate the dissociation constants for the binding of the inhibitors:

<table>
<thead>
<tr>
<th>n</th>
<th>$K_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$6.5 \times 10^{-2}$</td>
</tr>
<tr>
<td>2</td>
<td>$5.7 \times 10^{-2}$</td>
</tr>
<tr>
<td>3</td>
<td>$4.6 \times 10^{-2}$</td>
</tr>
<tr>
<td>4</td>
<td>$2.9 \times 10^{-2}$</td>
</tr>
<tr>
<td>5</td>
<td>$1.6 \times 10^{-2}$</td>
</tr>
<tr>
<td>6</td>
<td>$8.7 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

g. (2) What is another variable name for the $K_d$ values shown above?
   A. $K_I$
   B. $K'_I$
   C. $K_a$
   D. $K_m$

h. A. (3) Which of the inhibitors binds ADH with the highest affinity. (Give its n value.)
   B. (2) What differences in interactions best explain the differences in binding affinity?
      1) hydrogen bonding
      2) salt bridges
      3) van der Waals contacts
      4) steric hindrance
      5) charge repulsion
      6) covalent bond formation

14. (17) As described above, the conversion of aldehyde to acetate in ethanol catabolism is catalyzed by aldehyde dehydrogenase (ALDH). Of the two ALDH enzymes, the mitochondrial (matrix) enzyme is the primary one used in ethanol catabolism; its $K_m$ for acetaldehyde is lower than that of the cytosolic enzyme.

As with many proteins, there are sequence variants of ALDH in the human population. One variant, found in people of east-Asian descent, is nearly inactive. The reduced ALDH activity of this variant leads to build up of acetaldehyde when the drinker consumes alcohol. The higher levels of acetaldehyde can cause a set of uncomfortable symptoms, including facial flushing (reddening), nausea, rapid pulse, and sometimes more extreme symptoms. It is perhaps unsurprising, then, that both heterozygous and homozygous carriers of this ALDH variant have a much lower risk of alcoholism.

a. (2) There is a variant of alcohol dehydrogenase that induces similar side effects when drinking alcohol. How does the activity of this enzyme variant compare with the activity of the more common alcohol dehydrogenase?
   A. The variant has higher activity
   B. The variant is equally active
   C. The variant has lower activity

The inactive ALDH variant was initially characterized through purification of this variant and the active (more common) ALDH from human liver, followed by peptide sequencing (Yoshida et al, 1984, *PNAS* 81:258).
b. (3) Before the proteins were sequenced, they were first digested by trypsin. Briefly explain the role of proteolytic digestion in protein sequencing (20 words or fewer).

Purification and Edman degradation of the different tryptic peptides yielded the following sequences:

(inactiv variant) Glu-Leu-Gly-Glu-Ala-Gly-Leu-Gln-Ala-Asn-Val-Lys

c. (2) List the differences in structure and properties between Gln and Lys that might contribute to the difference in activities (15 words or fewer).

d. (2) How many ionizable groups does the ‘active form’ peptide have?

e. (4) Calculate the pI of the active form peptide (using the equation we learned in class).

f. (2) How does the pI of the ‘inactive variant’ peptide compare to that of the ‘active form’ peptide?

   A. It is higher
   B. It is the same
   C. It is lower

g. (2) Does the curve to the right accurately represents the titration of the active form peptide?

15. (6) Over-consumption of alcohol leads to numerous physiological changes and problems. For example, production of nitric oxide (NO), an important signalling molecule in mammals, increases in alcoholics. Recall that nitric oxide can bind to heme, very similarly to the way CO binds heme.

a. (5) How does NO affect oxygen binding by hemoglobin and myoglobin? Answer by drawing oxygen binding curves for:
   · Hemoglobin alone (Hb)
   · Hemoglobin + some NO (Hb + NO)
   · Myoglobin alone (Mb)
   · Myoglobin + some NO (Mb + NO)

   *Be sure to label your axes and label each curve.*

b. (1) True or False? Hemoglobin is an allosteric enzyme.

16. (4) Although most consumed alcohol is oxidatively broken down, some is used in other metabolic pathways. For example, some alcohol goes toward the synthesis of phosphatidyl ethanol.

a. (2) Phosphatidyl ethanol belongs to what class of biomolecules?
   A. Proteins
   B. Lipids
   C. Carbohydrates
   D. Nucleic acids
   E. Metabolites or intermediates

b. (2) What is the function (or role) of phosphatidyl ethanol in a cell? Be as specific as possible.
17. (36) Brewing Beer

Although fermentation is a key step in making beer, the brewing process involves multiple essential steps. (The following descriptions were adapted from Bering, CL. 1988. *J. Chem. Ed.* 65(6):519)

**Malting**

In the first step of making beer, called ‘malting,’ barley kernels (derived from the grass *Hordeum vulgare*, shown at right) are exposed to water and allowed to germinate (sprout). Germination leads to the production of many enzymes, including proteases and amylases, and the starting up of metabolic processes. Although the enzymes begin catalyzing reactions during the malting process, the majority of their beer-making work occurs during mashing (see below).

Once most of the barley has germinated, it is heated and dried in a process called kilning. Kilning burns off green shoots and roots and halts most metabolic activity.

a. (3) Why does kilning halt metabolic activity? Choose the most important reason:
   
   A. Some enzymes use water as a substrate
   B. Metabolites require water in order to diffuse to and from enzymes
   C. O₂ requires water in order to diffuse to the ETC
   D. Heating denatures all the enzymes
   E. The shoots and roots are destroyed

**Milling and Mashing**

Once dry, the malted barley is milled (ground) to promote the release of the products of the next step, mashing. In mashing, the milled barley is mixed with water, and the two are stirred in a large container. The temperature of the mash is controlled to promote optimal activity of the enzymes that were produced during malting. The proteases digest proteins that would otherwise make the beer hazy. The amylases, named α-amylase and β-amylase for the sugar anomer that results from their catalysis, break down the barley starch into mono- and oligosaccharide units. Sometimes additional sugars, from the breakdown of other, cheaper grains (like corn or rice, called adjuncts) are also added.

b. (5) Complete the following sentence, being as specific as possible. Note that for (b), you should name the monosaccharide units:

   Starch is composed of _**(a)**_, an *unbranched* polymer of _**(b)**_ units connected via _**(c)**_ linkages, and _**(d)**_, a related *branched* polymer containing additional _**(e)**_ linkages.

c. (2) Give the enzyme class and subclass for the amylases.

d. (3) α-Amylase and β-amylase both specifically cleave the major type of linkage in starch. Based on this specificity, which other molecules might they also cleave? (Choose all that apply.)

   A. Peptidoglycan
   B. Proteoglycans
   C. Maltose
   D. Cellulose
   E. Chitin
   F. Glycolipids
   G. Glycogen

e. (2) Which amylase, α or β, retains the anomic configuration of the substrate in the product?
The Wort
After mashing, the barely-water mixture is filtered to remove the solids. The remaining nutrient-rich mixture, or ‘wort,’ is boiled for a couple of hours, during which time the hops (the female flower cluster of the *Humulus lupulus* plant; see right) are added. This boiling serves several functions: (1) to concentrate the solutes; (2) to sterilize the wort; (3) to denature and precipitate out unwanted protein; (4) to extract and isomerize the flavor components from the hops; and (5) to darken the beer, through reactions (like the following) between sugars and amines:

![Diagram of sugar and amine reaction]

f. A. (2) Name the type of structure formed in the reaction shown above.
B. (2) What type of metabolite is most likely to be the amine in this reaction?
C. (2) Which enzyme of glycolysis forms this type of structure during catalysis?
D. (3) How does the formation of this type of structure allow the enzyme (of part c) to promote catalysis? Briefly explain in 20 words or fewer.

g. A. (3) How does boiling denature the barley proteins? Briefly explain in 15 words or fewer.
B. (2) Why do the boiled proteins precipitate? Briefly explain in 15 words or fewer.

Fermentation
After boiling, the wort is cooled and aerated to saturation, and the hops and precipitated proteins are filtered out. Yeast are added. The yeast rapidly multiply for a short time, then the fermentation begins. When the sugar concentrations drop below a certain level, the yeast clump together (in a process called ‘flocculation’) and either float to the surface or sink to the bottom, where they may easily be separated out.

h. (3) How does aerating the wort promote the initial rapid multiplication of yeast? Briefly explain in 20 words or fewer.

Aging and Finishing
In the last phases of brewing, the beer is chilled and aged, which allows the flavors to mellow, and yields more precipitated proteins to be filtered out. The beer is also carbonated, and may be pasteurized or filtered for sterilization.

i. (2) Why is it surprising that the beer must be carbonated after fermentation? Briefly explain in 10 words or fewer.

j. (2) In addition to producing enzymes, malting produces metabolites like sucrose and D-fructose. Which of the following structures represents L-fructose?

![Diagram of sucrose and fructose structures]

<table>
<thead>
<tr>
<th>a.</th>
<th>b.</th>
<th>c.</th>
<th>d.</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure a" /></td>
<td><img src="image" alt="Structure b" /></td>
<td><img src="image" alt="Structure c" /></td>
<td><img src="image" alt="Structure d" /></td>
</tr>
</tbody>
</table>