NOTES:

1. Where appropriate please show work – if in doubt show it anyway.
2. Pace yourself – you may want to do the easier questions first.
3. Please note the point value of questions – adjust your answers and effort accordingly.
4. Some questions may have more data than you need.
5. Please be brief – focus your answers to the space provided.
6. Please write CLEARLY – if I cannot read it – it is wrong.
7. You are welcome to detach the metabolic chart (carefully).
8. Good luck.
Question 1 (6 pts.). Draw clear accurate graphs to describe the behavior of the following systems. Clarity and accuracy rewarded.

a. only the form of the enzyme show below is active. Show its pH dependence at the right.

\[
\begin{align*}
&\text{NH}_2 \quad (\text{pK 9}) \\
&\text{S}^- \quad (\text{pK 5.0})
\end{align*}
\]

b. only the form of the enzyme show below is active. Show its pH dependence at the right.

\[
\begin{align*}
&\text{O}^- \quad (\text{pK 10.0}) \\
&\text{NH}_3^+ \quad (\text{pK 12.0})
\end{align*}
\]

Question 2 (4 pts.). Draw the structure of a chemically-credible reversible inhibitor of an HIV-protease. Circle the functional group that is the key aspect of the anticipated inhibition. Chemical accuracy important. For parts of the structure that you don't want/need to specify ... denote with an "-R"

\[
\begin{align*}
&\text{peptidic} \\
&\text{NORMAL}
\end{align*}
\]

Now explain the key feature of the proposed inhibitor:

So the cleavable amide (peptide bond) is replaced by a functional group that might resemble the tetrahedral intermediate. But it is not cleaved. 

R' & R'' are peptide-like (peptidic)
Question 3 (6 pts.). Given the following calculate $\Delta G^\circ$ for equation 1:

1. $C \leftrightarrow A$  \hspace{1cm} $\Delta G^\circ = \frac{+2}{\text{kcal}}$

2. $C \leftrightarrow B$  \hspace{1cm} $\Delta G^\circ = +5 \text{ kcal}$

3. $A \leftrightarrow B$  \hspace{1cm} $\Delta G^\circ = +3 \text{ kcal}$

$$\begin{array}{c}
B \leftrightarrow A \\
C \leftrightarrow B
\end{array}$$

$$\frac{-3 \text{ kcal}}{+5 \text{ kcal}} + 2 \text{ kcal}.$$ 

In equation 1... To make $\Delta G$ more negative I would: circle all of the following which will definitely accomplish this:

1. increase the concentration of $A$

2. decrease the concentration of $A$

3. increase the concentration of $C$

4. decrease the concentration of $C$

5. Double the concentration of both $C$ and $A$

6. Dilute the mixture of $C$ and $A$ with an equal volume of water.

Question 4 (6 pts.). Yield of ATP. In the space provided give the yield of ATP that would be formed in the following processes (enter a number from 0-10):

a. per molecule of glucose-1P phosphate to ethanol \hspace{1cm} 3

b. per molecule of fructose 1,6-diP converted to lactate \hspace{1cm} 4

c. per molecule of mannose converted to ethanol \hspace{1cm} 2

Question 5 (12 pts.). Quick calculations. Please note that most of the points are given for the correct numerical answer.

a and b: The osmotic pressure of 1 mL of a 0.1 M solution of glucose is 2.4 atmospheres.

a. Calculate the new osmotic pressure if the same amount of glucose were found in 1 mL of amylose (containing 10,000 glucose units per amylose molecule).

$$= \frac{2.4 \times 10^{-4}}{10} \text{ atmospheres}$$
b. Calculate the new osmotic pressure if the same amount of glucose were found in 1 mL of glycogen (again containing 10,000 glucose units per glycogen molecule).

\[ \frac{2}{4} \times 10^{-4} \text{ atmospheres} \]

e. What is the magnitude of the burst phase (i.e. the increase in absorbance at 405 nm when 30 \( \mu \text{M} \) of chymotrypsin is mixed with 5 mM of p-nitrophenylacetate at pH 8.0 in a 1 cm pathlength cell? The molar absorbIVITY (extinction coefficient) of p-nitrophenol at this pH is 11,000 M\(^{-1}\)cm\(^{-1}\)

\[ A = 11,000 \text{ M}^{-1}\text{cm}^{-1} \times (30 \times 10^{-6} \text{ M}) \times (1 \text{ cm}) \]

Absorbance: \(0.33\)

d. A competitive inhibitor shows a \(K_i\) of 2 mM. If the \(K_m\) for the substrate is originally 10 mM, what is the apparent \(K_m\) in the presence of 30 mM of the competitive inhibitor?

\[ K_{m,app} = K_{m,real} \left(1 + \frac{[I]}{K_i}\right) \]

Apparent \(K_m\) = \(160\) mM

Question 6 (6 pts). Tracing radiolabels and etc. Place asterisks indicating the position of the radiolabel in the molecules shown to the right – if the product contains no radiolabel write “NONE”.

a.

b.
Question 7 (5 pts.) Trehalose is a major energy storage compound in the circulatory systems of many insects. Answer the following questions

a. Name the glycosidic linkage: \( \alpha(1 \rightarrow 1) \)

b. Name the monosaccharide comprising the LEFT ring: \( D - \text{glucose} \)

c. Name the monosaccharide comprising the RIGHT ring: \( D - \text{glucose} \)

d. Circle the anomeric carbon atom in the RIGHT ring.

Question 8 (9 pts.) Freeze-clamping a tissue gave the concentrations of A and B in a metabolic pathway (A \( \rightarrow \) B \( \rightarrow \) C \( \rightarrow \) D \( \rightarrow \) E \( \rightarrow \) F) to be \([A] = 10^{-3} \text{M}\) and \([B] = 10^{-5} \text{M}\).

Calculate the mass action ratio for the reaction A \( \leftrightarrow \) B

\[
\text{MAR} = \frac{[B]}{[A]} = \frac{10^{-2}}{2} = \frac{10}{2}
\]

\( A \) and \( B \) are interconverted by \( E_1 \).
Compound \( A \) is mixed with a catalytic amount of \( E_1 \) in an appropriate buffer and the results shown to the right are obtained:

Calculate the equilibrium constant \([B]/[A] = \) \( 1.5 \)

\( A_{\text{init}} \rightarrow A_{\text{final}} = 10 \text{mM} - 4 \text{mM} = \frac{6 \text{mM}}{21 \text{mM}} \)

So \( B_{\text{final}} = 6 \text{mM} \)

Is this reaction at equilibrium in the cell? - Circle one answer: Yes \( \bigcirc \) No \( \bigcirc \) OR...

Cannot say (why "cannot say"?)

Finally draw an accurate representation of the progress of the reaction on the graph when the concentration of \( E_1 \) used was increased by a factor of 3.
Question 9 (12 pts) This question concerns glyceraldehyde-3P dehydrogenase.

This enzyme has a thioester intermediate that is shown incomplete in the top half of the box.

a. **complete the structure of the intermediate** found during catalysis by this dehydrogenase - put all the missing atoms in the box.

b. The next step in catalysis is **normally** attack of phosphate ion. At the bottom of the box is shown a phosphate analog (mimic) **arsenate**. Using the **arsenate drawn already**, show (by curved arrows) the next step of catalysis with release of a product.

c. In the box to the right show the structure of the **arsenate ester** released in "b"

d. Such arsenate esters are very rapidly hydrolyzed in water to regenerate arsenate and the carboxylic acid (before the compound could get to the next enzyme in glycolysis. Using this information - what would the yield of ATP per glucose be if all of the thioester intermediate were captured by arsenate (not phosphate). Assume no other steps are affected.

... **by-pass from 6 → 8 directly**

**Yield of ATP from glucose to lactate in the presence of arsenate**

Question 10 (6 pts.) Numbers: the answer to these question is a number from 0-20. Do not write abbreviations or words.

a. This numbered glycolytic intermediate is the first common intermediate between glucose and glycogen breakdown

b. phosphoenolpyruvate is compound number ___

c. how many phosphate atoms does a molecule of NADH have?

d. how many more electron does ethanol have than acetaldehyde?

e. conversion of one molecule of sucrose to ethanol releases ____ molecules of CO₂

f. the number of different enzymes needed to convert glucose to lactate?
Question 11 (12 pts) This question deals with the inhibition of chymotrypsin by the compound shown to the right. Please pay attention to concentrations of each component listed below.

5 μM chymotrypsin was mixed with 1 mM of the inhibitor at time zero. The graph below follows the release of chloride ion. A second 5 μM addition of chymotrypsin was made at 15 min.

a. what is the amount of the rapid phase seen below

b. explain the significance of the rapid phase seen to right. because 62 μM ≠ 5 μM this (4)

1) NOT a burst phase... The concentration of inhibitor over about 12 times (on average) before this mechanism-based inhibitor inactivates all enzyme. Inactivation is associated with release of Cl⁻.

(2 pts) explain what happens at the second addition of enzyme... and why NOT Graded "c"

The enzyme is inactivated already, so no further processing of inhibitor.

d. the inhibitor is radiolabelled at the asterisk * (top right). After 25 min (see graph) the mixture was subject to size exclusion chromatography. Draw clear curves to illustrate the elution profile for both protein and radioactivity.

e. after size-exclusion (gel-filtration) chromatography, the enzyme is assayed with a suitable substrate. What do you expect to see?

The enzyme is catalytically modified so shall still be inactive.
Question 13 (16 pts.). Fill in the blanks with not more than 3 legible words.

a. name an irreversible inhibitor of an enzyme
   many

b. and the enzyme that is the target of your answer in “a”
   many

c. these enzymes do not follow Michaelis-Menten kinetics
   allosteric

 d. the water-soluble vitamin incorporated into NAD+ is called
   niacin

 e. a negative allosteric regulator of glycolysis
   ATP, NADH

 f. a major regulatory enzyme in glycolysis
   hexokinase, phosphofructokinase

 g. the monosaccharides D-glucose and D-galactose are ___
   of one another

 h. a metal frequently associated with kinases
   Mg²⁺

 i. a hormone that indirectly activates phosphoryase
   epinephrine

 j. name an an acid protease
   papain, HVI protease

 k. a thiol protease
   papain / cathepsin

 l. inactive precursors of proteolytic enzymes are called
   zymogen

 m. a protease containing a bound zinc ion
   carboxypeptidase

 n. a technique used for studying rapid reactions in enzymology
   stopped flow

 o. how many times have you been taught “glycolysis”

 zz. the word that best describes this exam

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