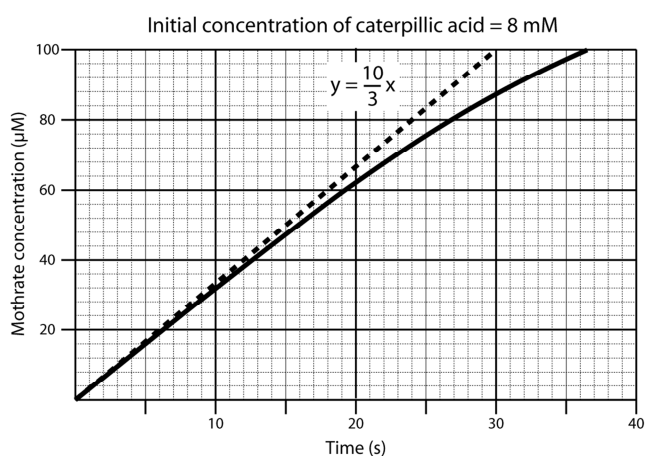
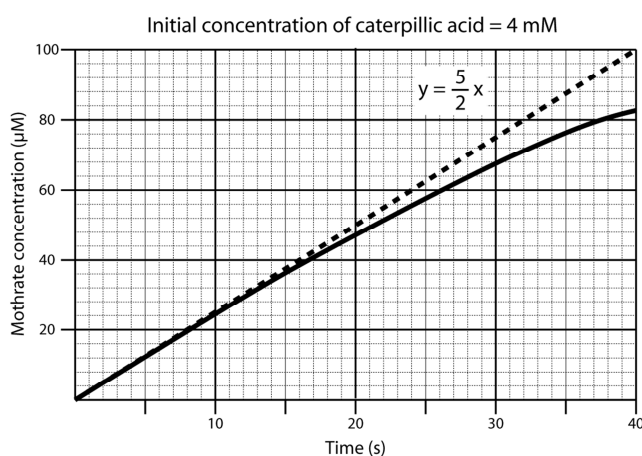
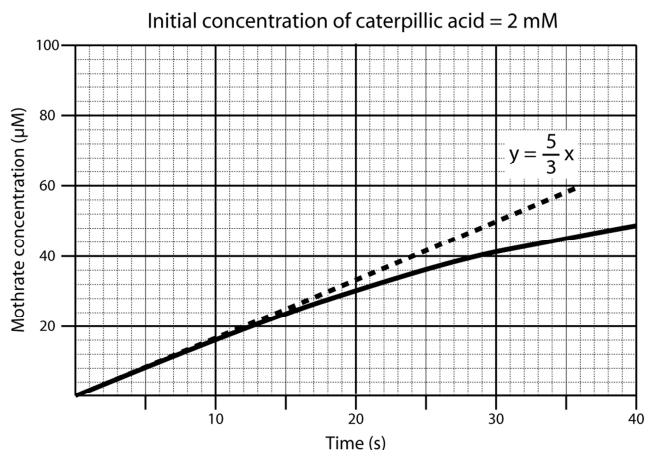
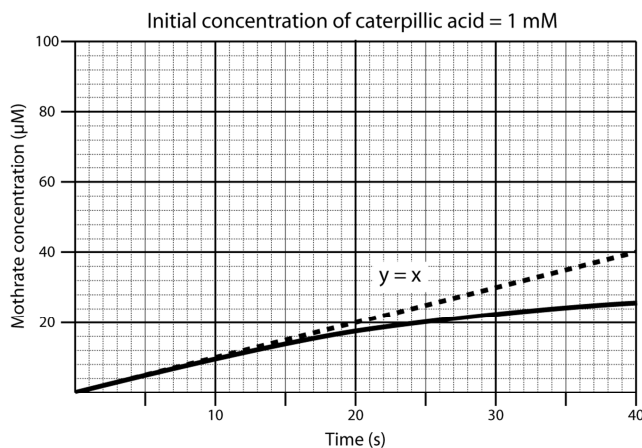
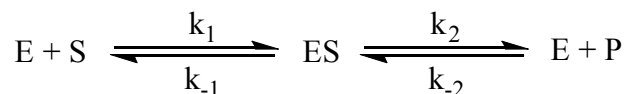


1. You have discovered a new enzyme, metamorphase, which catalyzes the one-to-one conversion of caterpillic acid into mothtrate. You analyze the kinetics of the enzyme by combining 0.8 nM metamorphase with varying concentrations of caterpillic acid, and measuring the rate of production of mothtrate, as shown in the plots below. The equations on each plot describe the dotted line that traces the initial slope of each curve.

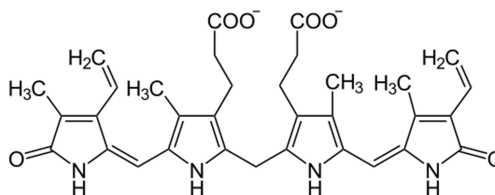


- Draw a Lineweaver-Burk plot for metamorphase acting on caterpillic acid. Label the axes with numerical values, labels, and units. Label your line 'M'.
- What is the  $K_m$  of metamorphase for caterpillic acid? Show your work.
- What is the  $V_{max}$  of metamorphase in these experiments? Show your work.
- What is the  $k_{cat}$  of metamorphase? Show your work.
- What is the catalytic efficiency of metamorphase? Show your work.
- The kinetic experiments with metamorphase are repeated, this time with the addition of 5 mM frigidol, an inhibitor. The apparent  $K_m$  and  $V_{max}$  are 20 mM and 5  $\mu\text{M/s}$ , respectively. Draw a line representing the results of this experiment on your plot, and label it 'M + F'.
- What type of inhibitor is frigidol (when acting on metamorphase)?
- What is the  $K_I$  of the frigidol-metamorphase complex? Show your work.
- Does metamorphase have a higher affinity for caterpillic acid or frigidol? (Assume the conversion of caterpillic acid to mothtrate is much slower than the binding and release of caterpillic acid to metamorphase.)

2. Given the enzyme catalyzed reaction:

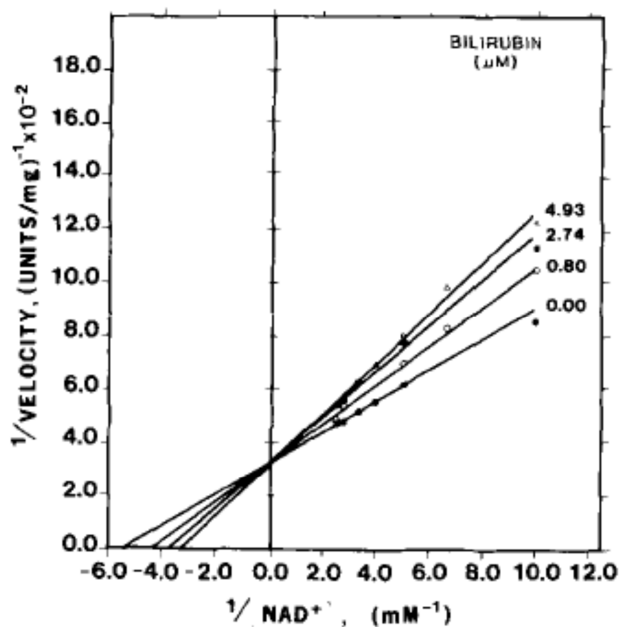


- What assumption must be made about this reaction in order for  $K_m$  to approach the  $K_d$  of the enzyme-substrate complex?
  - Briefly define 'first-order' as it applies to rate constants (15 words or fewer).
  - Of the rate-constants above, which are first-order?
  - Write two different expressions for the  $K_d$  of the enzyme substrate complex.
  - Under what condition is ES at steady state? Write an expression using concentrations and rate constants.
3. True or False?
- In a closed system, the rate of a catalyzed, spontaneous reaction will decrease over time.
  - In an open system, the rate of a catalyzed, spontaneous reaction will decrease over time.
4. Bilirubin is a product of the catabolism of heme, and it is the compound that gives urine its yellow color. The structure of bilirubin is shown:



- Circle the atom(s) that were bound the heme iron.
- Put an asterisk (\*) next to each atom that can act as an H-bond acceptor.
- Would you expect the carboxyl groups of bilirubin to have a higher or lower pKa than that of glycine? Explain why in 25 words or fewer.

Scientists have found that bilirubin can bind isocitrate dehydrogenase, as shown by the plot below:



- What kind of plot is this?
- How is  $NAD^+$  used (what is its role) in this experiment?
- What does this plot show about the interaction of bilirubin with isocitrate dehydrogenase? (5 words or less.)
- Which of the following values can be determined from this plot?
 

A. $[S]$	H. $K'_m$
B. $[E]$	I. $V'_{max}$
C. $[ES]$	J. $\alpha$
D. $K_m$	K. $\alpha'$
E. $V_o$	L. $K_1$
F. $V_{max}$	M. $K'_1$
G. $k_{cat}$	

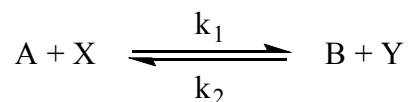
5. List all possible results that can be produced by each type of inhibitor:

- Competitive
- Mixed
- Noncompetitive
- Uncompetitive

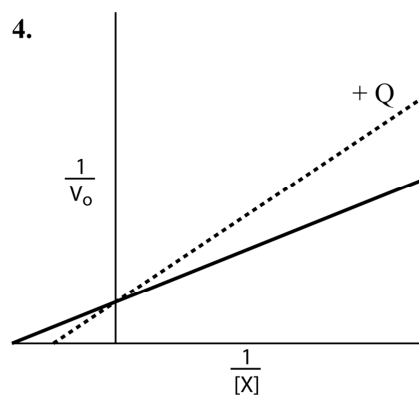
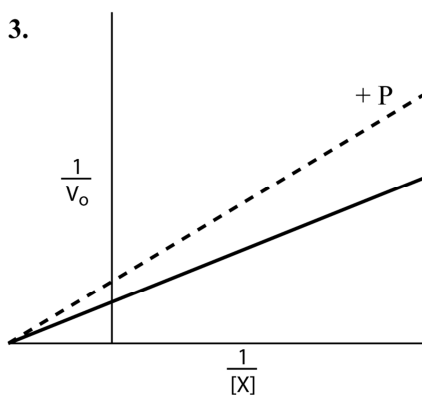
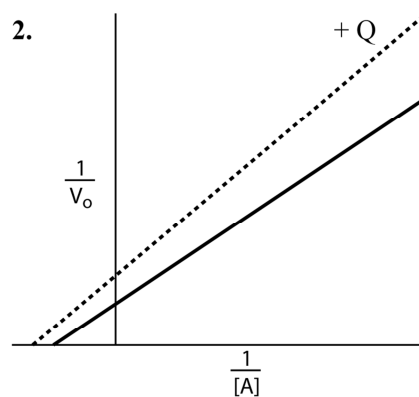
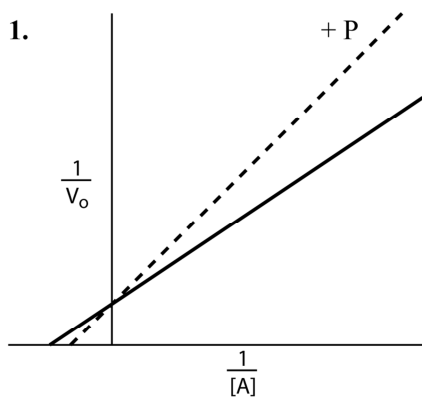
Choose from:

- An apparent decrease in  $K_m$
- An apparent increase in  $K_m$
- No apparent change in  $K_m$
- An apparent decrease in  $V_{max}$
- An apparent increase in  $V_{max}$
- No apparent change in  $V_{max}$

6. The enzyme 'convertase' catalyzes the following reaction:



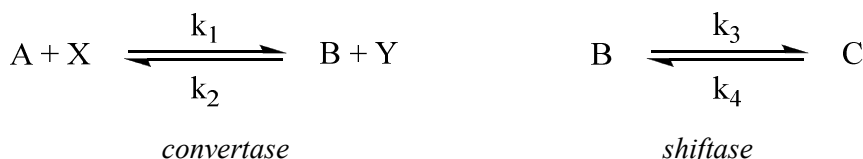
You have discovered two molecules, P and Q, that inhibit catalysis by this enzyme. You examine the effects of each inhibitor on convertase and produce the following plots. (Solid lines show the enzyme action in the absence of inhibitor; dotted lines show the enzyme action in the presence of the indicated inhibitor. In plots 1 and 2, X is present at saturating concentrations; in plots 3 and 4, A is present at saturating concentrations.)



- What type of inhibitor is P with respect to substrate A (plot 1)?
- What type of inhibitor is Q with respect to substrate A (plot 2)?
- What type of inhibitor is P with respect to substrate X (plot 3)?
- What type of inhibitor is Q with respect to substrate X (plot 4)?
- What effect(s) would increasing the concentration of *convertase* have on the dotted line in plot 1? (Circle your selections on the answer sheet.)
  - The slope would: (increase/decrease/not change)
  - The x-intercept would: (increase/decrease/not change)
  - The y-intercept would: (increase/decrease/not change)
- What effect(s) would increasing the concentration of Q have on the dotted line in plot 2? (Circle your selections on the answer sheet.)
  - The slope would: (increase/decrease/not change)
  - The x-intercept would: (increase/decrease/not change)
  - The y-intercept would: (increase/decrease/not change)
- Based on all four plots, would you expect P and Q to be able to bind *convertase* simultaneously? Explain your reasoning in 40 words or fewer.

You determine that the  $K_1$  for P binding to *convertase* is 1.8 mM, and the  $K_1$  for Q binding to *convertase* is 54  $\mu$ M.

- Does P or Q have a higher affinity for *convertase*? Briefly explain your answer (10 words or fewer).
  - Draw the binding curves for P and Q (independently) binding to *convertase*. Label your curves (P or Q), and label your axes with labels (axis names), units, and number values.
7. In addition to being a product of the reaction catalyzed by ‘*convertase*’ (from the previous question), molecule B is used in another cellular reaction catalyzed by ‘*shiftase*’:



- Write an expression (using concentrations and rate constants) describing the relationship between the reactions when B is at *steady-state*. Assume that B is not transported in or out of the cell, and these are the only two cellular reactions that involve B.
  - The  $\Delta G^\circ$  for the *shiftase*-catalyzed reaction (converting B to C) is -7.2 kJ/mol. A solution of 100 mM B and *shiftase* is left to reach equilibrium. What are the final (equilibrium) concentrations of B and C? Show your work.
  - In which direction will the reaction proceed if  $[\text{B}] < [\text{C}]$ ? Explain your answer in 25 words or fewer.
8. The  $\Delta G^\circ$  of the reaction  $\text{X} \rightarrow 2\text{Z}$  is 7.1 kJ/mol. Write the expression that relates the  $\Delta G$  of this reaction in a cell to that of standard conditions.
9. The  $\Delta G^\circ$  of the one-to-one conversion of X to Y is 4.9 kJ/mol. The concentration of X in *human brain cells* is 100  $\mu$ M. At what cellular concentrations of Y is the conversion of X to Y spontaneous? Show your work.

10. Below is the abstract for a research publication:

The isocitrate dehydrogenase of *Escherichia coli* is an example of a ubiquitous class of enzymes that are regulated by covalent modification. In the three-dimensional structure of the enzyme-substrate complex, isocitrate forms a hydrogen bond with Ser113, the site of regulatory phosphorylation. The structures of Asp113 and Glu113 mutants, which mimic the inactivation of the enzyme by phosphorylation, show minimal conformational changes from wild type, as in the phosphorylated enzyme. Calculations based on observed structures suggest that the change in electrostatic potential when a negative charge is introduced either by phosphorylation or site-directed mutagenesis is sufficient to inactivate the enzyme. Thus, direct interaction at a ligand binding site is an alternative mechanism to induced conformational changes from an allosteric site in the regulation of protein activity by phosphorylation.

- a. What enzyme is being studied in this article?
  - b. What organism does the enzyme come from?
  - c. The authors used X-ray crystallography to study the enzyme. What kind of information does this technique provide?
  - d. How many versions (or states) of the enzyme were examined?
  - e. How did the versions (or states) of the enzyme differ? Answer by listing the unique feature of each version or state.
  - f. The abstract states, "isocitrate forms a hydrogen bond with Ser113." Based on all of the information in the abstract, what functional group of isocitrate would you expect forms a hydrogen bond with Ser113? Draw this bond.
  - g. How does phosphorylation inactivate the enzyme? Explain in 25 words or fewer.
  - h. Is the enzyme an allosteric enzyme?
11. The reaction  $X \rightarrow Y$  has  $\Delta G^\circ = -3.7 \text{ kJ/mol}$ . If  $[Y] > [X]$ , which of the following can be concluded?
- a. the reaction will be spontaneous in the direction written
  - b. the reaction will be spontaneous in the reverse direction
  - c. the reaction is near equilibrium
  - d. the reaction is at equilibrium
  - e. none of the above
12. What metabolite is the first intermediate common to the breakdown of proteins, polysaccharides, and fatty acids?
13. True or False?
- a.  $K_I$  is equivalent to the  $K_d$  of an enzyme-inhibitor complex.
  - b. Allosteric inhibitors can change an enzyme's affinity for its substrate.
  - c. Some enzymes are regulated by how much substrate is present.
  - d. In the concerted model of homotropic positive cooperativity, the binding of substrate yields a higher-affinity (or more active) state of the bound enzyme.
  - e. Proteins that become functional after undergoing proteolytic cleavage are called zymogens.
  - f. Feedback inhibition can occur through heterotropic effects.