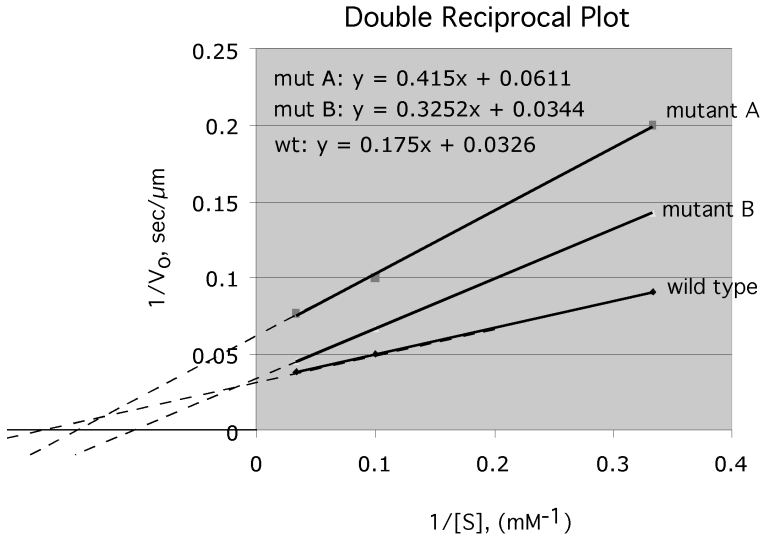
Problem Set 6 – Solution Key

1. i) **In this case, it is difficult to determine either Vmax or Km without a double reciprocal plot. It would be easier if the graph were extended to include the initial velocity at higher substrate concentrations. (1 pt)**
   ii) The double reciprocal plot, $1/v_o$ versus 1/[S], is shown below:



The V_{max} of each enzyme can be obtained from the y-int of each line equation, when [S] is in great excess and as 1/[S] approaches zero. At this point, V_{max} = 1/y-int. It should be apparent from looking at the graph that wt and mutant B have the same V_{max}, while mutant A has a significantly lower V_{max}. More precisely, the y-int for wt, A, and B are .0326, .0611 and .0344, respectively. Thus V_{max} for each enzyme is as follows: **wild type ~ 30 μm/sec**; **mutant A ~ 16 μm/sec**; for **mutant B ~ 30 μm/sec**.

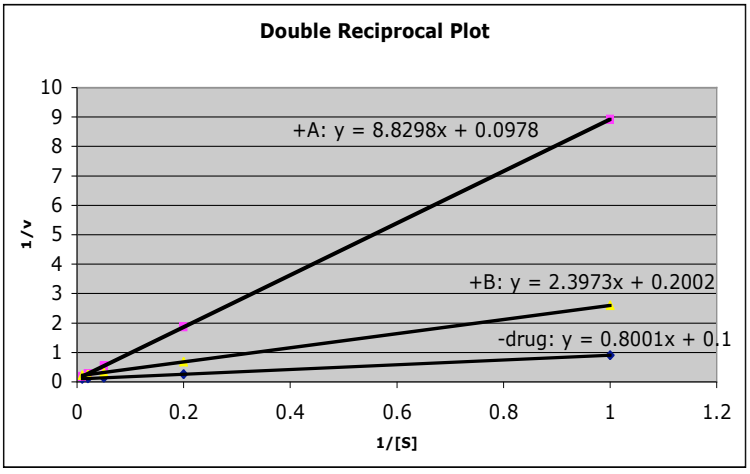
iii) The affinity of each enzyme for substrate can be obtained from the slope: Slope = Km/Vmax. For wt: slope = 0.175 = Km/30; **Km ~ 5.2 mM. (2 pts)**
For A: slope = 0.415 = Km/16; **Km ~ 6.6 mM. (2 pts)**
For B: slope = 0.325 = Km/30; **Km ~ 9.8 mM. (2 pts)**

iv) **Mutant A (1 pt)** is more likely to have an alteration of a residue critical for catalysis **because its V_{max} (and hence its k_{cat}) is reduced by nearly two-fold (2 pts)** while its affinity for substrate is only just slightly lower than that of wild type.

2. For the enzyme without inhibitor, V_{max} = 1/y-int = 1/0.1 = 10 μm/sec
   K_m = slope*V_{max} = 0.8*10 = 8 μM

i) In the presence of inhibitor A: V_{max} = 1/.0978 = 10.2 μm/sec;
K_m = 8.83*10.2 = 90 μM. **This inhibitor increases Km but does not affect Vmax; thus it is a competitive inhibitor**. **(3 pts)** The degree of inhibition, □, can be calculated by taking the ratio of the slopes of the two lines:
□ = slope (+ inh A)/slope (-inhibitor) = 8.83/.8 = 11.

K_I can then be calculated from α as follows: $\alpha = 1+[I]/K_I$; **$K_I = [I]/(\alpha-1) = 20\ \mu M/10 = 2\ \mu M$. (3 pts)** Thus A binds to the enzyme with a K_D of 2 μM.



ii) In the presence of inhibitor B: $V_{max} = 1/.2 = 5\ \mu$m/sec; $K_m = 2.4*5 = 12\ \mu$M. **This inhibitor both decreases V_{max} and increases K_m; thus it is a non-competitive inhibitor. (2 pts)** As in the above, the degree of inhibition, α, can be calculated by taking the ratio of the two slopes: $\alpha = 2.4/0.8 = 3$. K_I can be calculated from α as above: **$K_I = [I]/(\alpha-1) = 20\ \mu M/2 = 10\ \mu M$. (2 pts)** To calculate K_I', α' is first calculated by taking the ratio of the y-ints of the two lines:
α' = y-int (+B) / y-int (-inhibitor) = 0.2/0.1 = 2. **$K_I' = [I]/(\alpha'-1) = 20\ \mu M/1 = 20\ \mu M$. (2 pts)** Thus B binds to E with a K_D of 10 μM, and B binds to ES with a K_D of 20 μM.

iii) Because inhibitor A is a purely competitive inhibitor, it can be completely overcome by increasing the normal substrate concentration. **Inhibitor B, in contrast, is more difficult to overcome because it also binds to the ES complex and reduces V_{MAX}. (3 pts)**

2. **The protein purified was Glutathione Transferase (GT). (3 pts)** On the first cation exchange column step, phospholipase would have flowed through because it has no net charge at pH 6.0. **In contrast, Glutathione Transferase like the rest of the proteins with a large net positive charge would have bound to the negatively charged resin.** Of the bound proteins, Fatty Acid Binding Protein would have been the first to be eluted off the column because it is significantly less positively charged than the other bound proteins. **The remaining 5 proteins including Glutathione Transferase would have come off the column (together with the activity) with high salt. (3 pts)** Next, upon addition of 3M ammonium sulfate to the high salt eluate, Myoglobin, Hen Lysozyme, and Triose Phosphate Isomerase would have precipitated because the solubility of each of these proteins is at least 1M unit below 3M. **This would have left Quail Lysozyme and Glutathione Transferase in the supernatant. (3 pts)** In the final step, **Glutathione Transferase would have eluted from the gel filtration column prior**

to Quail Lysozyme because it is significantly larger, with 230 amino acid residues, than Quail Lysozyme, with only 129 residues. (3 pts)