

Part A: Multiple Choice (18 pts, 2 pts each)

1. Once a ligand dissociation constant (K_D) has been determined it is possible to calculate
 - a) the ligand binding constant (K_a). **(1 pt)**
 - b) the ΔG° for the binding interaction. **(1 pt)**
 - c) the concentration of ligand required for half-maximal occupancy. **(1 pt)**
 - d) All of the above are correct (2 pts)**
2. In both hemoglobin and myoglobin the oxygen is bound to.
 - a) the nitrogen atoms on the heme.
 - b) polar pocket in the protein.
 - c) histidine residues in the protein.
 - d) the iron atom in the heme group.**
3. A protein that binds two ligands in a non-cooperative manner will:
 - a) show a hyperbolic binding curve**
 - b) show a curved Scatchard Plot
 - c) show a curved Hill Plot.
 - d) show a sigmoidal binding curve
4. The active site of an enzyme differs from an antibody-antigen binding site in that the enzyme active site
 - a) contains modified amino acids.
 - b) catalyzes a chemical reaction.**
 - c) is complementary to a specific ligand.
 - d) contains amino acids without sidechains.
5. The steady-state assumption implies
 - a) ligands bind to their sites in a continuous fashion.
 - b) the concentration of the enzyme-substrate complex [ES] does not change during the reaction (1 1/2 pts)**
 - c) the concentration of substrate remains constant during the reaction.(+1 pt)
 - d) Both b and c are correct. (2 pts)**
6. A feature in common among *all* serine proteases is:
 - a) a hydrophobic specificity pocket.
 - b) a pair of reactive aspartic acid residues.
 - c) a cluster of reactive serine residues.
 - d) a single reactive serine residue.**
7. The major problem in the use of drugs to treat HIV infections is:
 - a) There are no problems, HIV is completely controlled by existing drugs.
 - b) Drugs that are good inhibitors cannot be synthesized.
 - c) The drugs are rapidly degraded.
 - d) Virus particles with altered (mutant) proteases arise.**
8. During any purification scheme, you would expect
 - a) the number of different proteins in the sample to decrease.
 - b) that the specific activity increases (1 1/2 pts)**
 - c) that the amount of target protein decreases.
 - d) all of the above are correct.**
9. An enzyme that produces ethanol from acetaldehyde is being purified from a complex mixture of proteins. What would be the best way to determine the location of this protein *during* the purification scheme?
 - a) UV absorption of fractions.
 - b) Measure the rate of ethanol synthesis of fractions (2 pts)**
 - c) SDS gel electrophoresis of fractions (1 1/2 pts)**
 - d) Mass spectroscopy of fractions.

A: _____/18

B1: _____/12

B2: _____/14

B3: _____/10

B4: _____/14

C: _____/16

D: _____/16

Tot: _____/100

Part B: Short Answer.

B1. (12 pts) Please do **one** of the following two choices:

Choice A: Briefly explain the concept of transition state stabilization in enzyme catalysis. Your answer should include a discussion of enthalpic versus entropic effects. Provide an example for one of these effects.

The transition state is a high energy intermediate in the reaction. By reducing the energy of the transition state, enzyme will increase the concentration of the transition state, thus increasing the rate of the reaction (6 pts).

The reduction in energy of the transition state can be due to two factors: enthalpy and entropy.

enthalpy: The enzyme forms some kind of interaction in the transition state that is an enthalpic one, such as the formation of hydrogen bonds, electrostatic interactions, van der Waals contacts. In the case of the serine proteases, the oxy-anion formed during the transition state is stabilized by two main-chain NH groups on the enzyme (3 pts).

entropic: The catalytic mechanism of all enzymes involves one or more amino acids. In the uncatalyzed reaction, these amino acids would be free in solution and would have to be assembled into a catalytically competent configuration, thus reducing the entropy of the system, which is unfavorable. In the case of the enzyme catalyzed reaction, there is no decrease in entropy because the amino acids are already pre-assembled, thus reducing the relative energy of the transition state. This occurs in all enzymes. (3 pts).

Choice B: Select **either** serine proteases **or** HIV protease and briefly discuss the role of important residues in the cleavage of the peptide bond. If you select serine proteases, you need not give the entire reaction mechanism to complete this question.

Serine Protease:

In the catalytic triad:

- Ser - the primary nucleophile that attacks the C=O (+4 pts)
- His - activates the Ser by deprotonating it (4 pts)
- Asp - Stabilizes the + charge on the His, thus indirectly aiding in the activation of the Ser. (+4 pts)

HIV Protease.

- Two Asp residues in the active site (+4 pts), one that is deprotonated (+1) one that is protonated (+1)
- One Asp activates water as the nucleophile (+5) pts
- Other Asp donates proton to the product (+1 pts)

B2. (14 pts) Using hemoglobin, or any other suitable protein as an example, provide a *brief* summary of the role of allosteric effects in the control of biological systems. Your answer should include a discussion of relaxed and tense states, and the importance of these terms. Illustrate your answer by selecting **one** of the following three molecules: O_2 , H^+ , or bisphosphoglycerate, and discuss its role in the regulation of oxygen transport in hemoglobin.

An allosteric molecule exists in two conformations (2 pts)

Tense state - low activity, the "off" state (2 pts).

Relaxed state - high activity the "on" state (2 pts).

An allosteric activator will convert the molecule from the tense to the relaxed state when binding, turning the system on (2 pts).

An allosteric inhibitor will convert the molecule from the relaxed to the tense state when binding, turning the system off (2 pts).

In the case of hemoglobin:

Oxygen: Allosteric homotropic activator. Binding of the first O_2 increases the affinity for subsequent oxygen binding, converting the T state to the R state. (2 pts) Enhancing the release of oxygen in the tissues (2 pts).

Protons & BPG: Allosteric heterotropic inhibitors. Both stabilize the T-state, reducing the oxygen affinity (2 pts). These both shift the O_2 binding curve to the right, in both cases the end result is to increase O_2 delivery. BPG is involved in altitude adjustment of oxygen delivery. H^+ are produced in active muscle, enhancing oxygen delivery to that tissue. (2 pts)

B3. (10 pts) Do **ONE** of the following two choices (The second choice is on the following page.).

Choice A: You wish to separate the following two proteins from each other, which type of column chromatography would you use? (2 pts) How would you elute the proteins off of the column? (2 pts) Briefly explain why the separation would occur (6 pts).

Protein A: isoelectric $pH = 7.0$.

Protein B: isoelectric $pH = 5.0$.

You would use an ion exchange column (3 pts) and exploit a different charge on each protein to cause separation (3 pts). Elution of the protein off of the column would be accomplished by either adding salt, or changing the pH . (2 pts)

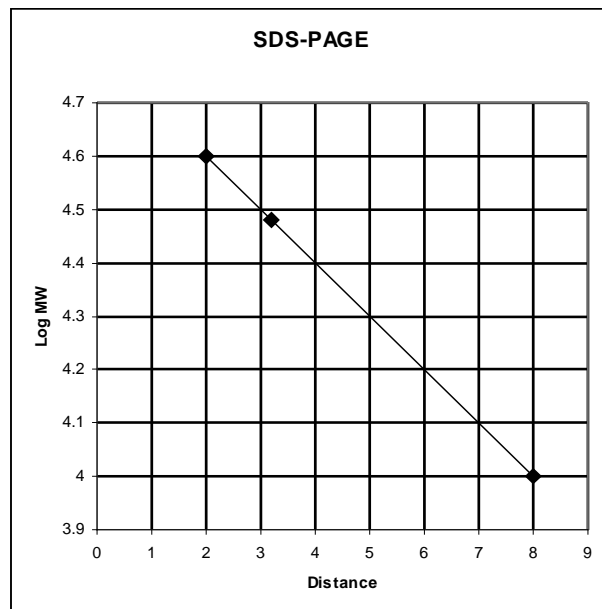
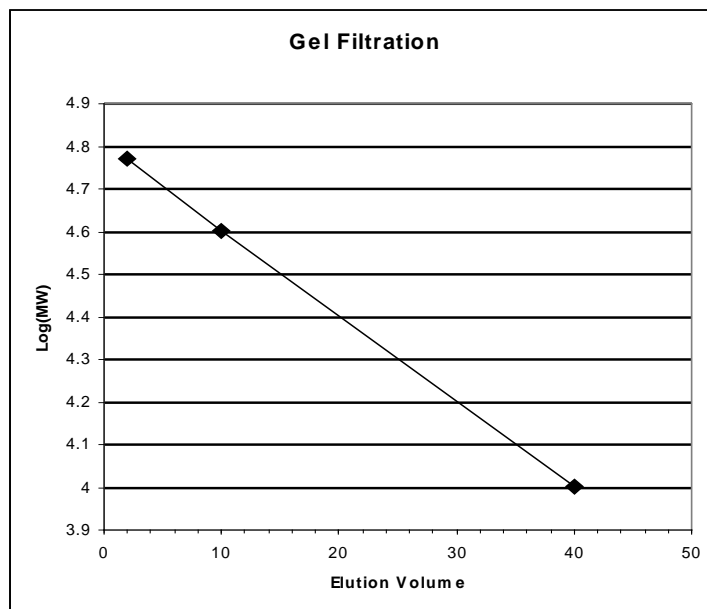
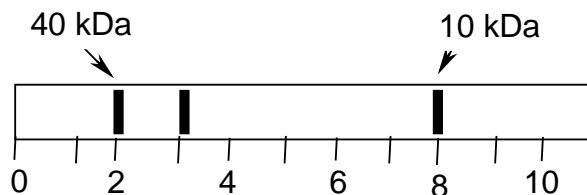
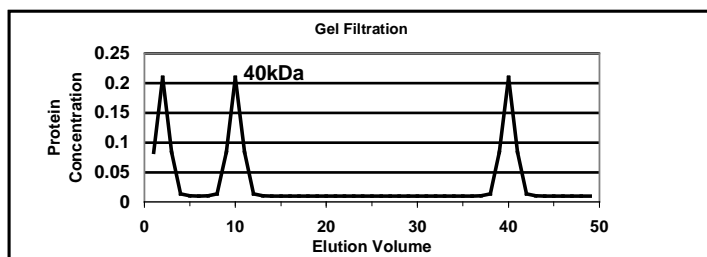
Some specific examples (2 pts):

@ $pH=7.0$, protein A would be uncharged and not stick to the column and protein B would have a neg charge and stick to an anion exchange column.

@ $pH=5.0$ protein B would be uncharged and protein A would be positively charged and would stick to a cation exchange column.

Choice B: You are trying to determine the quaternary structure of a protein. You mix the unknown protein with two molecular weight standards. The size of the standards are 10 and 40 kDa. The elution profile from the gel filtration column is shown to the left. An image of the SDS gel is also shown to the right.

i) Determine the quaternary structure of the protein using these data. Please justify your answer. A table of logs is provided on the face page.



The two standards are used to generate the calibration curve for both the gel filtration and SDS-PAGE, as indicated above. The calibration curves are used to determine the molecular weight of the native protein and the SDS-PAGE is used to determine the molecule weight of the subunits (+2 pts for calibration curves)

Gel Filtration: The elution volume for the unknown is approximately 2 mls, extrapolation of the calibration curve gives $\log(MW)=4.77$, or a molecular weight of 60,000 Da (+2 pts)

SDS-PAGE: the distance migrated, of 3.2 cm, gives $\log(MW)=4.48$, or a subunit molecular weight of 30,000 Da (+2 pts)

This is a **homodimer**, because both chains are identical and the native molecular weight is twice that of the subunits. (+2 pts)

ii) Do the above experiments give you any information regarding disulfide bonds in this protein? Why? No, because it was not stated whether β -mercaptoethanol (BME) was added or not (+2 pts). If we assume BME was absent then there are no disulfide bonds between the two subunits, otherwise the molecular weight from the SDS-PAGE experiment would equal that from the gel-filtration.

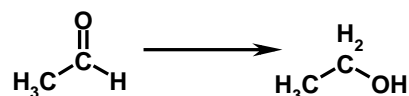
B4. (14 pts) Enzyme Inhibitors

i) Compare and contrast a competitive inhibitor to a non-competitive inhibitor. Your answer should include:

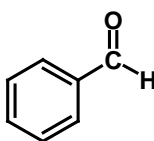
- A discussion of the location of the inhibitor binding site. (4 pts)
- How and why the inhibitor affects the enzyme kinetics (i.e. changes K_M or k_{CAT} , or both.). (4 pts)
- How to distinguish each type of inhibitor using a double reciprocal plot. (2 pts)

Competitive	Non-competitive
<ul style="list-style-type: none">• Binds to active site (+2 pts)	<ul style="list-style-type: none">• Binds elsewhere (+2 pts)
<ul style="list-style-type: none">• Does not change V_{MAX} (k_{cat}) because all of the inhibitor is displaced from the active site at high $[S]$. (+2 pts)• Changes <i>observed</i> K_M because it requires more $[S]$ to half saturate the enzyme.	<ul style="list-style-type: none">• The inhibitor leads to a <i>conformational</i> change in the active site, causing a change in V_{MAX}. (+2 pts)• The K_M may also change.
<ul style="list-style-type: none">• The y-intercept is unchanged, but the slope will increase (1 pt).	<ul style="list-style-type: none">• The y-intercept will increase and the slope <i>may</i> change (1 pt).

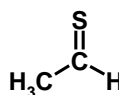
ii) An enzyme catalyzes the following reaction:



Which of the following compounds, A or B, would likely be a competitive inhibitor and which would be a non-competitive inhibitor of the enzyme? Briefly justify your answer (4 pts)



A



B

A is a non-competitive inhibitor. If it could bind to the active site, then it is likely that its aldehyde group could be converted to an alcohol. The phenyl group probably makes it too large to fit into the active site, so it binds elsewhere (2 pts).

B is the competitive inhibitor. It is almost exactly like the substrate, with the oxygen being replaced by the sulfur. Therefore there is nothing that restricts it from binding to the active site. Due to the differences in the electronic structure of the sulfur versus oxygen, the reaction cannot proceed (2 pts).

Part C: Oxygen Transport

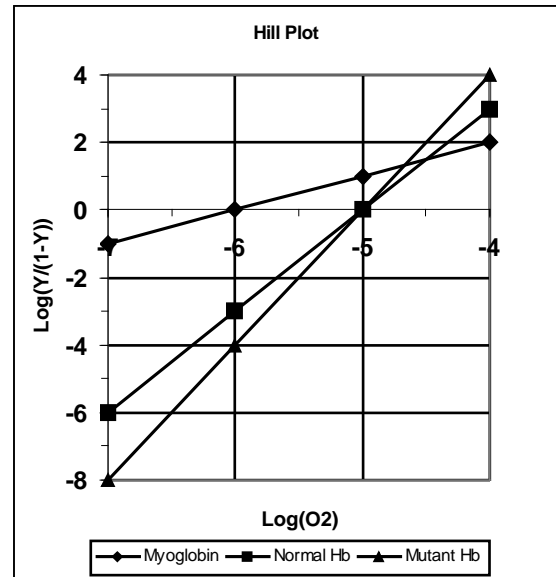
There are a large number of genetic alterations in the human hemoglobin gene that result in a change in the oxygen binding properties. The Hill plot for myoglobin, normal hemoglobin, and a mutant hemoglobin are shown in the diagram to the right.

i) Determine the Hill coefficient and K_D for the mutant hemoglobin. Please describe your approach (4 pts).

The Hill coefficient, n_h , is the slope of the line when it crosses the x-axis. In this case:

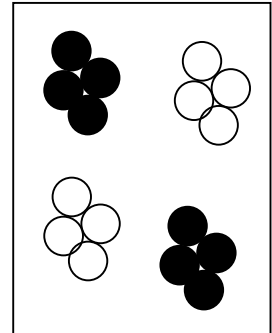
$$n_h = \Delta y / \Delta x = 4 / 1 = 4 \text{ (+2 pts)}$$

The K_D is the ligand concentration at $Y=1/2$, or the ligand concentration where the line crosses the x-axis: $\log(K_D) = -5$, therefore $K_D = 10^{-5} M$ (+2 pts)

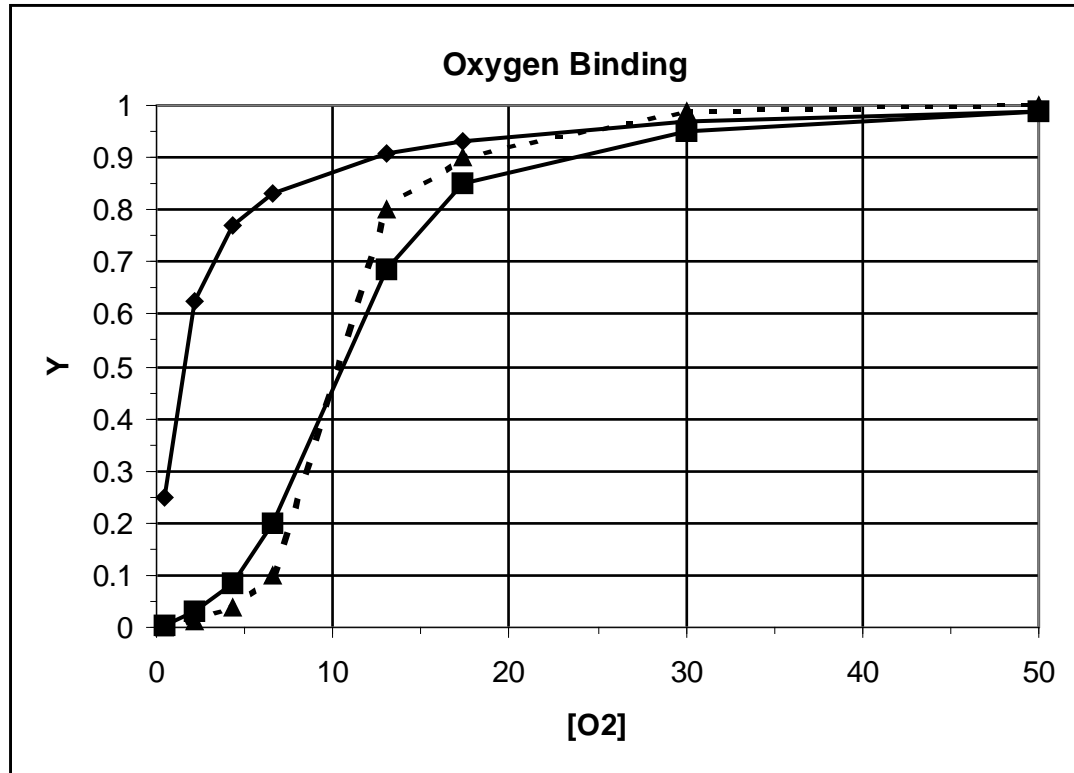


ii)

The Hill coefficient, n_h , is equal to the number of binding sites. Therefore the system shows infinitely positive cooperativity. Therefore the only species in solution are M and ML_4 , i.e. the concentration of all partially liganded species, such as ML_3 , are zero. (+3 pts). The diagram would show two tetramers that are fully loaded with O_2 and two that are empty (+1 pt).



- iii) On the basis of this Hill plot, sketch the oxygen binding curves for the mutant hemoglobin. The oxygen binding curves for myoglobin and hemoglobin are shown on the graph below. Draw the curves as accurately as possible, reflecting the K_D values as well as the degree of cooperativity.



The binding curve of the mutant hemoglobin is shown as the dotted line.

Since the K_D is the same as normal hemoglobin, both curves intersect at $Y=0.5$ when $[O_2]=10 \mu M$. (+2 pts)

The mutant hemoglobin is more cooperative than normal hemoglobin, so the binding curve rises more steeply (+2 pts).

- vi) Using your sketch from part iii, briefly discuss whether this mutant hemoglobin would be more or less efficient at delivering oxygen to the tissues. You can assume that the concentration of oxygen in the lungs is $50 \mu M$ and that in the tissues is $10 \mu M$ (4 pts).

The amount of oxygen delivered to the tissues is the difference in the fractional saturation in the lungs minus that in the tissues (+2 pts)

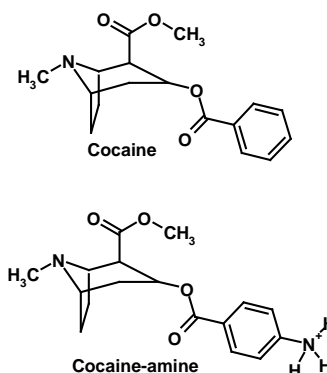
- For normal hemoglobin: $\Delta Y = 0.98 - 0.50 = 0.48$.
- For the mutant hemoglobin: $\Delta Y = 1.00 - 0.50 = 0.50$ (+1 pt)

The mutant hemoglobin is slightly more efficient (+1 pt)

Part D:

Choice A:

An antibody that is used clinically to treat cocaine (left structure) overdose **binds cocaine with a K_D of $1\ \mu\text{M}$** . The binding of a modified cocaine (cocaine-amine, right structure) to this antibody were tested using equilibrium dialysis. To do this experiment Fab fragment at a **concentration of $1\ \mu\text{M}$** was placed in the dialysis bag and different amounts of cocaine-amine were placed in the solution outside of the dialysis bag. After equilibrium was reached, the concentration of cocaine-amine inside and outside the dialysis bag was measured, giving the following values:



Experiment Number	[Cocaine-amine] Outside	[Cocaine-amine] Inside
1	$1.0\ \mu\text{M}$	$1.10\ \mu\text{M}$
2	$10\ \mu\text{M}$	$10.5\ \mu\text{M}$

i) Calculate the fractional saturation for each of the data points (2 pts).

The fractional saturation is: $Y = [ML] / ([M] + [ML])$.

$[ML]$ is the amount of excess ligand inside the dialysis bag. $[M] + [ML] = [M_T]$, which is $1\ \mu\text{M}$. Therefore:

Experiment 1: $[ML] = 1.1 - 1.0 = 0.1\ \mu\text{M}$
 $Y = 0.1 / 1.0 = 0.1$ (unitless)

Experiment 2: $[ML] = 10.5 - 10.0 = 0.5\ \mu\text{M}$
 $Y = 0.5 / 1.0 = 0.5$

ii) Determine the K_D for the binding of cocaine-amine to the antibody.

The K_D is equal to the ligand concentration that gives $Y = 0.5$. In this case $K_D = 10\ \mu\text{M}$ (+3 pts)

The K_D could also have been determined by (1/2 pt for any two)

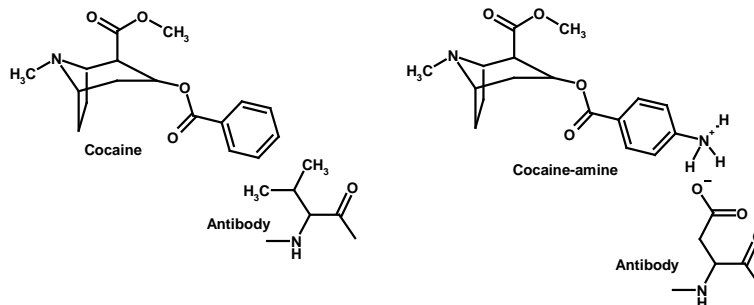
- Plotting a saturation curve and estimating K_D from the curve by interpolation.
- Plotting a Scatchard plot, the slope = $-1/K_D$
- Plotting a Hill plot, $\log(K_D)$ is the x-intercept.

iii) Based on your answer to part ii, does cocaine-amine bind more tightly, or weaker, to the antibody?

Since the K_D has increased, from 1 to $10\ \mu\text{M}$, the binding is weaker (2 pts).

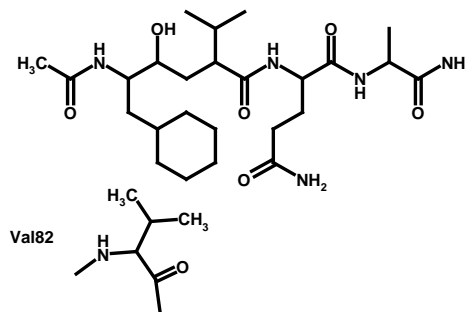
iv) How might you modify the structure of the antibody to enhance the binding to cocaine-amine and reduce binding to cocaine?

The only difference between the two compounds is the positively charged amine group. In cocaine, this group is missing and the antibody probably interacts with the benzene ring via interactions with a non-polar group on the antibody. Replacement of this non-polar group with a negatively charged residue, i.e. Asp or Glu, would enhance binding to cocaine-amine and likely reduce binding to cocaine.



Choice B:

The drawing to the right shows an HIV protease inhibitor. Val82 is a residue from the enzyme (HIV protease). The K_I for this inhibitor is 1 nM. A mutant HIV protease has arisen in an infected individual and inhibitor A and inhibitor B were tested to see which one would be the better drug to treat this patient. Initial velocity data was acquired as a function of substrate in the absence of inhibitor, or in the presence of 5 nM of inhibitor A, or in the presence of 5 nM of inhibitor B. These data were plotted on the double reciprocal plot shown to the right. The equation for each line are given on the chart. Please answer the following questions.



i) Obtain the K_I for each drug. Please show your work.

$$K_I = [I]/(\alpha - 1)$$

α is obtained from the ratio of the slopes.

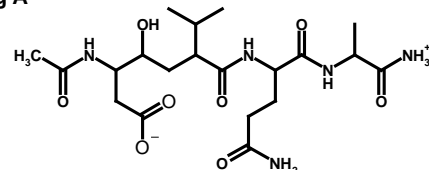
$$\text{Drug A: } \alpha = 0.2/0.1 = 2.0$$

$$K_I = 5 \text{ nM}/(2-1) = 5 \text{ nM} \text{ (+2 pts)}$$

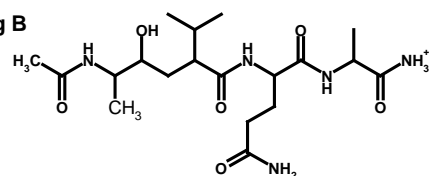
$$\text{Drug B: } \alpha = 0.6/0.1 = 6$$

$$K_I = 5 \text{ nM}/(6-1) = 1 \text{ nM} \text{ (+2 pts)}$$

Drug A



Drug B



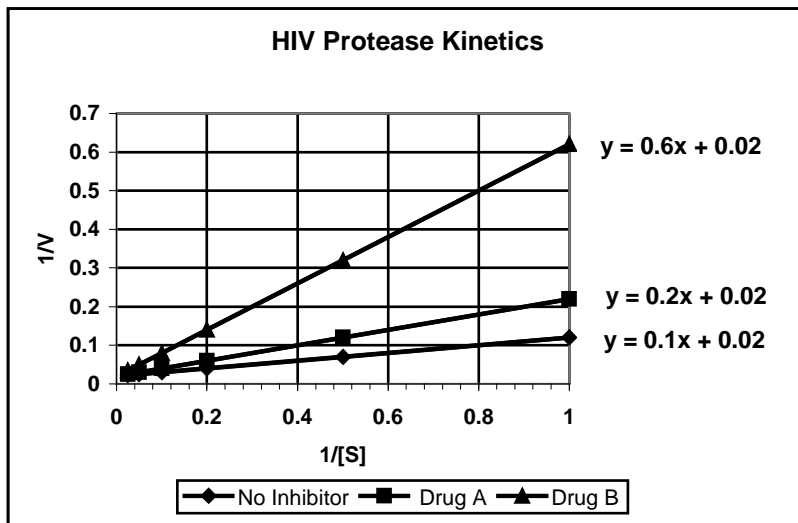
ii) Why are the K_I values meaningless for these drugs.

Both are competitive inhibitors since their structures are similar to the substrate. Competitive inhibitors cannot bind to the ES complex, and therefore K_I which describes that binding, is not defined (+2 pts)

iii) Which inhibitor binds to HIV protease more tightly? Justify your answer using your K_I values.

Drug B, because of its lower K_I value (+2 pts)

iv) On the basis of your K_I data, and the structures of the two drugs, provide a plausible suggestion for the amino acid side chain that has replaced Val82 in the enzyme.



Since drug B binds as more tightly than A, it is likely that the enzyme has maintained a non-polar residue at position 82. (+4 pts) However, the Ala sidechain on Drug B is much smaller than the cyclohexane group on the original drug, therefore Val 82 was replaced with a larger non-polar sidechain, such that the sidechain could reach the drug. (+4 pts) Two possible examples are shown below.

