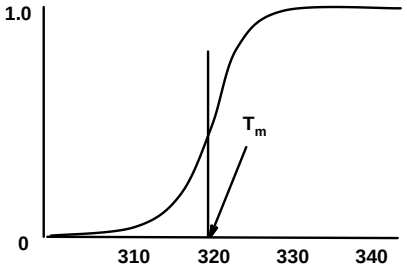
Instructions: This exam has 6 pages and 13 questions and is out of 100 points; you should allot 1 min/2 pts. *Please use the space provided or the back of the previous page*. On questions with more than one choice, all of your attempts will be graded and you will receive the grade for your best attempt.

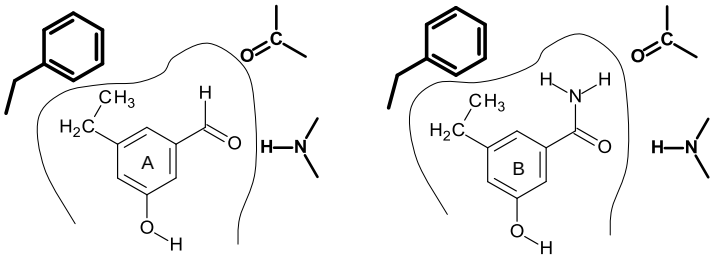
1. (6 pts) The melting curve for a protein is shown on the right. The enthalpy for unfolding is +200 kJ/mol.
   i) Briefly explain how the enthalpy would be obtained from the melting curve (3 pts).
   ii) Determine the entropy of unfolding. Briefly explain how you arrived at your answer. T_M = 320 K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



i) A plot of ln Keq versus 1/T will have a slope of $-\Delta H^0/R$.

ii) At T_M the energy difference between the folded and unfolded is zero:
$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ = 0$ at T_m. Therefore $\Delta S^\circ = \Delta H^\circ/Tm = 200{,}000/320 = 625$ J/mol-K

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).



i) B has an additional hydrogen bond with the protein (upper right interaction), therefore it would have the slower off-rate.

ii) Both ligands have a non-polar interaction with the protein (ethyl group on the ligand, phenylalanine group on the protein. Ordered water would be released during binding, so the entropy would be positive due to the increase in disorder of the water.

3. (6 pts) Please do **one** 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?

Choice A: $Y = [ML]/[M_{TOT}] = [L]/(K_D+[L])$. The fractional saturation is 0.5 since $[L]=K_D$. Therefore ½ of the protein will have ligand bound, [ML] = ½ uM. The total amount of ligand in the bag is 2 uM (free) + 1/2 um (bound) = 2.5 uM.

Choice B: $Y = (A - A_o)/(A_{SAT} - A_o) = (0.15-0.1)/(0.2-0.1) = 0.05/0.1 = 0.5$

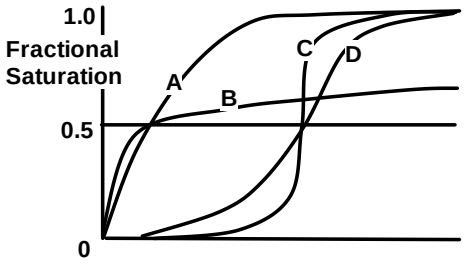
4. (12 pts) Using oxygen transport by hemoglobin as an example (please do both parts i and ii):
   i) Give the general properties of an allosteric system (8 pts).
   ii) please answer **one** of the following choices (4 pts).
   Choice A: How does the allosteric behavior of hemoglobin optimize O_2 delivery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

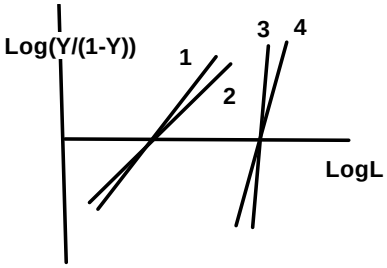
   i) Allosteric systems exist in two different shapes or conformations (3 pts).
   One is the tense (T) state, which has low activity or weak binding (1 ½ pts)
   Second is the relaxed state (R) which has high activity or strong binding of ligands (1 ½ pts)
   These are in equilibrium with each other (1 pt)
   Activators bind to and stabilize the R state., i.e. they shift the equilibrium to the R state (1/2 pt)
   Inhibitors bind to and stabilize the T state, i.e. they shift the equilibrium to the T state. (1/2 pt)

   Choice A: Oxygen shows positive cooperativity, meaning that the binding of one oxygen increases the affinity for subsequent oxygens. The higher affinity ensures that Hb will be fully saturated with oxygen in the lungs. As the Hb move out to the tissues, the affinity drops rapidly as oxygen is lost, causing the release of even more oxygen.

   Choice B: BPG is a heterotropic allosteric inhibitor, so it decreases oxygen affinity. But it changes the shape of the binding curve – making the system more highly cooperative with respect to oxygen binding. Although less oxygen is bound, more will be released as the Hb goes to the tissue because of the increased cooperativity.

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).



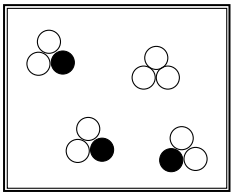


   A = 1
   B = 2
   C = 3
   D = 4

Curves A and B have a lower Kd and must be lines 1 and 2 on the hill plot. Line one has a slope of 1 which indicates non-cooperative binding, this is curve A which is hyperbolic. Curve B is not (difficult to saturate) indicating neg coop, with a slope less than one)
Curves C and D must correspond to 3 and 4. Curve C rises much faster than D showing greater cooperativity, so it should have the larger slope on the Hill plot – curve 3.

   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).

At Y=0.25, a total of three ligands would be bound. Because the cooperativity is negative, you would more likely see the bound ligand on different proteins, since the binding of the first ligand makes it more difficult to bind additional ones. 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.

i) Lowering the energy increases the amount of the transition state, which increases the rate of the reaction.

$V \propto [X^{\ddagger}]$

ii) The common method, which applies to all enzymes, is the fact that the functional groups on the enzyme are pre-ordered, so there is no unfavorable decrease in entropy as the system goes to the transition state.

OR

If the transition state is stabilized by a unique enthalpic interaction, that does not occur with the substrate, then the energy of the transition state will also be lowered. This occurs with certain enzymes, such as serine proteases.

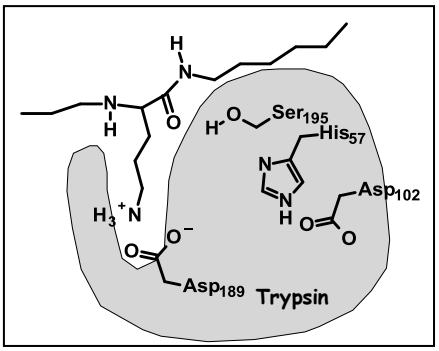
7. (8 pts) Please do **<u>one</u>** of the following choices:

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)

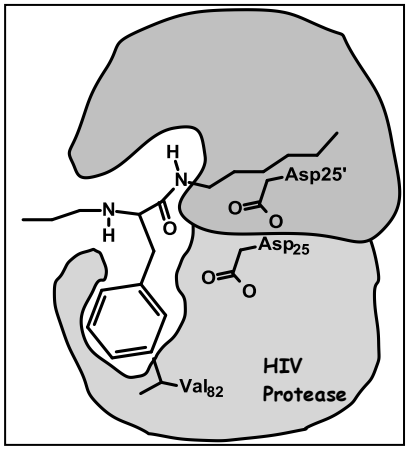


Choice B: A diagram of HIV protease is shown on the right, with substrate bound, cleavage would occur after the phenylalanine residue.

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)



Choice A:

Serine – nucleophile

Histidine – activates nucleophile by proton extraction.

Asp – Stabilizes + charge on His after activation of nucleophile

ii) k_{CAT}, since the serine is involved in the chemical step.

iii) It would contain non-polar residues and would be larger to accommodate the larger sidechain.

Choice B

i) The deprotonated Asp activates a water molecule (not shown) as a nucleophile.
The other Asp, will protonate the new amino terminus.

ii) k_{CAT}, since the serine is involved in the chemical step.

iii) The pocket would be much smaller, but still non-polar (e.g. replace Val82 with Phe, for example)

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?

Choice A: That the concentration of (ES) does not change during the measurement.

Choice B: In order to get K_M from the velocity curve and accurate value for V_{MAX} is required. This can only be obtained at high substrate. The double reciprocal plot extrapolates to high substrate, so it gives a more accurate value for V_{MAX}.

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}

Since [S] >> Km, both enzymes would be saturated, so kcat would be the deciding factor, B has the larger k_{CAT} so it would be best:

$V = E_T k_{CAT} \times [S]/(K_M + [S]) \approx E_T k_{CAT} \times [S]/([S]) \approx E_T k_{CAT}$

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?

i)

HIV RT – copies viral RNA to DNA

Integrase – inserts the viral DNA into the host cell chromosome.

HIV protease – responsible for cleavage of immature viral proteins.

ii) All of these steps are essential in the HIV life cycle, so inhibiting them will stop viral growth.

11. (8 pts)

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

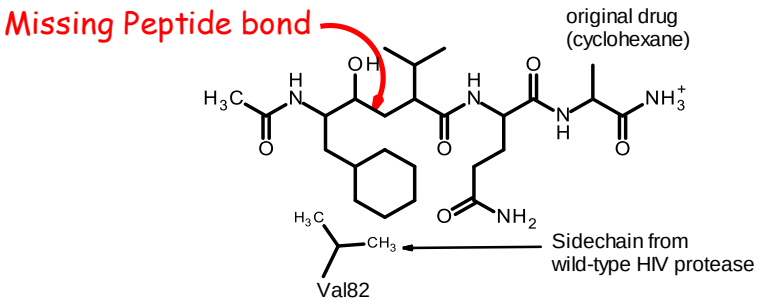
ii) Which type of inhibitor is potentially a better drug if the substrate concentration is low in the body? Why? (1 pt).

i) Competitive inhibitors bind to the active site and inhibit by preventing substrate from binding (3 ½ pts)

Mixed-type inhibitors bind elsewhere, causing allosteric changes that affect substrate binding and catalytic activity (3 1/2 pts)

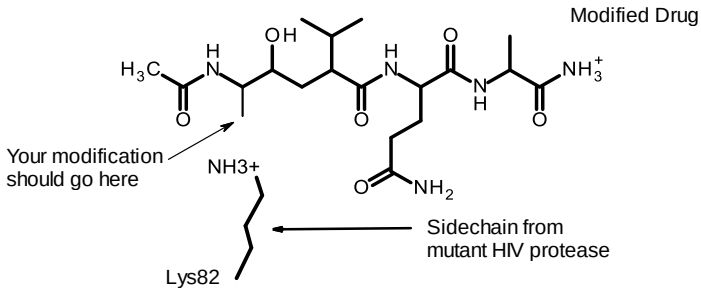
ii) A competitive inhibitor, because at low [S] it can effectively compete with the substrate for the active site. (Alternative answer – the drug with the lower K_I, since both competitive and mixed-type inhibitors might be equally effective.).

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)

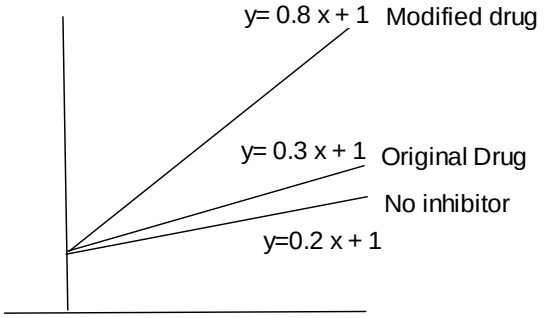
ii) How would you modify the drug such that it would effectively bind to the mutant enzyme, and therefore be effective at treating patients who have acquired this mutation? Justify your approach. (4 pts).



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).



i) It is a competitive inhibitor because it looks like the peptide substrate, but lacks a peptide bond for the "cyclohexane residue" (see diagram above). Therefore it will bind but will not be cleaved.

ii) Add a negative charge at that location, e.g. a carboxyl groups. This will have a favorable electrostatic interaction with the enzyme.

iii) $K_I = [I]/(a-1)$, a = ratio of slopes.

Original drug: K_I = 10 nM/ (1.5 -1) = 10 nM/0.5 = 20 nM

Modified drug: K_I = 10 nM/(4-1) = 10 nM/ 3 = 3.3 nM

iv) the K_I increased because we replaced a favorable non-polar interaction (cyclohexane-Val82) with an unfavorable non-polar – polar interaction (cyclohexane – lysine)

v) **This question was ambiguous since I didn't say which K_I explicitly (wild-type or mutant).**

If comparing to the 20 nM K_I for the binding of the cyclohexane drug to the mutant enzyme, the answer is that there is a favorable electrostatic interactions, so K_I is lower.

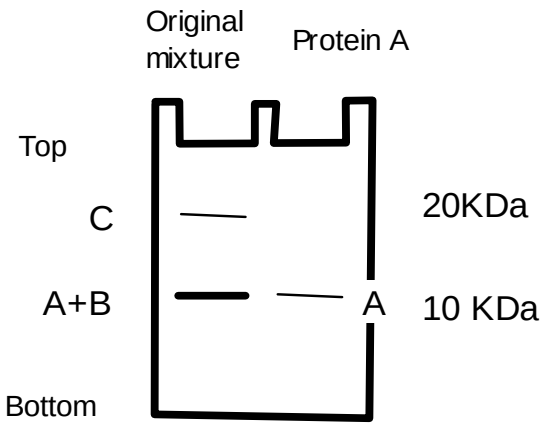
If comparing to the 1 nM K_I for the wild-type, the new interaction is weaker due to the higher K_I., i.e. the original cyclohexane-valine interaction was more favorable.

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).



i) Ammonium sulfate ppte cannot be used because the solubilities are to close. So….

Step 1 : Size exclusion (gel filtration) – separation by size. The resin consists of porous beads, and the smaller proteins enter the beads and take longer to elute.

A and B will elute together, C will elute first, since it is larger.

Step 2. Ion exchange. At pH=6, the charge on protein A is +5.5 while the charge on protein B is -4.5.
If you ran an anion exchange (positively charged beads) protein A would not stick while B would, so you could trap B on the column and have pure A. Opposite effect for a cation exchange (negative charge on beads) , A would stick and B would flow through.

ii) See i) above

iii) The specific activity is the amount of target protein/total protein. It should increase during the purification scheme.

iv) see diagram. The original mixture would have two bands, and the final mixture just one. Protein C will be the slower moving band in the original mixture. Smaller proteins migrate faster in the gel.

Bonus questions (2 pts each)
1) How does the nerve toxin sarin work?
It is a suicide inhibitor and forms a covalent bond with the serine in the active site of the enzyme that degrades the neurotransmitter acetylcholine.
2) How can antibodies be used in drug detoxification?
They can simply bind the drug or they can catalyze the decomposition of the drug (e.g. cocaine antibodies).