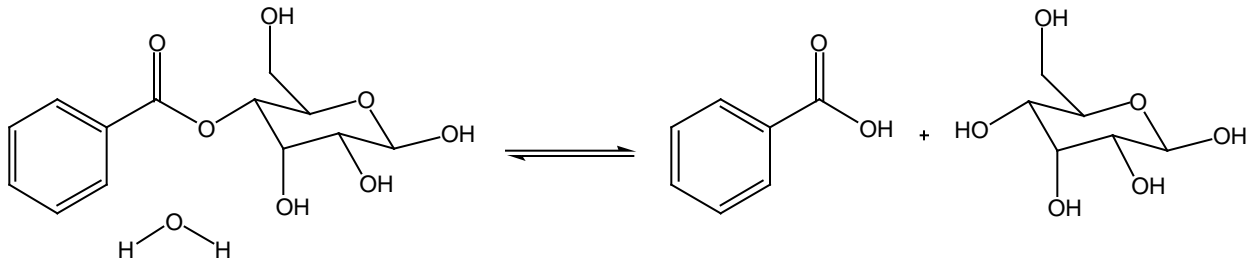


Problem Set VI - Due Thursday 8

- 1) (15 points) You have discovered an enzyme that catalyzes a reaction through general acid base catalysis, and additional studies have shown you that it uses the same amino acid residue both as an acid and as a base.

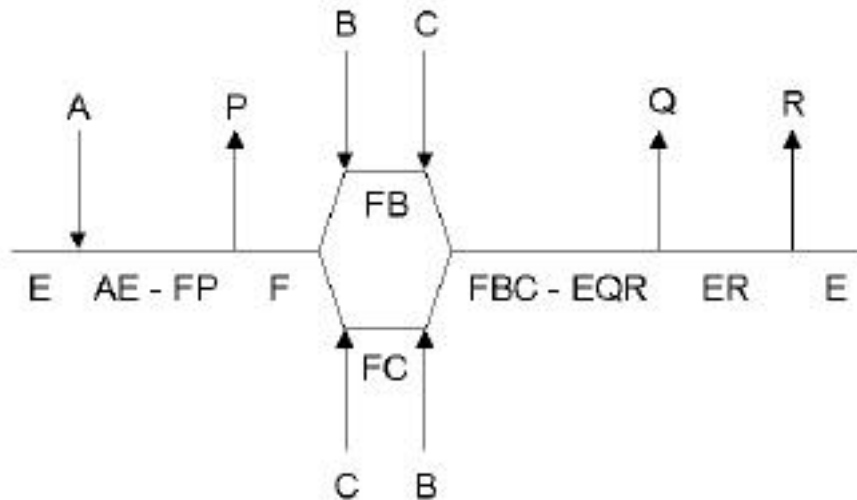
One of the students in your lab has done a study of the reaction rate (V_{max}) versus pH, and he found that the enzyme is active in a very narrow and unusually high range of pH: from 9.5 to 11.5. Outside of that pH range, the enzyme does not work at all. Which amino acid residue could be involved in acid-base catalysis? Justify your answer

- 2) (15 points) The reaction catalyzed by the enzyme you discovered (mentioned above) is the following:

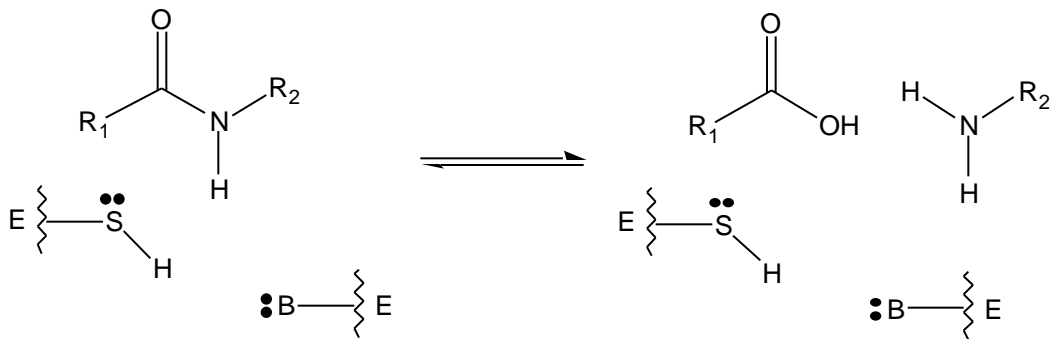


Propose a mechanism that uses solely the base you think is involved from question (1) that will catalyze this reaction. Please make sure that your electrons do what they are supposed to - It is one thing to be an electron *pusher*, another an electron *bouncer*...

- 3) (15 points) Using the following Cleeland diagram, write the equilibria involved in the transformation of substrates A, B, and C, into products P, Q, and R by enzyme E.

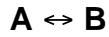


- i) How is the addition of the different substrates (sequential ordered, sequential random, or ping-pong)?
 - ii) How is the release of the different products?
 - iii) How many different enzyme forms are present at different stages?
- 4) (20 points) The mechanism of the cysteine proteases is very similar to that of the serine proteases. The main differences are that a cysteine residue is used instead of a serine residue in the initial attack to the peptide group, and that no evidence for a catalytic triad exists. There is, however, evidence that the cysteine residue is activated by general acid-base catalysis as with serine proteases. Consider the following peptide bond hydrolysis:

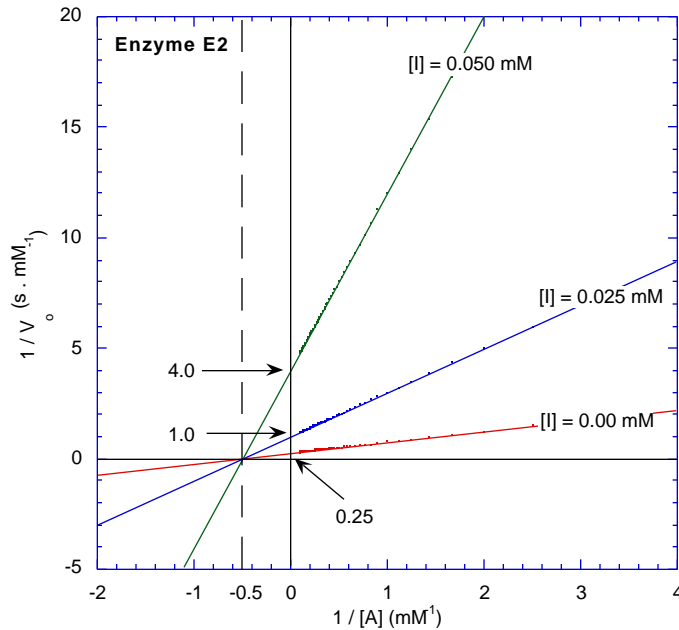
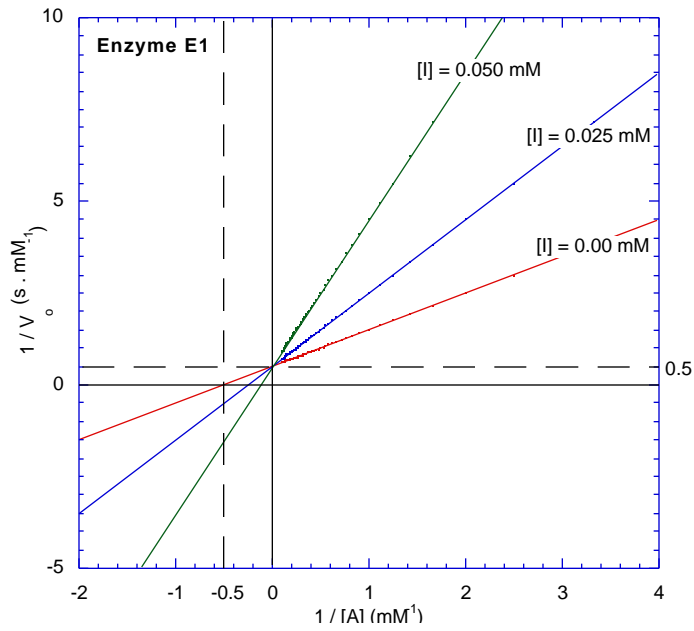


- i) Write a detailed mechanism for the enzymatic hydrolysis of the peptide bond presented above, accounting for all nucleophilic attacks and hydrogen abstractions ('push electrons wisely...')
 - ii) Indicate all the transition states that form during the reaction. Hint: There should be two of them...
 - iii) You investigated the pH dependence of this cysteine protease, and found that it is active in a narrow pH range, between 5.5 and 6.5. Considering this information, what could be the identity of the residue acting as a base in the reaction?
- 5) (15 points) You came across a cysteine protease while investigating a really nasty virus. This protease, which is crucial for the virus to self-replicate, cleaves peptide bonds flanked by small aliphatic residues (like A) to the left, and bulky aromatic residues (like F) to the right. Based on the mechanism of cysteine proteases you outlined in question (4), design a transition state analog that will inhibit this enzyme. Justify your design.

6) (20 points) You need to use an enzymatic reaction as part of a large chemical process. The reaction is the typical



where **B** is the product you want. You found two enzymes, **E1** and **E2**, that will catalyze your reaction. They have different K_M and V_{max} parameters, and they both show inhibition by **I**, which is a compound that is present as part of other reactions in your process. The double-reciprocal plots of $1/V_o$ vs $1/[A]$ at different concentrations of **I** for both enzymes are shown below:



- i) What type of inhibition does each enzyme exhibit vs **I**?
- ii) Calculate the *real* V_{\max} and K_M parameters from the graphs for both enzymes. Which enzyme is faster (i.e., has a higher V_{\max}) at $[I] = 0$?
- iii) You know that under the conditions of your experiment, $[I]$ will be 0.05 mM. Considering that you can use a large excess of substrate **A** in your system, which enzyme, **E1** or **E2**, would you buy to accomplish your process faster?