Chem 135: First Midterm

September 26th, 2008

*Please provide all answers in the space provided. Extra paper is available if needed. You may not use calculators for this exam, but you are free to use (previously unassembled!) molecular model kits. Including the title page, there should be **6** pages in this exam booklet.*

Good Luck!

Name: Key

(1) _____ (16 points)

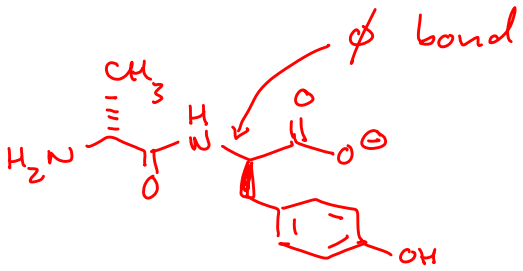
(2) _____ (12 points)

(3) _____ (40 points)

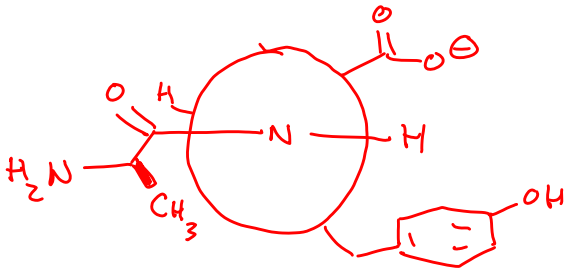
(4) _____ (32 points)

TOTAL _____ (100 points)

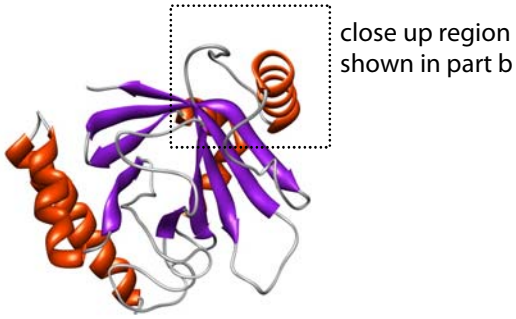
1. a) Draw the dipeptide AY using *all D-amino acids* (meaning the enantiomers of the naturally-occurring L-amino acids) in its most abundant protonation state at pH 9 (8 points).



b) Using your structural drawing in part (a), indicate the location of the phi (ϕ) bond in your dipeptide. In the space below, draw a Newman projection indicating the most stable conformation along this bond. Do not abbreviate the side chain groups in your structure (i.e. don't use 'R' groups) (8 points).



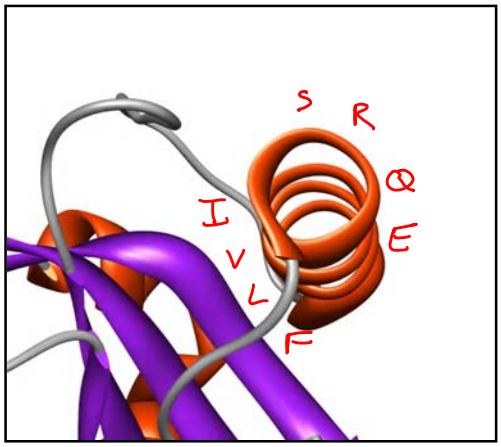
2. Many types of bacteria, such as *Staphylococcus aureus*, directly attach proteins to their surfaces in order to adhere to host tissues. This process is catalyzed by an enzyme called "sortase", which sorts through the proteins of the bacterial cell, chooses the ones that have a specific sequence at the C-terminus, and attaches them to the peptidoglycan cell wall directly (this reaction sequence is summarized in question 3b). As drug-resistant *Staph. aureous* infections have become more frequent in hospitals, many groups have studied the sortase enzyme in hopes of developing new types of antibiotics. The structure of a sortase enzyme is shown below:



sortase

(Question continued on next page.)

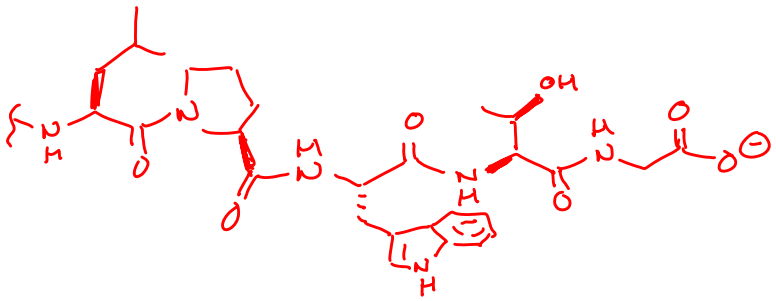
a) Shown below is a close up view looking down an alpha helix of this structure. The location of this helix is outlined with a box in the ribbon diagram on page 2. Note that there is no bulk water inside the protein structure. Place the following amino acids on the sides of the helix where they are most likely to be found: **I**, **S**, **R**, **Q**, **V**, **L**, **E**, **F**. Note that you do not have to draw any structures; just put the letters where you would expect the corresponding side chains to be (8 points).



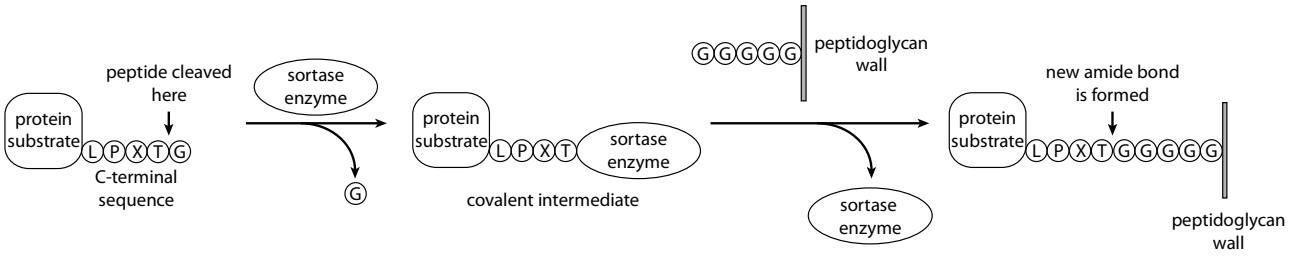
b) Considering that this 8-amino acid portion of the alpha helix is not at the N-terminus or at the C-terminus of the protein, what is the overall charge of this segment at pH 7 (4 points)?

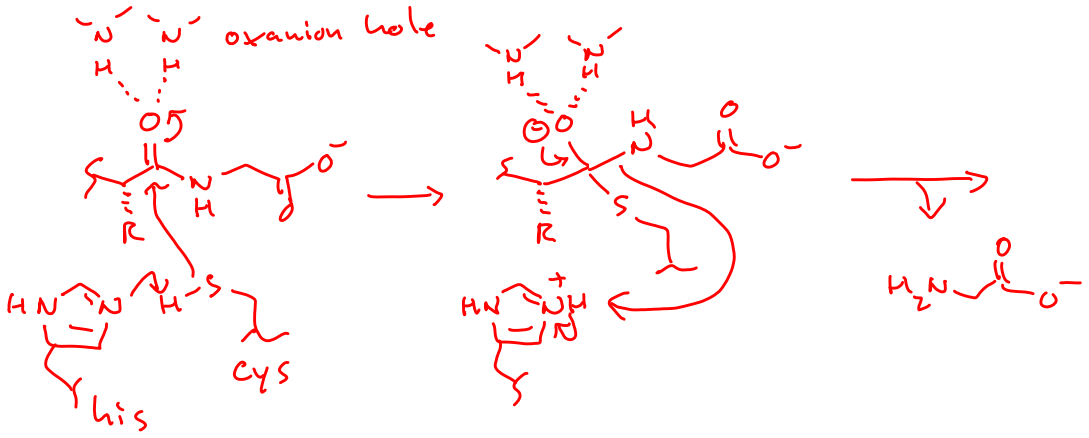
R: +1
E: −1
0 net charge

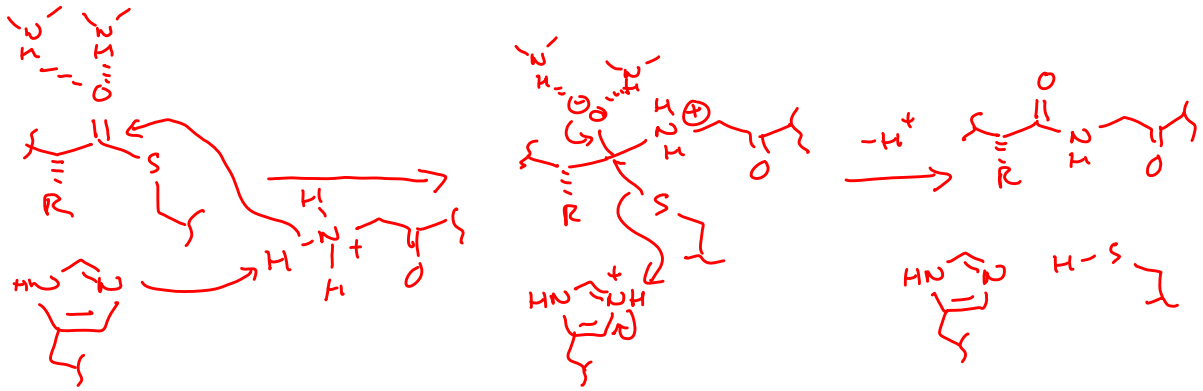
3. a) The sortase enzyme selects its protein substrate by recognizing an "LP***X***TG" sequence at the C-terminus, where "X" can be any amino acid. Draw a full chemical structure of one such sequence, in which "X" is a W residue. Include all appropriate stereochemistry and draw the residues in their most abundant protonation state at pH 7 (12 points).



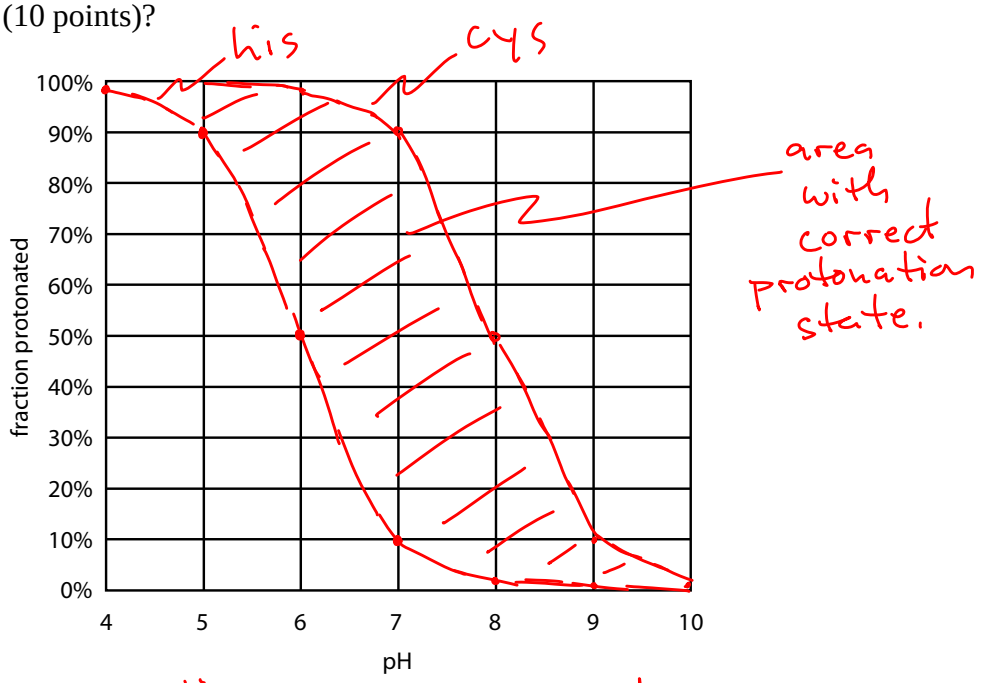
b) Once the substrate has been recognized, the sortase enzyme cleaves an amide bond between the T and G residues, much like a protease. However, *unlike* a normal protease, it goes on to form a new bond with the N-terminal amino group of a GGGGG sequence that is displayed on the peptidoglycan wall. The active site of the sortase enzyme is known to contain a cysteine residue and a histidine residue that are both essential to achieving catalysis. Based on this information and mechanisms you know from class, *propose a detailed arrow-pushing mechanism* for the overall reaction of the sortase enzyme that clearly indicates the role of these two residues. You may add any additional residues or portions of the protein that you feel are needed for your mechanism, and you may abbreviate the side chains of your substrate using 'R' groups (18 points).







c) On the graph below, sketch the percentage of histidine residues that would be protonated as a function of pH. On the same graph, complete the analogous curve for a cysteine residue. Indicate which curve corresponds to which residue. Based on your proposed mechanism, what does your graph predict about the behavior of this enzyme as the pH is altered (10 points)?

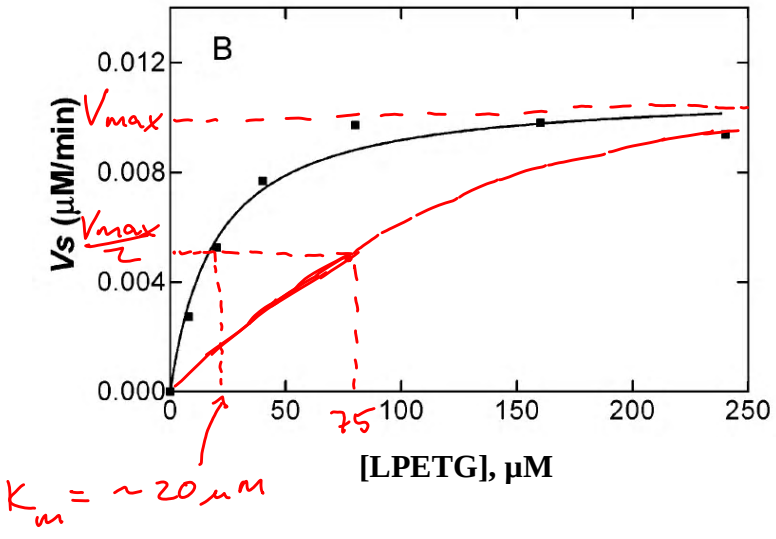


The optimum pH for this enzyme will be >6 and <8.

4. a) Several labs have shown that the kinetics of the *S. aureus* sortase enzyme exhibit Michaelis-Menten behavior. What is the rate law equation that applies to these situations (5 points)?

$$V = \frac{k_{cat}[E]_t[S]}{K_m + [S]}$$

b) Below is a plot of the rate of conversion for an LPETG substrate as a function of its concentration. The total enzyme concentration used in this experiment was 1.0 µM (which is $1x10^{-6}$ M). By inspecting the graph, provide estimates for the values of K_m and k_{cat} (including units!). Show how you arrived at your answers (8 points).



$k_{cat} = \frac{V_{max}}{[E]_T} = \frac{0.010\ \mu M/min}{1\ \mu M} = 0.010\ min^{-1}$

c) Assuming a substrate concentration of 0.01 µM, provide a *simplified rate law* for the sortase enzyme and provide a value for the effective rate constant (with units!) (5 points).

If $[S] << K_m$: $V = \frac{k_{cat}}{K_m}[E]_T[S]$

$k_{cat}/K_m = 0.0005\ \mu M^{-1}\ min^{-1}$

d) Now assume that you study a new substrate for this enzyme with the sequence LPE***S***G. You find that for this substrate, the sortase enzyme has the same value of k_{cat} as it does for the LPE***T***G substrate considered in (b). However, the K_m for LPE***S***G changes to a value of 75 in the same units you indicated above. Consider what the V/[S] plot would look like for this new substrate and draw it in on the graph provided in part (b) (8 points).

V_{max} is unchanged, but $\frac{V_{max}}{2}$ occurs at $[S] = 75\ \mu M$

e) Now consider what would happen if the enzyme was presented with a mixture of the two peptides (LPE***T***G and LPE***S***G) at equal concentrations. How would you describe the ability of the sortase enzyme to react with one of the peptides selectively as the total peptide concentration is altered? Briefly explain your answer (6 points).

The enzyme will be selective for LPETG at low [S] but the rates will be similar at higher [S].