Enzyme Kinetics:

$$v = \frac{\frac{V_{MAX}}{\alpha'}[S]}{\frac{\alpha}{\alpha'}K_M + [S]} \qquad \alpha = 1 + \left(\frac{[I]}{K_I}\right)$$

$\alpha' = 1$ for competitive inhibition

$\alpha' > 1$ for non-competitive (mixed) inhibition

α: ratio of slopes α': ratio of y-intercept

$V_{max} = k_{cat}[E_T]$ $K_M = (k_{off} + k_{cat})/k_{on}$

Double reciprocal plot: $\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$

Thermodynamics:

$\Delta G = \Delta G^o + RT\ln[Products]/[Reactants]$

$\Delta G^o = -RT\ln K_{eq}$

$\Delta G^o = \Delta H^o - T\Delta S^o$

$\mu = \mu^O + RT\ln[X]$

$S = R\ln W$

For $A \leftrightarrow B$: $f_A = 1/(1+K_{EQ})$ $f_b = K_{EQ}/(1+K_{EQ})$

Amino Acid Names:

Alanine: Ala	Arginine: Arg	Asparagine: Asn
Aspartic Acid:Asp	Cysteine: Cys	Glycine: Gly
Histidine: His	Isoleucine: Ile	Lysine: Lys
Leucine: Leu	Methionine; Met	Phenylalanine:Phe
Proline: Pro	Serine: Ser	Threonine: Thr
Tryptophan: Trp	Tyrosine: Tyr	Valine: Val
Glutamine: Gln	Glutamic Acid: Glu	

Acid-Base Chemistry:

$pH = pK_a + \log([A^-]/[HA])$

$pH = -\log[H^+]$

$[HA] = [A_T] / (1+R)$

$[A^-] = [A_T]\, R / (1+R)$

$R = [A^-]/[HA]$

Ligand Binding (L is the Ligand)

$Y = K_{EQ}[L]/(1+K_{EQ}[L]) = [L]/([L]+K_D)$

$Y = [ML]/([M]+[ML])$

Hill Plot: $\log(Y/(1-Y))$ versus $\log[L]$

Hill Equation: $\text{Log}(Y/(1-Y)) = -\log K_\pi + n_h \log[L]$

Miscellaneous Formula & Constants:

$A = \varepsilon C l$

R = 8.3 J/mol-deg RT = 2.5 kJ/mol @ 300K

log2 = 0.3 ln 2 = 0.69 $\ln X = 2.3\log_{10} X$

To convert from ^oC to K, add 273.

This exam contains 100 points on 7 pages. Use the back of a page if you need additional space.

1. (2 pts) Equilibrium dialysis was used to measure ligand binding. The *total* protein concentration inside the dialysis bag is *X* (e.g. 1 μM). The free ligand concentration outside the bag is equal to the K_D for this particular protein-ligand pair (e.g. 10 μM). Which of the following expressions gives the ***total*** ligand concentration inside the bag, L_{TOT}, at this particular ligand concentration ($[L] = K_D$), circle the correct answer:

a) $L_{TOT} = K_D$

b) $L_{TOT} = K_D + X$

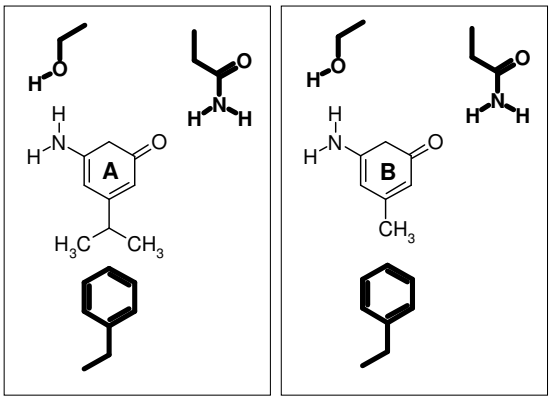
c) $L_{TOT} = K_D - X$

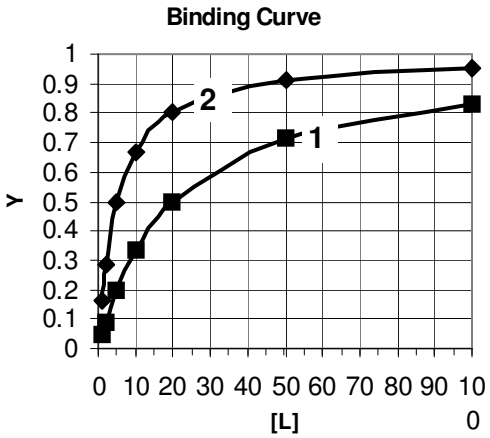
d) $L_{TOT} = \frac{K_D + X}{2}$

e) $L_{TOT} = K_D + \frac{X}{2}$

f) $L_{TOT} = K_D - \frac{X}{2}$

2. (12 pts) Two ligands (A and B) bind to the same protein. The structure of the protein–ligand complexes are shown on the right. The amino acid side chains are shown in bold. Binding curves were measured for each ligand and these are also shown on the right.



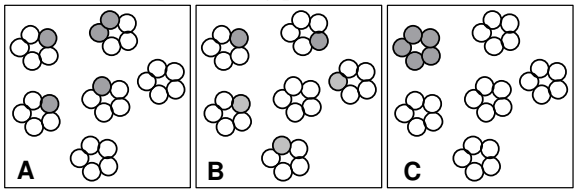


i) (8 pts) Determine the K_D values for both binding curves (1 & 2) and *assign* each of your K_D values to one ligand or the other. (*e.g. Ligand A binds with a K_D= xx.x, which was obtained from curve 1.*) Briefly justify your answer with reference to the structure of the ligand and the protein.

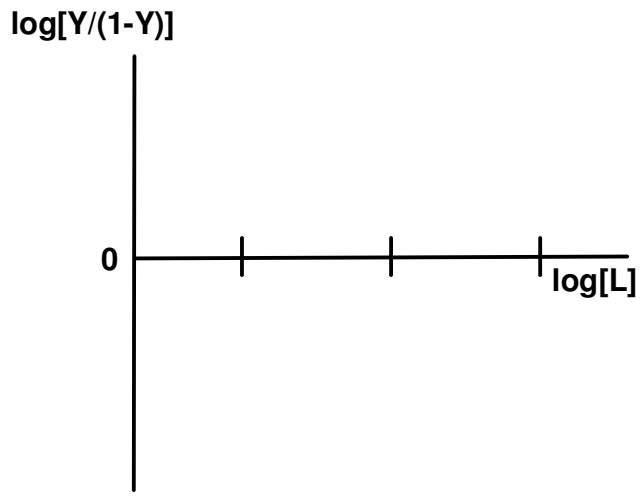
ii) (2 pts) Which ligand binding reaction is likely to show a *larger* increase in entropy (ΔS) on binding? A or B? Why? [Consider the direction of the reaction as: $M + L \rightarrow ML$].

iii) (2 pts) Now assume that A and B are substrates, and that the protein is an enzyme. Which substrate would have the lower K_M, A or B? Why?

3. (10 pts) The following diagram illustrates the distribution of ligands on three different pentameric proteins. Panel A represents a non-cooperative system while panels B and C represent cooperative systems. Sketch the central part of the Hill plot that you would obtain for each of these systems. You can assume that the K_D for all proteins is 10^{-6} M. J*ustify your answer on the back of the preceding page.*



Non-cooperative



4. (6 pts) List the key properties of a protein that are required for cooperative binding behavior. Feel free to us a diagram to answer this question. [Hint; Just relax and answer the question.]

5. (8 pts) Discuss the role of allosteric behavior in any ***one*** of the following processes. Feel free to use a diagram to answer this question.

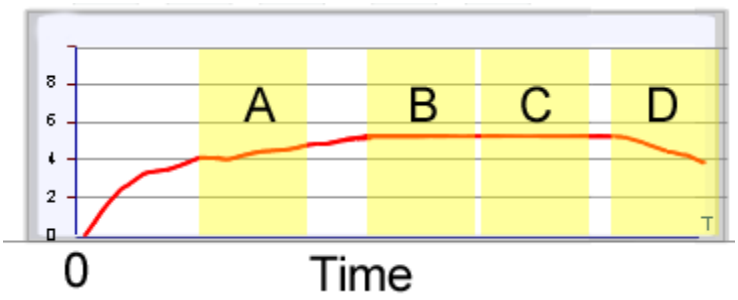
Choice A: Oxygen transport to the tissues.

Choice B: Adaptation of oxygen delivery at high altitude.

Choice C: Adaptation of oxygen delivery during vigorous exercise.

6. (14 pts) Using transition state theory, explain why enzymes catalyze reactions. Recall that there are two distinct methods by which this is accomplished, one common to *all* enzymes and one displayed by serine proteases. Briefly describe how *either* of these methods serves to increase the catalytic rate.

7. (5 pts) The image to the right shows the concentration of a species in an enzymatic reaction as a function of time, beginning with mixing the enzyme and substrate at t=0. Please answer the following two questions:



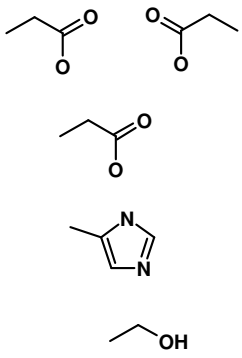
i) (2 pts) Which species in the reaction is represented by this curve? (circle your choice):
   a) free enzyme, (E).
   b) substrate (S)
   c) enzyme-substrate complex (ES)
   d) product (P)

ii) (1 pt) Which of the following periods, A, B, C, or D would be more suitable for measuring the initial velocity of the enzyme catalyzed reaction (circle your answer):
   A
   B
   C
   D

*Briefly justify your answer in the above space, or the back of the previous page. (**2 pts**).*

8. (3 pts) Fill in the blanks: At very low substrate concentration the measured velocity in an enzyme catalyzed reaction is essentially _____ with respect to substrate concentration ([S]), while at high substrate concentrations the velocity is _____ of substrate concentration, indicating that the enzyme is _____ with substrate.

9. (10 pts) Please do ***one*** of the following choices (labeled drawings are acceptable)



Choice A: Briefly explain the molecular basis for the substrate specificity of ***either*** trypsin, chymotrypsin, elastase, ***or*** HIV protease. Which kinetic rate-constant, k_{on}, k_{off}, or k_{cat} will be most affected by changing substrates? Why?

Choice B: Using ***either*** serine proteases ***or*** HIV protease as an example, discuss how functional groups on the enzyme lead to cleavage of the peptide bond. The diagrams to the right may be helpful.

Choice C: Explain how the pH dependencies of K_M ***or*** V_{MAX} (k_{CAT}) can be used to identify functional groups that are important for substrate binding ***or*** catalysis. Be sure to illustrate your answer with an example from ***either*** serine proteases ***or*** HIV protease.

10. (12 pts) Briefly explain why a competitive inhibitor only affects K_M but not k_{CAT} (V_{MAX}) while a mixed type inhibitor can affect both. Your answer should include a discussion/description of the general molecular structure of each type of inhibitor and its binding site on the enzyme.

11. (6 pts) Please answer ***one*** of the following two choices:

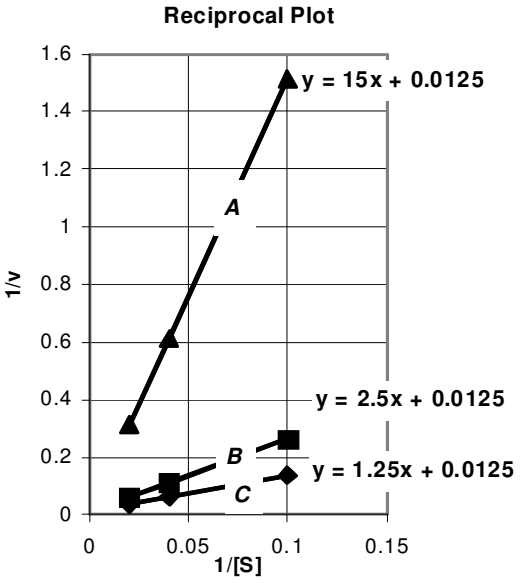
Choice A: Provide a definition for specific activity and describe its usefulness in protein purification.

Choice B: Select one method of column chromatography and briefly explain the basis for the separation of proteins by that method.

Place your answer on the back of the previous page.

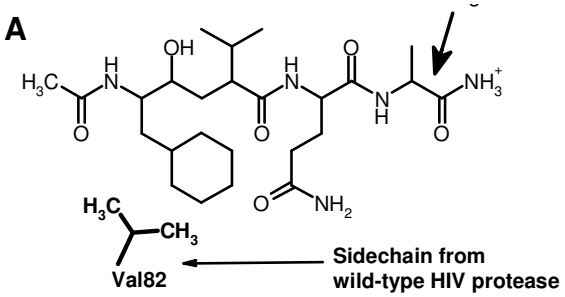
12. (12 pts) Please do ***one*** of the following three choices. Choices B and C can be found on the following page.

Choice A: The ability of a drug to inhibit the wild-type HIV protease and a mutant HIV protease (valine82 → serine) were tested using steady-state enzyme kinetics and the experimental data was plotted on the double reciprocal plot shown to the right. *Either enzyme was equally active against the substrate used in the assay, i.e. the mutation did not affect the steady state kinetics when the drug was absent.*

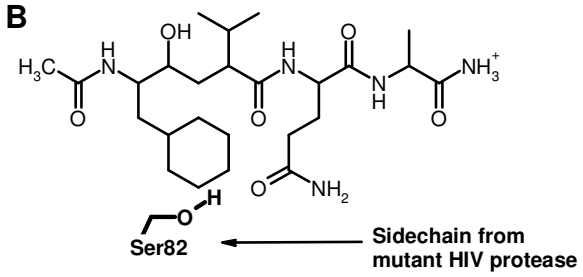


The structure of the drug-enzyme complexes are shown below. The drug is less active in individuals infected with the mutant virus.

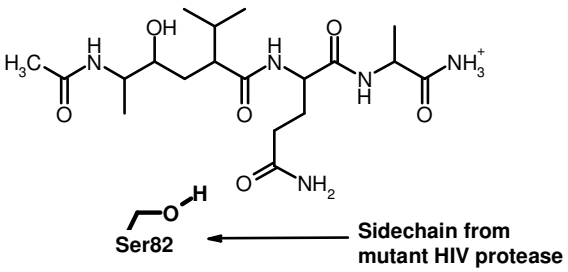
i) (4 pts) Which of the three lines (A, B, or C) corresponds to the data acquired without the inhibitor? *Briefly justify your answer.*



ii) (4 pts) Which of the three lines (A, B, or C) corresponds to the data acquired with the *wild-type* enzyme plus the drug. *Briefly justify your answer*.



iii) (4 pts) Briefly explain how you would modify the original drug such that it will be more effective against the virus with the mutant HIV protease. A sketch is acceptable, feel free to use the template on the right. *Briefly justify your answer on the back of the previous page.*



Choice B: Devise a purification scheme that will separate protein C from the following five proteins (A-E). You may summarize your purification scheme using a flow-chart, be sure to briefly indicate the logic you employed for each step.

Protein	[Ammonium Sulfate] that precipitates 50% of protein*	# Residues (Mol Wt)	#Asp (pK_a=4.0)	#His (pK_a=6.0)	#Lys (pK_a=9.0)
A	1.0 M	120 (13,200 Da)	2	0	10
B	1.5 M	120 (13,200 Da)	4	4	12
C	**4.0 M**	**120 (13,200 Da)**	**4**	**8**	**4**
D	4.5 M	125 (13,800 Da)	2	8	4
E	4.0 M	240 (26,400 Da)	2	0	10

*Assume an ammonium sulfate concentration that is 1 M above the 50% ppte concentration will precipitate all of that protein and 1 M below will leave all of the protein in solution; i.e. protein A is completely ppte by 2M ammonium sulfate.

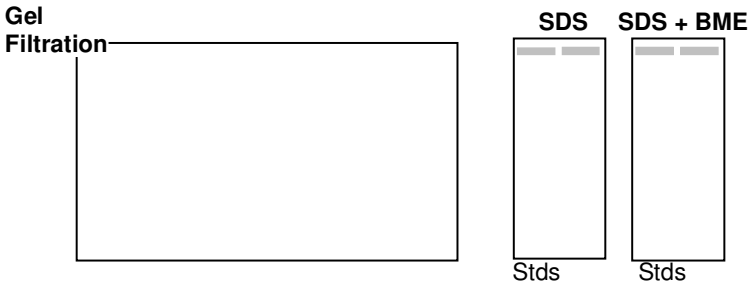
Choice C: You are verifying the quaternary structure of an antibody, which consists of two light chains (25 kDa) and two heavy chains (50 kDa). One light chain is crosslinked to one heavy chain via a disulfide bond. Assume that there are no disulfide bonds between the heavy chains.

i) (3 pts) *Sketch* the gel filtration profile you would observe for this protein. Assume that you included size standards of 10 and 100 kDa on the gel filtration column.

ii) (3 pts) *Sketch* the SDS gel that you would obtain from this sample, again including size standard of 10 and 100 kDa. You should assume that β-mercaptoethanol (BME) was not included. The left lane should be used to indicate the position of the standards (Stds).

iii) (3 pts) Sketch the SDS gel that you would obtain form this sample, assuming that BME was included.

iv) (3 pts) Briefly explain how SDS-PAGE separates proteins by size.



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