Name: _____Key_____

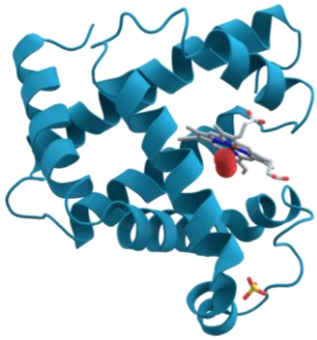
Chem 3511
Exam 3

The exam starts on the next page. It has 12 questions, worth a total of 100 points. Please write legibly and don't assume that long answers are required if there is a lot of space left for your response. No calculators are allowed.

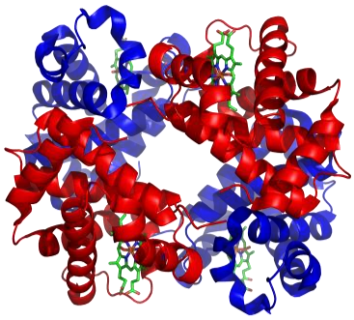
A test-taking tip: go through the whole exam and do the easy questions first. Then tackle the ones you find to be more difficult. Good luck.

Name: _____Key_____

1. (**12 points**) Below are the cartoon structures of myoglobin and hemoglobin.



myoglobin



hemoglobin

a. What levels of protein structure do we find in myoglobin? Name each of the levels and provide a one sentence definition for each. (**9 points**)

3pts- primary structure which is the entire linear sequence of amino acids from N to C terminus

3pts- secondary structure which is the formation of structures such as alpha helices, turns/loops, and beta-sheets

3pts- tertiary structure which is defined by the formation of particular motifs with one or more secondary structure. This is only present ion proteins that have multiple subunits.

b. What level of protein structure is present in hemoglobin but not in myoglobin? Name the level and provide a one sentence definition. (**3 points**)

3pt- Quaternary structure- folded protein that contains a multiple subunit complex

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2. (**3 pts**) Which of the following peptides is most likely to form a α-helix in the cytosol?

A. GSGAGAGSGSG
B. RKFYKRFYKFR
C. PSPAPAPSPSP
D. EKAVEMAVRAK
E. EFAVIMAVLAK (1.5 pts: not fully correct, too many hydrophobic residues, but you understand what makes a helix most likely to form)

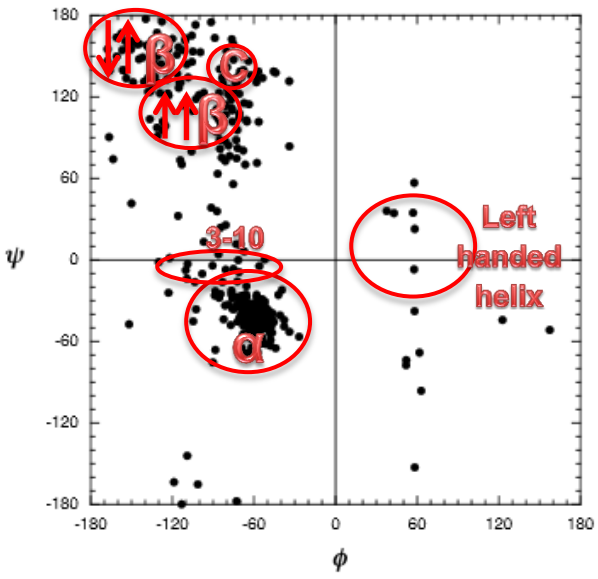
3. (**3 pts**) For β-sheets, the terms 'parallel' and 'antiparalllel' refer to _____.

A. the 'direction' of the associated peptide strands
B. the orientation of the amide cross-links
C. the quaternary structure of the protein
D. the orientation of the hydrogen bonding
E. the topology of the reverse turns

4. (**3 pts**) Native protein purifications often require multiple reaction steps in order to purify the protein of interest from other proteins. One method used for protein separation in purification procedures is a change from water to an organic solvent. Which of the following would be accomplished by this solvent change?

A. Proteins with hydrophobic groups on the interior would maintain their native state.
B. Proteins with hydrophilic groups on the exterior would denature and likely precipitate.
C. Proteins with exposed hydrophobic groups would maintain their structure and remain in solution.
D. Both A and B would occur.
E. Both B and C would occur.

5. (**6 pts**) Below is the Ramachandran plot for yeast hexokinase. Based on this data, what are the major secondary structures found in this protein? Circle and label each of the secondary structures on the Ramachandran plot.
2pts for beta-sheets, 2pts for alpha helices, 2pts for any other structure



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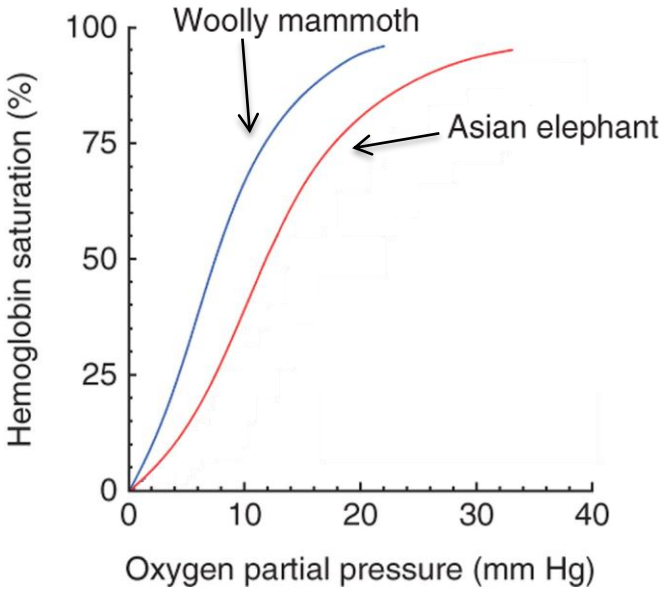
6. (**10 pts**) In the paper by Campbell et al. "Substitution in woolly mammoth hemoglobin confer biochemical properties adaptive to cold tolerance" Nature Genetics 42, 536–540 (2010), the authors determined the oxygen binding curves for hemoglobin of the woolly mammoth, which lived in artic environments, and that of the Asian elephant, which lives in tropical environments.



a) What is the p50 for woolly mammoth and asian elephant? (**4 pts**)

2pts: About 8 mm Hg for Woolly

2 pts: About 12 mm Hg for Asian

b) Does the hemoglobin of woolly mammoth or asian elephant have a higher affinity for oxygen? (**3 pts**)

2pts: Woolly

c) Assuming that the woolly mammoth evolved from the asian elephant via cold adaptation, based on this data, what has been the effect of cold adaptation on hemoglobin? (**3pts**).

3pts: Tighter binding of oxygen to hemoglobin in woolly. P_{50} wolly < P_{50} asian.

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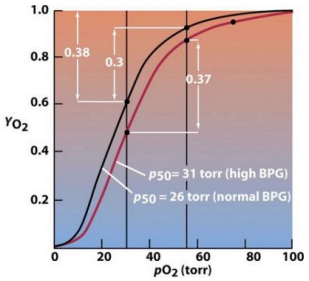
7. (**8 pts**) As one of the favorites to win the La Paz, Bolivia marathon (3700m altitude) you have been training there for several weeks. The company sponsoring your main running competitor has invited you and other runners to a pre-race beach party in Lima, Peru (sea level) two days before the race. The company has promised to get you all back to La Paz the night before to the race. If your goal is to win the race would you accept the invitation? **Explain** your decision using biochemistry. Make sure to use a graph to explain your answer.



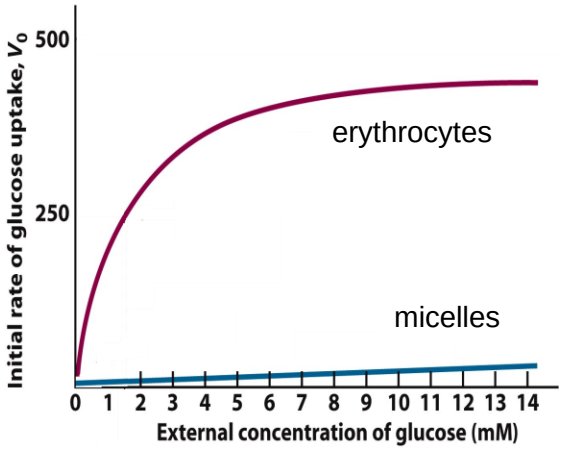
2pts No,

By training at higher altitudes your body has increased its levels of 2,3-BPG which would stabilize the deoxygenated state of hemoglobin thus allowing a faster release of O_2 to oxygen starved tissues. By going to the party in Lima, you have 2 days of sea level air exposure which will result in lower levels of 2,3-BPG in your body. This will make hemoglobin more stable in the oxygenated state and less susceptible to O_2 release.

3pts; for explaining the effect of 2,3-BPG

3pts for drawing the curves at sea level and high altitude

8. (**6 pts**) Below is the glucose uptake rate shown by erythrocytes and by a micelle. Explain what kind of glucose transport erythrocytes and micelles use.



3points: Erythrocytes: facilitated transport- due to the availability of certain transporters that are saturated with increasing [glucose]

3 points: Micelles: free diffusion, no sturation

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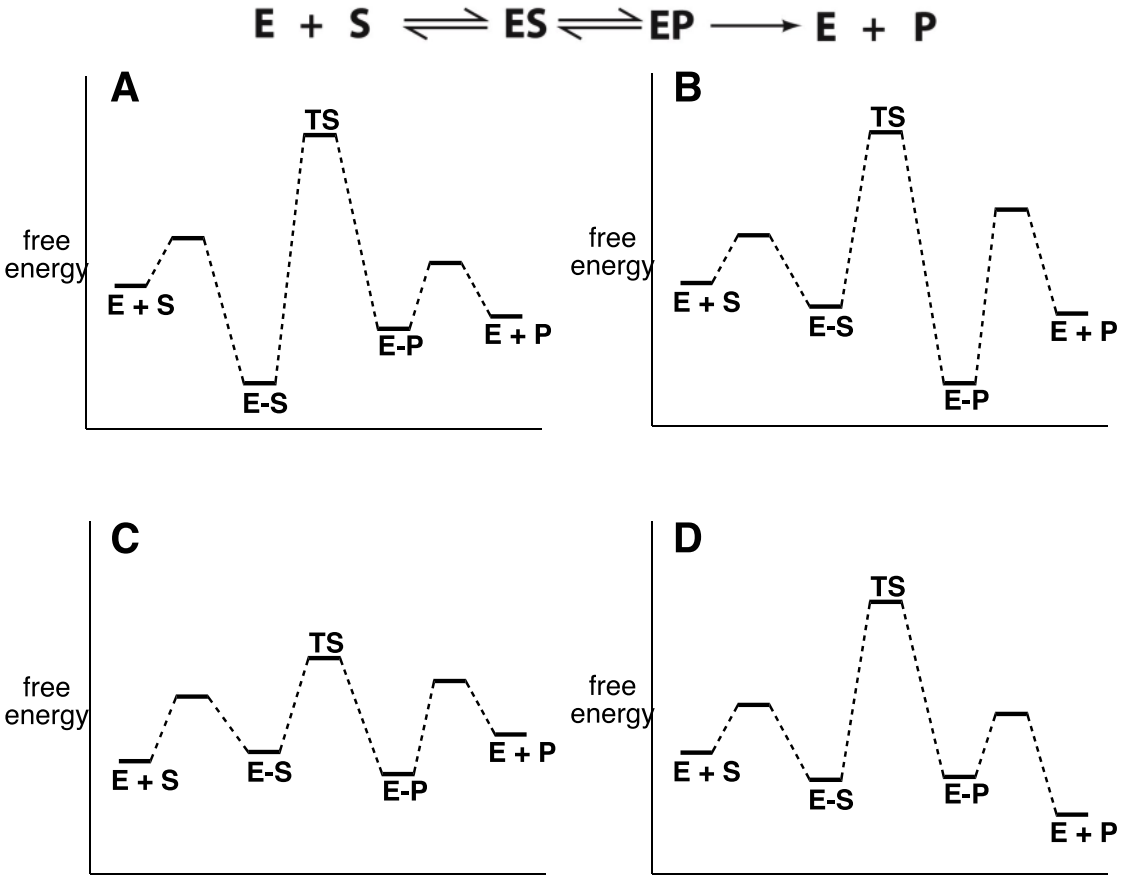
9. (**8 pts**) Here are four reaction coordinate diagrams for an enzyme catalyzed reaction.



Using the identifiers **A** – **D**, indicate which diagram corresponds to the following situations:

2 points per correct answer

- The reaction is thermodynamically disfavored (not "spontaneous") **C**

  Reaction where the E+P has a higher free energy E+S

- The reaction with the slowest product (P) formation **A**

  Reaction with the highest ΔG^{TS}

- The reaction with the fastest product (P) formation **C**

- Reaction with the lowest ΔG^{TS}

- The reaction with the slowest product (P) *release* **B**

  Reaction with the highest activation barrier between E-P and E+P

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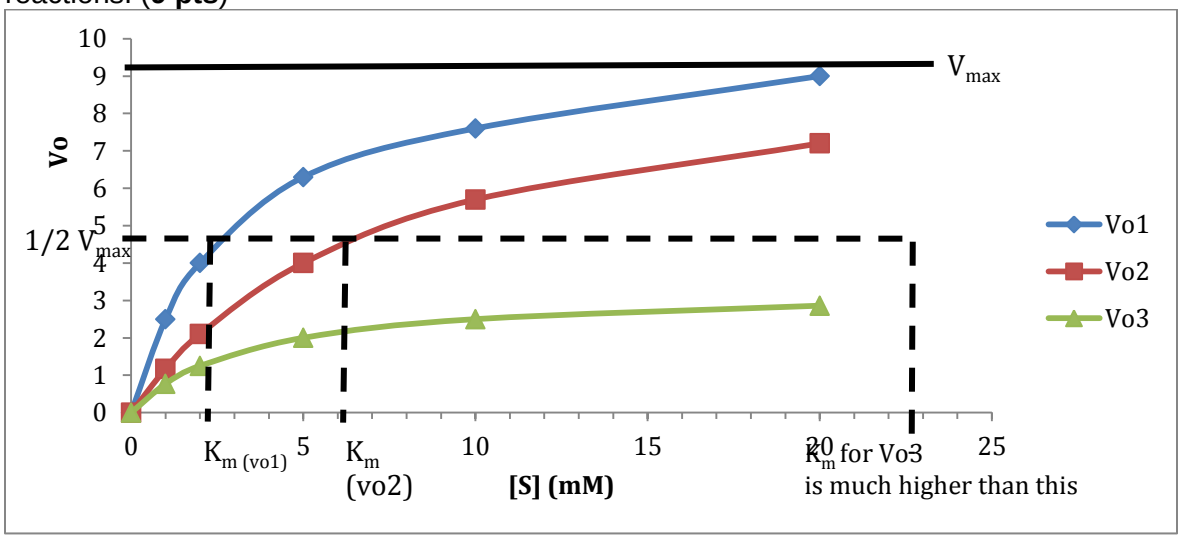
10. (**19 pts**) The following table indicates the rates at which a substrate reacts as catalyzed by an enzyme that follows Michaelis-Menten mechanism:

(1) in the absence of inhibition
(2) in the presence of inhibitor A
(3) in the presence of inhibitor B

[S] (mM)	(1) v_o (µM/sec)	(2) v_o (µM/sec)	(3) v_o (µM/sec)
1	2.5	1.17	0.77
2	4.0	2.10	1.25
5	6.3	4.00	2.00
10	7.6	5.7	2.50
20	9.0	7.2	2.86

a) Plot the initial velocity (v_o) versus the substrate concentration for each of the three reactions. (**9 pts**)



b) What is the V_{max} and K_m for the enzyme in the absence of inhibitor? (**4pts**)

V_{max} is Vo at very high [S]. Based on the graph, Vo is approaching 9.5. Vmax is about 9.5 **(2points)**

K_m is [S] at ½ V_{max}, Km= ~2.5 mM **(2points)**

c) What additional information would be required to calculate the enzyme turnover? (**2pts**)

Initial total concentration of enzyme → $[E_0]$ or $[E_t]$

d) What type of inhibition is seen for inhibitor A and inhibitor B? Give a one line justification for your choice of type of inhibition (**4pts**)

A: competititve inhibitor (2) K_M=~6, V_{max} = 9.5 **(1 point)**

Explanation 1point

V_{max} unchanged: Based on the graph both (1) and (2) eventually reach the same Vmax

K_M changes: K_M increases in the presence of inhibitor A when compared to non-inhibitor (1)

B: competititve inhibitor: (3) K_M= very high, Vmax= ~3 **(1 point)**

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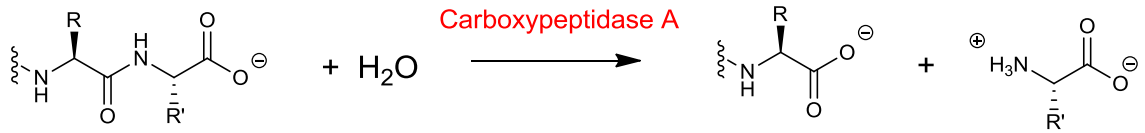
Explanation 1 point

Apparent Vmax and KM change when compared to (1) -> uncompetitive inhibitor

11. (**16 pts**) Carboxypeptidase A carries out the following reaction

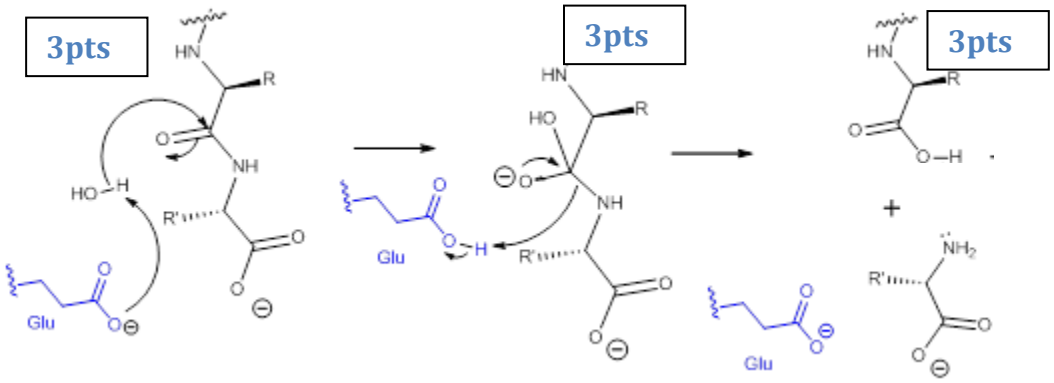


Polypeptide (n residues) | Polypeptide (n-1 residues) | Amino acid

Based on experimental data, the following is known about this enzymatic reaction:

- Replacing a Glu residue for Ala in the enzyme active site inactivates the enzyme.
- The molecular weight of the enzyme does not change during the reaction.

a) Draw the mechanism for the carboxypeptidase A reaction. Make sure to draw the three stages: before, during and after the reaction (**9 pts**)



b) What type of catalytic mechanism does the enzyme carry out? (**2 pts**)
Acid-base mechanism

c) Why does replacing a Glu with Ala result in enzyme inactivation? 2 sentences max. (**3 pts**)

The glutamate's carboxylate side chain deprotonates water which then attacks the carbonyl to form the tetrahedral intermediate. An alanine contains a $–CH_3$ side chain which is not able to deprotonate the water to initiate the attack.

d) What is the best residue to replace Glu with, and still achieve catalysis? (**2pts**)

Aspartate: It has a carboxylic acid. It is just one carbon shorter than Glu so it should be the best fit.

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12. For each of the cartoons below, name their 3^{ry} structure and one 2^{ry} structural motif (**6pts**).
1pt per 3ry structure, 1pt 2ry structural motif (x3)

Tertiary: alpha helical bundle (or mostly alpha)

β -α- β motif (or mixed alpha, beta)

β-hairpin or greek key (or mostly beta)

2ry structural motif: alpha helix

alpha helix or beta sheet

beta sheet