This exam contains **8 pages** and consists of **90** points. Allot 2 pts/min.

1. (15 pts) Provide a brief and *general* description of allosteric effects in biochemical systems (10 pts). Your answer should clearly define tense and relaxed states as well as homotropic and heterotropic compounds. Use *either* oxygen transport to the tissues *or* the adaptation of oxygen transport at high altitudes to illustrate your answer (5 pts).

Allosteric effects involve two inter-converting forms of the protein which differ in shape or conformation (+2 pts).

The tense (T) form is inactive (+2 pts)

The relaxed (R) form is active (+2 pts)

A homotropic allosteric compound affects its own binding. There must be more than one binding site. (+2 pts).

A heterotropic allosteric compound affects the binding of other ligands (+2 pts).

In hemoglobin, oxygen acts as an homotropic allosteric activator (+3 pts), increasing the affinity in the lungs so that it can be fully saturated with oxygen. In the tissues, where the oxygen concentration is low, it converts to the T, or low affinity state, releasing its oxygen (+ 2 pts).

OR

BPG is a heterotropic allosteric inhibitor that binds to, and stabilizes the T-state (+3 pts) This causes a change in the shape of the binding curve to enhance O2 delivery at high altitudes (+2pts).

1.	_____/15
2.	_____/12
3.	_____/ 8
4.	_____/12
5.	_____/ 5
6.	_____/10
7.	_____/10
8.	_____/18
Total	**_____/90**

2. (12 pts) Please answer **one** of the following three choices. Please indicate your choice

Choice A: Briefly describe in *general* terms, using the framework of transition state theory, how enzymes increase the rate of reactions.

- The transition state is a high energy intermediate in the reaction (+2 pts).
- The rate of a reaction is proportional to the concentration of the transition state (+ 2 pts)
- Enzymes catalyze reactions by lowering the energy of the transition state, increasing its concentration (+ 6 pts).
- The energy of the transition state is lowered by either pre-forming organized catalytic residues (therefore there is no entropy change in going to the transition state from the ES complex) or by forming direct contacts with only the **transition state (+ 2 pts)**.

Choice B: Most proteins (enzymes) are highly specific for their ligands (substrates). Briefly discuss why this is the case and illustrate your answer using any protein or enzyme that we have discussed in the course so far.

There are complementary interactions between the ligand and the protein (+4 pts).

These can involve hydrogen bonds, electrostatic effects, hydrophobic effect, van der Waals effects (+ 2 pts).

Examples (6 pts):

i) dinitrophenyl binds to its antibody due to the formation of a hydrogen bond and non-polar (hydrophobic interactions) with Trp residues.

ii) Trypsin cleaves after Lys or Arg resides because the +-charge on the substrate interacts favorable with a negative charge (Asp189) on the enzyme.

iii) Chymotrypin cleaves after Phe, Tyr, Trp residues because these large non-polar sidechains fit well into a non-polar pocket on the enzyme, enhancing hydrophobic effects and van der Waals interactions.

iv) In HIV protease, the non-polar substrate interacts favorable with Val82 in the enzyme.

Choice C: Most enzymes utilize specific residues to accomplish chemical catalysis. Discuss the role of such residues in either the mechanism of serine proteases or HIV protease.

All serine proteases contain the catalytic triad – Ser, His, Asp. The function of these three residues is as follows:

Ser – the nucleophile in the first stage of the reaction (4 pts)
His – activates the nucleophile (ser or water) (4 pts)
Asp – neg charge on Asp stabilizes the + charge on the His during the activation process. (4 pts).

HIV Protease. There are only two active site residues that are responsible for catalysis: Asp25 and Asp25′ (4 pts)

One Asp is deprotonated and activates the nucleophile (water) by proton abstraction. (8 pts).

3. (8 pts) Please do **one** of the following two choices.

Choice A: Enzyme kinetic measurements are usually performed under conditions of "steady-state". Briefly describe what "steady-state" means.

This means that the concentration of the [ES} complex is not changing during the reaction.

Choice B: In enzyme kinetic measurements it is customary to measure the initial rate of the reaction. Why is this important?

The possibility of interference due to the presence of the product is minimized *and* the substrate concentration is known.

4. (12 pts) Compare and contrast a competitive inhibitor and a mixed-type inhibitor. Your answer should include a discussion of the following points (4 pts each).

i) Binding site on the enzyme.
ii) Similarity to substrate
iii) Effect on steady-state kinetics.

A competitive inhibitor binds in the active site. A mixed inhibitor elsewhere (4 pts)

A competitive inhibitor is similar to the substrate, a mixed inhibitor is not (4 pts)

A competitive inhibitor will only affect Km, not Vmax (2 pts)

A mixed inhibitor can affect both Km and Vmax (2 pts)

5. (5 pts) Please do **one** of the following two choices. Please indicate your choice.

Choice A: Define specific activity. How is it utilized in protein purification schemes?

The specific activity is the ratio of the enzyme activity of the target protein divided by the total amount of protein (2 ½ pts). It should increase in a purification scheme (2 ½ pts).

Choice B: *Briefly* explain why gel filtration measures the native molecular weight of proteins.

Gel filtration separates proteins by size (2 pts) the smaller ones can enter the beads and elute later (2 pt). Since the chromatography is performed under conditions that do not denature the protein (1 pt), the native molecular weight is obtained.

Alternate Choice B: *Briefly* explain why SDS-PAGE measures the molecular weight of proteins.

■ In SDS-PAGE the proteins are denatured and given an uniform charge to mass ratio by the SDS. (+2 pts). They are then forced through a gel by an electric field, the smaller proteins migrate faster while the larger ones migrate more slowly, giving separation by size (+ 3 pts).

6. (10 pts) Select **one** of the following three choices. *Choice C is on the following page.*

Choice A: Select **two** of the following purification methods and *briefly* discuss why it can be used to separate proteins (e.g. what is the principle of separation.)

i) Ammonium sulfate precipitation.

Different proteins have different solubility in solutions of this salt. Those with low solubility can be precipitated out of solution (usually in active form) leaving others in solution.

ii) Cation exchange chromatography.

The beads in the column have negative charges on their surface. They bind positively charged proteins. Proteins with a smaller + charge will bind less tightly and elute from the column first.

Alternate part ii) Anion exchange chromatography:

The beads in the column have positive charges on their surface. They bind negatively charged proteins. Proteins with a smaller - charge will bind less tightly and elute from the column first.

iii) Affinity Chromatography.

The beads on the column have a ligand or antibody on the surface that will bind to the target protein. The target protein sticks to the column and all others can be washed out.

Choice B: You are given a mixture of 3 proteins with the characteristics described below.

Name	MW	Amm Suf. Sol.	#Asp/ Glu	#Arg/ Lys	Enzyme Activity
Fatty acid binding protein	15 kDa	1.00 M	10	15	Binds fatty acids.
Lysozyme	14 kDa	1.50 M	5	20	Degrades NAG-NAM poly-saccharides.
Glutathione oxidase	16 kDa	1.25 M	10	5	Oxidizes the tripeptide glutathione.

i) Briefly explain why is it not possible to use ammonium sulfate precipitation to separate these proteins (2 pts).

They all precipitate at almost the same concentration of ammonium sulfate.

Alternate part i) Briefly explain why is it not possible to use gel filtration to separate these proteins (2 pts).

Since the molecular weights are almost the same (~15 kDa) they cannot be separated.

ii) Devise a purification scheme to separate lysozyme from the other two proteins. Briefly justify your answer (8 pts).

Two possibilities:

i) Put NAG-NAM like polysaccharides on the column. The lysozyme will bind, the others won't.

ii) Ion exchange. At pH=6. The charge on the lysozyme is -5+20 = +15. Fatty acid binding protein has a charge of +5 at this pH and glutathione oxidase has a charge of -5. The lysozyme will bind more tightly to a cation exchange column.

Alternate part ii) Devise a purification scheme to separate glutathione oxidase from the other two proteins. Briefly justify your answer (8 pts).

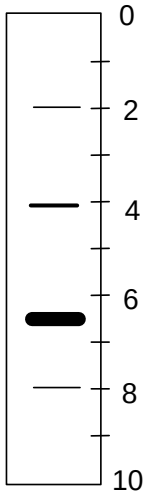
Two possibilities:

i) Put glutathione on the column. The target protein will bind, the others won't.

ii) Ion exchange. At pH=6. The charge on the lysozyme is -5+20 = +15. Fatty acid binding protein has a charge of +5 at this pH and glutathione oxidase has a charge of -5. The glutathione oxidase will bind to an anion exchange column, the others won't.

Q6 – Continued.

Choice C: A protein of unknown quaternary structure is subject to SDS-PAGE in the presence of beta-mercaptoethanol (BME). An image of the gel is shown to the right. Proteins with molecular weights of 10 kDa (log10=1) and 50 kDa (log 50=1.7) were used as standards. These are shown as thin bands on the gel.



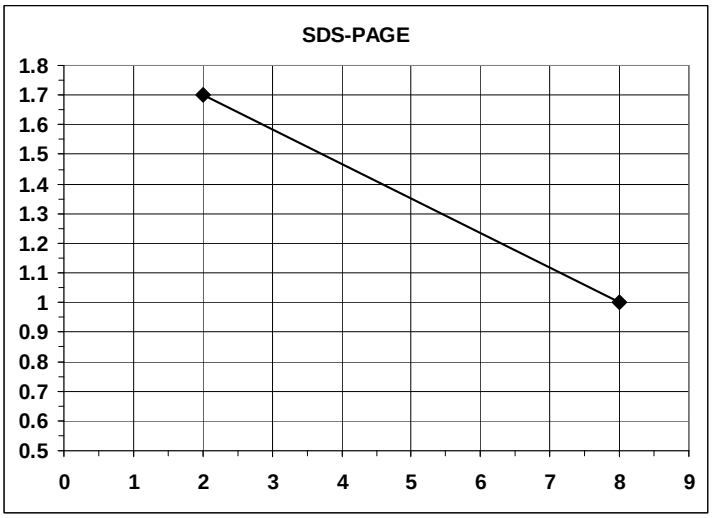
i) What quaternary structures are consistent with the SDS-PAGE gel? Your answer should include both estimates of molecular weight as well as the relative ratio of the subunits (6 pts).

The protein contains two different subunits.

One (α) migrates a distance of 4 cm, corresponding to a log MW of approximately 1.4, or a molecular weight of 25 kDa.

The (β) second migrates 6.5 cm, corresponding to a log MW of 1.15, or a molecular weight of 15 kDa. This band is about 3 times as intense, so a plausible quaternary structure is:

$(\alpha\beta_3)_n$ where 'n' is any integer is consistent with this data.

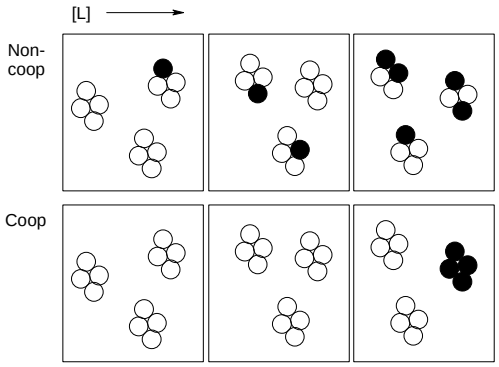


ii) How would you uniquely identify the correct quaternary structure (4 pts)?

Do gel filtration under conditions that give the native molecular weight. If it is 70 kDa, the quaternary structure is $\alpha\beta_3$

7. (10 pts) Please do **one** of the following two choices.

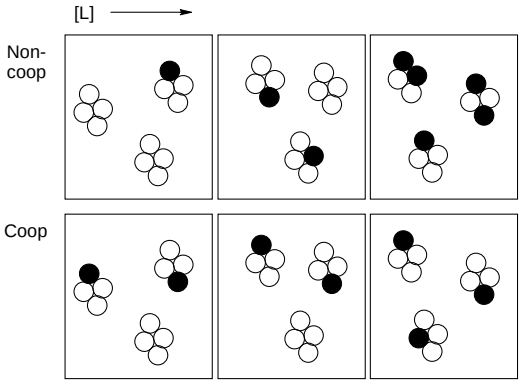
Choice A: The diagram illustrates a collection of homotetrameric molecules. The unfilled circles represent subunits with no ligand bound while the filled circles represent subunits with ligand bound. The top row represents a non-cooperative system while the bottom row represents a cooperative system. The ligand concentration increases from left to right. The top diagram was given to some students, the bottom to others.



i) Provide an estimate of the Hill coefficient for each system. Briefly justify your answer (6 pts).

The non-cooperative system must have a Hill coefficient of 1.

Top: The cooperative system is positive in cooperativity since all bound ligands in the right panel are found on the same tetramer. Since no intermediates are found, it must be infinitely in its cooperative with a Hill coefficient equal to the number of sites (n_h = 4).

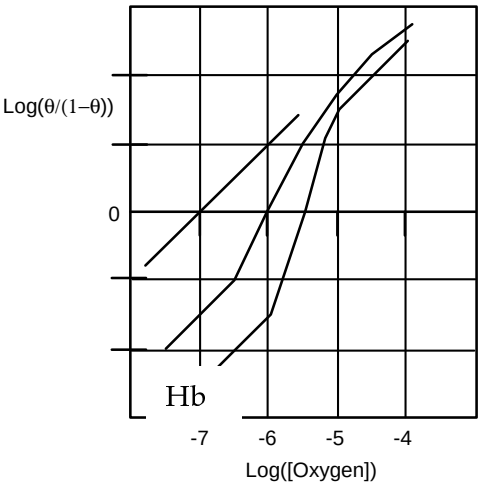


Bottom: The cooperative system shows negative cooperativity. It has bound more ligand at the low concentration, but is difficult to saturate. In addition, the ligands are distributed such that the singly ligand species is most common. (+5 pts for a general discussion along the above lines). The Hill cooefficient < 1. (+1 pt).

ii) Calculate the fractional saturation for the *right* panel for the non-cooperative protein. Please show your work (4 pts).

The fractional saturation is given by [ML]/{[M]+[ML]} = 5/12 = 0.41

Choice B: The Hill plot shows the binding curves for myoglobin (Myo), hemoglobin (Hb) and a mutant (Mut) hemoglobin. The curve for hemoglobin is the middle curve.



i) Label the two other curves with the correct name of the protein (e.g. Myo or Mut). Briefly justify your answer (4 pts).

Myoglobin is non-cooperative, so its Hill plot will have a slope of 1. Therefore it is the left curve. The mutant hemoglobin is therefore the right curve. (4 pts).

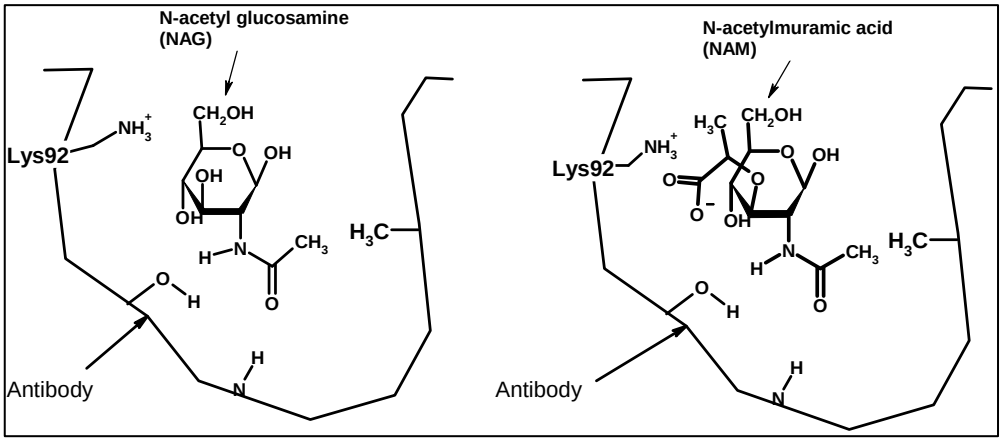
ii) Briefly describe, *quantitatively*, how the mutation has affected *both* the binding affinity *and* the degree of cooperativity of oxygen binding to hemoglobin (6 pts).

- The point of intersection on the x-axis is -5.5, giving a K_D of 1.3×10^{-5} molar, about 10 fold less than that of hemoglobin, with a K_D of 10^{-6} (3 pts).

- The slope of the mutant protein is steeper, almost equal to 4. Therefore the protein is more cooperative than hemoglobin. (3 pts)

8. (18 pts) Please do **one** of the following two choices. The second choice is on the following page.

Choice A: An antibody can bind *either* N-acetylglucose (NAG) or N-acetyl muramic scid (NAM). The structures of these two chemicals, when bound to the antibody are shown below.



The K_D for binding of NAG is 10 μM and the K_D for binding of NAM is 1 μM.

i) An equilibrium dialysis experiment was performed with NAG as the ligand. The concentration of the F_{ab} fragment of the antibody inside the dialysis bag was 1μM. The equilibrium concentration of *free* ligand outside the dialysis bag was 1μM. What is the equilibrium concentration of total ligand *inside* the bag (please show your work) (4 pts).

The fractional saturation, $\theta = [L]/\{K_D + [L]\} = 1\mu M/\{10\mu M + 1\mu M\} = 1/11 = 0.09$. Therefore the amount of the [ML] complex is 0.09 μM and the total amount of ligand in the bag is 1 + 0.09 μM = 1.09 μM.

ii) Which ligand binds more tightly to the antibody, NAG or NAM? Briefly justify your answer with reference to *both* the K_D values *and* to the molecular structure of the antibody-ligand complex (6 pts).

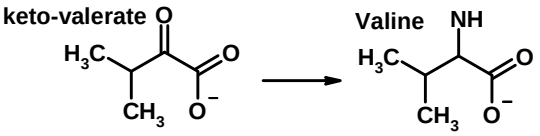
NAM because its K_D is lower (4 pts). This is probably due to the favorable electrostatic interaction between Lysine92 and the negative charge on NAM (2 pts).

iii) If lysine 92 on the antibody is changed to glutamic acid (sidechain=-CH2-CH2-COOH), how will the K_D values be affected for *both* ligands (8 pts)?

- It probably wouldn't change much with NAG, since there is no charge on the ligand near Lys92.
- The affinity for NAM would go down (higher K_D) because of electrostatic repulsion between the negative charge on the Glutamic acid and the charge on the NAM.

Q8 – Second Choice.

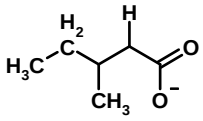
Choice B: A transaminase catalyzes the reaction shown to the right. Steady state kinetic measurements were performed and the velocity and double reciprocal plots are shown below.



i) *Estimate* the K_M for the substrate (keto-valerate) using the velocity curve. Describe your approach. (5 pts).

When the initial velocity = $V_{MAX}/2$, [S] = K_M.
V_{MAX} is approximately 100, so when v=50, [S]= 1 μM.

ii) The inhibitor shown to the right binds to the enzyme with a K_I of 0.1 μM (10^{-6} M). Why is this compound an inhibitor? (4 pts).

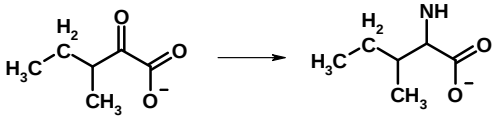


It looks very similar to the substrate, yet lacks the keto-oxygen that is converted to an –NH in the actual substrate.

iii) Sketch on the double reciprocal plot the line that you would expect to obtain if the concentration of the inhibitor in the reaction was 0.4 μM. Briefly justify your answer. (5 pts)

This is a competitive inhibitor, so only the slope will increase, by a factor a. a = 1 + (0.4μM/0.1μM) = 5, i.e. the line will have the same y-intercept, but will show a slope 5 times greater.

iv) Based on the above K_M and K_I values, predict the K_M value for the reaction shown on the right. Briefly justify your answer. (4 pts).



This substrate is almost identical to the inhibitor., the only difference is the substrate as a C=O group that has been replaced by a proton. Consequently the K_M for the substrate should be similar to the K_I for the inhibitor, or about 0.1 μM.