1. (14 pts) Allosteric effects:

i) *Briefly* discuss the general framework of allosteric effects in protein behavior. In your answer you should give concise definitions/descriptions of tense and relaxed states. In addition you should compare and contrast heterotropic and homotropic inhibitors and activators and describe how they affect allosteric behavior (12 pts).

An allosteric protein exists in two conformations which are in equilibrium with each other (2 pts)

The tense (T) conformation is less active (2 pts)

The relaxed (R) conformation is more active (2 pts)

A homotropic activator/inhibitor affects its own binding (2 pts)

A heterotropic activator/inhibitor affects the binding of another ligand (2 pts)

An activator will increase the concentration of the R state (1 pt)

An inhibitor will increase the concentration of the T state (1 pt)

ii) (2 pts) Give one example of either a homotropic or heterotropic allosteric activator/inhibitor and *briefly* describe its role in a biological process.

Homotropic : Oxygen, by increasing the oxygen affinity as more oxygen binds, Hb can become fully saturated in the lungs and release more oxygen in the tissues.

Heterotropic: Bisphosphoglycerate. This reduces oxygen affinity [by stabilizing the T state], but in doing so it increases the cooperativity, allowing a larger amount of oxygen to be released at high altitudes.

2. (3 pts) Please do **one** of the following choices, please indicate your choice.

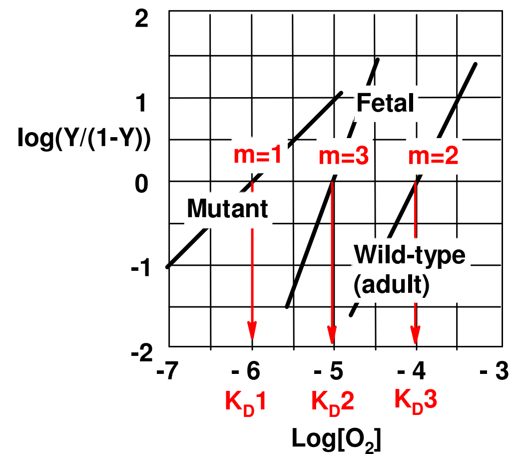
Choice A: What is the role of histidine residues in hemoglobin in oxygen transport?

Choice B: What is the general structure of the heme group and what is its involvement in oxygen binding?

Choice A: The proximal histidine residue contacts the iron atom and senses oxygen binding. This causes a conformation change in the protein, leading to positive cooperativity.

Choice B: Ring like structure with Fe^{2+} in the center. Fe^{2+} binds oxygen.

3. (20 pts) A rare species of mammals has a trimeric hemoglobin (Hb), formed from three identical subunits. The binding of oxygen to a fetal Hb, adult wild-type Hb, and a mutant Hb was measured and the binding data is plotted on the Hill plot shown on the right. These data have also been plotted as binding curves, shown below the Hill plot.



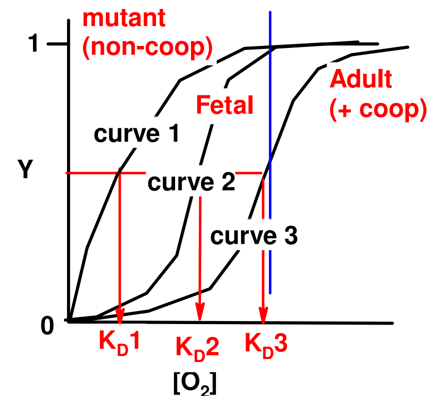
i) (7 pts) List the Hill coefficient and K_D for each of the three proteins in the following table:

Protein	Hill Coefficient (n_h)	K_D (M)
Mutant	1	10^{-6}
Fetal Hb	~3	10^{-5}
Wild-type Adult	2	10^{-4}

Briefly state how you obtained the above entries in the table (4 pts)

Hill coefficients from slope of line (as it crosses the x-axis.)

K_D from x-intercept, which is log K_D.



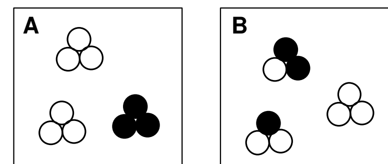
ii) (3 pts) Which of the binding curves shown in the lower diagram correspond to which protein? Briefly justify your answer.

Curve 1: Mutant, since it is non-cooperative, the binding curve should be hyperbolic in shape.

Curve 2: Fetal, positively cooperative with higher affinity than adult, therefore reaches ½ saturation at lower oxygen concentration.

Curve 3; Adult, positively cooperative, with lower affinity than fetal, so more oxygen is required to reach ½ saturation.

iii) (4 pts) Which of the diagrams on the right is an accurate representation of the distribution of bound oxygen for the adult hemoglobin? A or B? Which is an accurate representation for the fetal hemoglobin? *Briefly justify your answer.* (The solid circles are subunits with O_2 bound, the empty circles represent no O_2 bound.)



A represents fetal hemoglobin: Its Hill coefficient is equal to the number of binding sites so it is infinitely positive in cooperativity, no intermediates are present.

B represents the adult hemoglobin. Although it is positive in cooperativity, it is not infinite, so some intermediates (e.g. two oxygens bound) will be present.

Question 3 continues…

iv) (6 pts) Please answer only ***one*** of the following two choices.

Choice A: Explain how the binding properties of the adult and fetal Hb enhance oxygen delivery to the fetus.

Choice B: An animal that inherits one copy of the mutant Hb and one copy of the normal wild-type Hb survives. An animal that inherits both copies of the mutant Hb dies before birth. Why?

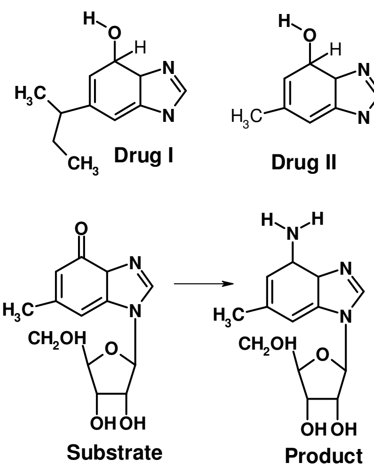
Choice A: The fetus has to obtain oxygen from the placenta and then transfer this oxygen to its tissues. It must become fully saturated under conditions where the maternal hemoglobin has released all of the oxygen it can at the placenta. This oxygen concentration is indicated by the vertical bar in the above binding curve.

Choice B: The mutant hemoglobin is non-cooperative, although it will become fully saturated at the placenta, it will not release enough oxygen to the fetal tissues.

4. (22 pts) The structure of two drugs that inhibit an enzyme are shown on the right. The normal reaction that this enzyme catalyses is shown below the structure of the drugs.

i) (2 pts) Are these drugs competitive or mixed-type inhibitors? Why?

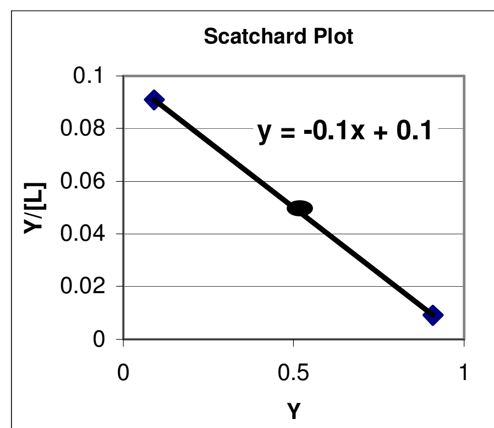
They seem to be competitive since they are similar to the substrate.



ii) (4 pts) Drug II binds with a K_D that is 10 fold lower than drug I. Briefly explain this observation with reference to the structure of the substrate and the two drugs.

The lower K_D indicates tighter binding of drug II (1 pt).

One possibility is that the additional iso-butyl group on drug 1 interferes with binding. The substrate just has a methyl group and is more similar to drug II. The larger butyl group would not fit into the binding site designed for the methyl. (3 pts)



iii) (6 pts) To obtain the binding constant of **drug I** to the enzyme you perform equilibrium dialysis at a number of different concentrations of the drug and a constant amount of enzyme (1 μM), giving the following data:

Free Drug Concentration	Drug Concentration inside Dialysis bag.
1 nM (0.001 uM)	0.091 uM
10 nM (0.01 uM)	0.510 uM
100 nM (0.1 uM)	1.00 uM

The first and last data points are plotted on the Scatchard plot shown to the right, the units of L are nM on this plot.

Place the point for the middle experiment ([L]= 10 nM) on the Scatchard plot (Hint: You need to calculate the fractional saturation.) (2 pts), and then determine the dissociation constant, K_D, for drug I (4 pts).

The amount of [ML] is the difference between the drug inside the bag and the free drug concentration:

[ML] = 0.51 uM – 0.01 uM = 0.5 uM.

The fractional saturation is: Y = [ML]/{[M] + [ML]} = 0.5 uM / 1 uM = 0.5.

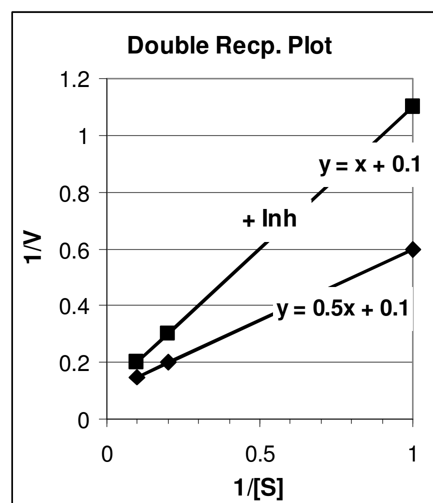
This point is the oval on the above graph.

There are two ways of getting the K_D:

From the binding data. Since Y=0.5 at 10 nM, the K_D is 10 nM.

From the Scatchard plot: The slope is $-1/K_D = -0.1$, therefore K_D = 10 nM.

iv) (10 pts) The steady-state enzyme kinetics are measured in the absence and in the presence of 10 nM of **drug I** and the data is plotted on the double reciprocal plot shown on the right.



a) (6 pts) Determine K_I, and if appropriate K_I'. Please explain why it may, or may not be, appropriate to obtain K_I'.

It is only necessary to obtain K_I since this a competitive inhibitor [K_I' refers to the dissociation of the inhibitor from the ES compled, which can only occur with a mixed inhibitor.]

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

α is the ratio of the slopes $\alpha = 1/0.5 = 2$

Therefore K_I = 10 nM

b) (2 pts) Why are the K_D (*part iii*) and the K_I for drug I essentially the same?

Both measure exactly the same thing, the dissociation of the inhibitor from the free enzyme.

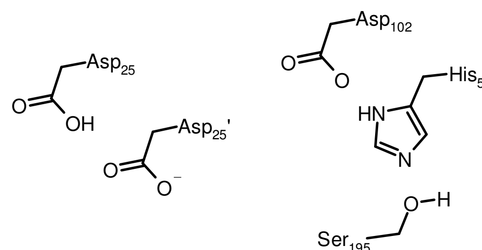
c) (2 pts) Sketch on the double reciprocal plot the line you would have obtained had you used **drug II** in your reaction. Explain your approach and show any calculations on the back of the *previous* page.

$\alpha = 1 + (10\ nM/1\ nM) = 11$. The line would have a slope of 5.5, same y-intercept since drug II is also a competitive inhibitor.

5. (12 pts) Please do one of the following two choices. Please indicate your choice.

Choice A: It is often said that enzymes increase the rate of the reaction by lowering the energy of the transition state. In the first part of your answer briefly describe why lowering the energy of the transition state increases the catalytic rate. In the second part of your answer describe in general terms how enzymes lower the transition state. Give one specific example.

Choice B: Peptide bond hydrolysis is catalysed by serine proteases (e.g. trypin) or aspartate proteases (e.g. HIV protease). Briefly describe the catalytic mechanism for **either** type of protease, including a brief description of the role of the catalytic residues in the reaction. If the reaction you have chosen proceeds in two phases, it is only necessary to describe the first phase. Please indicate your choice. The diagrams on the right may be useful.



Choice A:

The rate is proportional to the concentration of the transition state (3 pts)

The amount of the transition state increases if the energy difference between the substrate and the transition state is lower (3 pts)

Enzymes lower the transition state in two ways:

-preorganization of the catalytic residues due to the folding of the enzyme. Therefore there is no entropy decrease to bring the catalytic residues close to the substrate. This is true for all enzymes. (3 pts)

-direct interactions with the only the transition state. e.g. the oxy-anion hole that forms H-bonds to the tetrahydral intermediate in serine proteases. (3 pts)

Choice B:

In both cases an activated nucleophile attacks the electropositive carbonyl carbon (3 pts) generating a tetrahydral transition state (2 pts). The differences between the mechanisms are as follows:

Serine Proteases	**Asp Protease**
• Serine is the nucleophile (in phase I) • It is activated by proton abstraction by the His residue. • The Asp residue stabilizes the positive charge on the His residue.	• A water molecule is the nucleophile • It is activated by proton abstraction by the deprotonated Asp residue (25' in this diagram)

6. (6 pts) For any **one** of the enzymes we have studied, describe the substrate specificity of the enzyme in terms of molecular interactions between the enzyme and the substrate.

Trypsin likes to cleave Lys and arg and binds peptides containing these residues due to a favorable electrostatic interaction between the positive charge on the Lys or Arg and the negative charge on the additional Asp in the specificity pocket.

Chymotrypsin and HIV protease cleave after large non-polar residues. Their specificity pocket is large and non-polar.

Elastase cleaves after small non-polar residues, such as Ala. Its specificity pocket is small and non-polar.

Lysozyme cleaves b(1-4) linkages in N-acetyl glucosamine. It specifically recognizes the N-acetyl group.

7. (3 pts) The initial rate of an enzymatic reaction is usually measured experimentally so that (circle the best answer).
   a) the concentration of the [ES] complex is constant. **(2 pts)**
   b) there is no inhibition of the reaction by product.
   c) the concentration of the substrate is known.
   d) all of these answers are correct (3pts).

8. (8 pts) Please do **one** of the following two choices. Please indicate your choice.
   Choice A: How is ion exchange chromatography similar to affinity chromatography? In what way(s) are they different?
   Choice B: Briefly describe how the quaternary structure of a protein is determined. Feel free to use an example, such as hemoglobin, immunoglobulins, HIV protease, etc.

Choice A:

Both used the binding of proteins to the surface of beads as a means to retain desired proteins while washing away undesired proteins.

In the case of ion exchange chromatography the proteins bind via charge, in the case of affinity chromatography, they bind due to affinity for the ligand on the surface of the resin.

Choice B:

The native molecule weight (sum of all the individual chains) is obtained by gel filtration.

The molecular weight of each of the individual chains is obtained from SDS-PAGE.

This information is combined to give a quaternary structure that is consistent with both the native MW and that of each chain. For example, HIV would show a single band on SDS-PAGE, indicating that it is either monomeric or composed of subunits of equal size. The molecular weight in the native state would be twice the size as the denatured weights, so it must be a homodimer.

9. (12 pts) You are given a mixture of 4 proteins which have the following properties.
   i) (9 pts) Devise a purification scheme to purify protein C. Briefly justify your approach, use the back of the preceding page if you need additional room.

Protein	Solubility in Amm Sulfate	Molecular Weight	#Asp/Glu residues	#His residues	# Lys residues
A	1.0	10 kDa	2	6	5
B	3.0	5 kDa	5	8	3
C	5.0	20 kDa	4	3	8
D	5.5	21 kDa	4	3	10

There are probably other ways to do the separation, here is an example that would work.

Step 1 – gel filtration to remove A and B from C and D.

Step 2 – Cation exchange at any pH below the pKa of Lysine. Protein D will have a larger positive charge than C.

   ii) (3 pts) Do you expect the specific activity to increase or decrease during the purification? Why?

Increase (2 pts). The specific activity is the units (or activity) of the target enzyme/total protein. As we remove proteins A, B, and D, the number of units of C will only show a small decrease while the amount of total protein will decrease (1 pt)