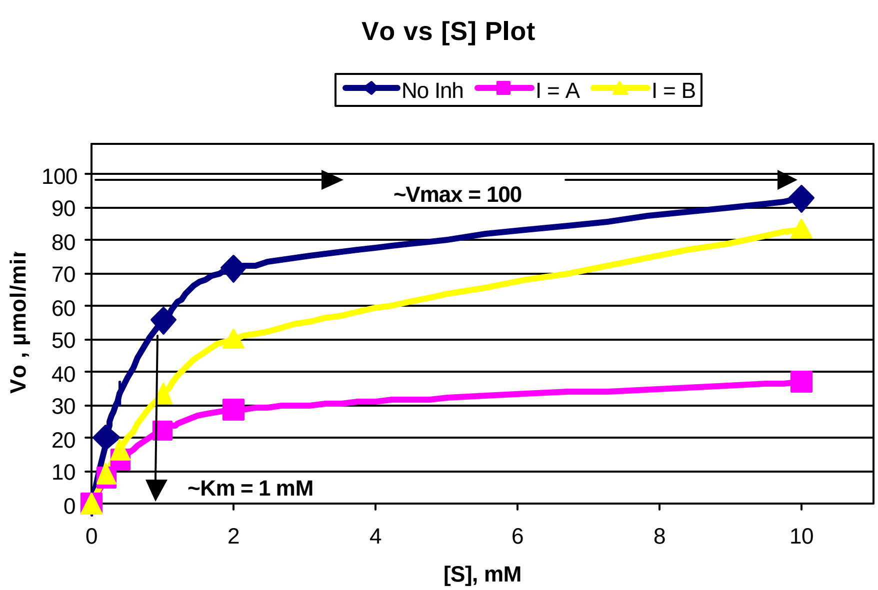
BL/CH401 EXAM 2 October 27th 1999

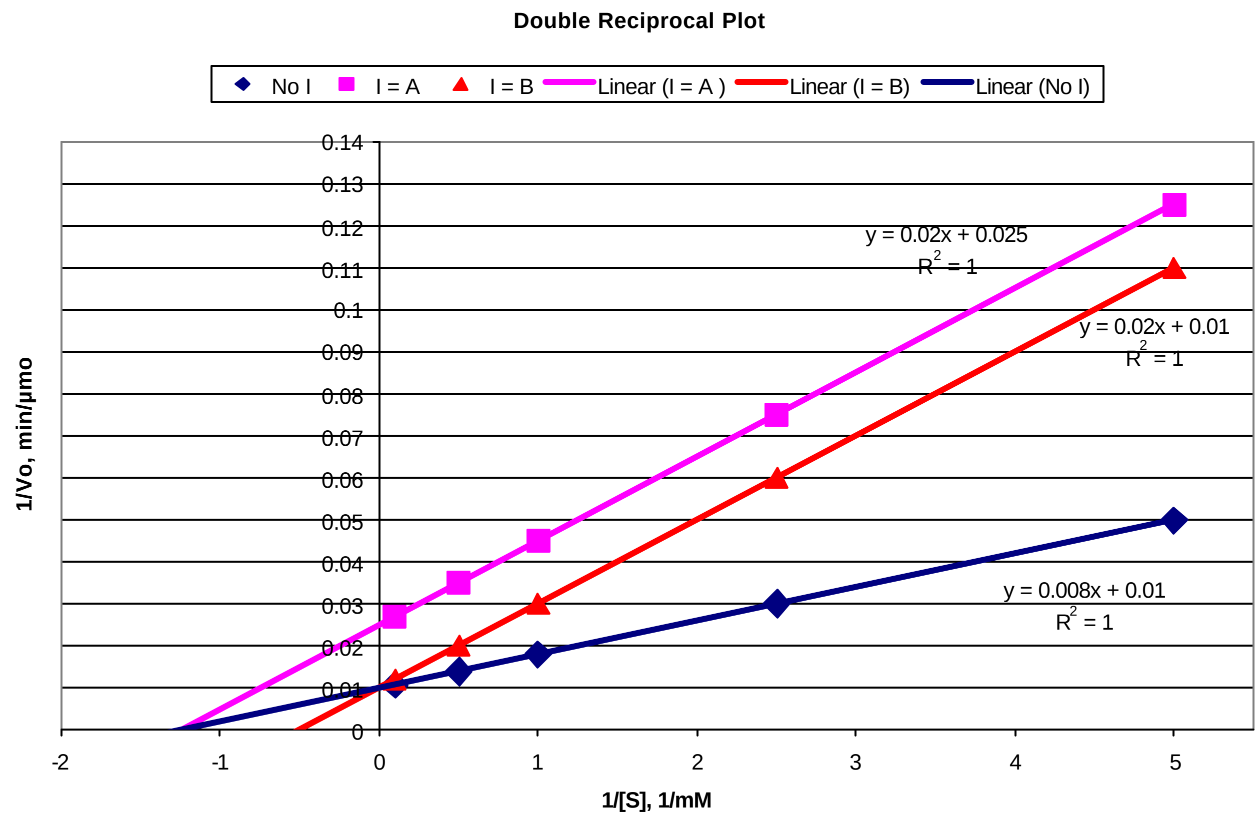
1. 50 Pts. Enzyme Kinetics Problem

- Draw plots of Vo vs [S] and 1/Vo vs 1/[S], using the data given below - 25 Pts
- Determine Km and Vmax for uninhibited reaction (include units) - 10 Pts
- Determine what type of Inhibitor A and B are using the Km' and Vmax' for the inhibitors (show your reasoning by comparing Vmax and Vmax' and Km and Km') - 10 Pts
- Calculate the Ki for binding of Inhibitor A and B to the enzyme (include units) - 5 Pts

[S]	Vo (No I)	Vo (I = 0.1 mM A)	Vo (I = 2.0 mM B)
mM	µmol/min	µmol/min	µmol/min
0.0	0.0	0.0	0.0
0.2	20.0	8.0	9.1
0.4	33.3	13.3	16.7
1.0	55.6	22.2	33.3
2.0	71.4	28.6	50.0
10.0	92.6	37.0	83.3

For Competitive Inhibitor, Km' = Km (1 + [I]/Ki)
For Non-Competitive Inhibitor, Vmax' = Vmax / (1 + [I]/Ki)





Summary Table of Kinetic Constants and Type of Inhibitor for A and B

Parameter	Vmax or Vmax'	Km or Km'	Type of Inhibitor
Units	µmol/min	mM	
No Inhibitor	100	0.8	
Inhibitor A	40	0.8	Non Competitive Vmax' ≠ Vmax Km' = Km
Inhibitor B	100	2.0	Competitive Vmax' = Vmax Km' ≠ Km

Calculation of Ki values for Inhibitor A and B:

Inhibitor A at I = 0.1 mM A
Ki = I/(Vmax/Vmax' - 1) = 0.1 mM/((100/40)-1) = 0.07 mM A

Inhibitor B at I = 2.0 mM B
Ki = [I]/(Km'/Km - 1) = 2.0 mM/((2.0/0.8)-1) = 1.3 mM B

2. 40 Pts. Protein Structure Problem (Use Words and Diagrams)

Describe the four levels of protein structure and bonding stabilizing these structures.
Be sure to illustrate each level of the structure of a protein with a drawing of the bonds or interactions involved among the polypeptide backbone and the amino acid side chains.

SEE Lectures 8 and 9

3. 10 Pts. Thought Question

Why is myoglobin a monomer and hemoglobin a tetramer, when both function as oxygen transport proteins? Explain in terms of the difference in the quaternary structure (4^{th} level of protein structure) in relation to their physiological environments and their functional roles in oxygen transport.

Myoglobin is an oxygen transport protein in muscle and its monomeric quaternary structure is all that is needed in this environment since it simply passes one oxygen molecule on along the concentration gradient from the blood where oxygen concentration is high to the mitochondria in the muscle cells, where the oxygen is consumed in respiration. The monomeric structure of myoglobin provides for only a fixed oxygen affinity, but this suits its role in muscle where the "flow" of oxygen is driven by the high concentration of oxygen in the blood and the low concentration in the mitochondria of muscle cells where oxygen is being rapidly consumed for respiration.

Hemoglobin is an oxygen transport protein in blood and its tetrameric quaternary structure is ideal for the adjustments it must make between the environment in the lung where oxygen pressure is high and hemoglobin gets loaded with oxygen and the peripheral tissues where oxygen pressure is low and hemoglobin must unload oxygen. In other words, the tetrameric structure of hemoglobin allows it to function in the 2 environments of the body where it operates, which could not easily be accomplished if hemoglobin were a monomeric protein. The tetrameric structure of hemoglobin allows adjustment in its affinity for oxygen which are needed to have efficient oxygen loading in the lung and efficient oxygen unloading in the peripheral tissues.