C483 Exam 1
Spring 2016

Name Key Seat Number _____

Student ID _____ AI _____

The last page of this exam contains pKa values and other information you might find useful.
This exam contains 110 points. The highest score you may earn on this exam is 100 points.

1 _____/20pts

2. _____/10pts

3. _____/20pts

4. _____/10pts

5. _____/10pts

6. _____/10pts

7. _____/10pts

8. _____/10pts

9. _____/10pts

Total:

Regrading: All requests for regrades must be submitted in writing within 48 hours of the return of the exam. You must explicitly state what has been misgraded and why it is an error. The entire exam will be regraded, which could result in points being added or deducted overall.

Section 1: Reading guides (50 points)

1. 20 pts. Fill in the blanks (2 points each.)

A. The Sanger sequencing method uses a polymerase, primer, and ddNTPs, which cause termination of the growing polynucleotide at various points.

B. An example of a regular secondary structure is α helix / β sheet.

C. Enzymes bind tighter to the TS (transition state) than the substrate in order to lower the activation energy of a reaction.

D. One way that serine proteases catalyze amide hydrolysis is by the presence of an oxyanion hole, which stabilizes the tetrahedral intermediate through hydrogen bonding.

E. The enzyme class which catalyzes bond formation coupled with ATP hydrolysis is ligase.

F. The saturation curve of hemoglobin is sigmoidal shaped because of cooperativity.

G. Amino acids are connected in a protein through peptide bonds.

H. Hydronium appears to move faster than the diffusion limit due to proton jumping, which makes acid-base reactions very fast in water.

I. A 0.01 M HCl solution has a pH of 2.

J. Protein tertiary structure is stabilized mainly by the hydrophobic effect.

2. 10 pts. Write True or False (1 points each)

A. False The conversion of an alkene into an alcohol is an example of an oxidation reaction.

B. True The first pKa of NaH_2PO_4 is about 7.

C. True DNA double helices with high G-C content have higher melting points than those with lower G-C content.

D. False In blue/white colony screening, white colonies are selected because they have intact galactosidase genes in their recombinant plasmids.

E. True Two peptides can form a disulfide bond with each other if they both contain the amino acid with the one letter abbreviation C.

F. True Phenylalanine is more likely than aspartate to be in the core of a protein.

G. True Micelles, vesicles, and lipid bilayers are all composed of amphipathic molecules.

H. False A protein sample was determined to have a mass of 60 kDa by size exclusion chromatography. After treating a sample of this protein with urea, SDS-PAGE analysis gave one polypeptide with a mass of 30 kDa. These data are consistent with a protein with homotrimeric quaternary structure.

I. True Polymerization of fibrous proteins like tubulin and actin is driven by ATP hydrolysis.

J. False The central cavity of myoglobin does not bind 2,3-bPG, giving myoglobin a greater affinity for oxygen than hemoglobin.

3. 20 pts. Short answer (5 points each)

A. What percent of phenol is ionized in a pH 10.5 buffer?

+3

$pH = pK_a + \log \frac{A^-}{HA}$

$10.5 = 9.9 + \log \frac{A^-}{HA}$

$4.0 = \frac{A^-}{HA}$

$A^- = \frac{4}{1.1} = 80\%$ (+2)

B. To which class of enzyme do each of the following enzymes belong?

Chymotrypsin hydrolase

Pyruvate kinase transferase

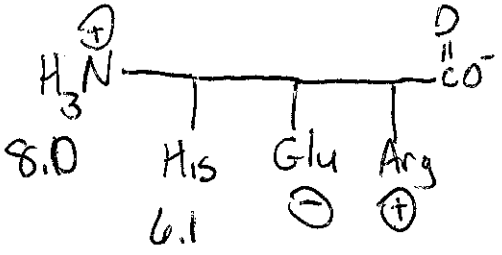
Alanine racemase isomerase

Malate dehydrogenase oxidoreductase

Alanine aminotransferase transferase

+1 each

C. Calculate the isoelectric point of the His-Glu-Arg tripeptide using data from the last page.



$IP = \frac{8 + 6.1}{2} = 7.1$ (+5)

D. A tetrapeptide was shown to be composed of alanine, lysine, phenylalanine, and threonine. Use the following data to determine its sequence.

1. Chymotrypsin digest leads to two dipeptides.
2. Trypsin cuts this tetrapeptide into lysine and a tripeptide.
3. Elastase doesn't digest this tetrapeptide.

Lys - Phe - Thr - Ala

(+1) (+1) (+1) (+1)

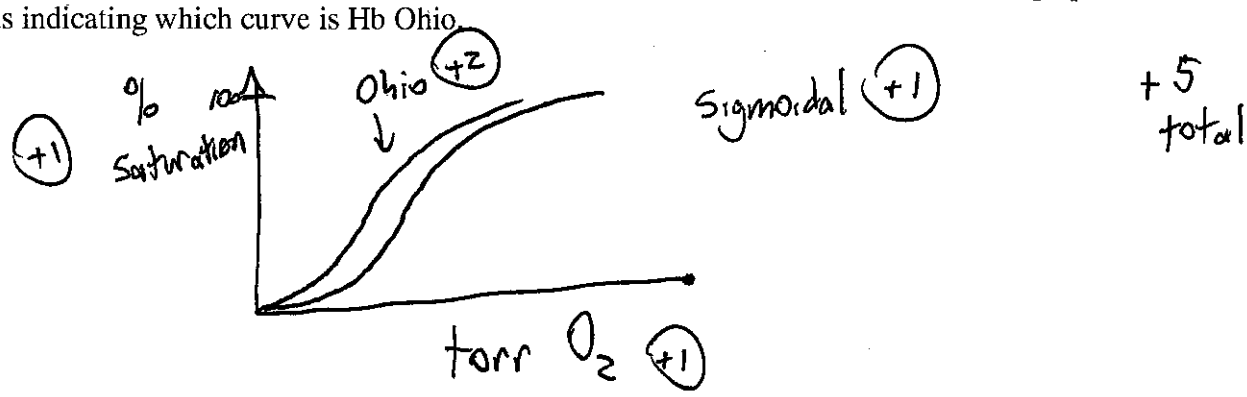
+5 if all right

Section 2: Problems (10 points each)

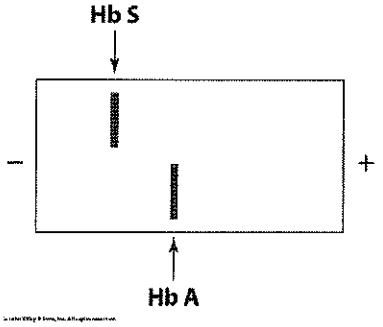
4. A mutant form of hemoglobin called Hb Ohio has a 142Ala→Asp mutation which causes a change in alpha helix structure, which in turn disrupts salt bridges in the central cavity of Hb Ohio. What effect does this mutation have on the oxygen binding affinity of Hb Ohio compared to normal hemoglobin (Hb A)?

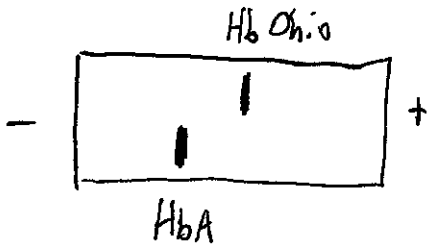
- Decreased stability of deoxy form
- leads to Hb Ohio having increased affinity (+3)

Draw an oxygen binding curve comparing Hb Ohio to Hb A. Label the axes of the graph, as well as indicating which curve is Hb Ohio.



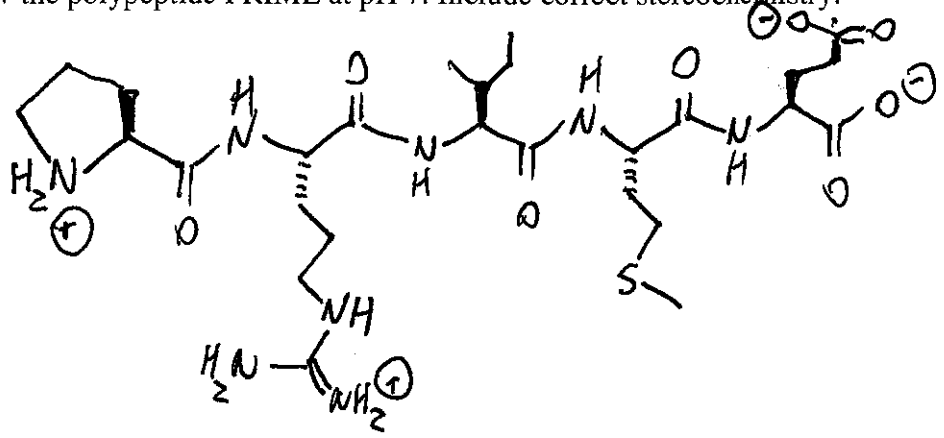
Hemoglobin can be analyzed by electrophoresis at pH 8.5, where the proteins have net negative charges. Samples are applied to the negative end of the gel and allowed to move. Example results are shown for sickle cell hemoglobin (HB S) and HbA. Draw a similar figure expected for the analysis of Hb Ohio and Hb A.





(+3)

5. Draw the polypeptide PRIME at pH 7. Include correct stereochemistry.



Each sidechain +1
Backbone +2
Stereochem +1
Charge +2

6. The coding strand of a short gene is CGTAATGTCGCC.

A. What is the sequence of the mRNA produced for this gene, written 5'→3'?

5' CGUAAUGUCGCC 3' (+3)

Common wrong answer → GCA|UUA|CAG|CGG

B. What is the sequence of the tetrapeptide produced from this gene? (You may use abbreviations.)

Arg - Asn - Val - Ala (+4)

Common wrong answer → Ala - Leu - Gln - Arg

C. Suggest a point mutation to the coding strand that would cause the tetrapeptide produced to have a decreased affinity for ion exchange chromatography and give the sequence of the mutant tetrapeptide.

* Change Arg. (+3)

CGU

U, A or G ↑ C or U (A acceptable

7. To an aqueous solution of the dipeptide Cys-Tyr was added 0.50 moles of Tris buffer sodium salt (the conjugate base of Tris.) 30.0 mL of 6.0 M HCl was added to the solution, then enough water was added to bring the total volume of the solution to 1.00 L. Draw the structure of the dipeptide as it exists in this buffer. Show all your work.

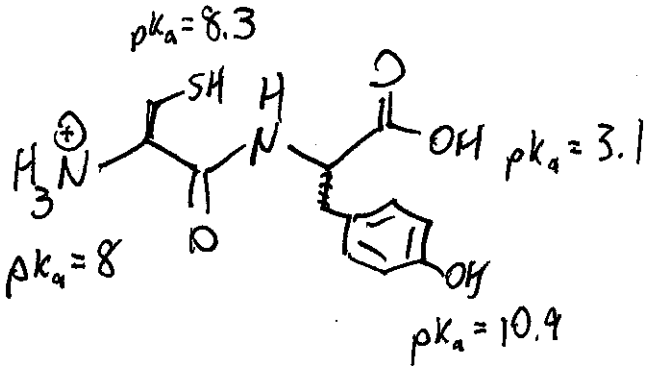
Buffer 0.50 M Tris with 0.18 M HCl added

Therefore, buffer is 0.32 M Tris (A^-)
0.18 M Tris (HA)

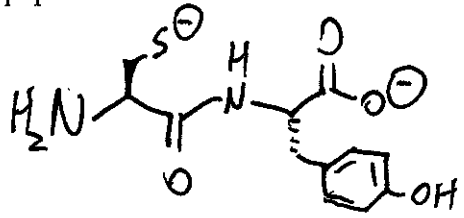
$$pH = pK_a + \log \frac{A^-}{HA}$$

$$pH = 8.3 + \log \frac{.32}{.18} = 8.55 \quad (8.6)$$

+6 buffer



dipeptide structure

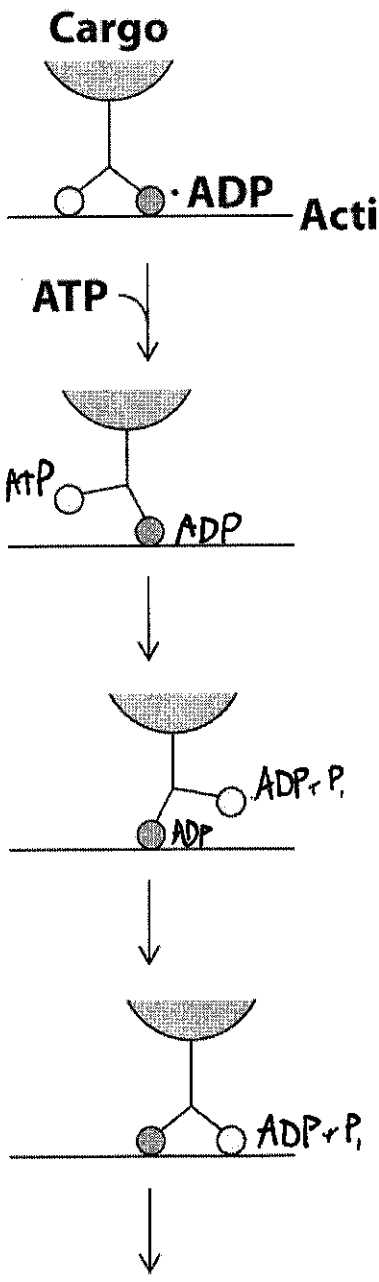


+4

+1 for each charge (assuming structure)

8. Myosin type V is a two-headed myosin that operates as a transport motor to move its attached cargo along actin filaments. Its mechanism is similar to that of muscle myosin, but it acts processively, like kinesin. The reaction cycle diagrammed here begins with both myosin V heads bound to the actin filament. ADP is bound to the leading head, and the nucleotide binding site in the trailing head is empty. Propose a mechanism for myosin V movement, starting with entry of ATP. Write a short description of what happens in each step.

+3 some mechanism
+5 mechanism using hydrolysis/binding
+8 coherent mechanism using hydrolysis



+10

ATP binding causes release of trailing head from actin

ATP hydrolysis causes the leading head to swing forward

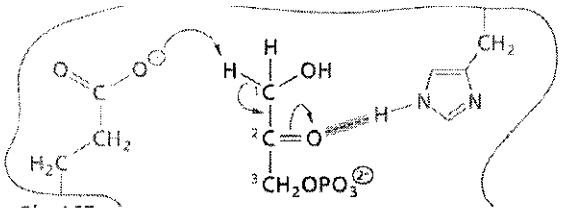
ADP release or P_i release causes new leading head group to bind actin

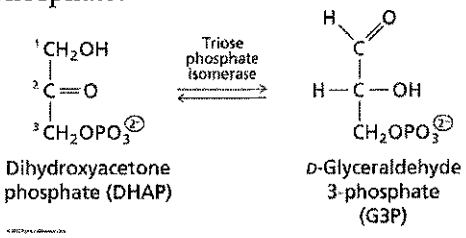
P_i release or ADP release allows ATP to bind in next step

some variation

Section 3: Case study (10pts)

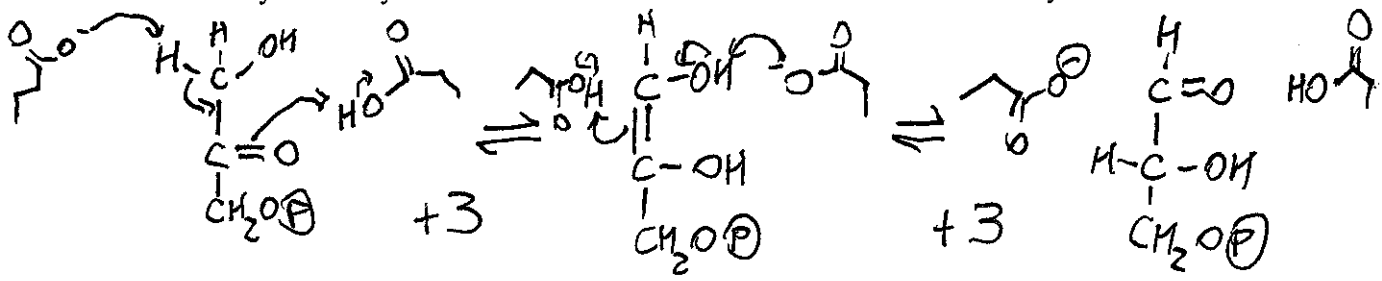
9. The active site of the enzyme triose phosphate isomerase is shown below. It catalyzes the isomerization of DHAP to glyceraldehyde 3-phosphate.





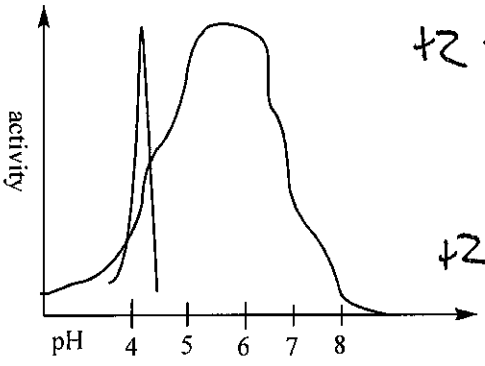
A mutant form of this enzyme has been produced in which the histidine residue has been replaced with aspartate. Remarkably, it has significant activity, but over a much narrower range of pHs. The pH profile of the normal enzyme is bell shaped with a maximum around pH 6, but the mutant has a bell shaped curve with a maximum around pH 4.

A. Draw an enzyme catalyzed mechanism for the reaction with the mutant enzyme.



* Can also be stepwise
* must include CA, CB and enediol

B. Explain why the pH profile of the mutant is so narrow and why it maximum has shifted from 6.0 to 4.0



+2 In the mutant, Glu residues are acting as both General acid and General base. They are only at

+2 appreciable concentration at their pH= pKa of 4.1. Much higher or lower pH will destroy activity.