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).

| | |
|---|---|
| 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.

**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.

**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.

**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.

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

**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.

**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?

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

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**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.

ii) Anion exchange chromatography.

iii) Affinity Chromatography.

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

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

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

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

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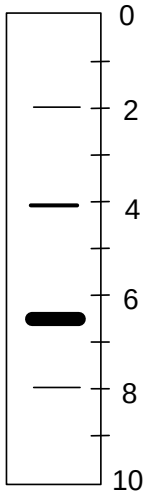
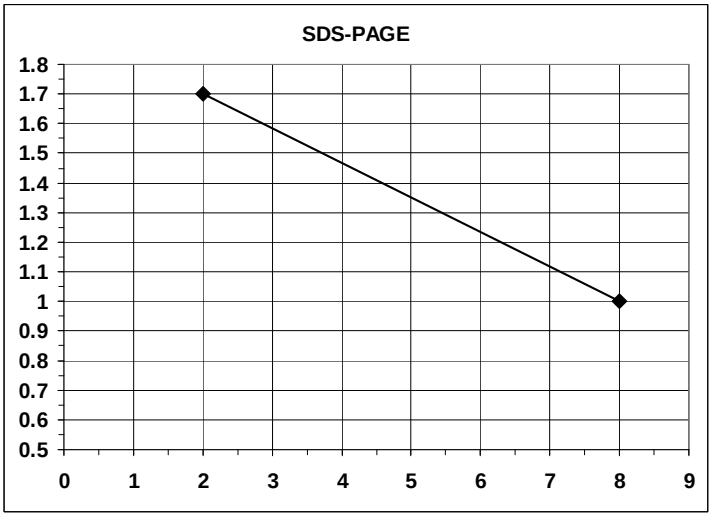
**Q6 – Continued.**

**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.



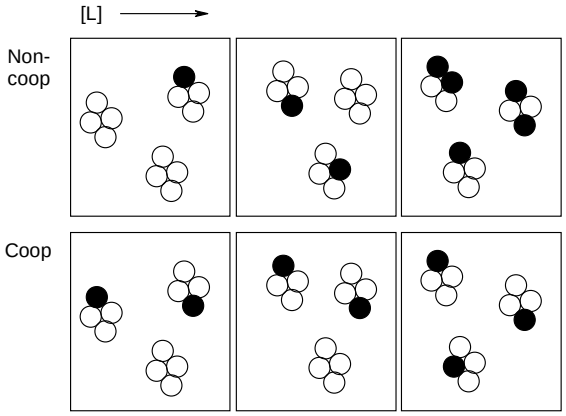

i) What quaternary structures are consistent with the SDS-PAGE gel? Your answer should include both estimates of molecular weight as well as the relative ratio of the subunits (6 pts).




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

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

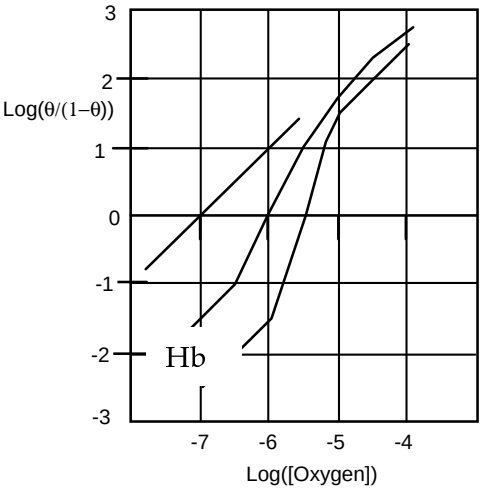
**Choice A**: The diagram illustrates a collection of homotetrameric molecules. The unfilled circles represent subunits with no ligand bound while the filled circles represent subunits with ligand bound. The top row represents a non-cooperative system while the bottom row represents a cooperative system.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




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

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

**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.

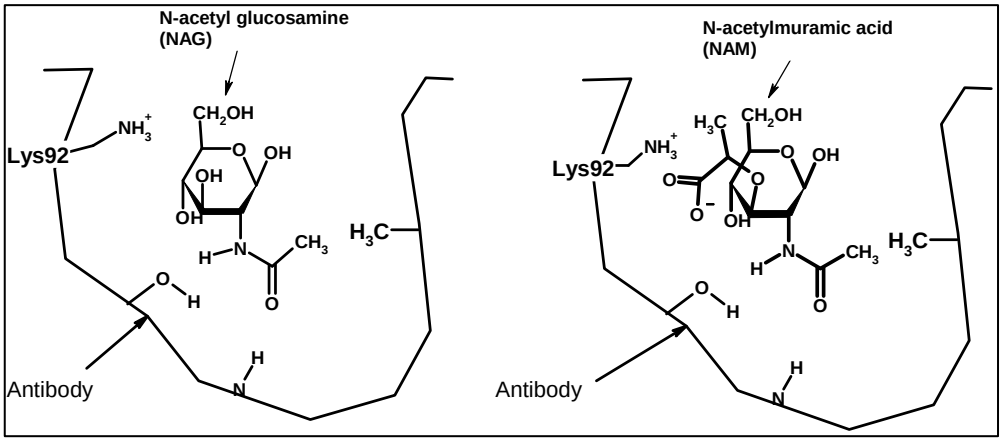



i) Label the two other curves with the correct name of the protein (e.g. Myo or Mut). Briefly justify your answer (4 pts).

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).

**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.

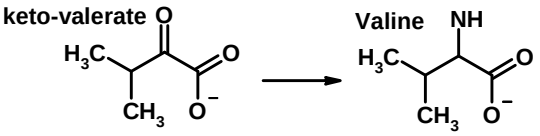
i) An equilibrium dialysis experiment was performed with NAG as the ligand. The concentration of the $F_{ab}$ fragment of the antibody inside the dialysis bag was 1μM. The equilibrium concentration of *free* ligand outside the dialysis bag was 1μM. What is the equilibrium concentration of total ligand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

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).

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 (NAG and NAM) (8 pts)?

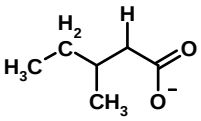
*Q8 – Second Choice.*

**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 (The substrate concentration is μM). Describe your approach. (5 pts).

ii) The inhibitor shown to the right binds to the enzyme with a $K_I$ of 0.1 μM ($10^{-7}$ M). Why is this compound an inhibitor? (4 pts).



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)

iv) Based on the above $K_M$ and $K_I$ values, predict the $K_M$ value that you would expect to find for the substrate in the reaction shown on the right. Briefly justify your answer. (4 pts).