Name _____

Exam II - Review Questions

1. In 1962 the Nobel Prize in chemistry was shared by two researchers, each who succeeded in determining the three-dimensional structure for a protein. Who were these two researchers and what protein did each determine a structure for?
    a. Researcher: _____ Protein Determined:_____
    b. Researcher: _____ Protein Determined:_____

2. Both myoglobin and hemoglobin function as oxygen binding proteins. Compare and contrast these two proteins on each of the following levels:
    a. Structural:

    b. Functional:

3. In 1949, Linus Pauling first proposed that genetically linked diseases were cause by molecular defects in the proteins that genes code for. One of the first examples to support this theory was provided by the sickle-cell hemoglobin disease. Describe the physiological effects of this disease along with the molecular defect that has been determined as its cause.

4. Hemoglobin functions to transport oxygen to the tissues where it is used to oxidize food molecules. The oxidation reactions release chemical energy to meet the energy needs of these tissues. The byproducts of these reactions include small molecular weight acids and CO_2. Describe how hemoglobin has evolved to respond to the presence of these byproducts in a way that helps the tissues meet their energy needs.

5. Earlier in the semester we discussed Christian Anfinsen's Nobel Prize winning experiment in which he showed that the enzyme *ribonuclease* could be reversibly denatured, elegantly demonstrating that the information for a correctly folding a protein is contained in its amino acid sequence. Anfinsen was able to determine when ribonuclease was renatured by observing when it regained its catalytic active. What characteristics of an enzyme's active site does Anfinsen's experiment nicely illustrate?

6. Which of the following effects can be brought about when an enzyme catalyzes the following simple reaction:

$$S \underset{k_b}{\overset{k_f}{\rightleftharpoons}} P$$
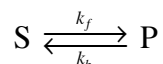
where $K'_{eq} = \dfrac{[P]}{[S]}$, and k_f and k_b are the forward and backward rate constants.

a. The enzyme increases k_f (Yes/No)._____

b. The enzyme increases K'_{eq} (Yes/No). _____

c. The enzyme increases k_b (Yes/No) _____

d. The enzyme increases the activation free energy change, $\Delta G^{\ddagger}$ (Yes/No) _____

e. The enzyme makes the overall free energy change, $\Delta G'$, more negative (Yes/No) _____

7. Shown below is a Lineweaver-Burke plot displaying the kinetics for an enzyme catalyzed reaction that was conducted with 800 pmol of enzyme in both the absence and presence of a 100 µM concentration of an inhibitor.
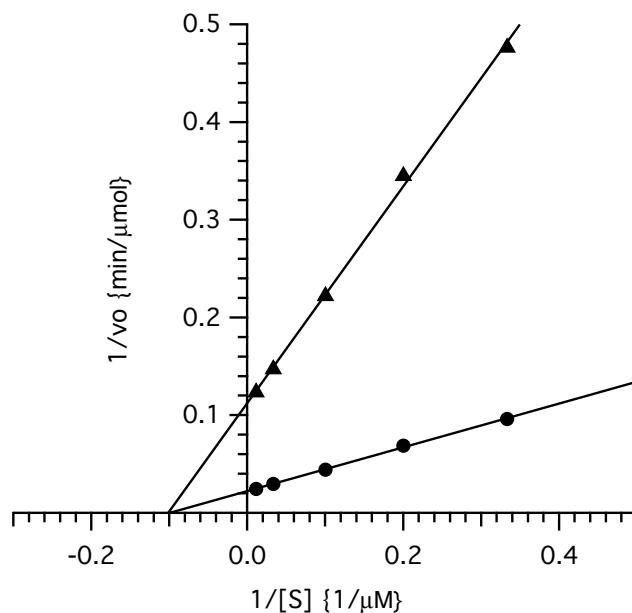
a. What type of inhibition is displayed by this inhibitor? _____

b. What are the values of V_{max} and K_M in the absence of the inhibitor? (Show your calculations and be sure to include units.)

c. How many reactions does each enzyme active site catalyze per second when saturated with substrate in the absence of the inhibitor? (Show your calculations and be sure to include units.)

d. Is the maximum rate for the uninhibited reaction diffusion limited? _____Explain:

e. Where on the enzyme molecule, relative to the active site, does the inhibitor most likely bind? _____. Explain:

f. What is the value for the dissociation constant, K_I, of the inhibitor-enzyme complex?

8. Shown below are kinetics data for an enzyme that were collected in both presence and absence of an inhibitor. The enzyme concentration used in both experiments was 1.5 μM. v_o is the initial rate of the catalyzed reaction, and [S] is the substrate concentration.

Lineweaver-Burk Plot
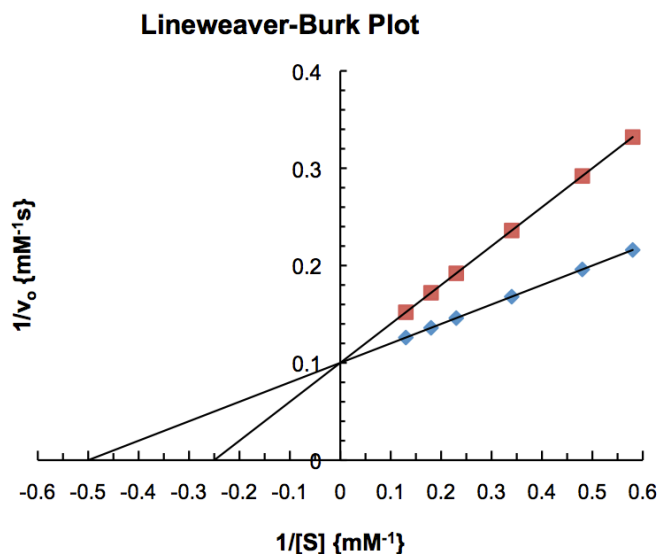
a. Does this enzyme obey Michaelis-Menten kinetics? _____Explain:

b. How many reactions does each enzyme active site catalyze per second when saturated with substrate in the absence of the inhibitor?_____

c. Is the maximum rate for the uninhibited reaction diffusion limited? _____Explain:

d. Where on the enzyme molecule does the inhibitor most likely bind? _____
    Explain:

9. In class we discussed several strategies used by enzymes to speed up the rate of a reaction. In one or two *sentences*, describe a *specific example* for each of the following drawing your examples from the four systems that we discussed in class. Include each system in at least of these.
    a. Catalysis by approximation:

    b. General acid/base catalysis:

    c. Metal ion catalysis:

    d. Covalent catalysis:

    e. Substrate specificity:

10. According the Michaelis-Menten equation, what is the v_o/V_{max} ratio when $[S] = 7\ K_M$?

11. If $K_M = 2$ mM, and $v_o = 100\ \mu mol/(mL \cdot s)$ when $[S] = 2$ mM, what is the velocity, v_o, for the reaction when $[S] = 18$ mM?

12. The following kinetic data were obtained for an enzyme in the absence of any inhibitor (1), and in the presence of two different inhibitors, (2) and (3), each at a concentration of 7.0 mM. Assume the total enzyme concentration, $[E]_T$, is the same for each experiment.

[S] {mM}	(1) v_o {μmol/(mL•s)}	(2) v_o {μmol/(mL•s)}	(3) v_o {μmol/(mL•s)}
1	3.8	2.2	3.3
2	7.3	4.3	5.7
4	13.3	8.2	8.9
8	22.9	14.9	12.3
12	30.0	20.4	14.1

a. Determine V_{max} and K_M for the uninhibited enzyme.

b. Determine the type of inhibition and the dissociation constant, K_I, for the inhibitor binding to the enzyme, for experiments (2) and (3).

13. Liver alcohol dehydrogenases (ADH) is relatively nonspecific. Its normal substrate is ethanol, however, it will oxidize other primary alcohols, such as methanol, to their corresponding aldehydes. In the case of methanol this produces formaldehyde, which is quite toxic and can lead to blindness. Mistaking it for the cheap wine she usually prefers, my dog Lulu ingested about 36 mL of windshield washer fluid, which is an aqueous solution of 50% v/v methanol. I knew that methanol would be eventually excreted by Lulu's kidneys if its oxidation to formaldehyde could be blocked; I also knew that ethanol could act as a competitive inhibitor of methanol oxidation by ADH. Not wanting to become a seeing-eye human to a blind pooch, I decided to offer my pooch some her favorite hooch, Miller Genuine Draft (MGD), a tasteless brew that contains 4.2% v/v ethanol.

   a. How much MGD, in mL, must my Lulu consume in order to lower the activity of her ADH on methanol to 5% of its uninhibited value, if the K_M values for canine ADH are 1 mM for ethanol and 10 mM for methanol? (Assume the K_I for ethanol in its role as a competitive inhibitor of methanol oxidation is the same as its K_M. Both methanol and ethanol will quickly distribute throughout Lulu's 17 L of body fluids. The densities of both methanol and ethanol are 0.79 g/mL.)

14. *Triose phosphate isomerase* (TIM) catalyzes the isomerization of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate:

glyceraldehyde 3-phosphate   dihydroxyacetone phosphate
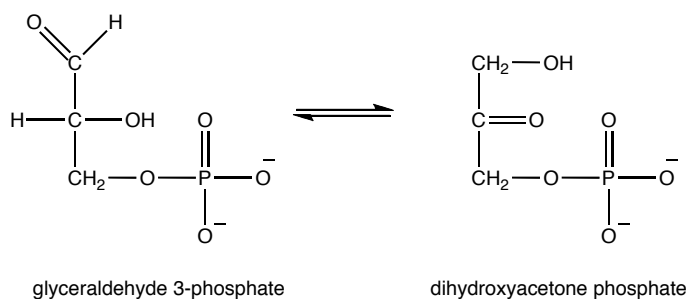
The K_M of TIM for its substrate glyceraldehyde 3-phosphate is 3.2×10^{-5} M. When [glyceraldehyde 3-phosphate] = 24 μM, the rate of the reaction , v_o, is 73.3 μmol/(mL•s).

   a. What class of enzyme is TIM?

   b. What is V_{max} for this enzyme?

   c. Assuming the TIM concentration in this experiment was 3 nmol/mL, what is k_{cat} for this enzyme?

   d. What is the catalytic efficiency (k_{cat}/K_M) for triose phosphate isomerase?

   e. Does the value of k_{cat}/K_M reveal that trisose phosphate isomerase approaches "catalytic perfection"?

   f. What determines the ultimate speed limit of an enzyme-catalyzed reaction? That is, what is it that imposes the physical limit on catalytic perfection?

15. The glycolytic enzyme *enolase* catalyzes the conversion of 2-phosphoglycerate to phosphoenol pyruvate::

2-phosphoglycerate   phosphoenolpyruvate
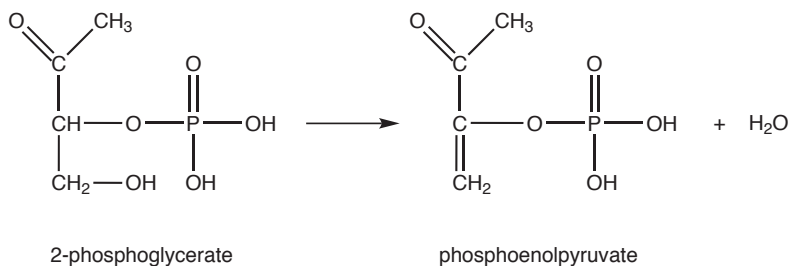
The turnover number for enolase is 950/s. The K_M of enolase for the substrate 2-phosphoglycerate is 8 μM.

a. What class of enzyme is enolase?

b. In an experiment using 1.32 nM enolase, what is V_{max}?

c. The cellular concentration of 2-phosphoglycerate is 47.5 μM. What is v_o under these conditions?

d. What is the catalytic efficiency of enolase?

e. Does enolase approach "catalytic perfection"?

16. There is a reaction in the citric acid cycle in which L-malate is converted to oxaloacetic acid:

L-malate   oxaloacetate
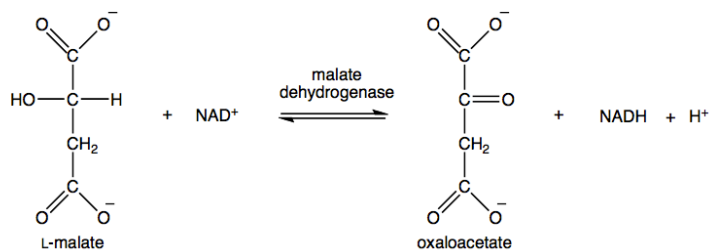
The standard free energy change for this reaction is $\Delta G°' = +29.7$ kJ/mol, which makes it an unfavorable reaction under standard conditions.

a. This reaction is catalyzed by the enzyme *malate dehydrogenase*. What enzyme class does this enzyme belong to? _____

b. What can malate dehydrogenase alone do to make this reaction favorable under standard state conditions?

c. The conditions found in most cells are not the standard state conditions. If the NAD^+ to $NADH + H^+$ ratio in a healthy cell at 37°C is 700, and the L-malate and oxaloacetate concentrations are 10 mM and 0.01 mM, respectively. What is the free energy change, $\Delta G'$, for this reaction under these conditions?

d. Is the reaction favorable under these conditions? _____. Explain:

17. In class we discussed the concepts of cooperativity and allosteric regulation with respect to both the activity of hemoglobin, which is an oxygen binding protein, and the activity of aspartate transcarbamoylase (ATCase), which is an enzyme.
   a. In both cases the terms "Tense (T)" and "Relaxed (R)" states were used to describe two different states for these proteins. Specifically, how do these terms relate to the cooperativity and allosteric regulation for these two proteins?

   b. Identify an example of an allosteric regulator for each protein. Describe where they bind and how they exert their effects in terms of the T and R states.

18. Some enzymes are activated by covalent modification. Give a short description of an example where activation is brought about by both a reversible and an irreversible covalent modification.
   a. reversible covalent modification:

   b. irreversible covalent modification: