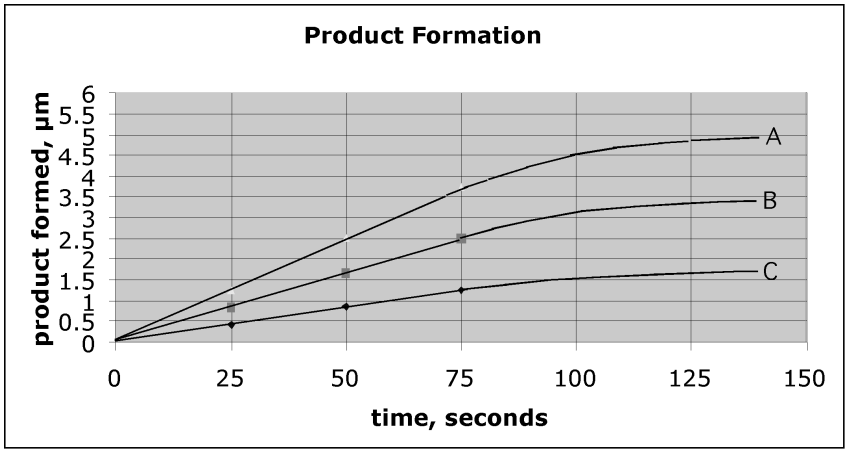
Biochemistry I Problem Set 5 *Due Oct 17, 2005* *Total Time required: ~80 min*

1. (20 points, 30 min) The plot below shows the amount of product formed (μmoles) as a function of time (sec) in an enzyme reaction. The substrate concentrations were 1, 2.5 and 5 mM.



i) Indicate which curve is associated with which substrate concentration. (1 pt)

ii) Determine the *initial* velocity, in units of μmoles/sec, of product formation for each substrate concentration. (3 pts)

iii) Suggest an explanation for why the rate of product formation decreases towards the end of the experiment (e.g. t > 100 sec). (2 pts)

iv) Plot the initial velocity versus substrate concentration for these data. Be sure to label the axes. (4 pts)

v) *Without* constructing a double reciprocal plot, estimate K_m and V_{max} from your plot done in part (iv). (2 pts)

vi) Now create a double reciprocal plot (1/v versus 1/[S]) to obtain V_{max} and K_m. (4 pts)

vii) How close was your estimate in part (v) to the 'true' value? What modification of the enzyme assay would have led to an estimate closer to the 'true' value? (2 pts)

viii) Assuming that the enzyme concentration was 1 nM and the volume of the reaction was 2 mls, calculate the turnover number, or K_{cat} of this enzyme. (2 pts)

2. (9 points, 20 min) The following problem requires that you visualize the active site of Trypsin using Chime. The URL can be found on the problem set page. The normal, wild type, structure is shown as "unmodified". In addition, there are 6 mutant versions, each of which contains a single amino acid change in the active site. Each mutation affects either substrate specificity or the catalytic mechanism, but not both (clearly an oversimplification!). Depending on your last name, select the appropriate mutant enzyme and answer the following questions:

A-C:	Trp 1	M-O:	Trp 4
D-H:	Trp 2	P-S:	Trp 5
I-L:	Trp 3	T-Z:	Trp 6

i) State which residue was altered and how (eg, Asp 189 to Phe). (3 pts)

ii) State whether the mutation will affect either the specificity of the enzyme (K_M) or the catalytic properties of the enzyme (V_{MAX}). Briefly justify your answer. (3 pts)

iii) Sketch the active site, indicating the location of the amino acid change in your particular structure. (You need only include the residues in the catalytic triad, the main-chain of the substrate, the side-chain of the Arg residue on the substrate, and the residue in the specificity pocket). (3 pts)

3. (9 pts, 15 min) Replacement of the Ser 195 residue of the catalytic triad in a serine protease with an Ala residue produces a relatively inactive enzyme. In contrast, replacement of Ser 195 with a Cys residue produces a functional, albeit less active, enzyme.

i) Provide an explanation for why Cys 195, but not Ala 195, can function in the place of Ser 195. (3 pts)

ii) Provide an explanation for why the mutant enzyme containing Cys 195 has a lower V_{MAX} than the wild type enzyme. [Hint: how do the side-chains of the two residues compare as nucleophiles?] (3 pts)

iii) Sketch the stable enzyme intermediate that would be found with the mutant (S195C) enzyme. (3 pts)

4. (9 points, 15 min) Alteration of Asp 102 in the catalytic triad of the serine protease trypsin to Asn (D102N) leads to a 10^4 fold reduction in the K_{cat} of the enzyme.

i) Taking advantage of what you know about the catalytic mechanism of serine proteases, explain the observed reduction in K_{cat} (i.e. describe the specific step in the catalytic cycle that is inhibited in the mutant enzyme). (3 pts)

ii) Analysis of the active site structure of the D102N mutant indicated that the specificity pocket, oxyanion hole and orientation of the nucleophilic Ser were identical to that of the wild type protease. Is the K_m of the D102N mutant likely to be higher, lower, or the same as the wild type protease? Explain. (3 pts)

iii) Remarkably, the Kcat of the D102N mutant was restored (to 6% of wild type) simply by raising the pH of the enzyme assay solution to 10.2. Explain this observation. (3 pts)