

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).

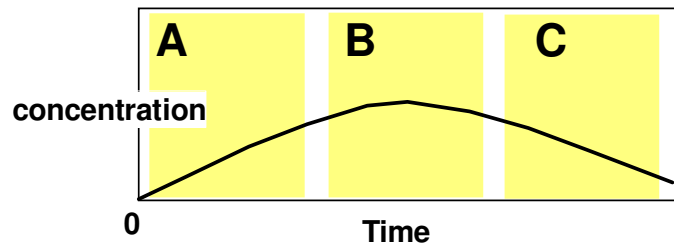
- a) hydrogen-bonds
- b) van der Waals
- c) electrostatics**
- d) conformational entropy
- e) hydrophobic effect

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 **1/2**.
- iii) To compare the rate of product formation by two enzymes, you would compare **k_{CAT}/K_M** at low substrate ($[S] \ll K_M$).
- iv) To compare the rate of product formation by two enzymes, you would compare **k_{CAT}** at high substrate ($[S] \gg K_M$).

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.]

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

None of these are suitable since the formula is derived using the steady state assumption that $[ES]$ is not changing (+2 pts).

If B was selected and you stated that the change in $[ES]$ was smallest during this period, full credit.

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?

Choice A:

- Protein is placed inside a dialysis bag.
- Ligand is added to the outside.
- The dialysis membrane allows the ligand to come to equilibrium.
- At equilibrium, the excess ligand inside the bag is equal to $[\text{ML}]$, the fractional saturation is $[\text{ML}]/[\text{M}_{\text{TOT}}]$

Choice B:

- $[\text{ML}]$ is obtained from the difference in the ligand concentration, $11 - 10 = 1 \mu\text{M}$. The fractional saturation is $[\text{ML}]/[\text{M}_{\text{TOT}}] = 1/3 = 0.33$ (unitless).
- (-2 pts if you used $[\text{M}] + [\text{ML}] = 4 \mu\text{M}$.)

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:

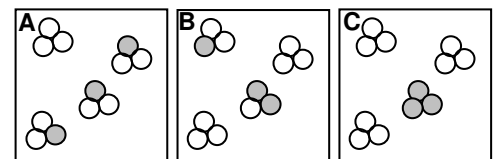
- i) Determine the Hill coefficient from the plot. Indicate how you arrived at your answer (4 pts).

The Hill coefficient is the slope as the curve crosses the x-axis, approximately 0.25.

- 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).

- The Hill coefficient is less than one, so the system is showing negative cooperativity.
- The binding of one ligand decreases the affinity for subsequent ligands (+4 pts).
- As ligand is added the high affinity R state is converted to the lower affinity T state (+4 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).



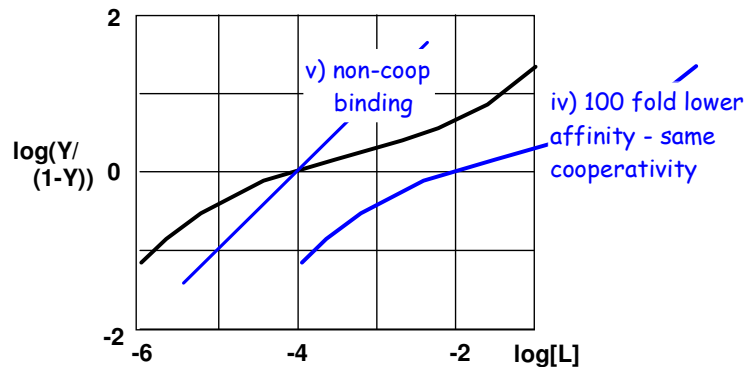
In the case of negative cooperativity, it is more likely for ligands to bind to un-occupied trimers, since the first binding event will make the remaining sites lower affinity. Therefore **panel A** is correct.

1 pt correct answer, 2 pts justification.

- 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).

The intersection of the X-axis is $\log K_d$. A 100 fold weaker binding would be 100x higher K_d , or 2 log units higher (curve shifted to the right, intersection at -2.)

- 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). This would intersect the x-axis at the same point as the original curve, but have a slope of one.



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?

Choice A (6 pts)

Homotropic - affects binding of the same ligand

Heterotropic - affects the binding of a different ligand.

Examples (4 pts).

Oxygen binding is an example of a homotropic allosteric activator, the affinity increases as more oxygen binds.

This gives a high affinity hemoglobin in the lungs so that it will be easy to saturate.

In the tissues, where the oxygen concentration is lower, the affinity will be lower, promoting the release of oxygen in the tissues.

Bisphosphoglycerate (BPG) is an example of a negative heterotropic allosteric inhibitor, its binding decreases the affinity for oxygen.

This compound is important for altitude adjustment. At high altitudes its concentration is increased. Although this reduces the overall affinity, it changes the shape of the binding curve as well, allowing the equivalent amount of oxygen to be released.

Choice B:

Myoglobin has only one sub-unit and shows non-cooperative binding for oxygen.

Hemoglobin has four sub-units and shows cooperative binding for oxygen.

Because the oxygen binding is cooperative, the affinity increases as more oxygen binds.

This gives a high affinity hemoglobin in the lungs so that it will be easy to saturate.

In the tissues, where the oxygen concentration is lower, the affinity will be lower, promoting the release of oxygen in the tissues.

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):

- confer substrate specificity **(+4 pts)**
- 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 pts)**

Serine Proteases:	HIV Protease:
Specificity: Trypsin likes Arg/Lys due to Asp in specificity pocket. Chymotrypsin: Large non-polar pocket makes Phe, Tyr and Trp good substrates. Mechanism: - Ser is 1st nucleophile, attacks peptide bond. - His activates nucleophile (Ser and water) by proton transfer. - Asp stabilizes + charge on His.	Specificity: Large non-polar binding pocket (similar to chymotrypsin) - Val82 forms part of the pocket. Mechanism: - Two Asp residues - one protonated, one deprotonated. - Deprotonated one activates water as nucleophile. (Water cleaves peptide bond) - Second Asp donates proton to product.

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.

Choice A:

The rate of the reaction is proportional to the concentration of the transition state (+ 3 $\frac{1}{2}$ pts)

The concentration of the transition state will be higher if the activation energy is lower. (+ 3 $\frac{1}{2}$ pts)

Some indication of quantitative relationships (1 pts)

$$v = k_{CAT} [EX]$$

$$[EX] = K_{EQ} [ES]$$

$$\Delta G = -RT \ln K_{EQ}$$

Choice B:

Entropy: The pre-ordered residues in the active site of the enzyme reduces the entropy loss that would otherwise occur to organize these groups around the substrate. In other words, the requirement to organize these groups in the uncatalyzed reaction raises the energy of the transition state (+ 6 pts). This effect occurs in all enzymes (+2 pts).

Enthalpy: If the enzyme makes energetically favorable contacts solely with the transition state it will lower the energy of the transition state (+ 6 pts). This is found on some enzymes (+2 pts).

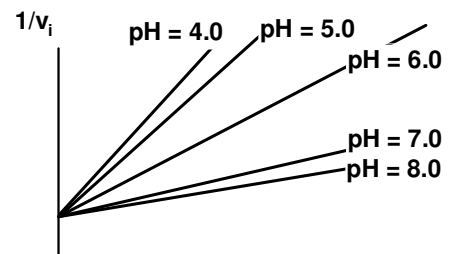
9. (7 pts) Enzyme reaction rates were measured at different pH values and the data is presented in a double reciprocal plot on the right. Please answer the following questions:

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 your 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?]



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

i) The plot shows that the slope of the line is changing, but not the y-intercept. Therefore K_M is changing (+3 pts) which implies substrate binding is being affected (+1 pt).

ii) The slope is larger at pH 4.0, meaning that the K_M is larger, or the substrate binding is low affinity. At pH 8 the slope has decreased, indicating a low K_M , or higher affinity. Therefore the deprotonated group is the most active (or effective) at binding substrate. (+2 pts)

iii) The largest change occurs around pH=6.0 which must be close to the pK_a for the group. So Histidine is the most likely residue. (+1 pts)

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.

- Are these inhibitors competitive or mixed-type? *Why?* (4 pts)
- Based on the enzyme-kinetic data which inhibitor binds with the *highest* affinity (4 pts)?
- 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).

i) Since the y-intercept ($=1/V_{max}$) changes, they must be mixed type. Competitive inhibitors would show the same y-intercept since a competitive inhibitor cannot affect V_{max} .

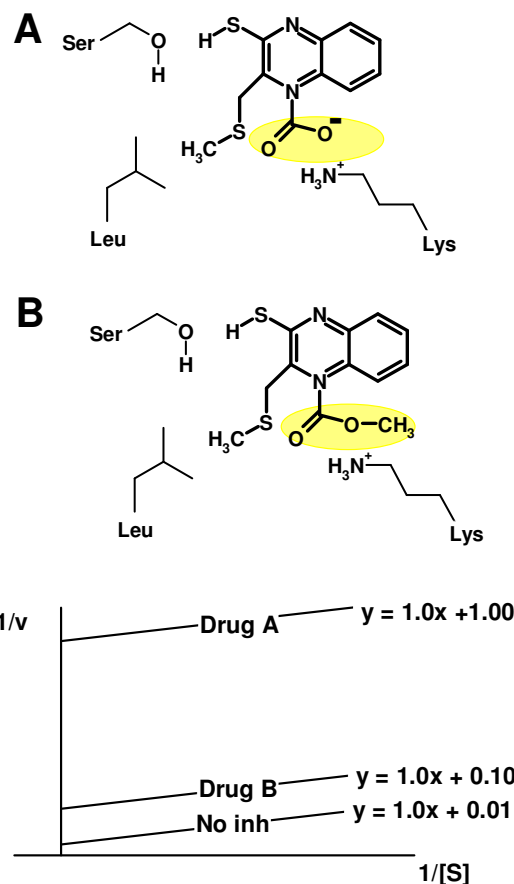
ii) Drug A gives the highest line, and since it is a reciprocal plot, the lowest velocity and therefore it is the best inhibitor. If you calculated α' values to get actual K_I' you should have gotten the following (this was not required):

Drug A: Ratio of Y-int = $1/.01 = 100$. $K_I' = 1\text{nM}/(100-1) \sim 0.01\text{nM}$.

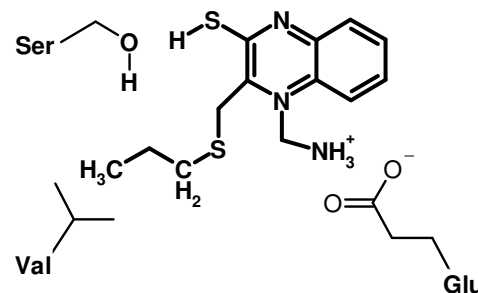
Drug B: Ratio of Y-int = $0.1/.01 = 10$. $K_I' = 1\text{nM}/(10-1) \sim 0.1\text{nM}$.

Drug A has the lower K_I' and therefore binds better.

iii) The only difference between the two drugs is that drug A has a carboxylate group where B has an ester. The negative charge on the carboxylate interacts favorably with the + charge on the Lysine, giving a lower K_I' .



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.



Replace the carboxylate group with a + charged amine. If you suggested Lys or Arg -1, since it is too large.

Make the thiol-methyl group larger, such as a thio-ethyl group..

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

Purification Scheme (8 pts)

Step 1:

A and B cannot be separated by either ammonium sulfate ppt or gel filtration. In both cases their properties are similar or equivalent. Either technique would be a good first step, removing protein C from the mixture.

Step 2: Separation of A from B can be accomplished by ion exchange chromatography.

A has one His and 5 Lys or Arg, thus it would be positively charged at pH ~ 7.0

B has one His and 5 Asp or Glu, thus it would be negatively charged at pH ~ 7.0

If you used anion exchange chromatography, B would bind to the column and A would flow through without binding.

If you used cation exchange chromatography, A would bind to the column, and B would flow through. A would then be eluted with continued washing or the use of higher salt.

Brief Description of Techniques (4 pts)

Gel filtration - separation by size. Porous beads cause smaller proteins to take longer to elute from the column.

Ammonium sulfate ppt - proteins have different levels of solubility, in this example, making the solution ~ 3 M in ammonium sulfate would ppt all of A and B, leaving C in solution.

Ion exchange chromatography:

Anion exchange - beads have + charge on them - bind negatively charged (anion) proteins.

Cation exchange - beads have a - charge on them - bind positively charged (cation) proteins.

Choice B: A protein has a native molecular weight of 180 kDa. SDS-PAGE obtained in the presence of β -mercaptoethanol (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).

i) Gel filtration (size exclusion) chromatography. Standards are used to generate a calibration line, the elution volume of the unknown protein is used to find the log MW from the calibration line.

ii) The three subunits add to 90 kDa, so there must be two copies of each subunit in the native structure $2 \times 90 = 180$ kDa. The quaternary structure is $\alpha_2\beta_2\gamma_2$.

iii) The band associated with the α - and β -chains would disappear and a new band, with a molecular weight of 40 kDa ($\alpha + \beta$) would appear because these two chains would be crosslinked with the disulfide bond.