**CHEM523 Test 2 Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Answer the following 9 questions in the 75 minutes allotted. DO NOT ANSWER THE QUESTIONS IN ORDER. Flip through the exam and answer the ones that you can answer immediately as soon as you identify them.

**1. (10 points total)** Draw the structure of:

1. **(6 points)** The repeating unit of peptidoglycan(You knew this one was coming!). You may give abbreviations only instead of full chemical structures.
2. **(4 points)** Draw the structure of and 3-letter abbreviations of two amino acids that are commonly used as nucleophiles in biological reactions. Draw the structures as they would be found at physiological pH.

**2. (5 points)** Samples of an unknown peptide are subjected to the two treatments below. After each treatment the resultant peptides are purified and sequenced by Edman's method to provide the data given. Determine the sequence of the unknown peptide using the overlapping peptide method.

i. Cyanogen bromide: ii. Trypsin:

 Asp-Ile-Lys-Gln-Hser Gln-Met-Lys

 Lys Gly-Met-Asp-Ile-Lys

 Lys-Phe-Ala-Hser Phe-Ala-Met-Lys

 Tyr-Arg-Gly-Hser Tyr-Arg

Bonus information. Treatment of the peptide with -chymotrypsin yielded 3 smaller peptides. One peptide had a single amino acid, the second peptide had three amino acids and the third peptide contained 10 amino acids

Sequence of unknown peptide:

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**3) (10 points total) Leupeptin**, also known as ***N*-acetyl-L-leucyl-L-leucyl-L-argininal** (it has an aldehyde instead of the normal carboxylate on the carboxy-terminus), is a naturally occurring compound that is a transition state-inhibitor of trypsin.

1. **(5 points)** Draw the complex that would be formed by trypsin and leupeptin. You only need to include atoms that are directly part of the acyl-enzyme intermediate.
2. **(5 points)** Why does this compound inhibit trypsin but not chymotrypsin? Give specific chemical reasoning for this observation in your answer.

**4) (20 points)** The serine proteases are a novel group of enzymes that have fascinated chemists and biochemists for years. Answer the following questions about this interesting group of enzymes.

1. **(10 points)** Explain how this family of enzymes serves to illustrate the two different types of molecular evolution.
2. **(2 points)** What are the catalytic residues of these enzymes?
3. **(4 points)** These enzymes employ a special method of stabilizing the transition state during the reaction. What is it and what is the chemical basis for it? Include all relevant amino acids and show all relevant chemical interactions.
4. **(4 points)** When we discussed the reaction mechanism in class, we mentioned a unique IMF between two of the catalytic residues. What is this IMF and how does it contribute to the reaction mechanism?

**5)** **(9 points total)** Describe the three types of non-covalent enzyme inhibition by:

a) **(6 points)** Drawing the double-reciprocal plots of the enzymatic reaction in the absence and presence of **two** concentrations of inhibitor. Place the name of each type above each plot.

b) **(3 points)** Describing the molecular species each type of inhibitor binds to (use reaction pathways (eg: A+B-> AB, in your answer)

**6) (18 points total)** You have identified a new lipase (an enzyme that hydrolyzes fatty acids of glycerophosphoslipids) from an organism growing in a Thompson Hall grease trap. After salting out, SDS-PAGE analysis showed the presence of four proteins in the sample with molecular weights of 15, 20, 35 and 55 kilodaltons.

1. **(6 points)** Describe a three-step purification protocol that will allow you to obtain a purified sample of the new enzyme. Be very specific in your answer or no credit will be given.
2. **(4 points)** Draw an image of the SDS-PAGE gel you would obtain if you ran the sample after salting out in one lane and after and your three steps in another lane. Be certain to label everything. How can you tell what the masses of the proteins are?
3. **(8 points)** If the enzyme catalyzes the hydrolysis of a stearic acid residue from the C-2 of phosphatidylcholine, what types of mechanism would you expect your enzyme might employ? Based upon the types of mechanism, what amino acids would you expect to find in the active site and why?

**7) (8 points total)** Write out:

a) **(4 points)** The Michaelis-Menton equation and show what a plot of V0 versus [S] would look like for an enzyme that follows Michaelis-Menton kinetics. Clearly label Vm and Km on the plot.

b) **(4 points)** The Lineweaver-Burk equation and show what a plot of 1/V0 versus 1/[S] looks like. Label the slope and intercept terms for the plot.

**8) (10 points)**  Draw the reaction coordinate for a non-catalyzed chemical reaction and an enzyme-catalyzed reaction. What are some features of an enzyme that account for the difference between the two coordinates? Hint: You can break an enzyme catalyzed reaction into 2 phases. One phase has to do with k1 and the other phase has to do with k2.

**9) (10 points)** Give a specific, complete chemical explanation for the observation that RNase A catalyzes the cleavage of RNA polymers but not polymers of DNA.

**Extra Credit 1. (2 points)** Explain how to make 5 L of 0.15 M acetic acid-sodium acetate buffer at pH 5.00 if you have 1.00 Molar acetic acid (60.05 g/mole) and crystaline sodium acetate (82.03 g/mole). The pKa of acetic acid is 4.76.

**Extra Credit 2. (10 points. All or nothing, no arguments, no discussions.)** Bacterial transpeptidases are responsible for cross-linking peptidoglycan chains in the cell wall. They are also called penicillin-binding proteins because they are inhibited by penicillin. Draw the reaction mechanism for the reaction catalyzed by a bacterial transpeptidase. You do not have to draw the sugar component of peptidoglycan or non-relevant residues of the substrate(s).