NAME: For GradeScope, please write your FIRST and LAST name in CAPITAL letters WITHIN the box:

Exam III
October 30, 2023
Biochemistry I
BI/CH 421/621

I. _____/48
II. _____/26
III. _____/ 6
IV. _____/20
TOTAL _____/100

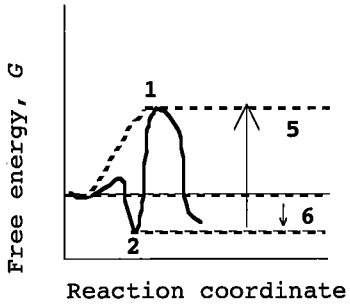
I. **MULTIPLE CHOICE**. (48 points; 3 pts each)
Choose the BEST answer to the question and write your answer in the box provided.

1. Which of the following is true of the binding energy derived from enzyme-substrate interactions during the catalytic cycle?

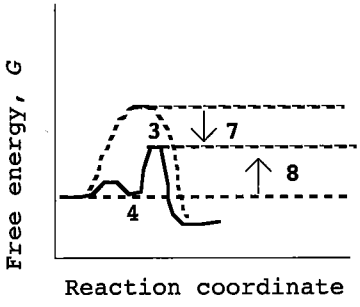
A. Most of it is used up simply binding the substrate to the enzyme.
B. Most of it is derived from covalent bonds between enzyme and substrate.
C. It is often used to hold two substrates in the optimal orientation for reaction.
D. It cannot provide enough energy to explain the large rate accelerations brought about by enzymes.
E. Reaching the transition state is not related to binding by the enzyme.

2. Compare the two reaction coordinate diagrams below and select the answer that correctly describes their relationship. In each case the single intermediate is the ES complex and the dashed curve is the *uncatalyzed* reaction coordinate.

(a)



(b)



A. The ES complex is given by #2 in (a) and #3 in (b).
B. The activation energy for the *catalyzed* reaction is #5 in (a) and is #7 in (b).
C. (a) describes tight binding to S, whereas (b) describes tight binding to the transition-state.
D. The activation energy for the *uncatalyzed* reaction is given by #6 in (a) and by #7 in (b).
E. The contribution of binding energy to the *catalyzed* reaction is given by #6 in (a) and by #8 in (b).

3. One of the assumptions stated by Michaelis & Menten is called the steady-state assumption. This assumption implies:

   A. $K_m = K_d$.
   B. the maximum velocity occurs when the enzyme is saturated.
   C. the ES complex is formed and broken down at equivalent rates.
   D. the K_m is equivalent to the cellular substrate concentration.
   E. the enzyme is regulated.

4. Which of the following statements about a plot of v_0 vs. [S] for an enzyme that follows Michaelis-Menten kinetics is *false*?

   A. K_m is the [S] at which $v_0 = 1/2\ V_{max}$.
   B. the shape of the curve is that of a hyperbola.
   C. the y-axis is a rate term with units such as μM/min.
   D. as [S] increases, the initial velocity of reaction, v_0, also increases.
   E. at high [S], the velocity curve becomes superimposed on a horizontal line which intersects the y-axis at V_{max}.

5. The most efficient substrate of an enzyme is usually considered to be the substrate with the _____.

   A. largest k_{cat}
   B. largest K_m
   C. largest k_{cat}/K_m
   D. smallest k_{cat}/K_m
   E. smallest K_m

6. How is trypsinogen converted to trypsin?

   A. Two inactive trypsinogen dimers pair to form an active trypsin tetramer.
   B. An increase in Ca^{2+} concentration promotes the conversion.
   C. Proteolysis of trypsinogen forms trypsin.
   D. Trypsinogen dimers bind an allosteric modulator, cAMP, causing dissociation into active trypsin monomers.
   E. A protein kinase-catalyzed phosphorylation converts trypsinogen to trypsin.

7. Which of the following statements about allosteric control of enzymatic activity is *false*?

   A. Allosteric proteins are generally composed of several subunits.
   B. Heterotropic allosteric effectors compete with substrate for binding sites.
   C. Binding of the effector changes the conformation of the enzyme.
   D. An effector may either inhibit or activate an enzyme.
   E. One explanation of allosteric control is the sequential model.

8. Protein kinase A

   A. is activated by ATP.
   B. consists of a tetramer of two catalytic (C) and two regulatory (R) subunits in the absence of the allosteric heterotropic positive effector molecule.
   C. upon binding the activator, dissociates into one C_2 and two R subunits.
   D. is inactive when C-subunits bind a pseudo-substrate sequence in the R-subunits that cannot be phosphorylated.
   E. is activated by trypsin.

9. The Lineweaver-Burk plot is used to:

A. determine the equilibrium constant for an enzymatic reaction.
B. illustrate the effect of temperature on an enzymatic reaction.
C. solve, graphically, for the rate of an enzymatic reaction at infinite substrate concentration.
D. solve, graphically, for the ratio of products to reactants for any starting substrate concentration.
E. extrapolate for the value of reaction rate at infinite enzyme concentration.

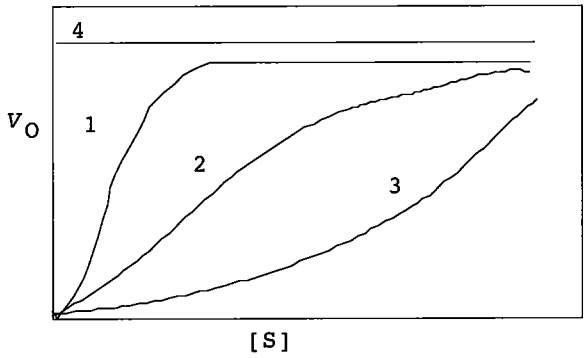
10. If an enzyme were able to stabilize the transition state ($\Delta\Delta G^{\ddagger}$) by -8 kcal/mole the rate of the catalyzed reaction would _____.

A. increase
B. decrease
C. increase by 10^{-3}
D. decrease by 10^{-3}
E. increase by 10^{6}
F. increase by 10^{2}

11. One benefit of measuring the *initial* rate of a reaction, v_0, is that at the beginning of a reaction:

A. changes in [S] are negligible, so [S] can be treated as a constant.
B. [ES] can be measured accurately.
C. $v_0 = V_{max}$.
D. changes in K_m are negligible, so K_m can be treated as a constant.
E. varying [S] has no effect on v_0.

12. Below is a plot of V_0 vs. [S] for a specific allosteric enzyme under different conditions.



Which of the following is *true* based on the graph?

A. Curve #3 will eventually cross over the asymptote given by the line #4 at very high [S].
B. Line 4 is valid exclusively for curve 1.
C. Adding a negative effector to #2 would result in curve 3.
D. Adding a positive effector to #1 would result in curve 2.
E. Curve 1 represents maximum inhibition.

13. Which of the following statements is *false*?

A. Each strand of the protein chain in collagen is a left-handed helix.
B. For S → P, a catalyst cannot shift the reaction equilibrium.
C. At the end of an enzyme-catalyzed reaction, the functional enzyme becomes available to catalyze the reaction again.
D. Substrate binds to an enzyme's active site.
E. Collagen is an abundant globular protein in mammalian organisms.

14. Which of the following interactions help stabilize the structure of collagen?

A. the H-bonds between residues in the sequence about every 4th residue.
B. the interstrand H-bonds
C. the van der Waals interactions between strands at glycine residues as the strands pack
D. ionic interactions (salt-bridges) between hydroxy-proline and hydroxy-lysine residues
E. the hydrophobic interactions between strands because every other residues is an alanine
F. B and C
G. A and E
H. all of the above

15. Addition of a phosphoryl group to a protein can change its conformation by ____.

A. providing additional H-bond acceptors
B. providing additional H-bond donors
C. providing a positive charge to create new van der Waals interactions
D. providing a negative charge to create new van der Waals interactions
E. provide a new peptide sequence

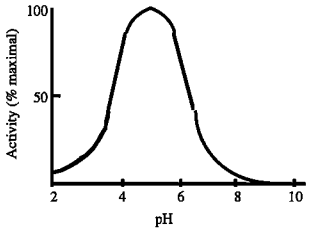
16. Which of the following pairs of strategies correctly identifies one catalytic strategy and one mechanistic strategy used by enzymes?

A. acid-base catalysis/metal ion catalysis
B. covalent catalysis/polarizing bonds
C. proximity/desolvation
D. bond strain/acid-base catalysis
E. metal catalysis/metal ion catalysis

II. **SHORT ANSWER.** (26 points)
Give a brief answer or fill in the blanks as directed in each problem or question below. Be sure to put your answer in the boxes provided.

17. What was the purpose of heating the BSA prior to digestion with trypsin in the Experiment in week 2 of the laboratory? (2 pts) *Note: answers with more than 5 words will receive NO credit.*

Would not heating have affected the results?



18. The active site of lysozyme contains two amino acid residues essential for catalysis: Glu-35 and Asp-52. The pK_a values of the carboxyl side chains of these residues are 6.1 and 4.0, respectively. What is the pH optimum of lysozyme based on the plot of pH versus rate, above? (4 pt)

pH optimum

What is the ionization state (HA or A^-) of each residue at this pH optimum?

Glu-35

Asp-52

Which of these residues might act as a base in the catalytic mechanism, consistent with these data.

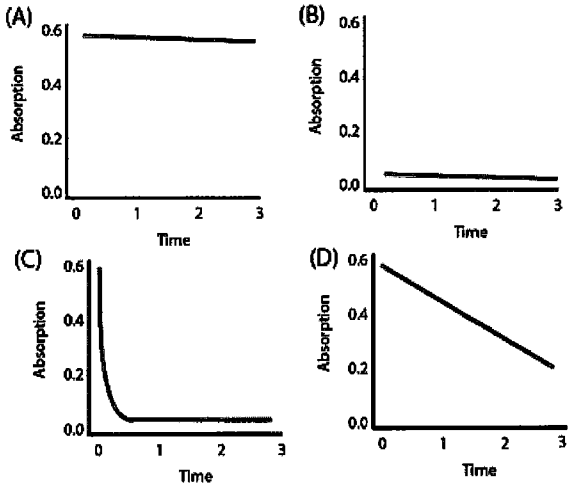
19. In collagen, several polypeptide helices are intertwined. The helices and the superhelical twist are in the opposite directions. What is the handedness of each collagen strand? (3 pts)

Why are they opposite?

Note: Answers with more than 8 words receive NO credit.

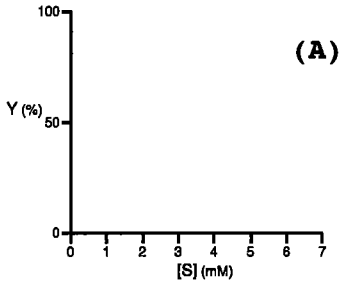
20. In the lab, you learned about assaying for LDH activity. Your partner and you collected the data shown below. Use these data to answer the following questions.

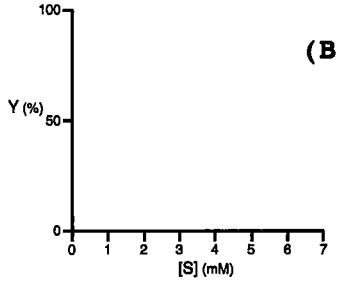
a) These data were collected using different dilutions of your stock enzyme and adding the same 50 μL volume to the assay. What is the order of the dilutions from most dilute to most concentrated?



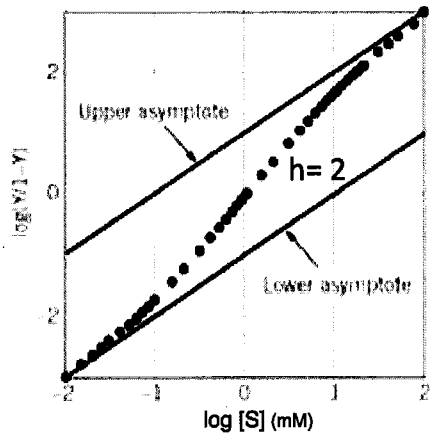
b) For sample B, your lab partner thinks that the lack of much change in absorbance means that you need to add more enzyme and repeat the assay. Do you agree or not? (3pts)

21. Draw the binding curves for (A) saturation binding and (B) cooperative binding with a K_d value of 2 mM using the lettered axes provided. (4 pts)





22. In the Hill Plot below, the substrate concentration alone is responsible for the cooperative behavior. What is the term for this type of allosteric effector (1 word)? (5 pts)



Is it positive of negative?

what is the degree of cooperativity (number)?

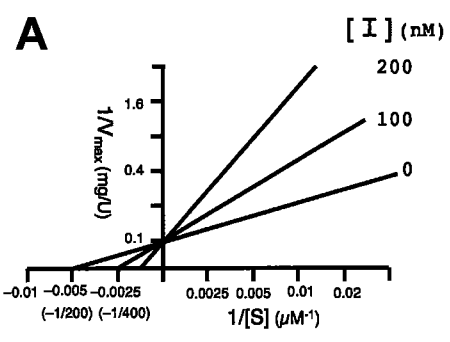
At very low [substrate], what is the K_d for the ES complex?

If the quaternary structure were a homotetramer, what is the percentage of cooperativity relative to a fully cooperative enzyme?

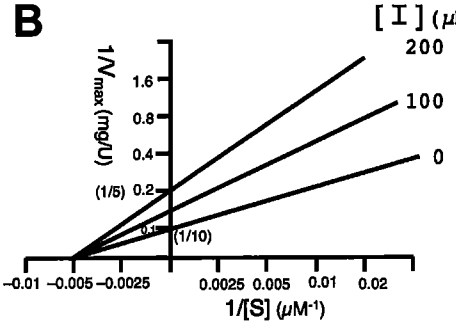
23. You are studying an enzyme that all of a sudden becomes an interesting target for a pharmaceutical company. You know your enzyme well, its assay, and its substrates. You were given two inhibitors of your enzyme by the company (A & B), and you performed steady-state kinetic assays with them. You obtain the results shown in the Lineweaver-Burk plots below. Provide answers requested in each box below. (5 pts)

Which is a stronger inhibitor, A or B?

A or B



K_i value



K_i value

Kind of inhibitor

Kind of inhibitor

III. **MECHANISMS**. (6 points)

Answer the following questions about enzyme mechanisms and CAREFULLY draw structures as described, but ONLY for what is asked.

24. Draw the tetrahedral intermediate in chymotrypsin that is produced just prior to deacylation (cleavage of F–Q (acyl-enzyme)) of the enzyme intermediate. Include only the tetrahedral carbon, those atoms covalently bonded to it, and labels for the substituents bonded to those atoms (enzyme, oxyanion, peptide, and hydroxyl). Subsequently, **draw arrows** indicating the electron movements that would occur in the next step, the formation of the second product (Q). Include His-57.
DO **NOT** DRAW THE ENTIRE MECHANISM.

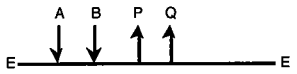
IV. **MATCHING**. (20 points)

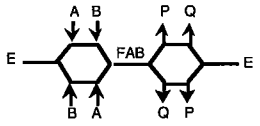
25. Match the items on the left with the BEST item for each on the right: (5 pts)

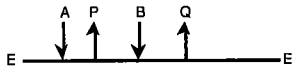
1. Trivial enzyme name (only)
2. Systematic enzyme name and EC number
3. sequential random bi bi
4. Enzyme pseudo-second order rate constant for E+S binding
5. Enzyme turnover rate

A. K_d

B. k_{cat}

C. 

D. 

E. 

F. k_{cat}/K_m

G. kinase

H. isomerase

I. EC 1.2.1.32 a lyase

J. EC 1.2.1.32 an oxidoreductase

26. The following phrases in the left-hand column best match an answer, feature, or value in the right-hand column. Put the letter corresponding to the **best** answer in the BOX to the left of each numbered phrase. A lettered answer may be used only once, and all but one will be used. (15 pts)

☐ 1.	distinct for ping-pong versus an sequential random mechanism	A. L-B plots intersect at y-axis vs. those intersection at x-axis
☐ 2.	distinct for competitive versus non-competitive inhibitors	B. 2.8
☐ 3.	pK_a of 4.5 in a plot of V_{max}/K_m vs. pH	C. indicates possibly an Asp involved in binding
☐ 4.	pK_a of 7.0 in a plot of V_{max} vs. pH	D. $V_{max}/[E]_T$
☐ 5.	Concerted model for allosteric control	E. indicates possibly a His involved in catalysis
☐ 6.	turnover number	F. L-B plots show parallel lines vs. those intersection near the x-axis
☐ 7.	collagen helix phi/psi angles	G. 1.0
☐ 8.	α-helix phi/psi angles	H. L-B plots intersect at x-axis vs. those intersection at y-axis
☐ 9.	Sequential model for allosteric control	I. K_m
☐ 10.	$(k_{-1} + k_2)/k_1$	J. $T_4 \leftrightarrows T_3R_1 \leftrightarrows T_2R_2 \leftrightarrows T_1R_3 \leftrightarrows R_4$
☐ 11.	measure of enzyme efficiency	K. K_d
☐ 12.	measure of enzyme proficiency	L. V_{max}/K_m
☐ 13.	Hill coefficient for a cooperative enzymes	M. $T_4 \leftrightarrows R_4$
☐ 14.	Hill coefficient for a non-cooperative enzyme displaying hyperbolic Michaelis-Menton kinetics	N. -65/+130
☐ 15.	Value of K_m under the rapid equilibrium assumption	O. -57/–47
		P. $k_{cat}/k_{uncatalyzed}$

No. on Test	Correct Answer
1	C
2	C
3	C
4	E
5	C
6	C
7	B
8	D or B
9	C
10	E
11	A
12	C
13	E
14	F
15	A
16	D

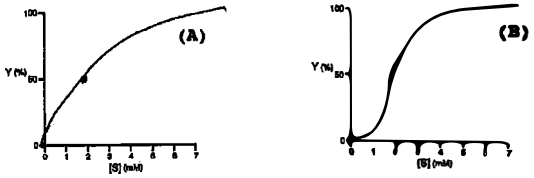
17.1 expose all Arg/Lys residues or denature protein
17.2 Yes

18.1 5
18.2 Glu-35 = HA
18.3 Asp-52 = A–
18.4 Asp-52

19.1 left-handed
19.2 opposite twist of coiled-coil is straight

20.a A-D-C-B
20.b No

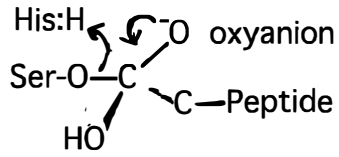
21.



22.1 homotrop[ic
22.2 positive
22.3 2
22.4 10 mM
22.5 50%

23.1 A
23.2 A is competitive
23.3 B is non-competative
23.4 A is 100 nM
23.5 B is 200 μM

24



25.
1. G
2. J
3. D
4. F
5. B

26.
1. F
2. A
3. C
4. E
5. M
6. D
7. N
8. O
9. J
10. I
11. L
12. P
13. B
14. G
15. K