BCH 4053 **Mini-Exam** **NAME**_____
August 3, 2001

Answer question 1 or 2 (20 points)

Page	Points
1	____
2	____
3	____
4	____
5	____
Total	_____

1. (a) How many mL of 0.1 M NaOH must be added to 100 mL of 0.05 M formic acid to make a buffer solution with a pH of 4.25?
   (b) How many mL of 0.1 M sodium formate must be added to 100 mL of 0.05 M formic acid to make a buffer solution with a pH of 4.25? (Formic acid has a pK of 3.75.)

2. Draw the structure of the following peptide, showing all dissociable functional groups in their fully protonated form, and indicate the **approximate** pK of each of the dissociable functional groups:

Lys.Cys.Arg.Tyr.Glu

(a) What is the charge on the **fully protonated** form of the peptide?
(b) What is the charge on the **fully unprotonated** form of the peptide?
(c) What is the approximate **pI** of the peptide?
(d) What is the net charge on the peptide at pH 6.0?

Answer question 3 or 4 (15 points)

3. You have isolated an octapeptide with the amino acid composition
(Lys_2, Asp, Tyr, Phe, Gly, Ser, Ala)
Reaction of the intact peptide with FDNB yields DNP-alanine. Cleavage with trypsin yields peptides with compositions (Lys, Ala, Ser) and (Gly, Phe, Lys) plus a dipeptide. Reaction with chymotrypsin releases free aspartic acid, a tetrapeptide with composition (Lys, Ser, Phe, Ala) and a tripeptide with composition (Gly, Lys, Tyr). What is the sequence? (Explain your reasoning).

4. Given the following data on three different proteins:

Protein	**hemoglobin**	**chymotrypsinogen**	**urease**
Molecular Weight (M)	64,500	23,250	482,000
Diffusion Coefficient (D)	6.9	9.5	3.5
Isolectric pH (pI)	6.8	9.5	5.0

Indicate in the blanks which of the three proteins will:

_____(a) Elute first from a gel filtration column.

_____(b) Elute first from a diethylaminoethyl cellulose ion exchange column.

_____(c) Have the smallest frictional coefficient (f).

_____(d) Migrate fastest upon electrophoresis in sodium dodecyl sulfate (SDS).

_____(e) Migrate fastest to the anode in an electrophoresis experiment at pH 6.0.

Answer question 5 or 6 (21 points)

5. Lactate dehydrogenase catalyzes the following reaction of anaerobic glycolysis:

$$\text{NADH} + \text{pyruvate} \rightleftarrows \text{NAD} + \text{lactate}$$

$\Delta G^{o'}$ for this reaction as written is –25.2 kJ/mol.

(a) Calculate the equilibrium constant for the reaction. (R = 8.314 J/mol-K; T = 310 K)
(b) What would ΔG be if the lactate/pyruvate ratio were 600 and the NAD/NADH ratio were 10?
(c) What do the letters NAD stand for?
(d) Draw the structure of NADH

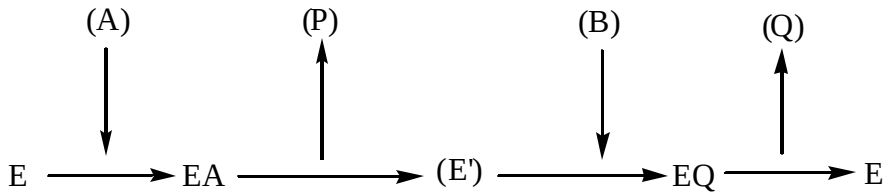
6. Give the structures of **seven** of the following:

maltose	phosphatidyl choline
linoleic acid	cyclic AMP
lipoic acid	biotin
fructose	thymidine

Answer question 7 or 8 (20 points)

7. The mechanism of chymotrypsin illustrates several of the factors that are believed to contribute to the rate acceleration obtained by enzymes. Describe each of the following aspects of the chymotrypsin mechanism.

   (a) A reaction model that shows ping-pong kinetics. (i.e., specify the identity of A, B, P, Q, E, and E', in the following scheme:)



   (b) Transition state stabilization by bonds formed between the enzyme and the transition state that are not found in the binding of substrate or product.
   (c) Acid-base catalysis mediated through a "catalytic triad". Describe how the triad assists in the formation of the covalently bound intermediate.
   (d) Substrate specificity provided by the nature of the substrate binding site. (Explain how chymotrypsin differs from trypsin in the binding site.)

8. (a) To illustrate the importance of tautomeric structure in the Watson-Crick base pairing, draw base pair structures showing how **cytosine** in the less stable tautomer can base pair with **adenine**, and how **guanine** in a less stable tautomer can base pair with **thymine**.
   (b) Both the Sanger and the Maxam-Gilbert methods of sequencing DNA generate sets of nested oligodeoxynucleotide fragments, each set ending at one of the four deoxynucleotides. The fragments are then separated according to size by electrophoresis. **Briefly** explain how the fragments are generated in each case.
   (c) DNA from a bacterial virus was analyzed and found to have the composition: 21% A, 28% G, 26% T, and 24% C. What is unusual about this composition, and what does it suggest to you about the structure of this DNA?

Answer question 9 (24 points)

9. Following is an alphabetical list of the glycolytic and TCA cycle enzymes plus a few other enzymes we have discussed. Choose enzymes from this list that are described by the statements below, and place the number or numbers of the enzyme in the blank to the left of the statement. In most cases, more than one enzyme will apply. Given in parenthesis after the statement is the **target number** of enzymes for you to identify.

(1) aconitase
(2) aldolase
(3) citrate synthase
(4) enolase
(5) fumarase
(6) glucose-6-phosphate isomerase (phosphoglucoisomerase)
(7) glyceraldehyde-3-phosphate dehydrogenase
(8) hexokinase
(9) isocitrate dehydrogenase
(10) á-ketoglutarate dehydrogenase complex
(11) malate dehydrogenase
(12) malic enzyme
(13) phosphoenolpyruvate carboxykinase
(14) phosphofructokinase
(15) phosphoglycerate mutase
(16) phosphoglycerate kinase
(17) pyruvate carboxylase
(18) pyruvate dehydrogenase complex
(19) pyruvate kinase
(20) succinate dehydrogenase
(21) succinyl-CoA synthetase
(22) triose phosphate isomerase

(a) _____ CO_2 is a substrate or a product. (six enzymes)

(b) _____ ATP or GTP is a substrate or a product. (six enzymes)

(c) _____ Coenzyme A is a substrate or a product. (four enzymes)

(d) _____ NADH is a substrate or a product. (five enzymes)

(e) _____ Thiamine pyrophosphate is a prosthetic group. (two enzymes)

(f) _____ Possible anaplerotic reactions. (three enzymes)

(g) _____ An oxidoreductase (seven enzymes)

(h) _____ Operates removed from equilibrium (eight enzymes)