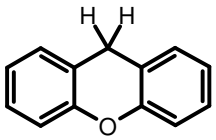
This exam consists of 9 questions on 6 pages. There are a total of 100 points. Allot 1min/2 pts. Please use the space provided for your answer. If necessary use the back of the preceding page for any intermediate calculations. Note that question 6 is worth 36 points.

1. (5 pts) An enzyme binds the substrate shown to the right. Name **one** molecular force/interaction that would stabilize the bound form of this substrate. Briefly justify your answer. Indicate whether this interaction would be predominantly enthalpic (ΔH) or entropic (ΔS).



2. (10 pts) Briefly describe allosteric effects and their relationship to cooperative ligand binding. Your answer should clearly state the role of relaxed and tense states in this effect.

3. (6 pts) Briefly describe the molecular features of oxygen binding that are common to both myoglobin and hemoglobin (4 pts). Why is oxygen binding to myoglobin non-cooperative (2 pts)?

4. (8 pts) Please do **one** of the following two choices (feel free to use a diagram).

Choice A: Discuss the role of homotropic allosteric effects in oxygen delivery to the tissues.

OR

Choice B: Discuss the role of heterotropic allosteric effects in altitude adaptation of oxygen delivery.

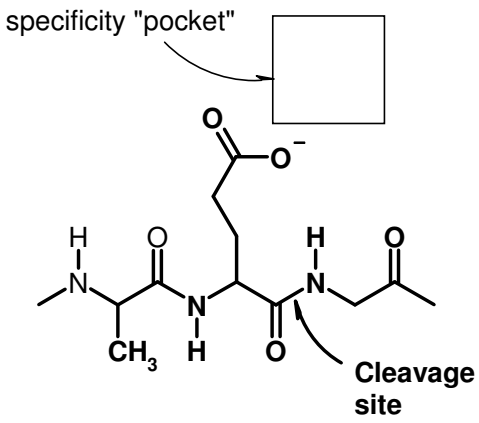
5. (8 pts) Please do **one** of the following two choices.

Choice A: Chemical reactions proceed through a high energy transition state. Using transition state theory, briefly describe two ways that enzymes increase the rate of reactions [Hint: one of the two ways is common to all enzymes].

OR

Choice B: What is the steady state hypothesis? What is its relevance to the experimental determination of K_M and V_{MAX}? How are K_M and V_{MAX} obtained?

6. (36 pts) Drippy nose virus requires a protease to complete its replication process inside cells. This protease cleaves peptides at the site shown in the diagram on the right. The box in this diagram represents the region of the **enzyme** that defines the substrate *specificity* of this enzyme.



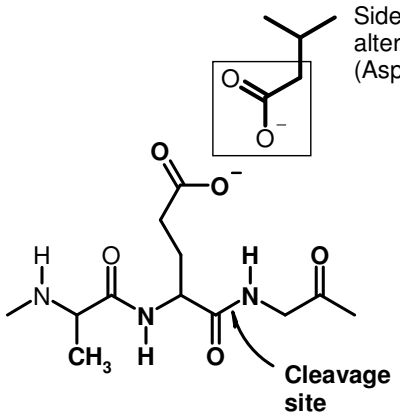
i) (10 pts) Indicate the residues in the active site region of this enzyme that are responsible for cleavage of the peptide bond. Your answer can be based on either the serine protease mechanism or the mechanism of HIV protease. Your diagram need not show the detailed structures of important residues, but you should indicate the role of each residue in the catalytic process and provide a *very brief* overview of the process of peptide bond cleavage.

ii) (2 pts) Write, in the box on the above figure, the name of the residue that would be responsible for the specificity of this enzyme. If you cannot recall the name of the amino acid, draw the appropriate functional group.

iii) (5 pts) Design a competitive inhibitor of this protease. State your design principles and briefly explain why it is a competitive inhibitor.

Question 6, continued….

iv) (6 pts) Although your drug is very effective at inhibiting the wild-type enzyme, mutations occur in the viral genetic material. These mutations result in a protease that is resistant to your drug. The amino acid sequence of the altered protease shows that the residue in the specificity pocket has been replaced by Aspartic acid (see structure of ES complex to the right). What change would you make in your inhibitor such that a modified inhibitor would bind equally well to the altered (mutant) protease?



v) (6 pts) Please do only **one** of the following two choices. *Please indicate your choice*. Note that both choices use the modified inhibitor from part *iv* and the altered (mutant) enzyme.

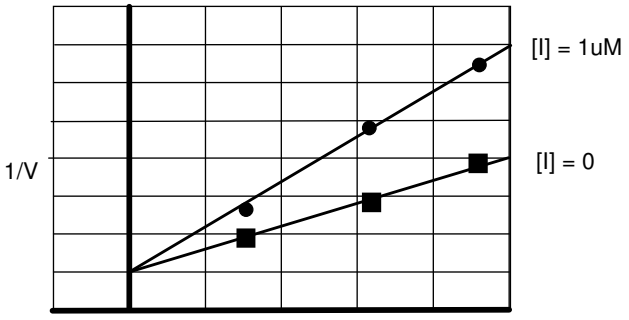
Choice A. The modified inhibitor from part *iv* is used in an equilibrium dialysis experiment. The total concentration of the altered (mutant) enzyme inside the dialysis bag is 1 μM. Several concentrations of inhibitors are used in separate experiments and the concentration of inhibitor outside (free inhibitor) and the total concentration of inhibitor inside the dialysis bag are given in the table to the right. What is the K_D for the binding of the inhibitor to the enzyme?

Experiment #	Free Ligand	Total Ligand in Bag
1	0.1 μM	0.2 μM
2	1.0 μM	1.5 μM

$$\theta = \frac{[ML]}{[M]+[ML]} = \frac{[L]}{K_D+[L]}$$

OR

Choice B. Steady state enzymatic data, using the altered (mutant) enzyme, was obtained in the absence of the inhibitor and in the presence of 1 μM of the inhibitor from part *iv*. These data were plotted on a double reciprocal plot shown on the right. What is the K_I for your modified inhibitor?



$$v = \frac{V_{MAX}[S]}{K_M+[S]}$$

$$\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$

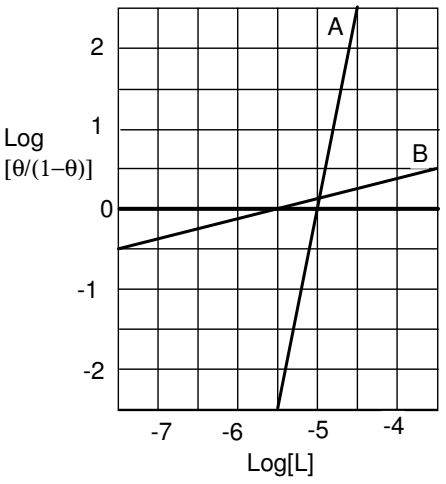
$\alpha = 1 + \frac{[I]}{K_I}$; α **from ratio of slopes.**

vi) (2 pts) Regardless of your choice for section *v* (equilibrium dialysis or steady state enzyme kinetics), your results would be identical, i.e. both methods give the same answer. Why is this so?

vii) (5 pts) How would the K_I for your modified inhibitor change if you measured its binding to the wild-type enzyme? Would the K_I increase, decrease, or stay the same? *Briefly justify your answer.*

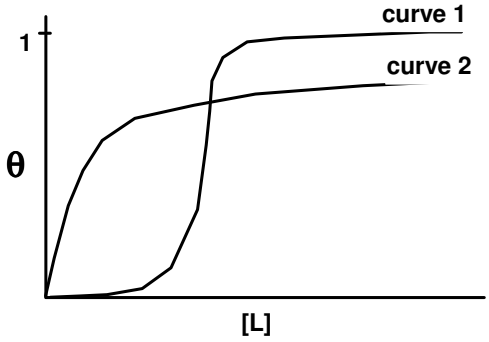
7. (14 pts) The binding of a ligand to two different pentameric proteins (A and B) was measured and the fractional saturation at different ligand concentrations was used to generate the Hill plot shown on the right.

$$\log \theta/(1-\theta) = \log\left[\frac{1}{K_D}\right]^{nh} + n_h \log[L]$$



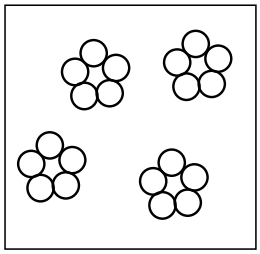
i) (6 pts) Which protein binds with higher cooperativity? A or B? *Briefly justify your answer.*

ii) (4 pts) Which of the two binding curves, "curve 1" or "curve 2", represents the binding of ligand to **protein A**? *Briefly justify your answer.*



Question 7, continued….

iii) (4 pts) The following box illustrates a collection of four **protein B** molecules in a solution where the ligand concentration is such that the fractional saturation, θ**, is equal to 0.25**. Show the distribution of ligands on these four molecules by shading in subunits that have ligand bound. *Briefly justify your answer*.



8. (10 pts)

i) (8 pts) Provide a purification scheme to separate protein C from a mixture of three proteins. The properties and amounts of all three proteins are given in the following table. *Briefly describe the basis of separation for each of your step(s).*

Protein	Amount	Size(# residues)	[Amm Sulfate] required for 50% ppte	# of Acidic residues (e.g. Aspartic Acid)	# of Basic Residues (e.g Lysine or Arginine)
A	100 mg	121	2.0 M	5	5
B	100 mg	120	1.1 M	5	6
C	**100 mg**	**122**	**1.0 M**	**5**	**10**

ii) (2 pts) Assuming that you started with 6000 units of enzyme activity for protein C. Calculate the specific activity of the starting material. What should happen to the specific activity during the purification?

9. (3 pts) Both SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and gel filtration provide molecular weights of proteins. How do they differ?