

Instructions: This exam consists of 100 points on 6 pages. Please use the space provided to answer the question, or the back of the preceding page. In questions with choices, all your answers will be graded and you will receive the best grade. Allot 1 min/2 points. **Note: An 18 pt question is on pg. 4.**

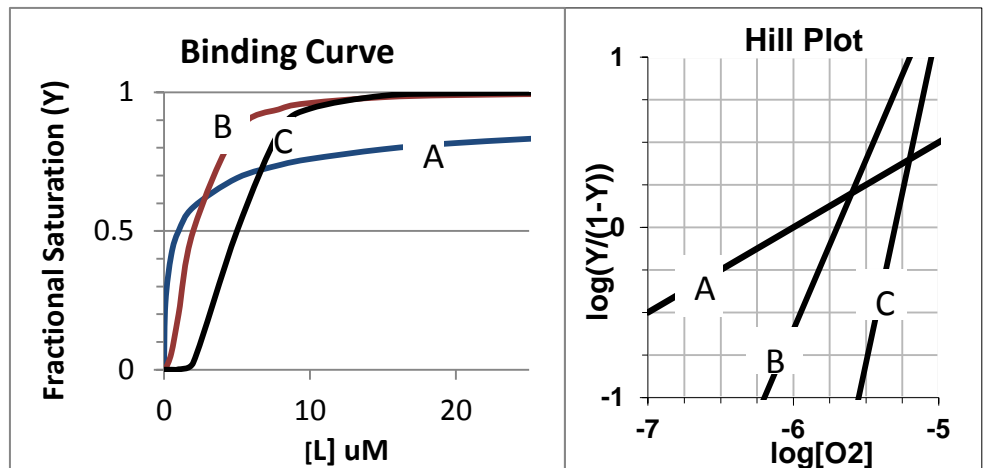
1. (4 pts) In an experiment to measure ligand binding, the concentration of the free ligand is adjusted to be equal to the K_D , i.e. $[L]=K_D=1\mu\text{M}$. Please complete one of the following two questions regarding measuring the binding:

Choice A: Assuming that you were measuring the binding with equilibrium dialysis, with a total protein concentration of 1 μM inside the bag. What is the total concentration of the **ligand** in the dialysis bag?

Choice B: The UV light absorbance of the unliganded protein (M) is 0.2 and that of the fully liganded (ML) protein is 0.6. What is the UV light absorbance when $[L]=K_D$? Why?

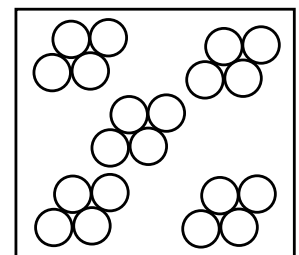
2. (10 pts) The cooperativity and binding affinity of three different tetrameric hemoglobins (A, B, C) is being studied. The binding curves and Hill plots for the three proteins are shown on the right.

i) Which hemoglobin shows the **highest** affinity for oxygen (A, B, or C), based on its K_D ? What is its $K_{D_{AVE}}$? (4 pts).



ii) Which hemoglobin shows the **lowest** cooperativity, A, B, or C? Briefly justify your answer. (4 pts)

iii) In the following panel, sketch the distribution of bound oxygen for the protein you identified in part ii), i.e. the one with the **lowest** cooperativity, by shading the circle. You should assume $Y=0.25$, i.e. only 5 of the 20 sites will be occupied. Briefly justify your answer (2 pts).



3. (4 pts) Compare and contrast the structure and oxygen binding capabilities of hemoglobin and myoglobin.

4. (16 pts)

i) Briefly discuss the major/general feature(s) of allosteric/cooperative behavior (10 pts), your answer should include a discussion of the properties of the tense (T) and relaxed (R) state.

ii) then discuss one of (6 pts):

Choice A: how this effect optimizes oxygen delivery to the tissues,

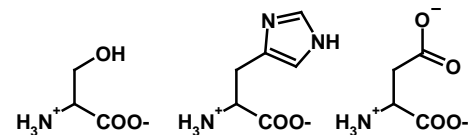
Choice B: how this effect is used to adapt oxygen delivery at high altitudes,

Choice C: how this effect could be used to regulate enzymes.

5. (6 pts) Briefly describe the “steady-state” assumption that is used in the analysis of enzyme kinetic data. Why is it useful to make this assumption?

6. (10 pts) This statement is often heard in biochemistry lectures: "Enzymes are catalysts because they lower the energy of the transition state".
- How does lowering the energy of the transition state increase the rate? (4 pts)
 - How do all enzymes lower the energy of the transition state. (4 pts).
 - What additional feature do serine proteases use to lower the energy of the transition state that is not used by HIV protease (2 pts)?

7. (8 pts) Select either serine proteases or HIV protease and: briefly discuss the role of the catalytic triad or the catalytic diad in peptide bond cleavage. The structures on the right may be useful. For serine proteases, it is not necessary to give the entire reaction mechanism, however you should do so for HIV protease.

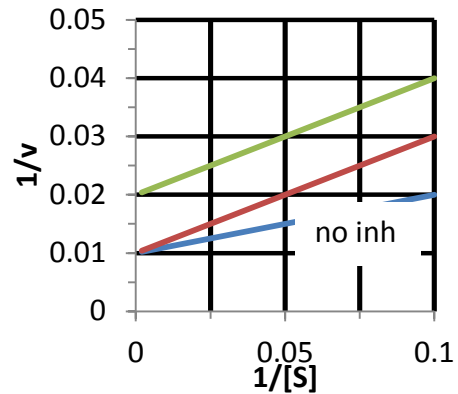


8. (3 pts) Name one enzyme that is inhibited in the treatment of HIV, besides HIV protease. What general properties of this enzyme make it a good drug target?

9. (6 pts) Please do one of the following two choices:

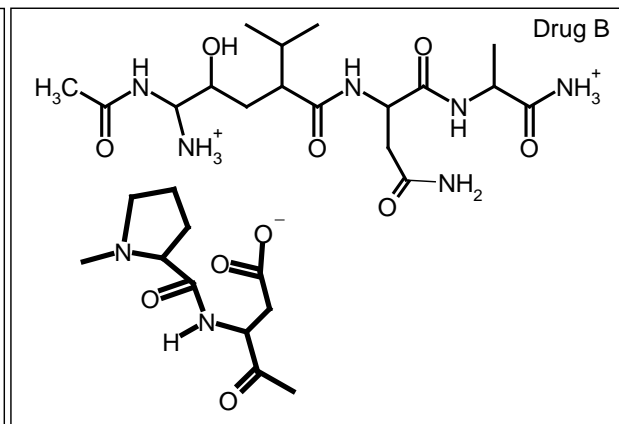
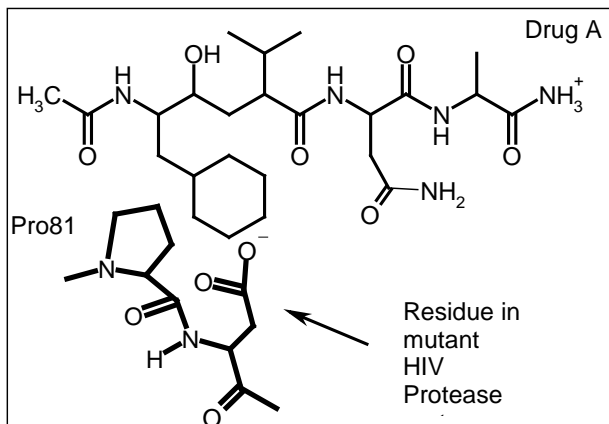
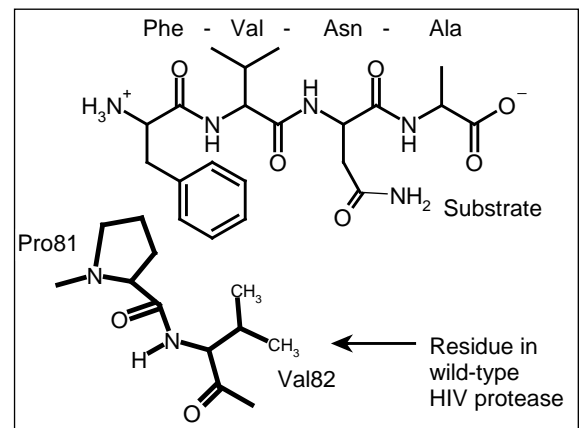
Choice A: Compare and contrast a competitive inhibitor to a mixed type inhibitor, in terms of their chemical structures and binding site on the enzyme.

Choice B: A double reciprocal plot showing the activity of a competitive and a mixed type inhibitor is shown on the right. Label the lines according to the type of inhibition. Briefly justify your answer.

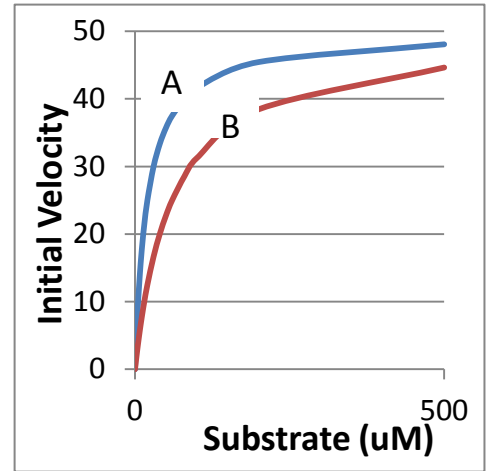


10. (18 pts) The structure of the complex between HIV protease and its substrate (Phe-Val-Asn-Ala) is shown on the right. The enzyme releases Phe from the substrate. The lower two panels below show a mutant HIV protease complexed with two different drugs, Drug A and Drug B

i) Why are these drugs inhibitors of the protease? Do you expect them to be competitive or mixed type? Why? (5 pts)

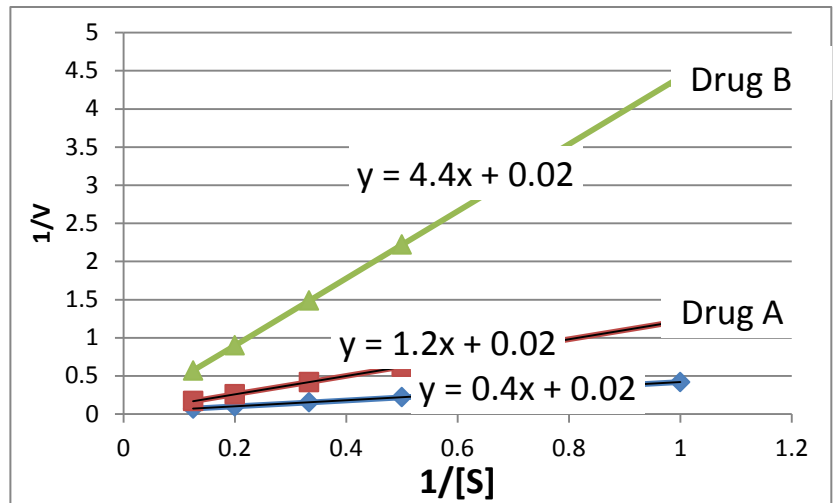


ii) Steady-state enzyme kinetic data was obtained for the **wild-type** and the **mutant** using the substrate Phe-Val-Asn-Ala, and these data were plotted on velocity curve. Which curve, A or B, is more likely to represent the data obtained on the wild-type enzyme? You can assume $V_{MAX}=50$. Briefly justify your answer (3 pts). [Hint: What are the K_m values for each enzyme?]



iii) To determine the effectiveness of drug A and drug B in inhibiting the **mutant enzyme** you obtained steady-state data in the presence of 10 nM of each inhibitor. These data are plotted on the double reciprocal plot on the right. Please answer the following questions:

- Calculate the K_i for each drug (2 pts)
- Based on the K_i which drug binds better to the protease? (2 pts)
- Explain the difference in the K_i values in terms of the interaction between the enzyme and the drug. (4 pts)



iv) How might you modify drug B to increase its ability to inhibit the enzyme? (2 pts).

11. (3 pts) Why does the specific activity increase during a protein purification?

12. (12 pts) You are given a mixture of 4 proteins with the characteristics described below.

- i) Devise a purification scheme to separate **maltose binding protein** from the other three proteins. Briefly justify your answer (8 pts).
- ii) Sketch the elution profile for **one** of your column chromatography steps and indicate where the target protein would elute (i.e. first peak, 2nd peak, etc.) and justify how you labeled the peak. Alternatively, discuss how the chromatography step works to separate the different proteins. (4 pts).

Name	Sol (AmmSul)	MW	#Asp/Glu (pK _a =4)	#Arg/Lys (pK _a =9)	Activity
Fatty acid binding protein	1.0	15 kDa	10	5	Binds fatty acids.
Lysozyme	1.5	14 kDa	5	10	Degrades NAG-NAM poly-saccharides.
Glutathione oxidase	4.5	30 kDa	10	5	Oxidizes the tripeptide glutathione.
Maltose binding protein	5.0	30 kDa	5	10	Maltose binding