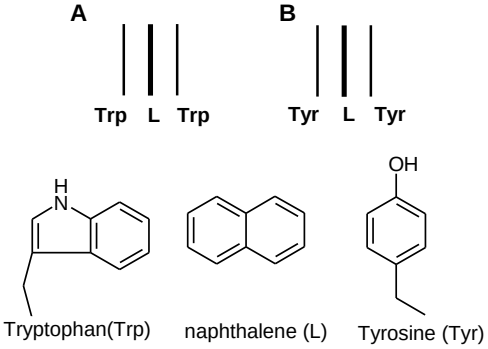
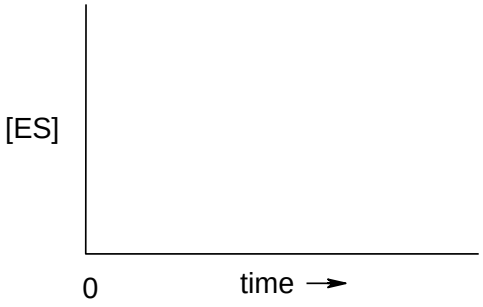
Instructions: This exam consists of 100 points on 6 pages. Please use the space provided to answer the question or the back of the preceding page. In questions with choices, all your answers will be graded and you will receive the best grade. Allot 1 min/2 points.

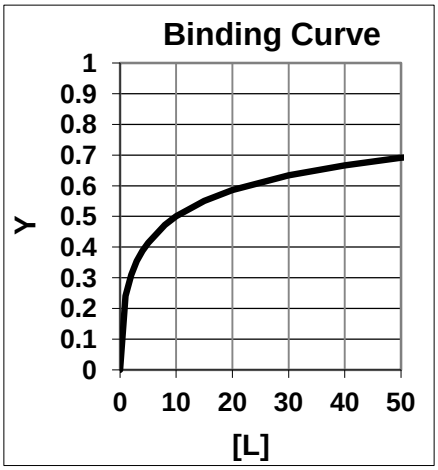


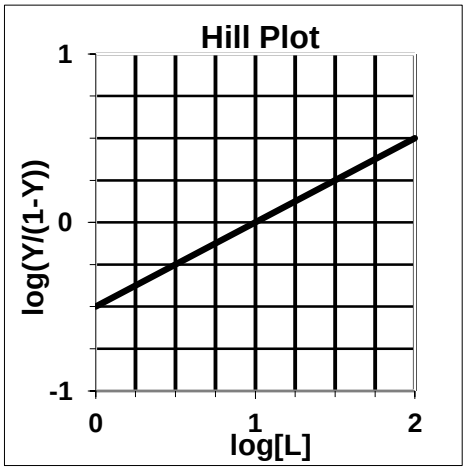
1. **(10 pts)** One protein (protein A) binds naphthalene (ligand) by sandwiching it between two tryptophan residues. Another protein (protein B) also binds naphthalene by sandwiching it between two tyrosine residues, however the binding is weaker. The structure of the protein-ligand complex is shown on the right (side view), the structures of tryptophan, naphthalene (ligand), and tyrosine are shown as well.
   i) What are the principal energetic factor(s) that are responsible for binding of naphthalene to these proteins? (4 pts)
   ii) Why does the tyrosine containing protein show weaker binding? (4 pts)
   ii) Which kinetic rate constant, the off-rate (k_{OFF}) or the on-rate (k_{ON}), would be most different between the two proteins? In what way would it differ? Why? (2 pts)

2. **(6 pts)**
   i) Sketch a graph that indicates the concentration of the enzyme-substrate [ES] complex as a function of time, where t=0 is when the substrate is first mixed with the enzyme (4 pts).
   ii) What time range would be most appropriate for measuring the reaction velocity for data analysis using the equation $v=V_{MAX}[S]/(K_M+[S])$. Justify your answer (2 pts).



3. **(14 pts)** Binding data (fractional saturation (Y) versus [L]) are plotted on the binding curve and the Hill plot shown to on the right. This protein has binding sites for **3** ligands. Please answer the following questions.



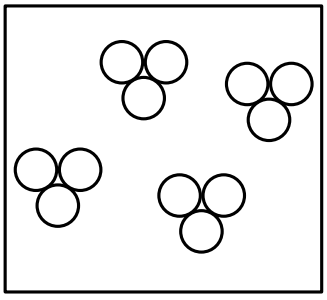


i) What experimental technique is used to obtain values of fractional saturation? (1 pt).

ii) What is the K_D for this ligand? Briefly indicate your approach (4 pts).

iii) Is the binding cooperative or not? Briefly justify your answer (4 pts).

iv) Four protein molecules are shown on the right. The three circles represent the binding sites on each protein. Fill in the circles to indicate the distribution of bound ligands for a fractional saturation of Y=0.33 (e.g. four ligands bound). Briefly justify your answer with reference to your answer to *part iii* (4 pts).



v) Sketch, on the Hill plot, the curve you would expect to find for binding if the protein showed the maximum possible amount of positive cooperativity, but with the same K_D. If your curve would overlap the existing curve, indicate that is the case. Briefly justify your answer (3 pts).

4. **(4 pts)** Briefly describe the molecular basis of oxygen binding to myoglobin and hemoglobin. In what ways are they similar and how do they differ?

5. **(14 pts)**

i) Briefly discuss the major/general feature(s) of allosteric behavior (8 pts),

ii) then discuss **<u>one</u>** of (6 pts):

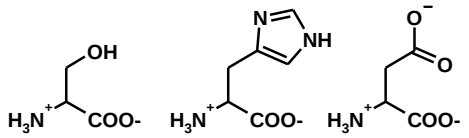
Choice A: how this effect optimizes oxygen delivery to the tissues,

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.

6. **(12 pts)** Select **either** serine proteases **or** HIV protease and:

i) Briefly discuss the role of the catalytic triad (Ser, His, Asp)/the catalytic diad (Asp) in peptide bind cleavage. The structures on the right may be useful. (5 pts)



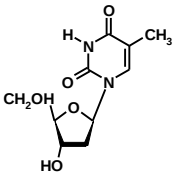
ii) What general feature of these enzymes (or any enzyme) results in an increase in the reaction rate (7 pts)?

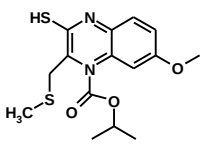
(Please answer part II on the back of the previous page.)

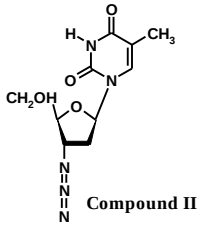
7. **(14 pts)** HIV Reverse Transcriptase – please answer all of the following questions.

i) What characteristics of HIV (human immunodeficiency virus) reverse transcriptase make it a suitable target for anti-HIV drugs? (1 pt)

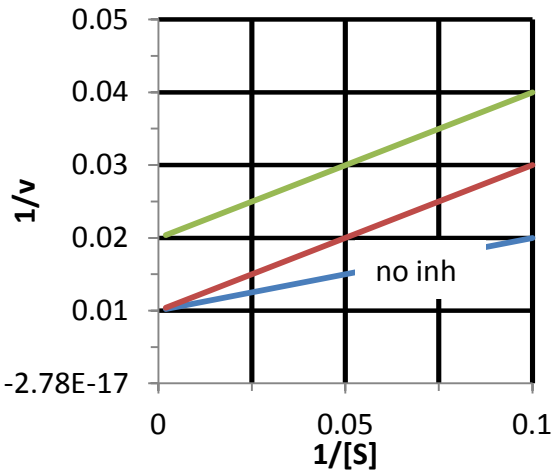
ii) The normal substrate of reverse transcriptase, and two different inhibitors of this enzyme, are shown on the right. Which of these two compounds is most likely to be a competitive inhibitor of the enzyme and which is most likely to be a mixed type inhibitor? Justify your answer, **including a brief description of the key properties of each type of inhibitor** (8 pts).



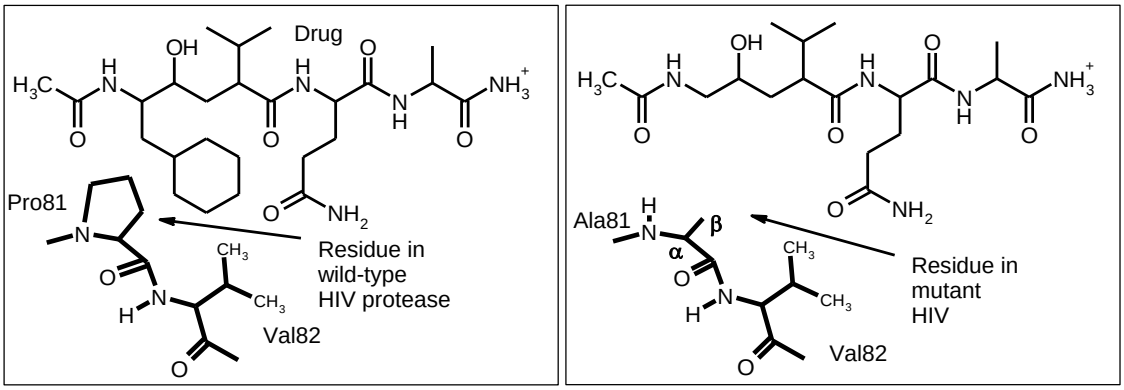




iii) A double reciprocal plots for the activity of reverse transcriptase in the absence and in the presence of both inhibitors are shown on the right. What is/are the dissociation constant(s) (K_I) for **compound II**? The inhibitor concentration is 10 nM. (5 pts)



8. **(11 pts)** The structure of the complex between HIV protease and an HIV protease inhibitor is shown in the left panel. This inhibitor is similar in structure to the substrate.



i) Why does this drug inhibit the enzyme? (1 pt)

ii) A mutant virus has arisen where proline (Pro) at position 81 has been replaced by an alanine residue. Complete the structure of the altered residue in the right panel (the α and β carbons are already shown), and describe/draw how would you redesign the original drug so that it would bind effectively to the mutant protease. Part of the structure of the inhibitor is present to aid your drawing. Justify your answer. (8pts)

iii) How would the mutation of the enzyme affect the K_M for binding of the ***original*** substrate to the protease from the ***mutant*** virus, would it increase it or decrease it? Why? (2 pts)

9. (3 pts) What is specific activity and how is it useful in protein purification?

10. (12 pts) Select **one** of the following **three** choices.

Choice A: For each of the following purification methods discuss why it can be used to separate proteins (i.e. what is the principle of separation.)

i) Ammonium sulfate precipitation.

ii) Anion exchange chromatography (either type).

iii) Affinity Chromatography.

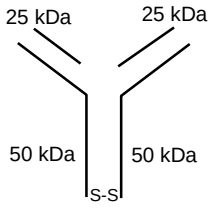
Choice B: You are given a mixture of 3 proteins with the characteristics described below.

Name	MW	#Asp/Glu	#Arg/Lys	Enzyme Activity
Fatty acid binding protein	15 kDa	10	15	Binds fatty acids.
Lysozyme	14 kDa	5	20	Degrades NAG-NAM poly-saccharides.
Glutathione oxidase	16 kDa	10	5	Oxidizes the tripeptide glutathione.

i) Briefly explain why is it not possible to use gel filtration to separate these proteins (2 pts).

ii) Devise a purification scheme to separate glutathione oxidase from the other two proteins. Briefly justify your answer (10 pts).

Choice C: Briefly discuss how you would determine the quaternary structure of an antibody (structure shown on the right) using a combination of gel filtration (size exclusion) chromatography and SDS-PAGE (polyacrylamide gel electrophoresis). Your answer should include sketches of the expected experimental data. You should assume that your standard molecular weights are 10 and 100 kDa.



Bonus. (2 pts) The enzyme lysozyme uses a deprotonated glutamic acid residue to hydrolyze the glycosidic bond. Sketch k_{cat} as a function of pH. Briefly justify your answer.