Instructions: This exam has 6 pages and 12 questions and is out of 100 points, you should allot 1 min/2 pts. Please use the space provided or the back of the previous page. On questions with more than one choice, all of your attempts will be graded and you will receive the grade for your best attempt.

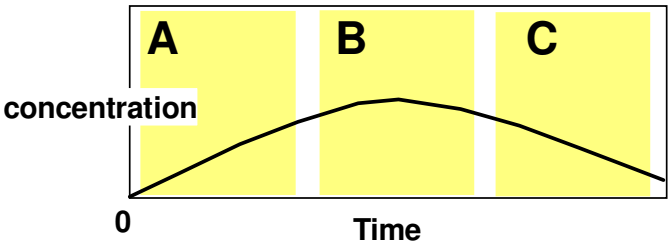
1. (2 pts) Which of the following energetic interactions can play a significant role in ligand binding, but is a *minor* contribution to the energetics of protein folding (circle single best answer).
   a) hydrogen-bonds
   b) van der Waals
   c) electrostatics
   d) conformational entropy
   e) hydrophobic effect

2. (4 pts) Complete **all** of the following. For *iii* and *iv*, the blanks are single kinetic constants (e.g. K_M, k_{CAT}), or a ratio of constants.

$$v_i = \frac{k_{CAT} E_T [S]}{K_M + [S]}$$

   i) Good substrates have low K_M values because their kinetic **on-rates** | **off-rates** are slow (circle correct answer.)
   ii) When the substrate concentration equals K_M, then [ES]/[E_{TOTAL}] equals _____.
   iii) To compare the rate of product formation by two enzymes, you would compare _____ at low substrate ([S] << K_M).
   iv) To compare the rate of product formation by two enzymes, you would compare _____ at high substrate ([S]>>K_M).

3. (4 pts) The image to the right shows the concentration of one species in an enzymatic reaction as a function of time, beginning with mixing the enzyme and substrate at t=0. Please answer the following questions:



   i) (2 pts) Which species in the reaction is represented by this curve (circle best choice)
   a) free enzyme (E). b) substrate (S) c) enzyme-substrate complex (ES)

   ii) (2 pts) Which of the following time-periods, A, B, C would be more suitable for measuring the initial velocity of the enzyme catalyzed reaction so that you could analyze the data using the equation shown on the right. *Briefly justify your answer.* [**Hint**: Consider any assumptions that were made in deriving the equation.]

$$v_i = \frac{k_{CAT} E_T [S]}{K_M + [S]}$$

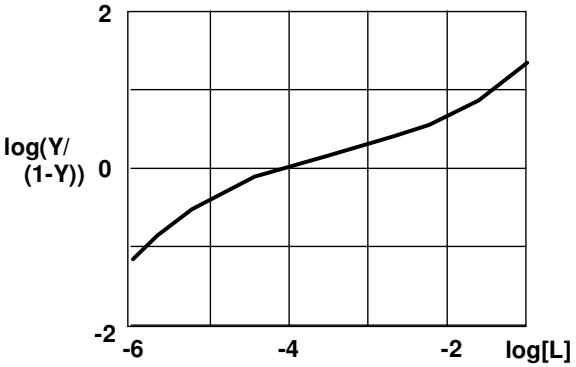
4. (6 pts) Please do **one** of the following choices.

Choice A: Briefly describe how fractional saturation can be measured using equilibrium dialysis.

Choice B: The concentration of a protein inside a dialysis bag is 3 μM. If the total ligand inside the bag is 11 μM and the free ligand outside the bag is 10 μM, what is the fractional saturation?

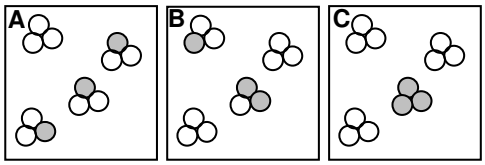
5. (17 pts) The following diagram shows a Hill plot for the binding of a ligand to a protein that has three binding sites. Please answer the following questions:

i) Determine the Hill coefficient from the plot. Indicate how you arrived at your answer (4 pts).



ii) Based on the Hill coefficient, is the binding of subsequent ligands stabilizing the relaxed (R) or tense (T) state of the protein? Be sure to briefly define/describe the properties of the R and T states in your answer (8 pts).

iii) Which of the three diagrams (A, B, C) best represents the distribution of bound ligands (shaded circles) for this protein? *Briefly justify your answer* (3 pts).



iv) Sketch on the graph the Hill plot you would expect to find if the affinity was 100-fold ***weaker,*** but with the ***same degree*** of cooperativity (1 pt).

v) Sketch on the graph the Hill plot you would expect to find if the system was ***non-cooperative,*** but with the **same K_D** (1 pt).

6. (10 pts) Please do one of the following two choices:

Choice A: Compare and contrast a homotropic to a heterotropic allosteric inhibitor (or activator). Give an example of **either one** from oxygen transport and state its importance or role in oxygen transport.

Choice B: What are the significant structural differences between myoglobin and hemoglobin and why is/are these difference(s) important in oxygen transport?

7. (12 pts) Using any enzyme that you wish, illustrate how the amino acid side-chain(s) of an enzyme (a *well labeled* drawing is a suitable answer):

i) confer substrate specificity

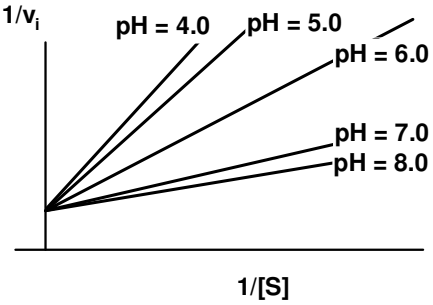
ii) catalyze a chemical reaction (**do not** give the entire reaction mechanism, just convince me that you understand the role of particular amino acid sidechains in the reaction.).

8. (8 pts) Enzymes lower the energy of the transition state. Please answer **one** of the following choices.

Choice A: Discuss why the decrease in activation energy leads to an increase in the reaction rate.

Choice B: Describe **one** thermodynamic feature that is responsible for the difference in the transition state energy between the uncatalyzed reaction and the enzyme catalyzed reaction. Be sure to indicate: i) whether the feature is related to enthalpy or entropy and, ii) whether it is common to all enzymes, or only a few.

9. (7 pts) Enzyme reaction rates were measured at different pH values and the data is presented in a double reciprocal plot on the right. Please answer the following questions:



i) Is the ionization of the group affecting substrate binding or the ability of the enzyme to perform the chemical reaction (4 pts)?

ii) Which form of this group – protonated or deprotonated - is most active? *Briefly justify you answer* (2 pts).

iii) What amino acid side chain is most likely involved in the reaction, Aspartic acid (pK_a=4), Histidine (pK_a=6), or Lysine (pK_a=9)? *Briefly justify your answer* (1 pts).

[**Hint**: Based on the plot, is V_{MAX} (k_{CAT}) or K_M changing as the pH changes? At what pH is the change the largest?]

$$\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$

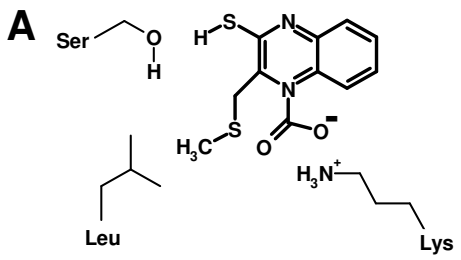
10. (12 pts) The diagram to the right shows the structure of two different inhibitors bound to HIV reverse transcriptase. The drug is drawn with thicker lines and interacts with sidechains from a Ser, Leu, and Lys residue in the enzyme.

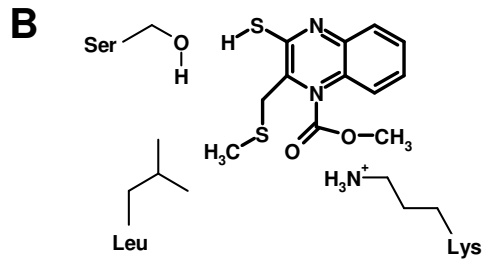
Double reciprocal plots of steady-state data were obtained for each inhibitor (concentration 1 nM) and these data are shown in the graph, along with data obtained in the absence of the inhibitor. Linear fits to the lines are given, but calculations are not necessarily required.

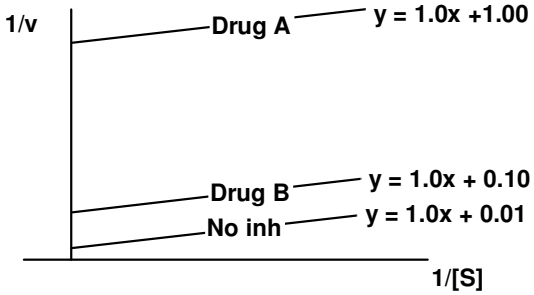
i) Are these inhibitors competitive or mixed-type? *Why*? (4 pts)

ii) Based on the enzyme-kinetic data which inhibitor binds with the *highest* affinity (4 pts)?

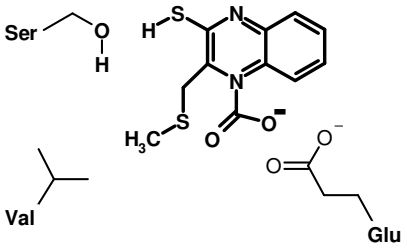
iii) Explain the basis of the difference in affinities for drug A and B with reference to the molecular structure of the enzyme inhibitor complex (4 pts).







11. (6 pts) A mutant HIV reverse transcriptase has arisen in a patient that has been treated with drug A from the previous question. This mutation replaces the Lys sidechain with a Glu and Leu with Val. The structure of the mutant enzyme-drug complex is shown on the right. The drug binds poorly to the mutant enzyme and is thus not effective. Suggest how you might modify the drug to restore its effectiveness.



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

Choice A: The properties of three different proteins are given in the table below. Devise a purification scheme that will purify **protein A** from the other two proteins. Briefly state how **each** step separates the proteins from each other.

	Solubility in Amm. Sulfate	Size	# Asp+Glu (pK_a=4.0)	# His (pK_a = 6.0)	# Lys + Arg (pK_a = 9)
A	1.0	10 kDa	0	1	5
B	1.2	10 kDa	5	1	0
C	4.0	20 kDa	0	1	5

Choice B: A protein has a native molecular weight of 180 kDa. SDS-PAGE obtained in the presence of β-mercatoethanol (BME) shows three bands of equal intensity, with molecular weight of 10 (α-chain), 30(β-chain), and 50(γ-chain) kDa.

i) What technique was used to determine the native molecular weight? How is the molecular weight obtained? (4 pts)

ii) What is the quaternary structure? *Briefly justify your answer*. (4 pts)

iii) Assuming that the α-chain was disulfide bonded to the β-chain, sketch (or describe) the appearance of the SDS-PAGE obtained in the *absence* of BME (4 pts).