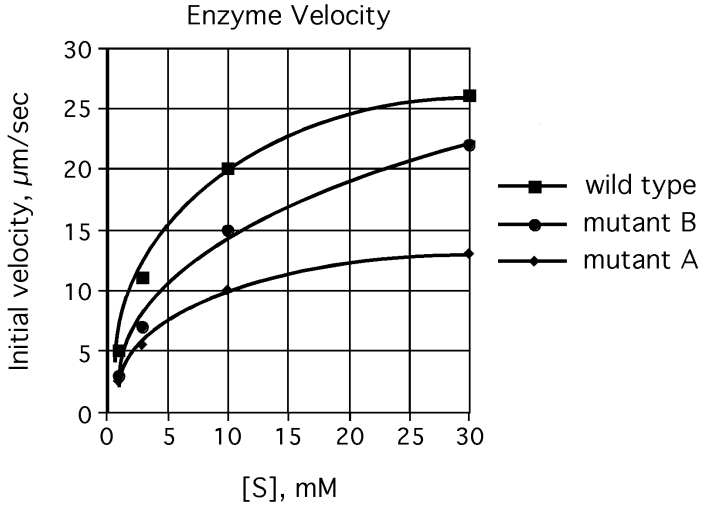
Problem Set 6 Total Time required: ~60 min

1. (16 points, 20 min) Mutagenesis has been performed in the lab to alter the activity of a protein phosphatase enzyme. Two versions of the phosphatase, mutant A and mutant B, have been produced and purified. You are required to determine whether the mutant enzymes are altered in their binding to substrate (K_m) or in their catalytic rate (V_{max}). The initial velocity of each enzyme at varying substrate concentrations is plotted below.



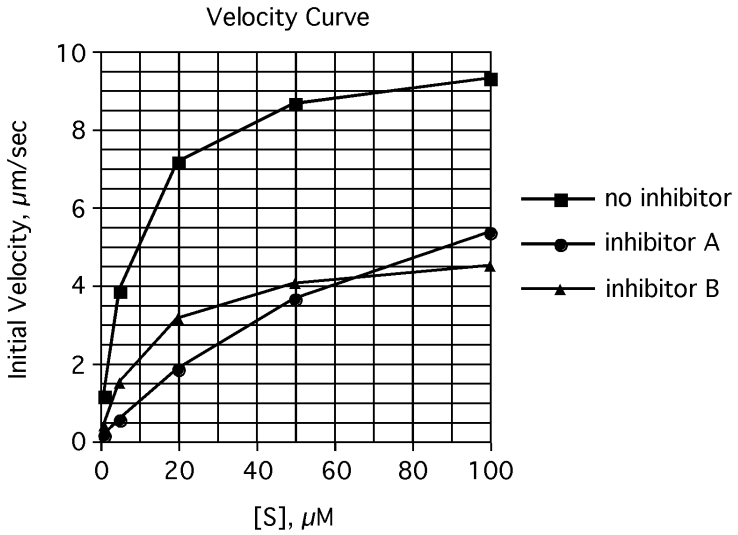
i) *Without* performing a double reciprocal plot, can you say which phosphatase version has the highest affinity (lowest K_m) for substrate with certainty? The highest V_{max}? (1pt)

ii) Plot the data on a double reciprocal plot. What is the V_{max} of each phosphatase version? (6 pts)

iii) What is the K_m of each phosphatase for its substrate? (6 pts)

iv) Which mutant, A or B, is more likely to have an alteration in an amino acid residue critical for catalysis? Please justify your answer. (3 pts)

2. (15 points, 25 min) The pharmaceutical company that you work for has just completed a large scale screen to identify a drug that might be used to inhibit an extracellular protease required for tumor metastasis. Two candidates have been identified, and your job is to further characterize the inhibitors. You perform three separate experiments, measuring the activity of the protease in the absence of inhibitor, in the presence of 20 μM inhibitor A, or 20 μM inhibitor B. The data are shown below. [S] is the substrate concentration and V is the rate of formation of the normal cleavage product of the protease.



[S], μM	v (μm/sec) No drug	v (μm/sec) [A] = 20 μM	v (μm/sec) [B] = 20 μM
0	0	0	0
1	1.111	0.112	0.385
5	3.846	0.538	1.470
20	7.143	1.852	3.125
50	8.621	3.623	4.032
100	9.259	5.319	4.464

i) Determine the type of inhibition (i.e. competitive, non-competitive) and the appropriate inhibition constants (K_I, $K_{I'}$) for inhibitor A. (6 pts)

ii) Determine the same parameters for inhibitor B. (6 pts)

iii) Which of the two inhibitors cannot be easily overcome by simply increasing the concentration of the normal substrate? Why? (3 pts)

3. (12 points, 15 min) Congratulations! You have just used an activity assay to purify a protein from a lysate composed of the following 7 proteins listed below:

Protein	AS Solubility (M) *	# amino acid residues	# of Charged Residues		
			Asp & Glu	His	Lys & Arg
Fatty Acid Binding Protein	0.5	131	10	1	15
Myoglobin	1.5	153	10	10	20
Hen Lysozyme	2.0	130	5	1	20
Quail Lysozyme	4.0	129	5	5	20
Phospholipase	4.0	133	10	1	10
Triose-phosphate isomerase	1.0	240	10	0	25
Glutathione Transferase	4.0	230	15	0	30

Based on the description of the procedure you used (see below), indicate the identity of your purified protein. Justify your choice by describing the behavior of your protein at each step of the purification procedure. (eg, alcohol dehydrogenase bound to the cation exhange column because it is….).

<u>The purification procedure</u>: The lysate containing your activity was first applied to a cation exchange column (negatively charged) at pH 6.0. After allowing unbound protein to flow through, a gradient of increasing salt was applied to the column. A small protein peak was observed to elute from the column early at low salt concentrations, but the majority of activity eluted together with the majority of protein at high salt concentrations. The high salt eluate was next subjected to ammonium sulfate fractionation. The majority of protein was found to precipitate with 3M ammonium sulfate, but luckily your activity remained in the supernatant. In the final step, the supernatant was subjected to gel filtration chromatography. Your activity was found to elute with the first of two peaks of protein.