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 - unfocused, rambling answers won’t receive as much credit as a few short appropriate phrases.

6. Please write CLEARLY - if I cannot read it - it is wrong.

7. Good luck
Question 1. (29 pts.) Short problems. Show work, but most credit goes to the correct numerical answer. Some questions contain more data than you will need.

a. A pure rat enzyme shows a rate of 2.6 μmol/min for a certain concentration of substrate at 20 °C. The substrate shows a $K_m$ of 3 mM and a molecular weight of 280 g/mol. At saturating substrate concentration the rate is 8.55 μmol/min. What is the substrate concentration?

$$v = \frac{V_{max} [S]}{K_m + [S]}$$

$$2.6 \text{ μmol/min} = \frac{(8.55 \text{ μmol/min}) [S]}{3 \text{ mM} + [S]}$$

$$[S] = \frac{2.6(3 \text{ mM} + [S])}{8.55} = 1.31 \text{ mM}$$

b. In "a" above the molecular weight of the substrate and enzyme were 280 and 48,000 g/mol respectively and the amount of enzyme used was 40 μg. What is the maximal turnover number (at substrate saturation) at 20 °C?

$$TN = \frac{8.55 \times 10^{-6} \text{ mmol/min}}{40 \times 10^{-6} \text{ g} / 48,000 \text{ g/mol}} = 10,260 \text{ /min}$$

Calculate, or discuss, what turnover number would be expected at 80 °C.

$$TN = ? \text{ cannot predict, but likely enzyme denatured at 80 °C}$$

(c) Tetrameric human hemoglobin has a total molecular weight of 64,000 g/mol. The atomic weight of iron is 56 g/mol. A human contains 500 g of hemoglobin. Calculate (no partial credit)

- How many moles of oxygen can be bound by 500 g hemoglobin

$$100781 \text{ mol}$$

- How many moles of DPG (BPG) can be bound by 500 g hemoglobin

$$7.8 \times 10^{-3} \text{ mol}$$

- How many grams of iron are there in 500 g of hemoglobin

$$4 \times 0.4374 \text{ g} = 1.75 \text{ g}$$
d. No partial credit. If a SINGLE human glycogen molecule contains 120,000 monosaccharide units. About how many:

i.) monosaccharide units in this molecule have their “4” position NOT ATTACHED via a glycoside linkage to another monosaccharide unit number

ii.) monosaccharide units have their “1” position NOT ATTACHED to another monosaccharide unit number

iii.) monosaccharide units have their “3” position NOT ATTACHED to another monosaccharide unit number

e. An enzyme has a Km of 20 mM for substrate S. What concentration of the substrate would give 90% of the maximal rate? 

\[ [S] = \frac{180}{1800 + 9S} \]

f. The Km for a substrate is observed to be 38 μM in the presence of 1.6 mM of a competitive inhibitor. In the absence of the inhibitor it is 3 μM. What is the K_i for the inhibitor.

\[ K_m(\text{pp}) = \frac{K_m}{1 + \frac{I}{K_i}} \]

\[ K_i = \frac{0.137 \text{ mM}}{12.67} = 1 + \frac{1.6 \text{ mM}}{K_i} \]

g. The reaction: \( A + B \leftrightarrow C + C \)

shows a standard free energy change of -4 kcal. Calculate the free energy change for the reaction at 300 K using these concentrations: A = 10 mM; B = 7 mM; and C = 0.01 mM. The gas constant is 2 cal/degree/mol.

\[ \Delta G = -4000 + 2 \times 2 \times 2 \times 300 \times \log \left( \frac{0.01 \times 0.01}{10 \times 7} \right) \]

\[ = -4000 - 8086 = -12,086 \text{ cal} = -12.1 \text{ kcal} \]

The original solution is diluted by 10-times. What is the new free energy change

\[ \Delta G = \frac{-12.1}{10} \text{ kcal} \]
Question 2 (12 pts.) For the disaccharide shown to the right:

a. Name the glycosidic bond: \( \times (2-1) \) (3)

b. Circle the anomeric carbon in ring B (1)

c. Give the molecular formula (e.g. C\(_2\)H\(_6\)O\(_5\)) for the disaccharide

\[ \text{C} \quad 12 \quad \text{H} \quad 21 \quad \text{O} \quad 11 \quad \]

d. Number carbon atoms 1, 3 and 6 in ring A (1)

(1)

e. After hydrolysis of the glycoside, ring A is a (circle one) ketohexose, ketopentose, aldohexose, aldopentose, none of these (1)

and ring B is a (circle one) ketohexose, ketopentose, aldohexose, aldopentose, none of these (1)

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Question 3 (9 pts) Short answers. Just a few phrases. Please stay within the area allotted.

a. Normal paper loses most of its strength when wet with water but \textit{retains} most of its strength when wet with olive oil. Clearly explain the basis for this.

Cellulose chains of \( p(1-4) \) linkages are held together by H-bonds (inter-chain H-bonds). Water tends to disrupt these linkages by competition - oil cannot provide these competing H-bonds.

b. Human red blood cells contain a single membrane. Carefully describe a method that would allow you to find that 80% of the phosphatidyl-ethanolamine was located on the inner leaflet and only 20% on the outer leaflet of the bilayer.

Use a phospholipase that removes the polar head group (like "C" and "D"). Treat red blood cells (intact) with phospholipase, quantitate ethanolamine released. Repeat with broken RBC to get total content. Evaluate results.

c. An enzyme is reported to have a \( k_{\text{cat}} \) of 80,000/sec and a \( K_m \) for its substrate of 0.5 \( \mu \)M. Briefly comment.

\[
\frac{k_{\text{cat}}}{K_m} = \frac{80,000 \text{sec}^{-1}}{0.5 \times 10^{-6} \text{M}} = 1.6 \times 10^9 \text{M}^{-1} \text{sec}^{-1}
\]

Much faster than the upper limit for diffusion of \( 10^8 \text{M}^{-1} \text{sec}^{-1} \)

So data must be wrong.
Question 4 (5 pts.) What is the effect of the following on hemoglobin. Circle the most appropriate answer. NC = no change

Increasing pH on the oxygen affinity of hemoglobin  
Circle: increase  NC  decrease

Increasing oxygen concentration on CO₂ binding to hemoglobin  
Circle: increase  NC  decrease

Decreasing DPG on oxygen affinity of hemoglobin  
Circle: increase  NC  decrease

Increasing DPG levels on CO₂ affinity of hemoglobin  
Circle: increase  NC  decrease

Decreasing pH on the ratio of [T]/[R] state of hemoglobin  
Circle: increase  NC  decrease

Question 5 (3 pts) What is the most appropriate answer?

a. the higher the Kₘ, the higher the affinity
b. at [S] = 2 Kₘ, v = Vₘₐₓ
C. at [S] = 2 Kₘ, doubling the enzyme concentration would exactly double the rate
d. Kₘ is exactly one half of the maximal velocity
e. all of the above are false

Question 6 (3 pts) What is the most appropriate answer?

a. bacteria raise their temperature by increasing the proportion of unsaturated fatty acid chains in their membranes
b. Increasing the percentage of unsaturated chains helps keep reindeer legs close to bulk body temperature.
c. unsaturated chains are only found in the inner leaflet of biological bilayers
d. cholesterol is not found in mammalian cell membranes
e. all of the above are false

Question 7 (9 pts.) Draw the pH activity curves for the following situations.

a. Only this protonic form is active.

b. Only this protonic form is active
c. Only this protonic form is active

\[ \text{Hi} \quad \text{PK 7} \quad \text{pK 5} \]

Question 8 (10 pts) Suppose that an enzyme is a tetramer of identical subunits in equilibrium with monomers. The substrate of the enzyme, A, binds 10 times more tightly to the monomer. The monomer is 20-times more active than the tetramer. An allosteric molecule binds preferentially to the tetramer. Monomers react 8-times more rapidly with iodoacetate.

\[ \text{Substrates} \quad \text{tetramer} \quad \text{monomer} \]

What is the effect of:

- Increasing [A] on the proportion of monomers: increase, NC, decrease
- Raising the concentration of the allosteric molecule on enzyme activity: increase, NC, decrease
- Lowering total enzyme concentration on the percentage of monomer: increase, NC, decrease
- Increasing total enzyme concentration on the binding of A: increase, NC, decrease
- Increasing the concentration of the allosteric molecule on the reactivity with iodoacetate: increase, NC, decrease
Question 9 (8 pts) Vesicles are spherical droplets of buffer enclosed by a continuous lipid bilayer. A cross-section is shown to the right. The bilayer is formed from the phospholipid, phosphatidylcholine, shown at the far right. When this phospholipid is hydrolyzed by phospholipase D the bond shown with an arrow is broken.

In the spaces provided draw accurate chemical structures of the two products of this reaction.

\[ \text{H}_2\text{C-O-P-O-CH}_2\text{CH}_2\text{-N(CH}_3\text{)}_3 \]

Suppose that you have 100 vesicles in buffer and then you add 20 enzyme phospholipase D molecules to the buffer. They either

(A) bind to the surface of the first vesicle they encounter, catalyze the reaction as above, but never let go of the vesicle.

Or (B) hop on and off vesicles during their catalytic action. Vesicles don’t break/burst in either A or B.

Pay careful attention to the axes of the graph (percentage of the total phospholipid hydrolyzed versus time) and plot the release of choline with time after the addition of enzyme at time zero.

\textbf{Note} The enzyme can only hydrolyze \textit{HALF} the outer leaflet ONLY.
Question 10 (12 pts.) Fill in the blanks with not more than 3 legible words.

a. Name an irreversible inhibitor of an enzyme

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

c. give the NAME of an enzyme that hydrolyzes alpha 1-6 glycosidic bonds

d. What compounds can be used to increase the viscosity of solutions in enzymology

e. these enzymes do not follow Michaelis Menten Kinetics

f. Name a saponifiable lipid

g. a technique used to measure lateral diffusion in membranes

h. this particular polysaccharide forms a blue color with iodine

i. the T state is less soluble in this hemoglobin mutation

j. these inhibitors cannot be dialyzed away from the enzyme they inactivate

k. these inhibitors change K_m not V_max

zz. the word that best describes this exam

Life is a struggle with equilibrium that we all eventually lose