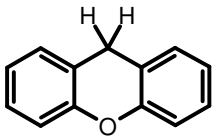
This exam consists of 9 questions on 6 pages. There are a total of 100 points. Allot 1min/2 pts. Please use the space provided for your answer. If necessary use the back of the preceding page for any intermediate calculations. Note that question 6 is worth 36 points.

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).



i) hydrophobic effect. (2 pts) The substrate is largely non-polar and so when it binds it will release ordered water (2 pts). This is an entropic effect (1 pt)

ii) Van der walls. The substrate is likely to form close contacts with residues in the active site, optimizing van der waals effects. This is enthalpic.

iii) H-bonds. The electronegative oxygen could accept a hydrogen bond from the enzyme. This is enthalpic.

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.

- **An allosteric effect is a change in the structure of a protein in response to binding of a ligand. (+4 pts)**
- **The protein exist in two states, an inactive tense form (T) and an active relaxed form (R). These two forms are in equilibrium with each other. (+2 pts)**
- **Positive cooperativity is seen when binding of ligand enhances the R state, increasing the binding affinity (+2 pt)**
- **Negative cooperativity is seen when binding of the ligand enhances the T state, reducing the binding affinity (+2 pt)**

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)?

- **Both myoglobin and hemoglobin bind heme (2 pts)**
- **The heme group has a central Fe atom that binds oxygen (2 pts)**
- **Oxygen binding to myoglobin is non-cooperative because cooperative binding requires at least two binding sites, myoglobin only binds one oxygen. (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.

- **The binding of one oxygen increases the affinity of subsequent oxygens (homotropic) (2 pts)**
- **Thus the curve is 'S' shaped, rising slowly and then more steeply.**
- **In the lungs, at high oxygen levels, the hemoglobin is fully saturated and in the R-state.**
- **At the lower oxygen levels in the tissue hemoglobin releases more oxygen because as it loses oxygen the binding goes from the high affinity state of the fully saturated hemoglobin to the lower affinity state of partially saturated hemoglobin, which is in the T-state.**

+6 pts for a reasonable discussion of oxygen delivery.

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

- **Bisphosphoglycerate is a heterotropic allosteric inhibitor. It binds to the T state of hemoglobin, reducing the affinity and changing the shape of the binding curve. (2 pts)**
- **At high altitudes the amount of BPG is increased.**
- **This increase changes the binding curve such that although less is bound to the hemoglobin, just as much oxygen is released as would be at sea level.**

+6 pts for a reasonable discussion of how higher levels of bpg restore delivery.

5. (6 pts) Please do **one** of the following two choices.

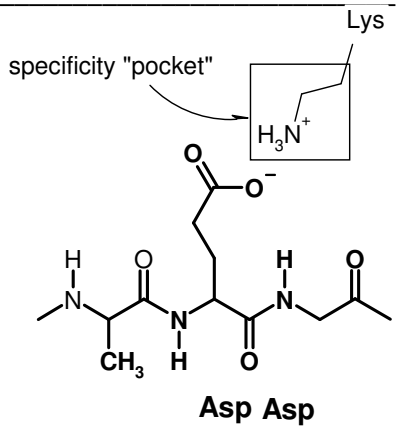
Choice A: Chemical reactions proceed through a high energy transition state. Using transition state theory, briefly describe two ways that enzymes increase the rate of reactions [Hint: one of the two ways is common to all enzymes].

- **Enzymes lower the energy of the transition state, therefore increasing the rate of the reaction since k_{CAT} is proportional to the amount of the transition state (4 pts)**
- **The energy of the transition state is stabilized by two methods:**
  - **a) The pre-ordering of active site residues in the folded protein reduces the entropy change that would occur in the absence of the enzyme.(2 pts)**
  - **b) Direct enthalpic interactions (e.g. hydrogen bonds) to the transition state also stabilize it.(2pts)**

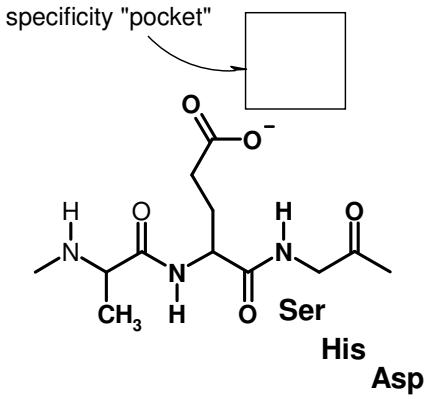
Choice B: What is the steady state hypothesis? What is its relevance to the experimental determination of K_M and V_{MAX}? How are K_M and V_{MAX} obtained?

- **The steady-state hypothesis is that during the course of measurement of product formation, the amount of the enzyme-substrate complex remains the same, d[ES]/dt =0 (4 pts)**
- **This provides a simple solution relating the reaction velocity to the substrate concentration and the total amount of enzyme present: $v = \frac{k_{CAT} E_t [S]}{K_M + [S]} = \frac{V_{MAX}[S]}{K_M + [S]}$ (2 pts)**
- **Measuring the reaction velocity at high [S] gives V_{MAX}, and the substrate concentration that gives a velocity of $V_{MAX}/2$ is equal to K_M. (2 pts)**

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 is 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 detailed structures of important residues, but you should indicate the role of each residue in the catalytic process and provide a brief overview of the process of peptide bond cleavage.



Full credit if most of what is listed below is discussed.

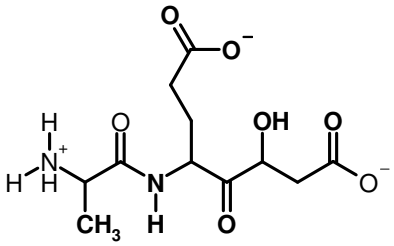
The region near the cleaved bond would contain either two Asps (HIV protease, top) or Ser, His, Asp (serine Proteases, bottom). The role of each of these in the overall mechanism is:

HIV Protease:	Serine Protease:
• One Asp is deprotonated, the other protonated. • The deprotonated Asp activates a water molecule by removing a protein. • The activated water molecule attacks the carbonyl oxygen of the peptide bond, causing cleavage. • The second Asp donates a proton to the product	• The Histidine residue removes a proton from the serine residue, generating the active nucleophile. • The now positively charged His is stabilized by the nearby negatively charged Asp that is part of the triad. • The deprotonated serine will attack the carbonyl carbon, causing peptide bond cleavage and the formation of an acyl-enzyme intermediate.

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.

The most likely residue would be a lysine or arginine, with the positive charge forming a favorable interaction with the negative charge on the substrate. (2 pts for correct functional group).

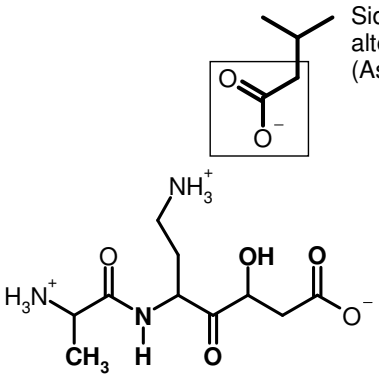
iii) (5 pts) Design a competitive inhibitor of this protease. State your design principles and briefly explain why it is a competitive inhibitor.



A competitive inhibitor would look very much like the substrate so that it would bind in the active site (3 pts) The inhibitor must have a non-cleavable peptide bond (2 pts).

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 to the right). What change would you make in your inhibitor such that the modified inhibitor would bind equally well to the altered (mutant) protease?



The restore the binding of the inhibitor it would be necessary to replace the carboxylic group with a positively charged amine, such as a lysine or arginine sidechain. This will lead to a favorable electrostatic interaction with the inhibitor.

Full credit for some indication of complementary interactions, either electrostatic or hydrogen bonding.

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

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

[ML] is obtained from the difference in the ligand concentration at each ligand concentration:

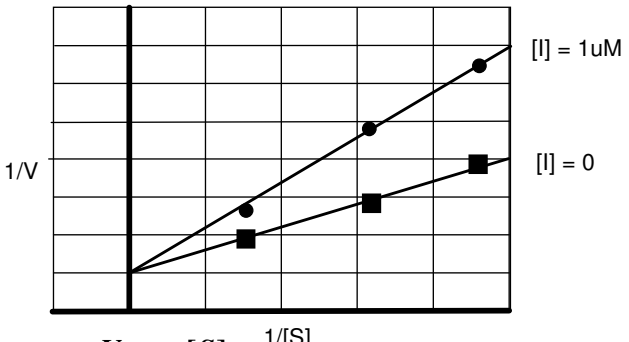
Experiment 1: [ML] = 0.2 - 0.1 = 0.1 μM, θ = 0.1 μM/1 μM = 0.1.

Experiment 2: [ML] = 1.5 - 1.0 = 0.5 μM, θ = 0.5 μM/1 μM = 0.5 (3 pts for one of these)

Since the K_D = [L] when θ=0.5 the K_D = 1 μM. (3 pts)

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?



The slope in the absence of inhibitor is 3/5.

The slope in the presence of 1 μM inhibitor is 6/5.

Therefore, the ratio of the slopes is α=2.(3 pts)

K_I = [I]/(α-1) = 1μM /(2-1) = 1μM.(3 pts)

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

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

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

vi) (3 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?

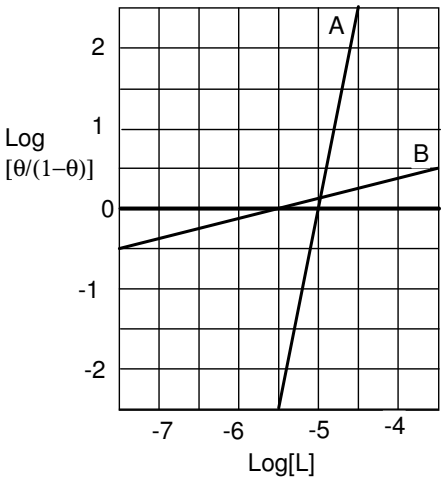
Both methods report the dissociation constant from the free enzyme, i.e. EI → E + I, i.e. $K_D = K_I$.
(3 pts)

vii) (4 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.*

The modified inhibitor is designed to bind to the enzyme with the negativity charged sidechain by virtue of the positive charge on the inhibitor. The original enzyme binds a negatively charged substrate, therefore it will interact less favorably with the modified inhibitor. The K_I would increase.
(4 pts)

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.

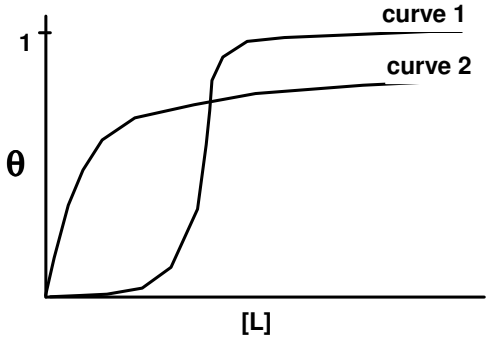
$$\log \theta/(1-\theta) = \log\left[\frac{1}{K_D}\right]^{nh} + n_h \log[L]$$



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

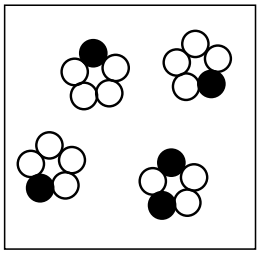
- **The slope of the curve as it crosses the x-axis gives the Hill coefficient. (3 pts)**
- **For protein A the slope is 5, while for protein B the slope is 1/4.**
- **Since protein A has the higher slope, its cooperativity is higher. (3 pts)**

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.*



- **Protein A shows positive cooperativity since the Hill coefficient is greater than 1. (1 pt)**
- **Therefore you would expect weak binding at low [L] and then a rapid increase in fractional saturation. Thus curve 1 is the correct answer (3 pts).**

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.*

- **Protein B shows negative cooperativity since the Hill coefficient is less than 1, therefore the binding of subsequent ligands to the same pentamer will be impaired.**

- **A fractional saturation of 0.25 implies that 1/4 of the 20 binding sites will be filled, or 5 will have ligand bound (2 pts). The first four would go to separate pentamers and the fifth would be found on one pentamer (2 pts).**

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 (Aspartic acid)	# of Basic Residues (Lysine)
A	100 mg	121	2.0 M	5	5
B	100 mg	120	1.5 M	5	6
C	**100 mg**	**122**	**1.0 M**	**5**	**10**

- **Gel filtration cannot be used since all three proteins have similar sizes.**
- **Although there is some difference in solubility in ammonium sulfate solutions it is not enough to cleanly separate B from C, i.e. making the solution 1.5 M in ammonium sulfate will ppte all of C and 50 % of B.**
- **Protein C has more + charged lysine residues than the other two, so it will stick to a cation exchange column (6 pts) more tightly due to interaction with the negative charges on the separation media (2 pts)**

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?

The initial specific activity is 6000 units/300 mg = 20 units/mg of protein. (1 pt)

The specific activity should increase as the enzyme becomes purer. (1 pt)

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

- **SDS-PAGE gives the denatured weight of each polypeptide chain. (1 1/2 pt)**
- **gel filtration provides the native, or non-denatured molecular weight. (1 1/2 pt)**