Chapter 8

Case Study: Enzyme kinetics

Because biochemical reactions take place at essentially room temperature (as compared to the highly elevated temperatures of most chemical manufacturing processes), catalysts are needed to allow these reactions to proceed at biologically relevant rates. Enzymes are bio-catalysts that bind with a reactant chemical species (called the substrate) and then either rearrange or break the substrate species to form a new product species.

An important conceptual model of the enzyme action on the substrate is given by the lock and key model (see Fig. 8.1); the enzyme has a binding site that is configured so that only molecules of a specific configuration (and composition) can bind with that site. Once the substrate is bound to that receptor, the chemical reaction takes place, and the products of the reaction are released. While conceptually attractive, this model has been supplanted by others (e.g., the induced fit model) that give a more refined understanding of the overall process.

![Diagram of enzyme action](image)

Figure 8.1: The role of an enzyme in catalyzing the decomposition of a substrate chemical species into a product through the lock and key mechanism.

Given this physical description of the reaction process, we can begin to quantify the rate at which the reactions take place by first defining the chemical species $E = \text{enzyme}$, $P = \text{product}$, and $S = \text{substrate}$ (reactant). With this notation, the reactions to form the enzyme-substate complex $ES$ and the subsequent product $P$ can be written as

$$E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P.$$
8.1 Michaelis-Menten kinetics

The total rate at which the substrate S is consumed and product species P is produced will depend on the concentration of the relevant chemical species in the solution. To distinguish the species (e.g., S) from its concentration value, the [ ] notation frequently is used, and so the concentration of S will be denoted [S]. It is important to keep clear the differences between this notation and the use of the brackets to denote MATLAB matrices.

We now consider a case where the enzyme and substrate are mixed initially in a flask and some means of measuring the instantaneous substrate concentration and product production rate are available (as an approximation, one may accurately measure the initial substrate concentration and then estimate the initial reaction rate from data taken shortly after the reaction proceeds). The overall system is homogeneous - the enzyme is not fixed to a solid substrate, but is well-mixed with the reactants. In this experiment, the initial enzyme concentration [E₀] also is known.

A critical assumption in analyzing the kinetics of the process is that the reaction between the substrate and enzyme is extremely fast relative to the reverse reaction or the product-forming reaction; under this assumption the concentration of the ES complex is at quasi-steady state with respect to substrate concentration [S]:

\[ k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 \]

or

\[ [ES] = \frac{k_1[E][S]}{k_{-1} + k_2} = \frac{[E][S]}{K_m} \]

with

\[ K_m = \frac{k_{-1} + k_2}{k_1}. \]

Determining a total molar balance on the enzyme:

\[ [E_0] = [E] + [ES] \quad \text{so} \quad [E] = [E_0] - [ES] \]

and

\[ [ES] = \frac{([E_0] - [ES])[S]}{K_m} \quad \Rightarrow \quad [ES] = \frac{[E_0][S]}{K_m + [S]} \]

So the production rate of species P (mol/time or mass/time) is

\[ R_p = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} = \frac{V_m[S]}{K_m + [S]} \]

Having found

\[ R_p = \frac{V_m[S]}{K_m + [S]} \quad (8.1) \]

we can invert the equation to find

\[ \frac{1}{R_p} = \frac{K_m}{V_m[S]} + \frac{1}{V_m} \]

and so the traditional method to determining the rate parameters \( V_m \) and \( K_m \) is to create a plot of \( 1/R_p \) versus \( 1/[S] \) in the form of a Lineweaver-Burk plot, where the intercept is used to determine \( V_m \), and the slope is used to subsequently determine \( K_m \).
8.2. REVIEW PROBLEMS

Given a set of data corresponding to measured values of $R_p$ versus $[S]$, we can use the least squares technique to fit a straight line $\hat{y} = b_0 + b_1 x$ through the plotted data $y = 1/R_p$ versus $x = 1/[S]$. Determine $V_m$ from $b_0$, and then $K_m$ from $b_1$ and $V_m$. However, because $V_m$ is used to compute $K_m$, any error in determining the former is propagated in computing the latter. One approach that may result in a more balanced distribution of error among the identified coefficients is to rearrange (8.1) in the following way:

$$R_p K_m - [S] V_m = -R_p [S]$$

(8.2)

8.1.1 Reaction data

We demonstrate our approach using data taken from Table 1.1 of [7] and reproduced in Table 8.1. The first column of the data represents the substrate concentrations (experiment inputs) and the remaining four columns represent four sets (a-d) of experimental data. Using (8.2), we identify a set of $K_m$ and $V_m$ values for each of the four data sets (a-d) and plot the results in Fig. 8.2. We see that in each case, we achieve a reasonable degree of accuracy in terms of the model fit. In particular, case (a) appears to be the best in that the residuals (the difference between the model prediction and measured values) are normally distributed and are not a function of $R_p$. However, we see that one data point in (b) appears questionable, there is a strong dependence of the residual on $R_p$ in case (c), and the lack of precision in data set (d) strongly affects the residual for that case.

8.2 Review Problems

1. Determine the Michaelis-Menten kinetics constants $V_m$ and $K_m$ for the following set of data:

$$[S] = [0.138, 0.220, 0.291, 0.560, 0.766, 1.46]$$

and

$$R_p = [0.148, 0.171, 0.234, 0.324, 0.390, 0.493]$$

Table 8.1: Enzyme kinetic data taken from [7].

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Figure 8.2: Plots of demonstration data compared to rate curves fitted by linear regression (left) and residual plots ($\hat{R}_p - R_p$ versus $\hat{R}_p$).