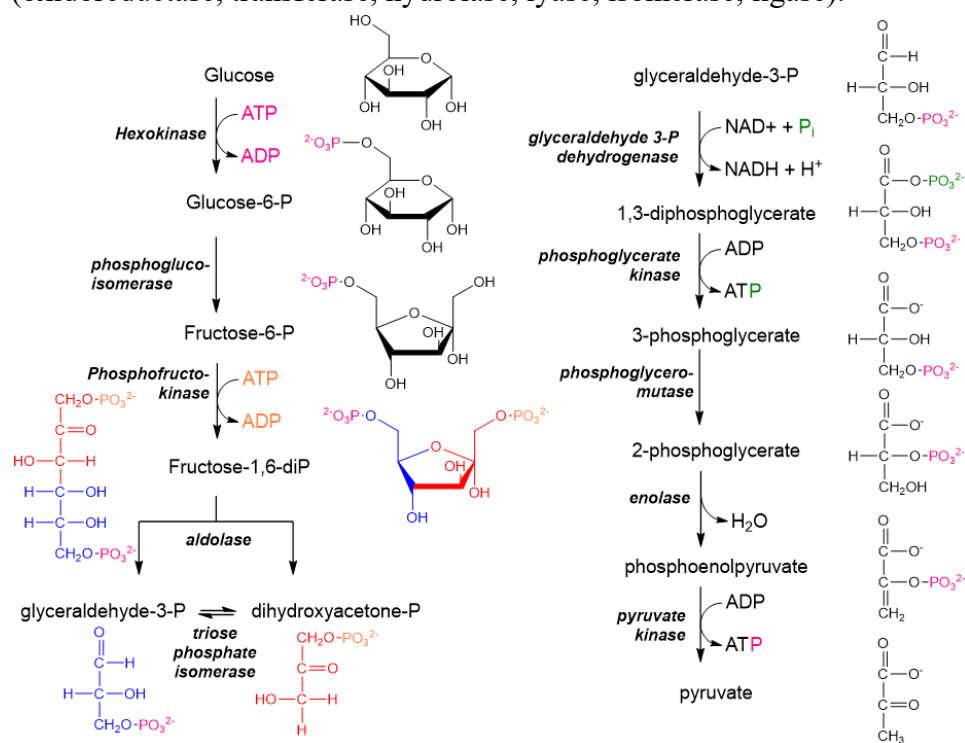


Here are the quiz questions you will answer online using the quiz tool in canvas instructor. Be sure to note the quiz deadline in canvas to be sure you get your answers submitted by the deadline.

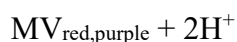
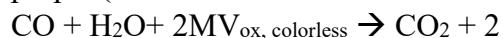
1. Classify each of the enzymes of glycolysis to the proper one of the 6 EC classes (oxidoreductase, transferase, hydrolase, lyase, isomerase, ligase).



2. Carbon monoxide dehydrogenase catalyzes the reversible two-electron oxidation of CO to CO₂. The physiological electron acceptor in the reaction is ferredoxin, an iron-sulfur protein that accepts a single electron:



For conducting assays in the lab, the artificial electron acceptor methyl viologen is typically used. The oxidized form of methyl viologen is colorless, while the one-electron reduced form is purple (note two MV_{red} are formed for every CO oxidized):



To conduct an enzyme assay, a small amount of enzyme is added to a cuvette containing buffer and the substrates (1 mM CO and 10 mM methyl viologen) to initiate the assay.

The increase in absorbance at 578 nm vs. time is used to calculate the rate of CO

oxidation in units of $\mu\text{mol CO}$

oxidized/min/mg protein. The curve at the

right was generated as follows: A stock solution of enzyme with concentration of 3.0

mg/ml was diluted 1000-fold to give a

working stock solution. Of this working

stock solution, 2.0 microliters was added to

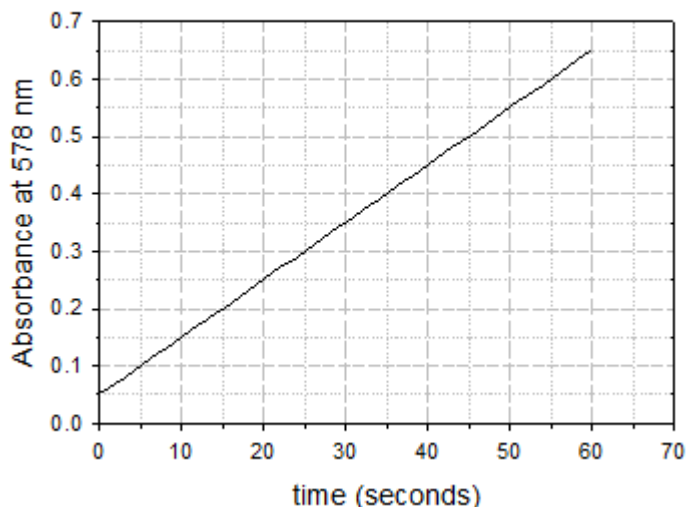
998 microliters of assay mixture in the cuvette. The cuvette was then placed in a

spectrophotometer. The absorbance at 578 nm was then recorded over time as shown in the graph.

Determine the **specific activity of CO dehydrogenase** in units of $\mu\text{mol CO}$ oxidized/min/mg enzyme (units CO oxidized /mg) using the data in the graph. Make sure you account for the assay volume, dilutions, and amount of enzyme added, as well as the stoichiometric ratio of methyl viologen to CO, and convert your units properly. To complete this part you will need to use Beer's law: $A = \epsilon cl$

Where: **A** = absorbance of the solution; **ϵ** = extinction coefficient for reduced methyl viologen ($9.78 \text{ mM}^{-1} \cdot \text{cm}^{-1}$), **c** = concentration of reduced methyl viologen in the solution, and **l** = the cuvette path length (assume it is 1 cm).

CO dehydrogenase-catalyzed methyl viologen reduction



3. Malate dehydrogenase (MDH) catalyzes the reversible oxidation/reduction of malate/oxaloacetate with NAD^+/NADH . The enzyme is most commonly assayed in the direction: $\text{OAA} + \text{NADH} \rightarrow \text{L-malate} + \text{NAD}^+$

You have a 15.0 mg/mL suspension of MDH you purchased from Sigma Chemicals, and you want to know the specific activity of the enzyme. You dilute the stock solution of MDH to 1.50×10^{-3} mg/mL in phosphate buffer containing 1 mg/mL BSA to make a working enzyme solution for addition to assays. You then mix the following components in an assay cuvette:

0.1 M phosphate buffer +BSA	0.867 mL	time (s)	A340
0.00375 M NADH	0.0667 mL		
0.006 M oxaloacetate	0.033 mL	0	1.556
You allow the reagents in the cuvette to equilibrate for 3 minutes, then		15	1.33
add 0.0330 mL of your working enzyme solution to initiate the assay,		30	1.104
recording the change in absorbance at 340 nm over time. You collect		45	0.8772
the data to the right on the spectrophotometer:		60	0.651
The extinction coefficient for NADH at 340 nm is $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$.		75	0.425
What is the specific activity of your enzyme in units/mg?		90	0.199

4. Referring back to the previous problem, why did you include BSA in the enzyme assay?

5. An enzyme can accelerate the rate of a reaction through which of the following strategies (select all the correct answers, this is a multiple response question).

- destabilize substrate(s)
- stabilize transition state(s)
- lower the free energy of the substrate(s)
- lower the free energy change for the reaction
- raise the free energy of transition state(s)
- alter the reaction mechanism

6. One “unit” of enzyme activity is defined as:

- the amount of enzyme catalyzing the conversion of one millimolar of substrate to product per minute
- the amount of enzyme catalyzing the conversion of one millimole of substrate to product per minute
- the amount of enzyme catalyzing the conversion of one micromole of substrate to product per minute
- the amount of enzyme catalyzing the conversion of one micromolar of substrate to product per minute
- a. the amount of enzyme catalyzing the conversion of one millimolar of substrate to product per second

7. The standard “unit” for enzyme activity has units of:

- a. mmol/min
- b. mM/min
- c. $\mu\text{mol/min}$
- d. $\mu\text{M/min}$
- e. $\mu\text{M/s}$

8. The rate law for a chemical reaction:

- a. can always be deduced from the stoichiometry of reactants
- b. can always be deduced from the stoichiometry of products
- c. can always be deduced from the stoichiometry of reactants and products
- d. is never related to the stoichiometry of reactants and products
- e. must be determined experimentally

9. Match the units with the correct type of rate constant. The options are:

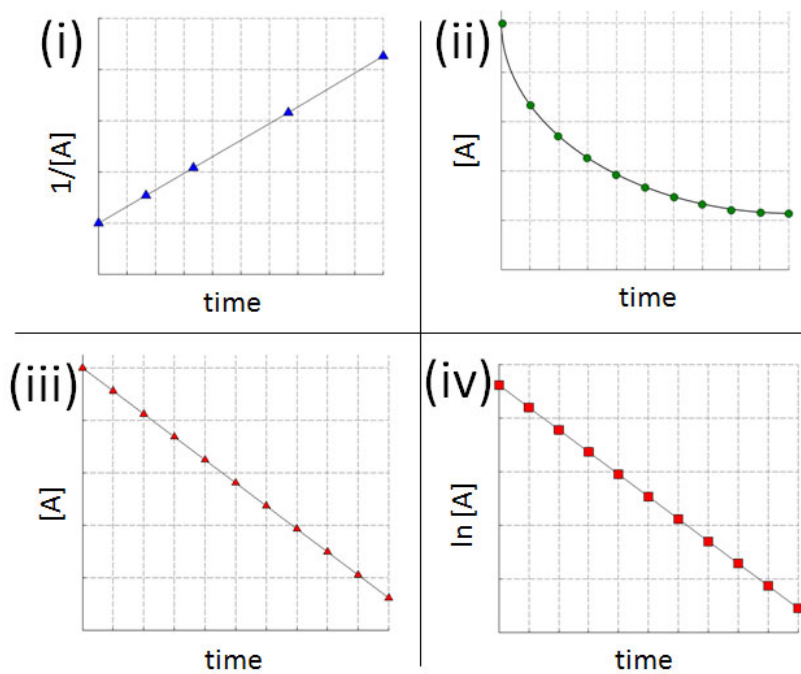
- (i) s^{-1} (ii) $\text{M}^{-1} \text{s}^{-1}$ (iii) $\text{M}^{-3} \text{s}^{-1}$ (iv) $\text{M}^{-2} \text{s}^{-1}$ (v) M s^{-1}

- a. zero order =
- b. first order =
- c. second order =
- d. third order =
- e. no match =

10. The reaction $\text{A} + \text{B} \rightarrow \text{C}$ is first order in A and zero order in B. What is the rate law for the reaction?

11. What is the overall order for a reaction following the rate law $\text{rate} = k[\text{A}]^2[\text{B}]$?

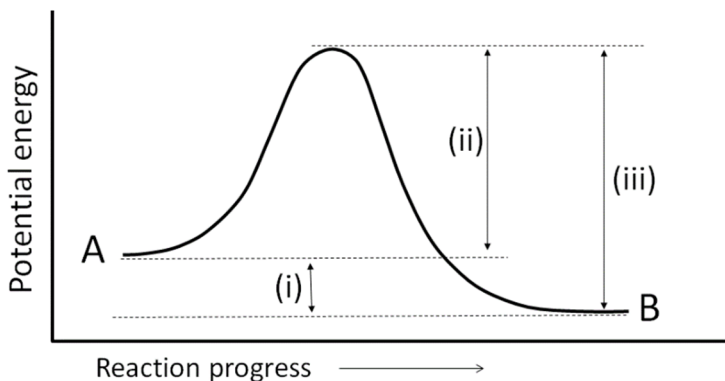
12. The following graphs show the relationships between [reactant] and time plotted in different formats for four reactions that occur by different kinetics. Pay special attention to what is plotted on the y axes. What can you tell about the order (i.e. zero order, 1st order, 2nd order, etc) of the reactions from each of these graphs?



13. Chemical reaction rates are dependent on which of the following?

- the frequency of collisions
- the energy with which molecules collide
- the orientation of reacting molecules
- the magnitude of the rate constant k
- all of the above

14. Consider the reaction $A \rightarrow B$. The following reaction coordinate diagram represents a ____ reaction in the forward direction, where line ____ corresponds to the activation energy barrier for the forward direction.

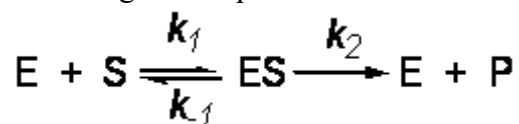


- a. thermodynamically unfavorable (ii)
- b. thermodynamically favorable (ii)
- c. thermodynamically unfavorable (iii)
- d. thermodynamically favorable (iii)
- e. thermodynamically favorable (i)

15. Are the following statements correct descriptions of the steady state assumption used to derive the Michaelis-Menten equation? Check ALL the correct answers.

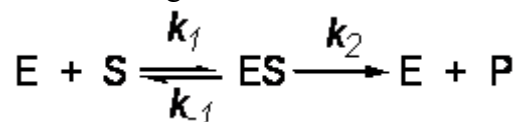
- a. The concentration of enzyme in an assay is much lower than the concentration of substrate
- b. the equilibrium between E, S, and ES is rapidly established
- c. The concentration of the ES complex does not change during the time frame where enzyme activity is being measured
- d. ES breaks down faster towards E and P than towards E and S
- e. product formed during the reaction does not react with E to reform ES at a significant rate
- f. for conversion of S to P, the rate of the forward reaction must be greater than the rate of the reverse reaction

16. Which of the following statements are true about the catalytic efficiency of an enzyme following the simple kinetic scheme shown here? Check ALL the correct answers:



- a. a low K_m and high k_{cat} increase catalytic efficiency.
- b. a high K_m and high k_{cat} increase catalytic efficiency.
- c. the theoretic upper limit to catalytic efficiency is controlled by the activation energy barrier for the slowest step in converting ES to E + P.
- d. the theoretic upper limit to catalytic efficiency is diffusion controlled.
- e. catalytic efficiency is a second order rate constant with a value close to k_1 for a “perfect” enzyme.
- f. catalytic efficiency is a first order rate constant with a value close to k_2 for a “perfect” enzyme.
- g. catalytic efficiency is a first order rate constant with a value close to k_1 for a “perfect” enzyme.
- h. catalytic efficiency is a second order rate constant with a value close to k_2 for a “perfect” enzyme.

17. Which of the following are true concerning K_m for an enzyme-catalyzed reaction that follows the following kinetic mechanism? Check ALL the correct answers:

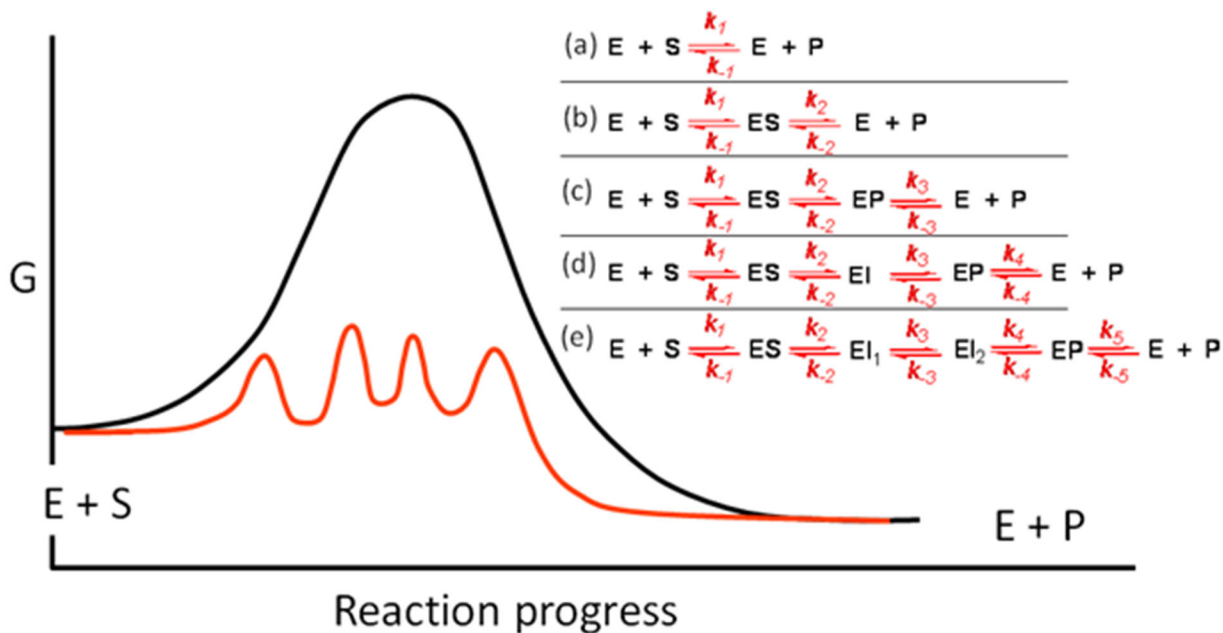


- a. K_m is always numerically greater than the value for the dissociation equilibrium constant for the equilibrium between E, S, and ES
- b. K_m is always numerically smaller than the value for the dissociation equilibrium constant for the equilibrium between E, S, and ES
- c. K_m is numerically equal to the concentration of substrate that results in a rate that is one half of V_{max} .
- d. K_m is the sum of the rate constants for dissociation of the ES complex in the forward and reverse directions divided by the rate constant for formation of the ES complex from E and S
- e. K_m is the ratio of the rate constant for dissociation of the ES complex in the reverse direction divided by the rate constant for formation of the ES complex from E and S
- f. K_m and [S] have units of concentration (e.g. mM)

18. Which of the following are ALWAYS true concerning K_m for an enzyme-catalyzed reaction that follows Michaelis-Menten kinetics, regardless of how many steps are in the actual kinetic mechanism? Check ALL the correct answers:

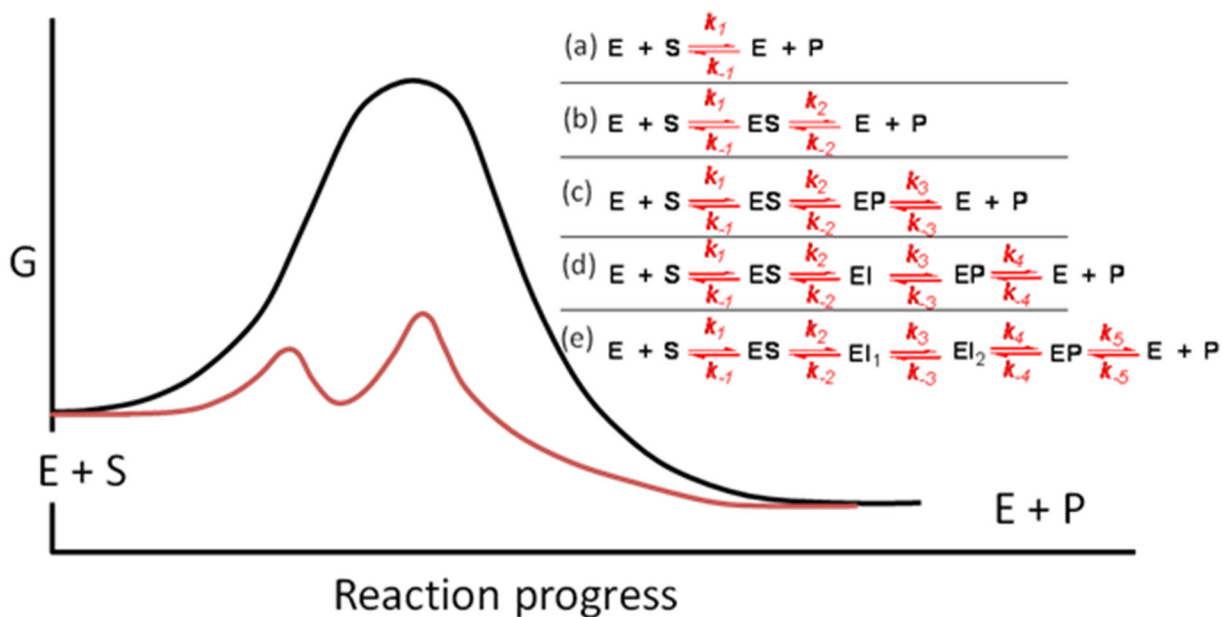
- a. K_m is always numerically greater than the value for the dissociation equilibrium constant for the equilibrium between E, S, and ES
- b. K_m is always numerically smaller than the value for the dissociation equilibrium constant for the equilibrium between E, S, and ES
- c. K_m is numerically equal to the concentration of substrate that results in a rate that is one half of V_{max} .
- d. K_m is the sum of the rate constants for dissociation of the ES complex in the forward and reverse directions divided by the rate constant for formation of the ES complex from E and S
- e. K_m is the ratio of the rate constant for dissociation of the ES complex in the reverse direction divided by the rate constant for formation of the ES complex from E and S
- f. K_m and [S] have units of concentration (e.g. mM)

19. The red line on the following reaction coordinate diagram is consistent with which kinetic mechanism?



- a. (a)
- b. (b)
- c. (c)
- d. (d)
- e. (e)

20. The red line on the following reaction coordinate diagram is consistent with which kinetic mechanism?



21. An enzyme that follows Michaelis-Menten kinetics has a $V_{\max}$ of 200.0 units and a K_m of 0.100 mM. What will be the activity for this enzyme when the substrate concentration is 0.500 mM?

22. Algebraically solve the Michaelis-Menten equation for $[S]$ to answer the following question: what concentration of substrate gives an initial rate of 1200 units for an enzyme that exhibits a $V_{\max}$ of 5000 units and a K_m of 2 mM?

23. Kinetic analysis of a homodimeric enzyme of 350,000 molecular weight (that is the M_r of the α_2 dimer) catalyzing conversion of substrate S to product P provides the following kinetic constants: $V_{\max} = 5,000$ U when 1 mg of protein is assayed, and $K_m = 0.15$ mM. Assume each subunit contains an active site; i.e. there are two active sites per dimer. Calculate the catalytic efficiency (units of $M^{-1} \cdot s^{-1}$) of the enzyme on the basis of monomeric units (i.e. per active site).

24. Given the following equations for a reversible enzyme-catalyzed reaction of substrate S to product P:

$$v = \frac{\frac{V_{\max}^f [S]}{K_m^S} - \frac{V_{\max}^r [P]}{K_m^P}}{1 + \frac{[S]}{K_m^S} + \frac{[P]}{K_m^P}} \quad K_{eq} = \frac{[P]}{[S]} = \frac{V_{\max}^f K_m^P}{V_{\max}^r K_m^S}$$

The equilibrium constant for the reaction $S \rightleftharpoons P$ is 5.00. An enzyme catalyzes this reaction with $K_{m,s} = 3.00 \times 10^{-5}$ M, $V_{\max,f} = 2.00$ $\mu\text{mol}/\text{min}/\text{mg}$ and $V_{\max,r} = 4.00$ $\mu\text{mol}/\text{min}/\text{mg}$.

What is the value for $K_{m,p}$ for this enzyme?

25. Continuing the previous problem, suppose you have a mixture of $[S] = 2.00 \times 10^{-4}$ M and $[P] = 3.00 \times 10^{-4}$ M. In which direction will the reaction proceed on addition of enzyme

- _____ (i) _____, and at what initial velocity will the reaction start towards equilibrium
 _____ (ii) _____?