**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. **Note: An 18 pt question is on pg. 4.** 

- **1.** (4 pts) In an experiment to measure ligand binding, the concentration of the free ligand is adjusted to be equal to the  $K_D$ , i.e. [L]= $K_D$ =1 $\mu$ M. Please complete one of the following two questions regarding measuring the binding:
  - **Choice A:** Assuming that you were measuring the binding with equilibrium dialysis, with a total protein concentration of 1 uM inside the bag. What is the total concentration of the **ligand** in the dialysis bag?
  - **Choice B:** The UV light absorbance of the unliganded protein (M) is 0.2 and that of the fully liganded (ML) protein is 0.6. What is the UV light absorbance when  $[L]=K_D$ ? Why?
  - Choice A: Since the ligand concentration equals K<sub>D</sub>, <sup>1</sup>/<sub>2</sub> the protein will be saturated with ligand. The total ligand inside the bag is 1 uM (free) plus what is bound to the protein (0.5 uM), giving a total concentration of 1.5 uM.
  - **Choice B:** The total range of absorption change is 0.6-0.2 = 0.4. Since the fractional saturation is 0.5, half of the absorption change will have occurred, the total absorption is 0.2 (absorption of free protein) + 0.2 (amount of change due to ligand binding = 0.4.) Check by calculating fractional saturation:  $Y = (A - A_0)/(A_0 - A_0) = (0.4 - 0.2)/(0.4) = 0.5$ .
- 2. (10 pts) The cooperativity and binding affinity of three different tetrameric hemoglobins (A, B, C) is being studied. The binding curves and Hill plots for the three proteins are shown on the right.
  - i) Which hemoglobin shows the highest affinity for oxygen (A, B, or C), based on its K<sub>D</sub>? What is its K<sub>D-</sub> AVE? (4 pts).



<u>"A"</u> shows the highest affinity since its  $K_d$  is lowest, log  $K_d = -6$ ,  $K_d = 10^{-6}$ Note that the  $K_{D-AVE} = K_D$ , the "ave" indicates that it does not represent binding to a single site. In this case:  $K_{D-AVE} \approx \sqrt[4]{K_{D1}K_{D2}K_{D3}K_{D4}}$ .

ii) Which hemoglobin shows the lowest cooperativity, A, B, or C? Briefly justify your answer. (4 pts)

## "A" shows the lowest cooperativity since the slope of the Hill plot is smaller than B or C.

- iii) In the following panel, sketch the distribution of bound oxygen for the protein you identified in part ii), i.e. the one with the **lowest** cooperativity, by shading the circle. You should assume Y=0.25, i.e. only 5 of the 20 sites will be occupied. Briefly justify your answer (2 pts).
- Since the slope of the Hill plot for "A" is less than one it is negatively cooperative, so it is more likely to see one filled site/tetramer. Since the binding of the first ligand will reduce the affinity at the other sites.



3. (4 pts) Compare and contrast the structure and oxygen binding capabilities of hemoglobin and myoglobin.

Both use heme to bind oxygen via the Fe atom in the heme (2 pts) Myoglobin binds only one oxygen, Hemoglobin binds four oxygens. (2 pts).

# 4. (16 pts)

- i) Briefly discuss the major/general feature(s) of allosteric/cooperative behavior (10 pts), your answer should include a discussion of the properties of the tense (T) and relaxed (R) state.
- 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.

The protein exists in two states:

Tense (T) state - inactive (enzyme) or lower affinity (ligand binding) (2  $\frac{1}{2}$  pts) Relaxed (R) state - active (enzyme) or higher affinity (ligand binding) (2  $\frac{1}{2}$  pts)

The T and R state are in equilibrium with each other. (2 pts)

Allosteric activators cause the conversion from T to R, enhancing activity or binding (1 pt) Allosteric inhibitors cause the conversion from R to T, reducing activity or binding. (1 pt)

Homotropic - ligand affects its own binding at other sites (1/2 pt) Heterotropic - one ligand affects the binding of a different ligand. (1/2 pt)

## Choice A:

Hemoglobin show positive cooperativity with respect to oxygen binding.

This makes it easier to saturate Hb in the lungs due to the high O2 content, binding of the last O2 is very favorable.

As the Hb moves to the tissue, it loses oxygen, once the oxygen begins to leave, the remaining occupied sites have lower affinity and the oxygen leaves more readily, increasing the delivery.

## Choice B:

Bisphosphoglycerate (BPG) is an allosteric inhibitor of O2 binding.

Its concentration is increased at higher altitutes.

It changes the cooperativity of Hb to become more cooperative.

Thus, although less O2 is bound in the lungs, more is released in the tissue, compensating for lower oxygen levels at high altitude.

## Choice C:

An allosteric inhibitor would turn an enzyme off by causing it to be in the T state An allosteric activator would turn an enzyme on by causing it to be in the R state. **5. (6 pts)** Briefly describe the "steady-state" assumption that is used in the analysis of enzyme kinetic data. Why is it useful to make this assumption?

The steady state assumption is that during the measurement the concentration of (ES) does not change: d(ES)/dt=0 (5 ntc)

d(ES)/dt=0 (5 pts)

It simplifies the equation that describes the relationship between velocity, [S] and [Et], giving: v=Vmax[S]/(Km+[S])

(1 pt)

- **6. (10 pts)** This statement is often heard in biochemistry lectures: "Enzymes are catalysts because they lower the energy of the transition state".
  - i) How does lowering the energy of the transition state increase the rate? (4 pts)
  - ii) How do <u>all</u> enzymes lower the energy of the transition state. (4 pts).
  - iii) What additional feature do serine proteases use to lower the energy of the transition state that is not used by HIV protease (2 pts)?

i)

The rate is proportional to the amount of the transition state.

If the energy of the transition state is lower, then there will be more of it, and a higher rate will occur.

ii) The folding of the enzyme pre-orders the functional groups (aa sidechains) in the active site,

so there is no unfavorable decrease in entropy.

## iii) The formation of hydrogen bonds to just the transition

## state in the oxyanion hole.

**7. (8 pts)** Select <u>either</u> serine proteases <u>or</u> HIV protease and: briefly discuss the role of the catalytic triad or the catalytic diad in peptide bond cleavage. The structures on the right may be useful. For serine



proteases, it is not necessary to give the entire reaction mechanism, however you should do so for HIV protease.

## Serine proteases (a diagram is an acceptable answer)

Serine - nucleophile that attacks the C=O (3 pts)

Histidine – activates the nucleophile (2 1/2 pts ) and protonates the new amino terminus (1/2 pt) Aspartic acid – stabilizes the + charge on Histidine that develops by activation of the nucleophile (2 pts)

## HIV Proteases:

Deprotonated Asp: Activates nucleophile ( $H_2O$ ) that attacks C=O (6 pts) Protonated Asp: Protonates the new amino terminus. (2 pts)

- **8. (3 pts)** Name <u>one</u> enzyme that is inhibited in the treatment of HIV, besides HIV protease. What general properties of this enzyme make it a good drug target?
  - Reverse transcriptase or Integrase (1 pt)
  - Required for viral replication. (1 pt)
  - Enzyme activity is not found in humans, minimizing side effects (1 pt)

- 9. (6 pts) Please do one of the following two choices:
  - **Choice A:** Compare and contrast a competitive inhibitor to a mixed type inhibitor, in terms of their chemical structures and binding site on the enzyme.
  - **Choice B:** A double reciprocal plot showing the activity of a competitive and a mixed type inhibitor is shown on the right. Label the lines according to the type of inhibition. Briefly justify your answer.

#### Choice A:

- A competitive inhibitor binds to the active site and is therefore similar to the substrate in structure.
- A mixed-type inhibitor binds elsewhere and is therefore likely to be different in structure.

#### Choice B:

- The upper curve is the mixed-type since it shows a change in Vmax only mixed type inhibitors change Vmax.
- The middle line is the competitive inhibitor since it shows the same Vmax, the Vmax is the same because a high substrate the inhibitor cannot bind.
- **10. (18 pts)** The structure of the complex between HIV protease and its substrate (Phe-Val-Asn-Ala) is shown on the right. The enzyme releases Phe from the substrate. The lower two panels below show a mutant HIV protease complexed with two different drugs, Drug A and Drug B
  - i) Why are these drugs inhibitors of the protease? Do you expect them to competitive or mixed type? Why? (5 pts)
    - They do not have a peptide bond, so they cannot be cleaved (the C=O - NH has been replaced by C-OH - C) (3 pts)
    - The drugs are similar to the substrate so the should be competitive inhibitors. (2 pts)





Name:



- ii) Steady-state enzyme kinetic data was obtained for the **wild-type** and the **mutant** using the substrate Phe-Val-Asn-Ala, and these data were plotted on velocity curve. Which curve, A or B, is more likely to represent the data obtained on the wild-type enzyme? You can assume  $V_{MAX}$ =50. Briefly justify your answer (3 pts). [Hint: What are the Km values for each enzyme?]
- Curve A has the lower Km, indicating better binding of substrate (2 pts)
- The valine sidechain in the wild-type will show a non-polar (hydrophobic effect) interaction with the Phe on the substrate. This is more favourable than the interaction with the Aspartic acid in the mutant (1 pt)
- iii)To determine the effectiveness of drug A and drug B in inhibiting the <u>mutant</u> <u>enzyme</u> you obtained steady-state data in the presence of 10 nM of the each inhibitor. These data are plotted on the double reciprocal plot on the right. Please answer the following questions:
  - a) Calculate the K<sub>1</sub> for each drug (2 pts)
  - b) Based on the  $K_1$  which drug binds better to the protease? (2 pts)
  - c) Explain the difference in the K<sub>1</sub> values in terms of the interaction between the enzyme and the drug. (4 pts)



Name:

#### a)

The K<sub>i</sub> values are obtained from the ratio of the slopes (a):  $K_I = [I]/(a-1) = 10 \text{ nM}/(a-1)$ 

Drug A: a = (1.2/0.4) = 3,  $K_I = 10 \text{ nM}/(3-1) = 5 \text{ nM}$ Drug B: a = (4.4/0.4) = 11,  $K_I = 10 \text{ nM}/(11-1) = 1 \text{ nM}$ 

- b) Drug B, since it has the lower  $K_{\ensuremath{\text{I}}}$
- c) Drug B has a + charge which will interact favorably with the charge on the enzyme.

iv) How might you modify drug B to increase its ability to inhibit the enzyme? (2 pts).

Increase the length of the  $-NH_3^+$  group to bring it closer to the negatively charged Aspartic acid residue on the enzyme.



11. (3 pts) Why does the specific activity increase during a protein purification?

Specific activity = amount of target protein/total protein

During a purification scheme the total protein decreases, therefore specific activity should increase.

**12.** (**12 pts**) You are given a mixture of 4 proteins with the characteristics described below.

- i) Devise a purification scheme to separate **maltose binding protein** from the other three proteins. Briefly justify your answer (8 pts).
- ii) Sketch the elution profile for <u>one</u> of your column chromatography steps and indicate where the target protein would elute (i.e. first peak, 2<sup>nd</sup> peak, etc.) and justify how you labeled the peak. Alternatively, discuss how the chromatography step works to separate the different proteins. (4 pts).

| Name                       | Sol      | MW     | #Asp/Glu             | #Arg/Lys             | Activity                             |
|----------------------------|----------|--------|----------------------|----------------------|--------------------------------------|
|                            | (AmmSul) |        | (pK <sub>a</sub> =4) | (pK <sub>a</sub> =9) |                                      |
| Fatty acid binding protein | 1.0      | 15 kDa | 10                   | 5                    | Binds fatty acids.                   |
| Lysozyme                   | 1.5      | 14 kDa | 5                    | 10                   | Degrades NAG-NAM poly-saccharides.   |
| Glutathione oxidase        | 4.5      | 30 kDa | 10                   | 5                    | Oxidizes the tripeptide glutathione. |
| Maltose binding protein    | 5.0      | 30 kDa | 5                    | 10                   | Maltose binding                      |

#### **Best Scheme:**

- i) Make an affinity column with maltose attached to the beads (8 pts).
- ii) All the other proteins will elute first, maltose binding protein will elute last, requiring free maltose to elute it. (4 pts)

#### Acceptable Scheme 1:

Step 1: Ammonium sulfate @ 3 M. - Precipitate out 1<sup>st</sup> two proteins.

Step 2: Anion or cation exchange chromatography at pH =7.

 $q_{Glutathione oxidase} = -10 + 5 = -5$ 

 $q_{\text{Maltose binding protein}} = -5 + 10 = +5$ 

Anion exchange - Maltose binding protein will elute first since its charge is the same as the beads.

Cation exchange - The maltose binding protein will bind and elute second, perhaps requiring the use of NaCl.

#### Acceptable Scheme 2:

Step 1: Gel filtration (size exclusion) - First two protein elute last (smaller), last two elute first. Step 2: Ion exchange chromatography as above.