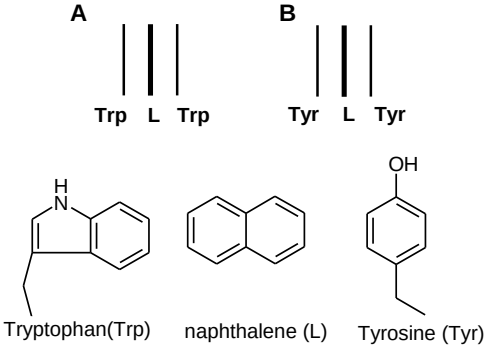
Instructions: This exam consists of 100 points on 6 pages. Please use the space provided to answer the question or the back of the preceding page. In questions with choices, all your answers will be graded and you will receive the best grade. Allot 1 min/2 points.



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)

   i) van der waals (ΔH) and the hydrophobic effect (ΔS) (3 pts for one, 4 points for two).

   ii) van der waals is reduce because the contact between the tyrosine and the ligand will be smaller.

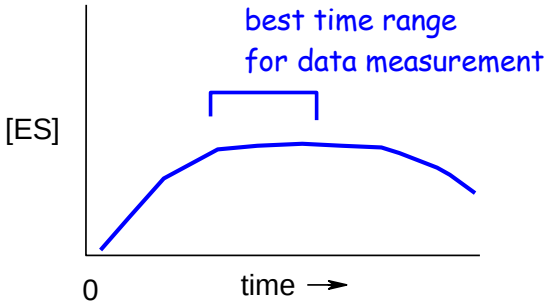
   There will be a smaller hydrophobic effect as well because the non-polar surface area of tyrosine is smaller than tryptophan. Therefore in protein B, a smaller number of ordered water molecules will be released.

   iii) koff will be affected because it depends on the number of interactions/stability of the complex. It will be higher for protein A.

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).

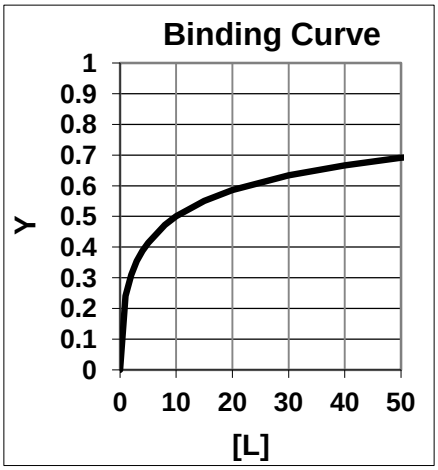
   See plot.

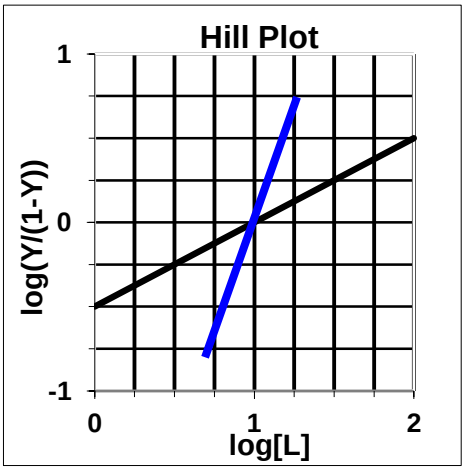


   ii) What time range would be most appropriate for measuring the reaction velocity for data analysis using the equation $v=V_{MAX}[S]/(K_M+[S])$. Justify your answer (2 pts).

   When the concentration of (ES) no longer changes, d[ES]/dt=0 – **steady-state conditions**. The earlier part of the steady-state region is best, because the substrate concentration is closer to the initial amount.

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).

Equilibrium dialysis

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

The K_D is the ligand concentration to $\frac{1}{2}$ saturate the protein, Y=0.5.

The binding curve shows that when [L]=10, Y=0.5, so K_D=10.

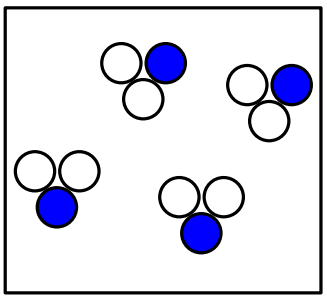
You could also get the K_D from the Hill plot, when the curve crosses the x-axis. $\log K_D$ = 1, $K_D=10^1=10$.

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

The Hill coefficient, which is obtained from the slope of the line when it crosses the x-axis, is 0.5.

Since the Hill coefficient is less than one, the binding is negatively cooperative.

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).



Since the cooperativity is negative, the binding of one ligand should reduce the probability of the binding of additional ligands to the same protein (+3 pts)

The distribution will look something like that on the right, i.e. the single-liganded state will be the most likely. (+1 pt)

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).

Since the protein binds 3 ligands, the highest Hill coefficient can be 3, the number of binding sites. Since the K_D is the same, it must intersect the x-axis at the same point.

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?

In both proteins the oxygen binds to an iron atom (Fe) in the center of a heme group (2 pts)
In the case of hemoglobin, there are four binding sites, and the binding of oxygen to one affects the affinity at the other sites (2 pts)

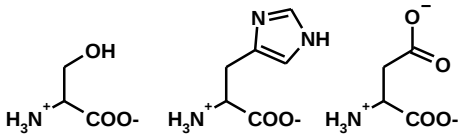
5. **(14 pts)**

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

i)
Protein exists in the Tense (T) or Relaxed (R) state, which differ in conformation.
The T state is the lower affinity/inactive state
The R state is the high affinity/active state
The T and R states are in equilibrium, and the equilibrium is affected by allosteric modulators.
ii)
Choice A: Oxygen acts to increase the affinity of the other oxygen binding sites in the protein.
This helps Hb load oxygen in the lungs, because once a few oxygens bind, additional oxygens will bind more easily and the Hb will become fully saturated.
As this Hb moves to the tissues it begins to lose oxygen. The loss of oxygen converts the other sites, which still have oxygen bound, to the T-state. The T-state loses oxygen more readily, enhancing the release of oxygen in the tissues.
Choice B:
BPG is elevated at high altitudes.
BPG stabilizes the T-state, making it more difficult for oxygen to bind.
It also changes the shape of the binding curve such that an equivalent amount of oxygen is released.
Choice C:
A mixed-type inhibitor could stabilize the Tense state of a enzyme, reducing its activity.
An allosteric activator could stabilize the relaxed state, increasing the activity of the enzyme.

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.)

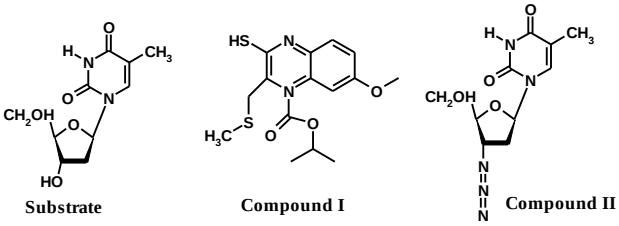
i)
Serine Proteases:
Serine is activated to become a nucleophil by transfer of the –OH to the histidine.
Histidine activates the Serine
Aspartic acid stabilizes the developing positive charge on the histidine.
HIV Protease:
The more acidic Asp deprotonates water, forming the nucleophil the attacks the peptide bond.
The second Asp protonates the newly formed amino terminus.
ii) The energy of the enzyme-transition state complex (EX) is decreased due to pre-ordering of the functional groups required for catalysis (4 pts). This increases the concentration of the transition state (2 pts), which increases the rate of the reaction since the rate is proportional to the transition state. (1 pt)

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)

This step in the viral lifecycle is unique to the virus, inhibiting it by a drug should not interfere with the biochemistry of the patient.

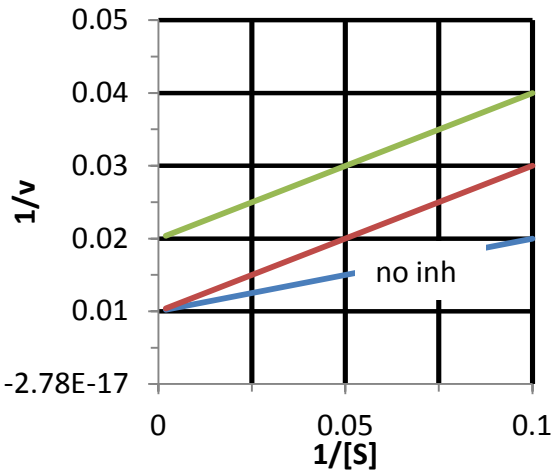
ii) The normal substrate of reverse transcriptase, and two different inhibitors of this enzyme, are shown on the right. Which of these two compounds is most likely to be a competitive inhibitor of the enzyme and which is most likely to be a mixed type inhibitor? Justify your answer, **including a brief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



Compound I is the mixed-type inhibitor because it does not look like the substrate and therefore likely binds elsewhere on the enzyme (3 pts). Because it doesn't bind to the active site it can affect both K_M and k_{CAT} (1 pt).

Compound II is the competitive inhibitor, because it looks like the substrate it most likely binds in the active site (3 pts). Because it binds in the active site it can only affect substrate binding (K_M). V_{MAX} will be unaffected since all of this inhibitor can be displaced by high substrate. (1 pt)

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)



Since compound II is a competitive inhibitor, the middle curve would be the data obtained in its presence.

$K_I=[I]/(a-1)$

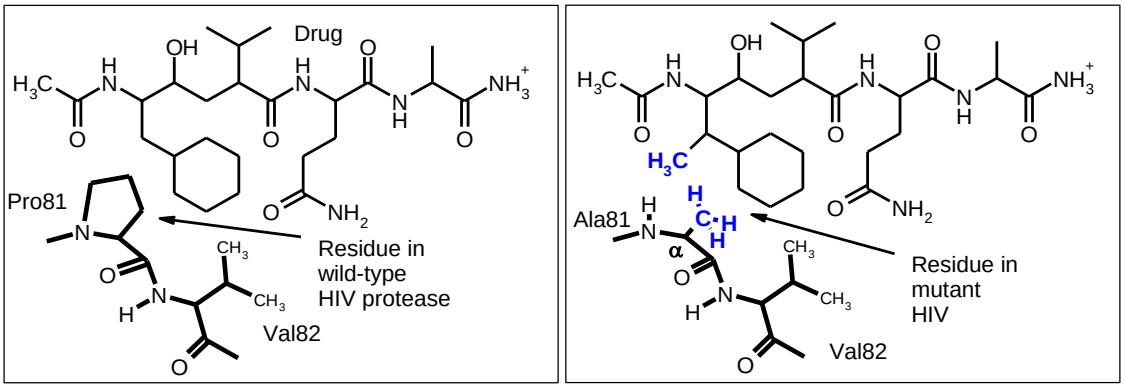
a is the ratio of the slopes. The slope w/o inhibitor is .01/.1 =0.1

The slope with inhibitor is 0.02/.1=0.2

Therefore a=2

$K_I=[I]/(a-1)$ = 10 nm / (2-1) = 10 nM.

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)

HIV protease is a protease – it cleaves peptide bonds. **The drug does not have a peptide bond – competitive inhibitors bind to the active site, but cannot undergo a chemical reaction.**

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)

The right hand structure is complete as is, alanine only has a methyl group as a side chain. I've just added the three hydrogens to the β-carbon in the above diagram. (1 pt)

Since the alanine sidechain is smaller, it would be necessary to make the cyclohexane ring on the original drug larger so that it could contact the alanine sidechain. I just added an additional methyl. (6 pts for reasonable argument on complementarity, 1 pt for reasonable modification to drug, i.e. if the student didn't get alanine correct, but gave a convincing argument for their protease-drug combination, that is fine).

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)

The original substrate, which would have a phenylalanine residue, would form weaker contacts with the mutant enzyme, increasing K_M.

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

Specific activity is the ratio of the activity of the target protein divided by the total amount of protein (1 ½ pts)

During a purification it should increase after each step, since the total amount of protein decreases while the amount of the target protein remains essentially the same. (1 ½ pts)

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.

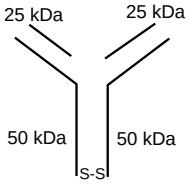
Choice B: You are given a mixture of 3 proteins with the characteristics described below.

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.



Choice A:

i) Ammonium sulfate precipitation – proteins differ in their solubility in ammonium sulfate. At a certain ammonium sulfate concentration some proteins will ppte and some will largely stay in solution. The target protein can be separated by keeping the solid or liquid fraction, depending on its solubility.

ii) Anion exchange chromatography (either type). The resin beads can be either positively (anion exchange) or negatively charged (cation exchange). The target protein will stick if its charge is opposite to that of the beads. Since different proteins will have different charges, they will stick to the column with different affinities and elute out at different volumes.

iii) Affinity Chromatography. The beads have a ligand attached that the target protein will bind to and be retained on the column. The contaminating proteins can be washed away, and the target protein eluted with free ligand.

Choice B:

i) The three proteins are too similar in size to be separated by gel filtration.

ii) Purification.

a) Make an affinity column with glutathione attached to the beads, apply the mixture to the column, the glutathione oxidase will stick, and the contaminating proteins can be washed off. The oxidase can be eluted by applying free glutathione to the column.

b) Separate by ion exchange. At pH=7.0, the charges on the proteins will be:

Name	#Asp/Glu	#Arg/Lys	Charge
Fatty acid binding protein	10	15	-10 + 15 = +5
Lysozyme	5	20	-5 + 20 = +15
Glutathione oxidase	10	5	-10 +5 = -5

If the mixture of proteins is run over an anion exchange column, the glutathione oxidase will bind and the contaminating proteins can be washed off. The oxidase can be eluted lowering the pH, which will reduce the charge on the protein.

Choice C:

Gel filtration will give native molecular weight of 150 kDa. Elution profile will have three peaks, order of which is 150 kDa, 100 kDa, 10 kDa.

SDS-PAGE without BME will show two bands, one at 100 kDa, the sum of the two heavy chain, since they are connected by a disulfide bond, and one at 25 kDa, corresponding to the light chain.

SDS-PAGE with BME will show two bands, one at 50 kDa, the heavy chain, and one at 25 kDa.

The standards should be drawn to the appropriate location.

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.

The pKa of glutamic is 4.0. The kcat will be 50% of its value at the pH. The kcat will increase as the pH is raised, since more of the glutamic acid will be deprotonated, giving the curve shown on the right.