

YOUR NAME: KEY

SECTION (circle one)      Morning      or      Afternoon

NOTES:

1. where appropriate please show work - if in doubt show it anyway.
2. pace yourself - you may want to do the easier questions first.
3. please note the point value of questions - adjust your answers and effort accordingly.
4. some questions may have more data than you need.
5. please be brief - unfocused, rambling answers won't receive as much credit as a few short appropriate phrases.
6. Please write CLEARLY - if I cannot read it - it is wrong.
7. A glycolysis chart is included at the back of this exam (on page 11).  
Detach (carefully) if you wish. Please make sure you have all 11 pages.
8. Good luck

Question 1. (10 pts) What is the yield of ATP per molecule of the following converted to lactate. Insert a number from 0-100 in the space provided.

Fructose

2 ea

2

Sucrose in the diet

4

Maltose in the diet

4

Dihydroxyacetone-P in the presence of arsenate

1

For a typical glucose molecule released from glycogen intracellularly

3

Question 2. (6 pts.) Short problems. Show work, but **most** credit goes to the correct numerical answer.

a. an enzyme has a  $V_{max}$  of  $0.55 \mu\text{mol}/\text{min}$  and a rate of  $0.12 \mu\text{mol}/\text{min}$  as  $260 \mu\text{M}$  substrate. What is the  $K_m$  for the substrate

$9.32 \times 10^{-4} \text{ M}$   
 $K_m = 932 \mu\text{M}$

~~Handwritten scribbles~~

$$v = \frac{(0.55 \mu\text{mol}/\text{min})(260 \mu\text{M})}{K_m + (260 \mu\text{M})} = 0.12 \mu\text{mol}/\text{min}$$

$$K_m = \frac{.55}{.12}(260 \mu\text{M}) - 260 \mu\text{M}$$

b. a reaction:  $A \leftrightarrow B + C$  has a standard free energy of  $+4 \text{ kcal}$ . If A is maintained at  $10 \text{ mM}$ , what concentration of B and C (assume  $[B] = [C]$ ) would be in equilibrium with A? Assume  $27^\circ\text{C}$ ,  $R = 2 \text{ cal}/\text{mole}/^\circ$ .

$3.55 \times 10^{-3} \text{ M}$   
 $[B]=[C] = 3.55 \text{ mM}$

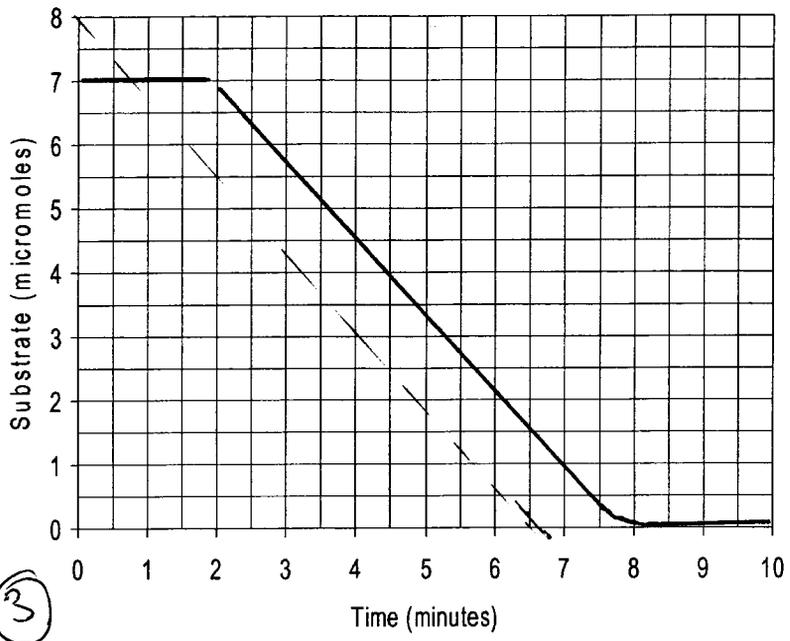
$$\Delta G = 0 = 4000 + 2.3(2)(300) \cdot \log K$$

$$\log K = -2.899$$

$$K = 1.263 \times 10^{-3} = \frac{[B][C]}{10^{-2} \text{ M}}$$

① 0.9    ② 1.0    ③ 1.01    ③ 1.02    ③ 1.03    ② 1.4    ① 1.5

Question 3 (6 pts.) The graph to the right shows an enzyme assay converting a single substrate into a single product (S → P). It was started at time 2 minutes by the addition of 7 micrograms of enzyme to a solution of 1 mL of substrate containing the amount of substrate shown in the graph. The pH was 7.5 and the temperature 25 °C.



Answer the following questions - there is more information than you need.

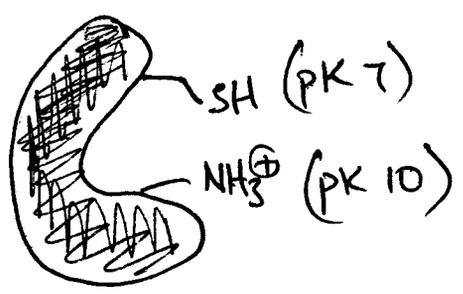
$\frac{8 \mu\text{mol}}{6.5 \text{ min}}$     1.01 - 1.03    ③  
1.0 to 1.04

- a. Calculate the rate of the enzyme assay ~ 1.02 micromole substrate/min    ③
- b. What is the rate in the absence of enzyme? ~ 0 micromoles/min    ①
- c. what is the *concentration* of substrate in the assay before the addition of enzyme  

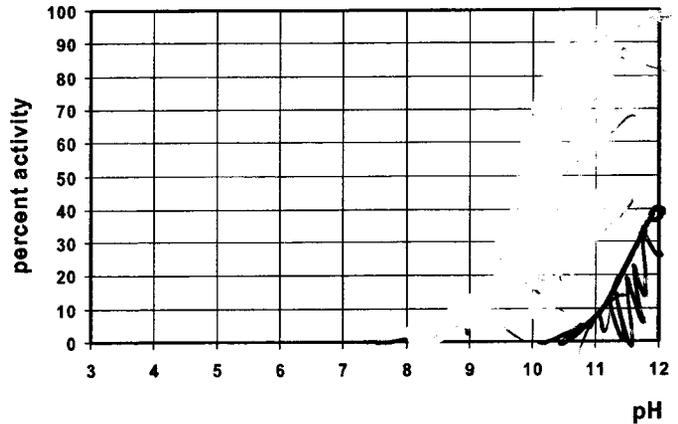
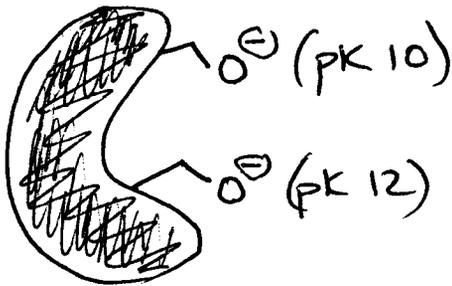
$$\frac{7 \text{ } \mu\text{M}}{7 \times 10^{-3} \text{ M}}$$
    ②

Question 4 (9 pts.) Draw the pH activity curves for the following situations.

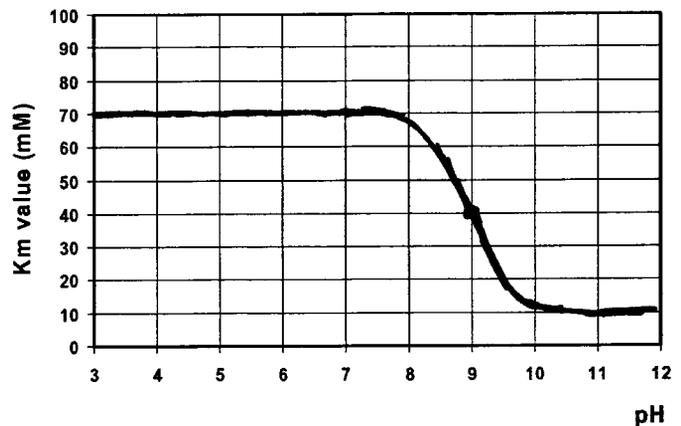
a. Only this protonic form is active.



b. This enzyme is only active as shown



c. An enzyme has a lysine side chain (pK of 9 which solely determines the  $K_m$  for the substrate. The protonated side chain shows a  $K_m$  of 70 mM and the deprotonated form shows a  $K_m$  of 10 mM. Complete the graph.



Question 5 (5 pts) Draw an equation to illustrate the reaction stoichiometry of adenylate kinase:



(3)

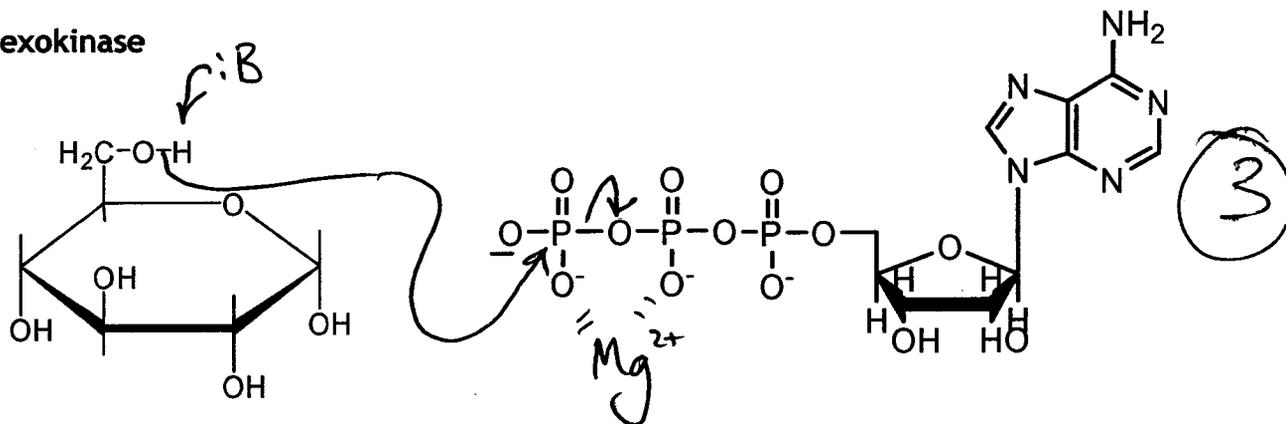
You add phosphofructokinase to a tube containing these (initial) concentrations of substrates/products: [Compound #3] = 5 mM [Mg ATP] = 5 mM

Why is the production of compound #4 accelerated as the reaction progresses by adding small amounts of adenylate kinase?

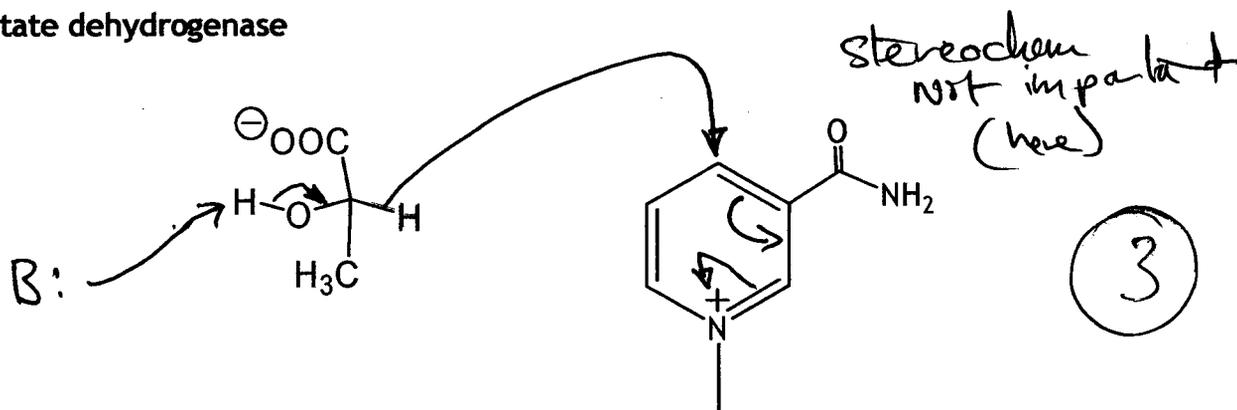
(2) Because AMP is a much more potent activator of PFK & can relieve ATP inhibition. So as ADP begins to accumulate adenylate kinase would start to generate AMP ←

Question 6 (6 pts) Fill in the initial series of curved arrows that start the reactions of the following enzymes. The curved arrows should make chemical sense. Don't draw any more detailed structures. (If you need to deprotonate or protonate something draw general base/acids as appropriate). Add any additional critical catalytic groups as appropriate.

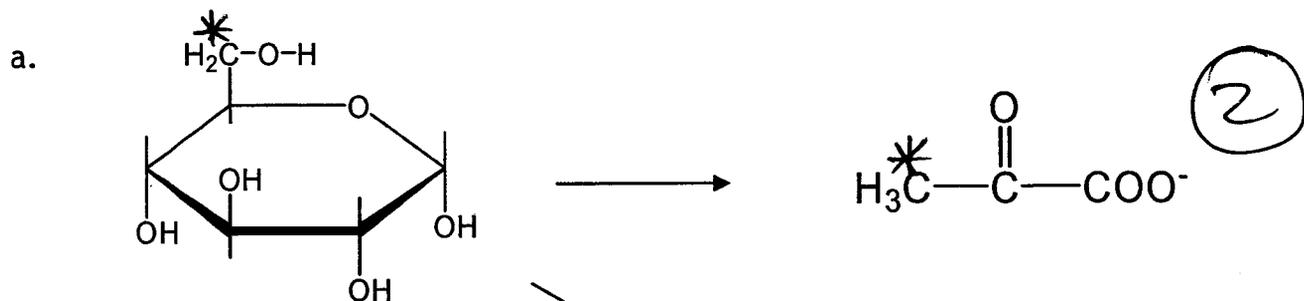
a. hexokinase



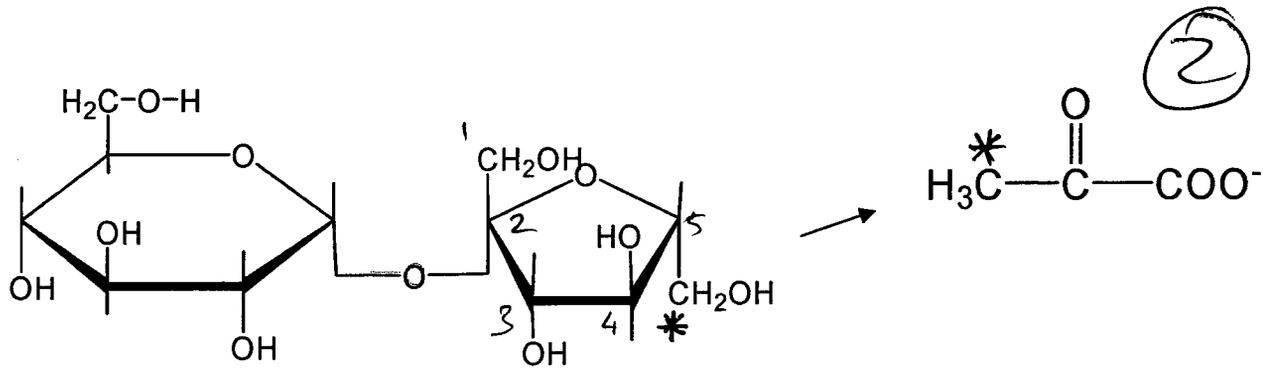
b. lactate dehydrogenase



Question 7 (6 pts) Tracing radiolabels. Place asterisks indicating the position of the radiolabel in the molecules shown to the right - if the product contains no radiolabel write "NONE".

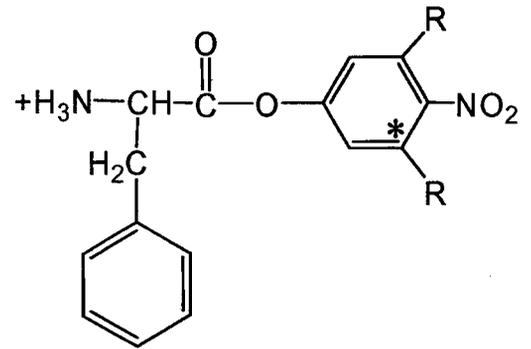


c.



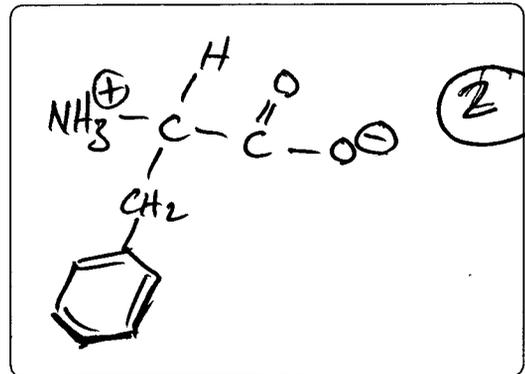
Question 8 (12 pts) Chymotrypsin is mixed with the radiolabeled ester substrate shown to the right ( $C^{14}$  at the asterisk) to give concentrations of  $30 \mu\text{M}$  and  $10 \text{mM}$  respectively.

The first product to be released shows a molar extinction coefficient of  $10,000 \text{M}^{-1}\text{cm}^{-1}$  at  $410 \text{nm}$  under the conditions of the experiments ( $\text{pH } 8.5$ ).



Answer the following questions:

a. In the box at the right draw the second product to be released from the enzyme:



b. The burst phase is completed in less than 1 min. Calculate the absorbance increase at  $410 \text{nm}$  in a  $1 \text{cm}$  pathlength expected for the burst phase:

$$\text{Burst} \equiv 30 \times 10^{-6} \text{M}$$

$$A = (10,000 \text{M}^{-1}\text{cm}^{-1})(1 \text{cm})(30 \times 10^{-6} \text{M})$$

Absorbance increase 0.3

c. Suppose the turnover number of the enzyme in the steady state was  $20/\text{min}$  what is the increase in absorbance at  $410 \text{nm}$  that would be observed between 1 and 2 min after mixing?

$$20 \times 30 \times 10^{-6} \text{M}/\text{min}$$

Absorbance increase 6

(If spectrophotometer can measure)!

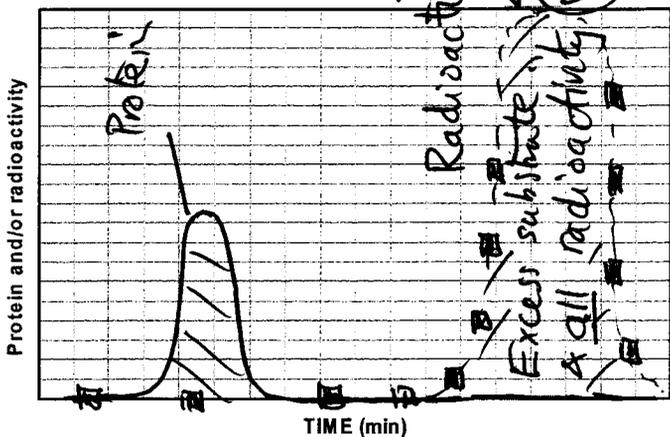
radioactivity + small MW frtn  
before protein - 1

Question 8 (continued)

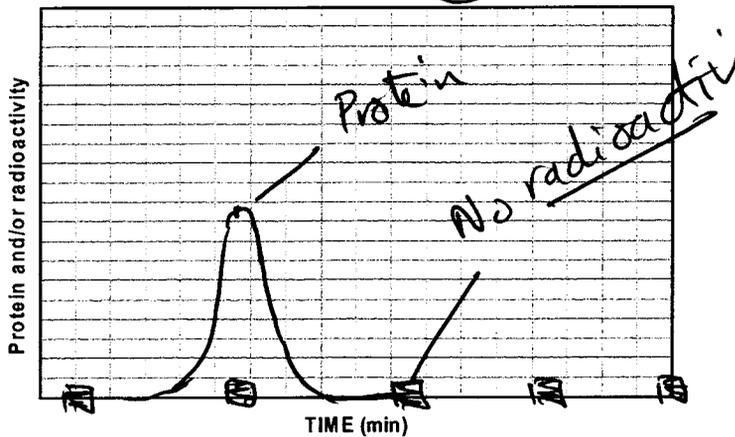
d. After 2 min the mixture is cooled rapidly and gel-filtered (size exclusion chromatography) at 4°C. Using the graph below left draw a representative trace of the chromatogram clearly indicating where protein and radioactivity would emerge.

e. The protein-containing fractions are collected and combined. They are allowed to warm to 25 C and then the chromatography is repeated. Again the fractions were followed for protein and radioactivity. Show the expected result at the right (above). if radioactivity included (2)

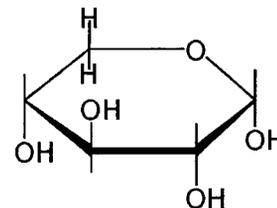
first chromatography



second chromatography (2)



Question 9 (9 pts) Alpha-D-xylose (shown) is dissolved in buffer and mixed with ATP/Mg<sup>2+</sup> and hexokinase. The concentration of selected compounds is shown below at time zero and after 5 min



Time	xylose	ATP	ADP	AMP	Pi
0 min	1mM	10 mM	0 mM	0 mM	0 mM
5 min	1 mM	6 mM	4 mM	0 mM	4 mM

a. show a chemical equation to describe this overall reaction in the presence of xylose:

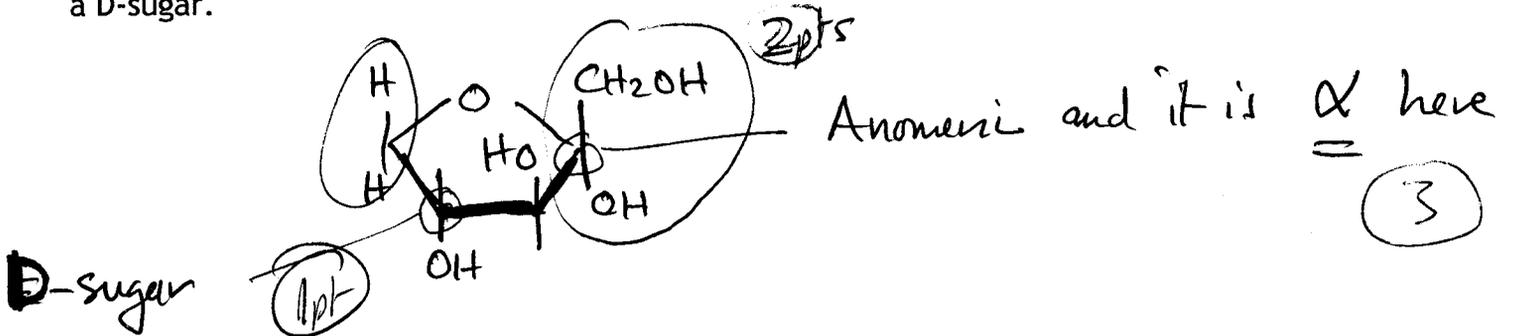


3

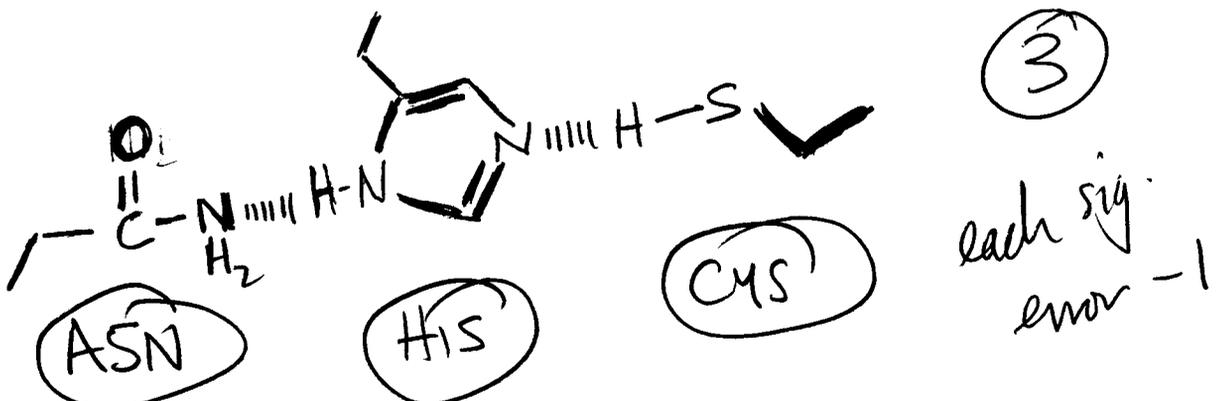
b. Explain concisely what is happening in this example:

xylose does not have a hexose's C6. So there is space where that  $-CH_2OH$  would be a water  $\begin{matrix} O-H \\ | \\ H \end{matrix}$  assumes corresponding position in E-xylose complex & hydrolyzes ATP. Xylose accelerated rxn w/o becoming phosphorylated.

c. Finally, draw D-xylose in a furanose ring form. Label the configuration of the anomeric carbon atom that you chose and circle the C atom in this furanose form that designates this as a D-sugar.



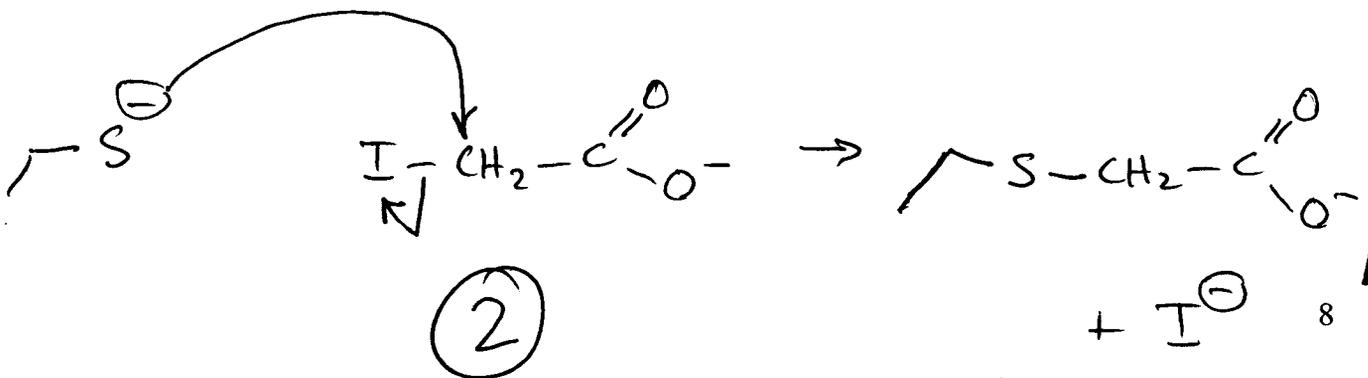
Question 10 (7 pts) Draw the catalytic triad of a thiol protease. Include side chain structures and interactions.



Suggest a simple covalent inhibitor (less than 12 atoms) of the enzyme that you depict.

Name Iodoacetate etc Chemical structure  $I-CH_2-C(=O)O^-$  etc

Then in the space below draw the essential chemistry using appropriate curved arrows.



Question 11 (24 pts.) Fill in the blanks with not more than 3 legible words.

- a. name the glycosidic bond formed in amylose  $\alpha(1-4)$
- b. name the glycosidic bond at the branch points in amylopectin  $\alpha(1-6)$
- c. These compounds bind equally to both free enzyme and the enzyme substrate complex NON-competitive
- d. This process sets a physical upper limit on an enzymes catalytic efficiency DIFFUSION
- e. The reagent used to diagnose *Helicobacter pylori* infections discussed in class  $^{13}\text{C}$ -labelled UREA
- f. The method for determining the in vivo concentrations of metabolites discussed in class Freeze clamping  
iodoacetate (etc)
- g. A chemical inhibitor of glycolysis discussed in class G3P dH
- h. And the enzyme it inhibits Zymogen
- i. Inactive precursors of proteolytic enzymes TPCK
- j. Give an example of an affinity label for an enzyme Chymotrypsin } any thing appropriate
- k. And the enzyme it inhibits NAD<sup>+</sup> / NADH
- l. The epimerization step converting a galactose derivative to a glucose derivative utilizes what coenzyme G3PDH
- m. this glycolytic enzyme generates a thioester intermediate

- n. One form of this enzyme uses a Schiff base intermediate
- o. if you could make yeast thiamine deficient, what compound would accumulate when yeast are fed fructose
- p. Name a metalloprotease
- q. Name an aspartyl protease
- r. Name a thiol protease
- s. reactions with positive free energies are called
- t. Name one component responsible for the brown color of aerobic avian muscle
- u. the absence of which enzyme in humans prevents us from converting dietary starch to ethanol
- v. the vitamin incorporated into  $\text{NAD}^+$  is called
- w. The human deficiency disease caused by the vitamin in "v"
- zz. the word that best describes this exam

Aldolase

Pyruvate

Carboxypeptidase

pepsi

papain

endergonic

myoglobin etc.

pyruvate decarboxylase

NIACIN / Nicotinic

PELLAGRA

OVER

*"Life is a struggle with equilibrium that we all eventually lose"*