QUESTION (30 points)

The following reaction is catalyzed by an enzyme:

 $A \rightarrow P$

The ES complex is a colored molecule whose formation can be monitored by spectrophotometrically.

Your best friend has studied this enzyme by assaying the formation of the ES using a rapid reaction technique, ascertaining the *instantaneous initial velocity*

[S]	Moles/minute	
2.50E-03	7.5	
5.00E-03	15	
1.00E-02	30	
2.00E-02	60	
4.00E-02	120	
8.00E-02	240	
1.20E-01	360	

a) Calculate k₁ for his results. What assumptions did you need to make and why (be explicit) (4-5 sentences) (5 points)

Your advisor suggests that it might be interesting to study this enzyme reaction by rapid reaction technique in the presence of 10 mM of a known inhibitor and you obtained the following results:

[A] M	d[P]/dt	
	Inh=10 mM	
2.50E-03	9.16E-01	
5.00E-03	1.76E+00	
1.00E-02	3.26E+00	
2.00E-02	5.69E+00	
4.00E-02	9.05E+00	
8.00E-02	1.29E+01	
1.20E-01	1.50E+01	

b) Can you calculate k_1 for the reaction under these conditions? Why or why not (3 sentences) (5points)?

You then performed the same reaction at the same enzyme concentration at steady state in the presence of a known inhibitor of the reaction and obtained these results:

[A]	d[P]/dt	
	Inh=10 mM	
2.50E-03	9.16E-01	
5.00E-03	1.76E+00	
1.00E-02	3.26E+00	
2.00E-02	5.69E+00	
4.00E-02	9.05E+00	
8.00E-02	1.29E+01	
1.20E-01	1.50E+01	

c) Calculate Km and Vmax for the enzyme reaction under these conditions (5 points).

Slope

1.200E-03

0

5

10 15 [Inhibitor] mM 20

25

The replots of the Lineweaver-Burk data obtained at several inhibitor concentrations look like this:



d) What kind of kinetic mechanism and inhibition scheme best reconciles <u>all</u> (and I do mean ALL) these results (6-8 sentences)? In answering this question, please point out how the equation for that scheme describes the results (15 points).

4.000E-03

0

5

10

[Inhibitor] mM

15

20

25

QUESTION 4 (25 points)

a) (12 points) Substance A is consumed by a reaction that <u>only</u> occurs in the presence of substance B. The role of substance B is unknown (i.e., it could be a reactant or a catalyst) and the reaction that consumes A is of unknown order. The initial concentration of A is 2.0 mM and the concentration of A as a function of time is:

Time (min)	[A] remaining	
1	1.6	
2	1.44	
4	1.12	
8	0.76	
16	0.48	

(i) Define what is meant when a reaction mechanism is called 'first order' or 'second order' (3 sentences). (ii) Is the consumption of A a first order reaction and how do you know (4 sentences and a graph)? (iii) If not how would you repeat this experiment such that 'first order' kinetics would be observed and why (4 sentences).

b) (13 points) The temperature dependence for the dissociation rate constants for the substrate S and inhibitor I are given in the table below. (i) Which of these compounds dissociates more slowly (2 sentences)? (ii) Indicate the thermodynamic origin for the difference in dissociation rate constant (Be quantitative please!-also please pay attention to UNITS!). (iii) Why do you think the inhibitor compound was designed (use your answer to part (ii) to guide your thinking) (4-5 sentences)?

	Substrate	Inhibitor
Temperature	k _{off} s⁻¹ (X10³)	k _{off} s⁻¹ (10 ⁶)
4	0.5	0.5
14	1.1	1.1
25	2.4	2.4
40	6.1	6.1
55	15	15