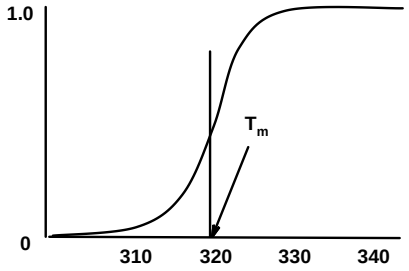
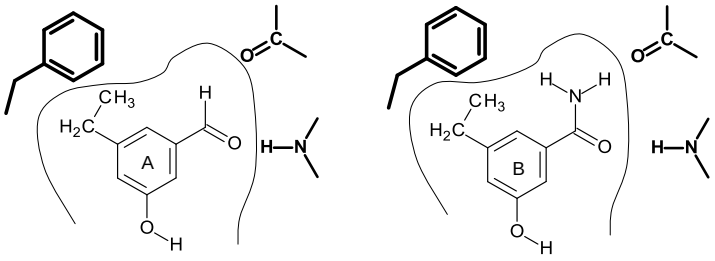
Instructions: This exam has 6 pages and 13 questions and is out of 100 points; you should allot 1 min/2 pts. *Please use the space provided or the back of the previous page*. On questions with more than one choice, all of your attempts will be graded and you will receive the grade for your best attempt.

1. (6 pts) The melting curve for a protein is shown on the right. The enthalpy for unfolding is +200 kJ/mol.
   i) Briefly explain how the enthalpy would be obtained from the melting curve (3 pts).
   ii) Determine the entropy of unfolding. Briefly explain how you arrived at your answer. T_M = 320 K (3 pts).



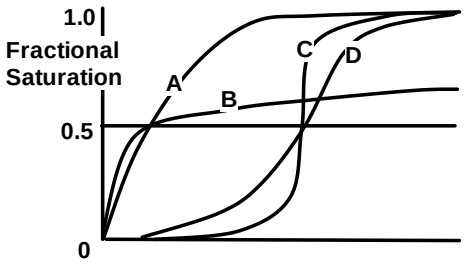
2. (8 pts) Two ligands (A, B) bind to a protein. The complexes of ligand A and B with the protein are shown on the right. The bold atoms and bonds indicate groups from the protein that interact with the ligand. Please answer all of the following:
   i) Which ligand would have the slower off-rate, A or B? Briefly justify your answer (4 pts).
   ii) What is the likely sign of the **entropy** of binding (e.g. reaction direction M + L → ML), positive or negative? Why? (4 pts).

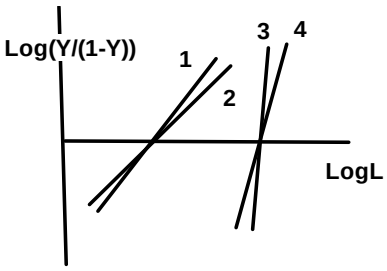


3. (6 pts) Please do **<u>one</u>** of the following choices:
   Choice A: In an equilibrium dialysis experiment, the concentration of protein inside the dialysis bag is 1 uM. Sufficient ligand is added outside the bag to give a concentration of 2 uM. What is the ligand concentration inside the dialysis bag? The K_D for this protein-ligand combination is 2 uM.
   Choice B: The UV absorption of unliganded protein is 0.1 while the absorption for the fully liganded (saturated) protein is 0.2. After adding some ligand to a solution of the unliganded protein, the absorption was found to be 0.15. What is the fractional saturation?

4. (12 pts) Using oxygen transport by hemoglobin as an example (please do both parts i and ii):
   i) Give the general properties of an allosteric system (8 pts).
   ii) please answer **one** of the following choices (4 pts).
   Choice A: How does the allosteric behavior of hemoglobin optimize O_2 delivery to the tissues?
   Choice B: How does the allosteric binding of BPG (bisphophoglycerate) to hemoglobin facilitate adaptation of oxygen delivery at high altitudes?

5. (8 pts)
   i) Match the following binding curves to the corresponding Hill plot. *Briefly justify your answer* (Note: Line 1 on the Hill plot has a slope of 1.)(5 pts).





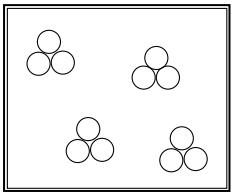
   A =

   B =

   C =

   D =

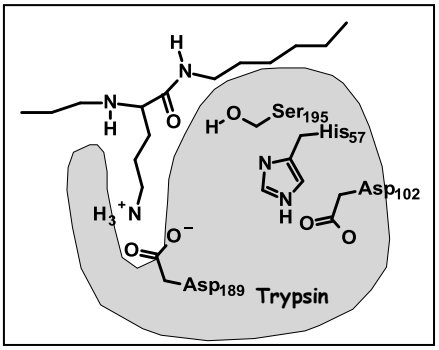
   ii) Indicate the distribution of bound ligands for the trimeric protein shown on the right for binding curve "B" assuming a fractional saturation of Y=0.25. Shade subunits that have bound ligand. *Justify your answer* (3 pts).



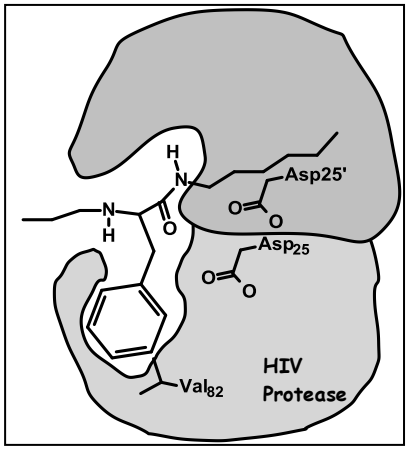
6. (8 pts) Enzymes increase the rate of reaction by "stabilizing the transition-state".
   i) Why does stabilizing the transition state increase in the rate of the reaction?
   ii) Give one way by which enzymes lower the energy of the transition state, be sure to indicate whether this is an enthalpic or an entropic term and whether it applies to all enzymes or just some.

7. (8 pts) Please do **<u>one</u>** of the following choices:

Choice A: A diagram of trypsin is shown on the right, with substrate bound. Cleavage would occur after the lysine residue.
   i) Explain the role of the serine, histidine, and the aspartic acid in the reaction mechanism (3 pts)
   ii) If the serine was replaced by alanine ($-CH_3$), which would change, the K_M or the k_{CAT}? Justify your answer. (3 pts)
   iii) Chymotrypsin cleaves after Phe, Tyr, or Trp residues (large non-polar). How does the active site of chymotrypsin differ from trypsin? (2 pts)



Choice B: A diagram of HIV protease is shown on the right, with substrate bound, cleavage would occur after the phenylalanine residue.
   i) Explain the role of the two Asp residues in the reaction mechanism.(3 pts)
   ii) If one of the Asps was replaced by alanine ($-CH_3$), which would change, the K_M or the k_{CAT}? Justify your answer. (3 pts)
   iii) What would you change in the active site that would allow the enzyme to cleave after small non-polar residues, such as alanine? (2 pts)



8. (4 pts) Please do **one** of the following choices:

Choice A: The "steady-state" assumption is important in the analysis of enzyme kinetics. What is this assumption?

Choice B: In obtaining K_M and V_{MAX} one can either use a velocity curve (v versus [S]) or a double reciprocal plot (1/v versus 1/[S]), why is the double-reciprocal plot preferred?

9. (5 pts) Two enzymes are available that can be used in commercial manufacture of lactate, the kinetic parameters for each of these enzymes is given in the table on the right. Given that the substrate concentration is 100 mM (i.e. $>>K_M$), which would be the better enzyme, A or B? *Briefly justify your answer.*

Enzyme	K_M	k_{CAT}
A	0.1 mM	10 s^{-1}
B	1 mM	100 s^{-1}

10. (5 pts)

i) Select **one** of the following three enzymes and briefly describe its role in the HIV lifecycle.

a) HIV reverse transcriptase b) Integrase c) HIV protease

ii) Why are inhibitors of any of these three enzymes effective drugs for the treatment of HIV?

11. (8 pts)

i) In what way(s) do competitive inhibitors differ from mixed-type inhibitors? (7 pts)

ii) Which type of inhibitor is potentially a better drug if the substrate concentration is low in the body? Why? (1 pt).

12. (12 pts) The compound on the right is a competitive inhibitor of HIV protease. It is shown interacting with Val82, which is part of the specificity pocket of the enzyme. This inhibitor binds to the wild-type enzyme with a K_I of 1 nM. A mutant form of the HIV protease is also shown on the right, with Val82 replaced by lysine, in this case the cyclohexane ring has been removed from the drug (see part ii).

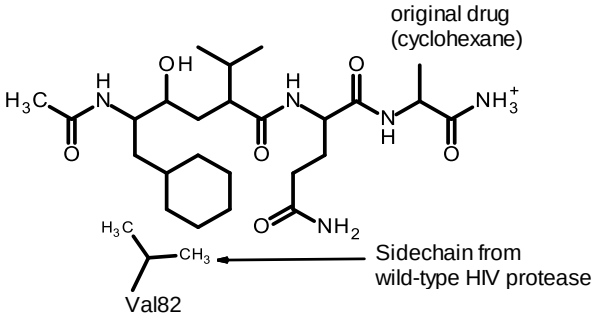
i) Why is this a competitive inhibitor of HIV protease? (1 pt)

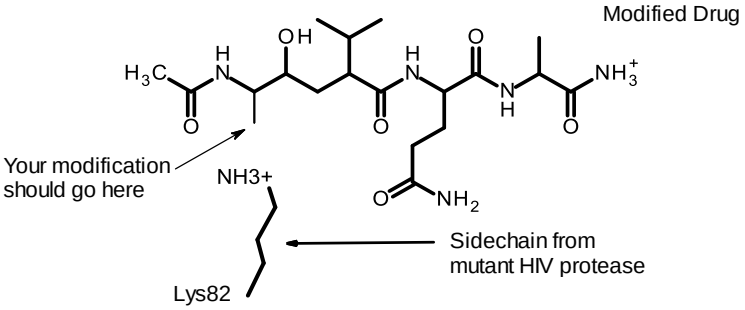
ii) How would you modify the drug such that it would effectively bind to the mutant enzyme, and therefore be effective at treating patients who have acquired this mutation? Justify your approach. (4 pts).

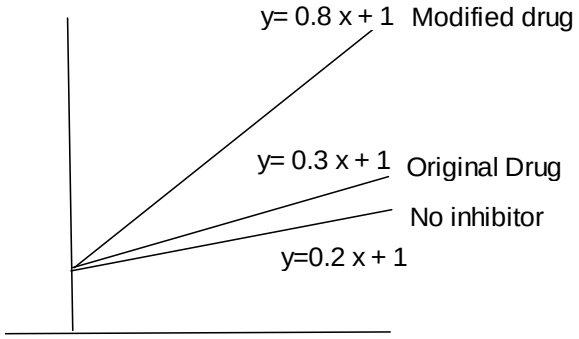
iii) You measure steady state enzyme kinetics with the original drug (cyclohexane), and your modified drug, ***using the mutant enzyme***. The double reciprocal plots are shown below. What are the K_I values for the binding of the original drug and your modified drug to the mutant enzyme ([I]=10 nM) (3 pts).

iv) Briefly explain why the K_I has changed for the original drug, from its value of 1nM for binding to the wild-type enzyme (2 pts).

v) Briefly explain why the K_I value for your drug is different (higher or lower) than that for the original drug (2 pts).





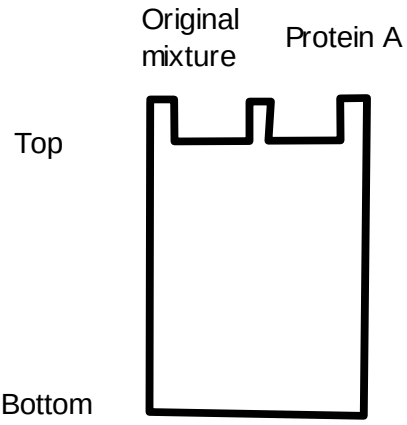


13. (10 pts) You are presented with a mixture of the following three monomeric proteins:

	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	1.1	20 kDa	0	1	5

*Concentration at which 50% of the protein will precipitate. Assume that concentrations 0.5 below that value will leave proteins in solution, and concentrations 0.5M above that value will precipitate all of the protein.

i) Outline a purification scheme that will **separate protein A** from the other 2 proteins (5 pts).
ii) Briefly describe how **one** of your purification steps works (3 pts)
iii) What is specific activity and what should happen to the specific activity during an ideal purification scheme, should it increase, decrease, or stay the same? (1 pt)
iv) Sketch the SDS-PAGE gel that you would expect to see for the mixture of the proteins and the final purified protein. The gel is shown on the right, one lane corresponds to the original mixture, and the second lane to the final purified protein (1 pt).



Bonus questions (2 pts each)
1) How does the nerve toxin sarin work?

2) How can antibodies be used in drug detoxification?