Enzyme kinetics problems

1. A. Use the serine protease reaction scheme to derive an initial rate law for product formation. Show that it has a form equivalent to the Michaelis-Menten model.

$$E + S \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} ES \underset{\hookrightarrow Q}{\overset{k_2}{\rightharpoonup}} EP \underset{H_2O \rightsquigarrow}{\overset{k_3}{\rightharpoonup}} E + P$$

$$\frac{d[P]}{dt} = k_3[EP]$$

$$\frac{d[EP]}{dt} = k_2[ES] - k_3[EP]$$

$$\frac{d[ES]}{dt} = k_{on}[E][S] - (k_2 + k_{off})[ES]$$

Conservation of matter requires:

$$[E]_0 = [E] + [ES] + [EP]$$

B. Define K_m and k_{cat} in terms of the fundamental rate constants in the scheme above.

C. Define the catalytic efficiency k_{cat}/K_m.

D. Determine the dissociation constant for the enzyme substrate complex ES. The data are presented as fraction bound (fb) as a function of the substrate concentration. The substrate in this case is a methoxy tyrosine modified peptide.

[S]	fb
0.00012	0.0232
0.00024	0.092
0.00048	0.1925
0.00092	0.3235
0.00184	0.6105
0.00368	0.8108
0.00734	0.8705
0.01468	0.983
0.02248	0.9958

E. For the same methoxy tyrosine peptide in part D. the k_{cat} = 28 s^{-1} and $K_m = 0.095\ M$. Use the analysis you have done to calculate $k_{on}, k_{off}, and\ k_2$. To make this calculation feasible with the given data, you may assume that $k_{off} << k_2$.

2. A pharmaceutical company is investigating a new lead for a drug known as Cureall. The drug binds to the active site of enzyme Blahase, which tends to make sick people feel lousy if it is not inhibited. The binding equilibrium is known to be:

Cureall + Blahase ←→ Complex (1)

The substrate for Blahase is the carbohydrate Yuckose. Cureall is a competitive inhibitor of Yuckose and prevents the formation Hyperyuckose in the reaction:

Yuckose + Blahase ←→ Hyperyuckose (2)

A chemist reports that the association constant for Cureall is greater than that for Yuckose and that it has a high binding enthalpy. You are an analyst for the investment firm Smartmoney, Inc. and you are asked to examine the thermodynamic data. To determine whether Cureall will be a success in clinical trials you should do the following:

A. Determine the binding (association) enthalpies for Cureall and Yuckose, respectively, with Blahase. Use the data in the van't Hoff plot provided

van't Hoff plot data

1/T (K^{-1})	ln(K_a) for (1)	ln(K_a) for (2)
0.00357	22.34	21.49
0.00345	21.16	20.74
0.00333	20.05	20.05
0.00322	19.01	19.41
0.00312	18.05	18.83

ΔH^o (1) = _____. ΔH^o (2) = _____.

B. Determine the binding (association) entropy for Cureall and Yuckose, respectively, with Blahase.

ΔS^o (1) = _____. ΔS^o (2) = _____.

C. Cureall is a mimic for a carbohydrate, but it is a floppy molecule (i.e. it has many ether linkages and there any many possible conformations of the drug in solution). Your boss asks you to explain the sign of the entropy of binding of the drug.

D. Based on the data in the table, ascertain whether the drug binds more tightly than the native substrate Yuckose at body temperature.

Temperature (K)	K_a for (1) $10^9\ M^{-1}$	K_a for (2) $10^9\ M^{-1}$
280	5.08	2.15
290	1.55	1.02
300	0.51	0.51
310	0.18	0.27
320	0.069	0.15

E. The pharmaceutical company states that the concentration of the drug Cureall can be as high as 10^{-8} M while the native substrate has a concentration of 10^{-6} M (1 micromolar). Assuming these concentrations and an enzyme concentration of 10^{-6} M determine the effect of inhibitor on the enzyme kinetics at 290 K. The enzyme turnover number (i.e. the rate constant k_b) is 1000 s^{-1} at 290 K. The binding half-life for the substrate Yuckose is 69.3 milliseconds. NOTE: The binding constants above are association constants. K_I for inhibition is usually reported as a dissociation constant ($K_I = 1/K_a$ for 1).

K_M = _____. V_{max} = _____.

V = _____ for [S] = 10^{-6} M.

V_I = _____ for [S] = 10^{-6} M and for [I] = 10^{-8} M.

The rate slows by a factor of:

V/V_I = _____.

Assuming the same concentrations as above determine the effect of inhibitor on the enzyme kinetics at 310 K. At this temperature the enzyme turnover number (i.e. the rate constant k_b) is unchanged at 10^3 s^{-1} and the binding half-life for the substrate Cureall is 6.93 milliseconds.

K_M = _____. V_{max} = _____.

V = _____ for [S] = 10^{-6} M.

V_I = _____ for [S] = 10^{-6} M and for [I] = 10^{-8} M.

F. Experts suggest that an inhibitor should lower the enzyme rate by at least a factor 100 to be an effective drug. Should Smartmoney, Inc. invest in Cureall?