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).

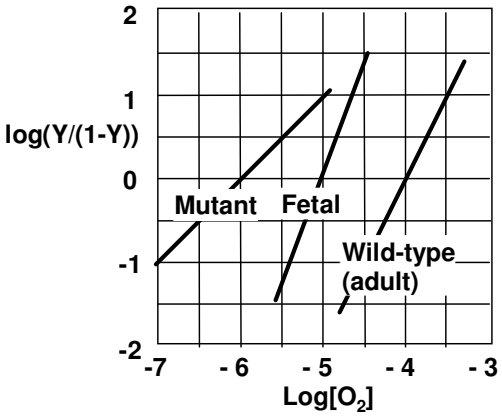
   ii) (2 pts) Give one example of either a homotropic or heterotropic allosteric activator/inhibitor and *briefly* describe its role in a biological process.

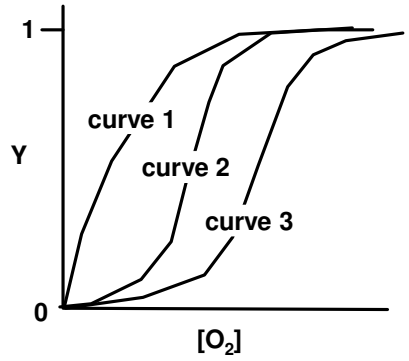
2. (3 pts) Please do **one** of the following choices, please indicate your choice.

   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?

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		
Fetal Hb		
Wild-type Adult		

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

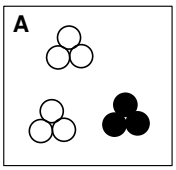
ii) (3 pts) Which of the binding curves shown in the lower diagram correspond to which protein? Briefly justify your answer.

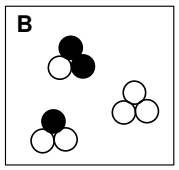
Curve 1:

Curve 2:

Curve 3;

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.)





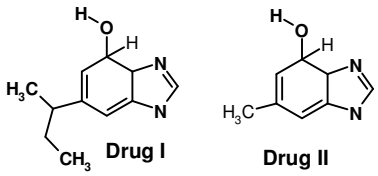
Question 3 continues…

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

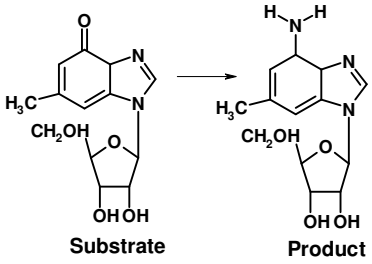
Choice A: Explain how the binding properties of the adult and fetal Hb enhance oxygen delivery to the fetus.

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?

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.



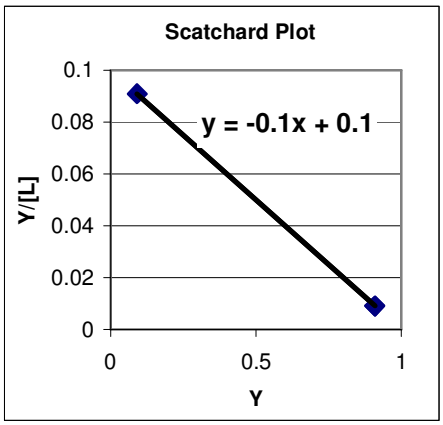
i) (2 pts) Are these drugs competitive or mixed-type inhibitors? Why?



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.

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:

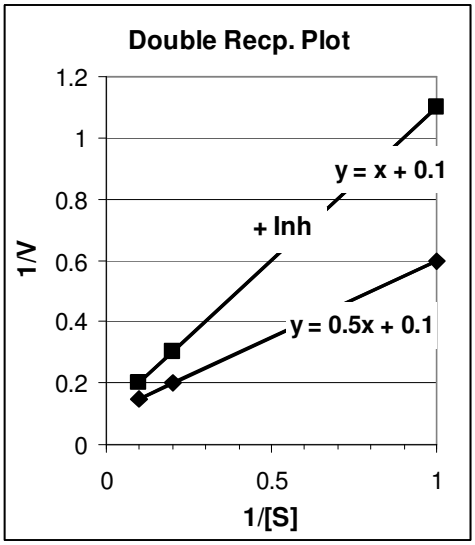
Free Drug Concentration	Drug Concentration inside Dialysis bag.
1 nM (0.001 uM)	0.091 uM
10 nM (0.01 uM)	0.510 uM
100 nM (0.1 uM)	1.00 uM



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).

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'.

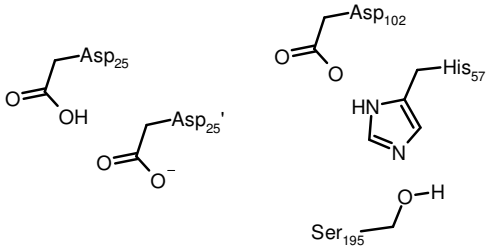
b) (2 pts) Why are the K_D (*part iv*) and the K_I for drug I essentially the same?

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.

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.



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.

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.
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.

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.

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

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