Problem 1: Product Inhibition (Ingalls 3.7.4, 20 points)

Many enzymatic reactions that are irreversible are nevertheless subject to *product inhibition*, meaning that the product readily rebinds the free enzyme. To describe product inhibition, consider the scheme:

$$\mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C}_1 \xrightarrow{k_r} \mathrm{C}_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{P} + \mathrm{E}$$

From this reaction scheme, derive the rate law:

$$\frac{d[P]}{dt} = V = \frac{V_{\max}[S]}{[S] + K_M\left(1 + \frac{[P]}{K_P}\right)}$$

Note: This is a specific example of the general phenomenon of competitive inhibition.

Problem 2: Non-competitive Inhibition (20 points)

Non-competitive inhibition occurs when an inhibitor prevents catalysis from occurring by binding to the enzyme independently of the substrate:

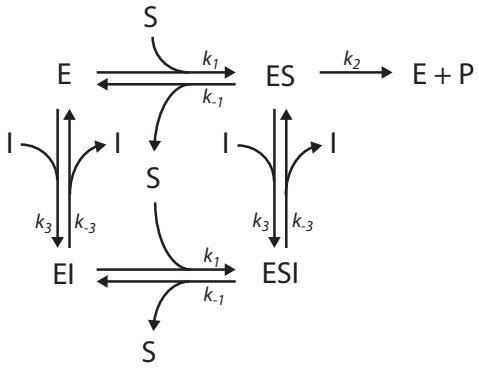

Figure 1: Binding scheme for non-competitive inhibition.

By applying quasi-steady-state approximations to all three complexes and accounting for conservation of the enzyme moiety, show that:

$$\frac{d[P]}{dt} = V = \left(\frac{V_{\max}}{1 + \frac{[I]}{K_I}}\right)\frac{[S]}{[S] + K_M}$$

Problem 3: Lineweaver-Burk Plots (30 points)

K_M and $V_{\max}$ can be determined from measurements of the rate of product formation vs. initial substrate concentration. It is possible to estimate these values from a plot of production formation rate V vs. substrate concentration $[S]$, but only if the substrate concentrations tested were chosen appropriatley.

a) Describe how you would roughly estimate K_M and $V_{\max}$ for the data given in figure 2.

When this approach is not possible due to an inappropriate choice of data points or noise, K_M and $V_{\max}$ can still be estimated using a Lineweaver-Burk (aka double reciprocal) plot of $1/V$ vs. $1/[S]$.

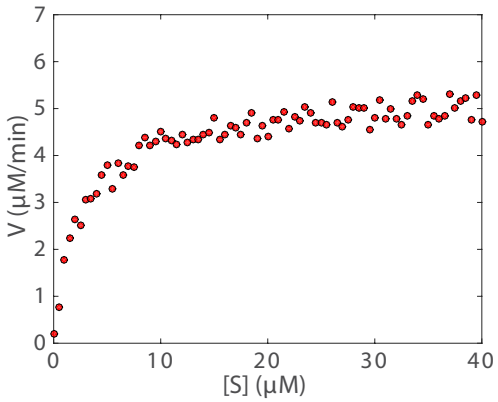

Figure 2: An example plot of reaction rate vs. substrate concentration.

b) Rearrange the Michaelis-Menten expression,

$$\frac{dP}{dt} = V = \frac{V_{\max}[S]}{K_M + [S]},$$

to find a linear expression for $1/V$ in terms of $1/[S]$. Describe how you can determine K_M and $V_{\max}$ from the slope and intercept of the line.

c) Lineweaver-Burk plots can also be used to determine the general mechanism of enzymatic inhibitors. Describe how you would expect the plot to differ in the presence vs. absence of:

(a) A competitive inhibitor

(b) A non-competitive inhibitor

You may find it helpful to refer to the rate laws derived in problems 1 and 2.

d) Apply your approach to the data provided on the course website in the file `rate_data.tsv`. Estimate K_M and $V_{\max}$ in the presence and absence of inhibitor. What type of inhibitor is this?

Problem 4: Concerted Model of Cooperativity (Ingalls 3.7.11, 30 points)

In 1965, Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux proposed a mechanistic model of cooperativity (Monod et al., 1965). Their model addresses a multimeric protein composed of identical subunits, each with one ligand binding site. They supposed that each subunit could transition between two conformations: a tensed state T and a relaxed state R. For each protein molecule, all of the subunits are presumed to have the same conformation at any given time: transitions between the relaxed and tense states are *concerted*.

In the absence of ligand, the tensed state is more stable than the relaxed state. The relaxed state, however, has a higher affinity for ligand. Thus, at a sufficiently high ligand concentration, ligand binding causes the protein to adopt the relaxed state. This increases the protein's affinity for ligand, triggering a positive feedback, and resulting in a sigmoidal binding curve.
This mechanism is called the *MWC model*, or the *concerted model*. The ligand binding scheme for a dimer is shown in figure 3, where R_2 is the dimer of two relaxed monomers, and T_2 is the dimer of two tensed monomers.

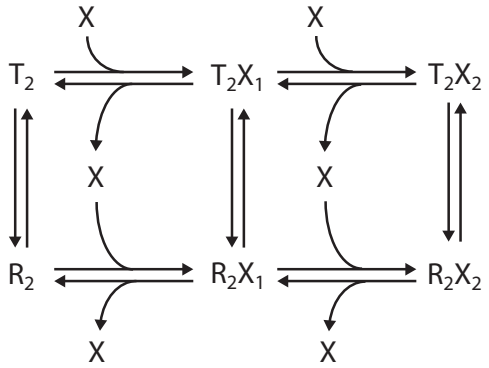

Figure 3: Binding scheme for concerted model of cooperativity (Ingalls problem 3.7.11).

a) Let K be the equilibrium constant for the $R_2 \leftrightarrow T_2$ conversion, i.e.:

$$K = \frac{[T_2]}{[R_2]} \text{ at steady state}$$

Suppose that the dissociation constant for ligand binding to R_2 is K_R, and that the dissociation constant for ligand binding to T_2 is K_T. (The dissociation constants for the first and second binding event are the same, but the association/dissociation rates will depend on stoichiometry factors that reflect the number of sites.)

Confirm that in steady state, the concentrations satisfy

$$[R_2] = \frac{[T_2]}{K} \qquad [R_2X_1] = \frac{2[X][R_2]}{K_R} \qquad [R_2X_2] = \frac{[X][R_2X_1]}{2K_R}$$

$$[T_2X_1] = \frac{2[X][T_2]}{K_T} \qquad [T_2X_2] = \frac{[X][T_2X_1]}{2K_T}$$

(The stoichiometric prefactors reflect the availability of binding sites.) Use these equilibrium conditions to verify that in steady state, the fractional saturation is given by

$$Y = \frac{K\frac{[X]}{K_T}\left(1+\frac{[X]}{K_T}\right) + \frac{[X]}{K_R}\left(1+\frac{[X]}{K_R}\right)}{K\left(1+\frac{[X]}{K_T}\right)^2 + \left(1+\frac{[X]}{K_R}\right)^2} \tag{1}$$

Plot the corresponding binding curves for $K_T = 1000$, $K_R = 1$, and $K = 500$, 1000, and 2000. Verify that although this is not a Hill function, the curves are nevertheless sigmoidal.

b) Consider the special case of the concerted mechanism in which $K = 0$. Interpret the resulting binding mechanism and use formula 1 to verify that the resulting binding curve is hyperbolic. Repeat for the case when $K_R = K_T$.

c) Verify that when the concerted model is applied to a tetramer (such as hemoglobin), the resulting fractional saturation is

$$Y = \frac{K\frac{[X]}{K_T}\left(1+\frac{[X]}{K_T}\right)^3 + \frac{[X]}{K_R}\left(1+\frac{[X]}{K_R}\right)^3}{K\left(1+\frac{[X]}{K_T}\right)^4 + \left(1+\frac{[X]}{K_R}\right)^4}$$