Systems Biology Tutorial 3: Enzyme-catalyzed kinetics

1. Consider the Michaelis-Menten equation

$$v = \frac{V_m \frac{s}{K_s}\left(1 - \frac{\Gamma}{K_{eq}}\right)}{1 + \frac{s}{K_s} + \frac{p}{K_p}}, \tag{1}$$

with $K_s = 1$, $K_p = 10^4$, $V_m = 2$, $K_{eq} = 10^4$ and $p = 0$.

   (a) Plot the rate as a function of substrate concentration ($0 \leq s \leq 10$).

   (b) Use the Manipulate function in Mathematica to plot the rate as a function of substrate concentration ($0 \leq s \leq 10$) and vary the following parameters (use PlotRange to fix the y-axis scale):

      i. $0 \leq p \leq 10000$

         A. What happens at low substrate concentrations when the product concentration is high? Why?

      ii. $0.1 \leq K_s \leq 10$

         A. What happens to the rate as K_s increases? What property of the reaction are you varying and does this agree with your result?

      iii. $0 \leq V_m \leq 10$

         A. Explain the behaviour of the rate as V_m increases.

   (c) At what substrate concentration is the enzyme most sensitive to a change in substrate concentration? Answer this by plotting the elasticity as a function of substrate concentration ($0 < s \leq 50$).

      i. Plot $\frac{s}{v} \cdot D[v, s]$ as a function of s.

      ii. Perturbation analysis (use the notebook Tut3_Q2_d_iii.nb)

         A. Calculate the rate at a chosen substrate concentration (call this v_{wild}),

         B. Increase the substrate concentration by 1% (multiply by 1.01). Calculate the new rate (call this v_{up}),

         C. Decrease the substrate concentration by 1% (multiply by 0.99). Calculate the new rate (call this v_{down}),

         D. Calculate the elasticity at the specific substrate concentration chosen in A by using

$$\varepsilon_s^v = \frac{v_{up} - v_{down}}{2 \times 0.01 \times v_{wild}}.$$

         E. Repeat this for a number of substrate concentrations to obtain a plot of the elasticity as a function of substrate concentration.

2. Consider the reversible Hill equation

$$v = \frac{V_m \frac{s}{s_{0.5}}\left(1 - \frac{\Gamma}{K_{eq}}\right)\left(\frac{s}{s_{0.5}} + \frac{p}{p_{0.5}}\right)^{n-1}}{1 + \left(\frac{s}{s_{0.5}} + \frac{p}{p_{0.5}}\right)^{n}}, \tag{2}$$

with $s_{0.5} = 1$, $p_{0.5} = 1$, $V_m = 2$, $K_{eq} = 10^6$ and $p = 0$.

   (a) Use the Manipulate function in Mathematica to plot the rate as a function of substrate concentration ($0 \leq s \leq 5$) and vary the Hill coefficient $1 \leq n \leq 4$ (use PlotRange to fix the y-axis scale).

   (b) At what substrate concentration is the enzyme most sensitive to a change in substrate concentration for

      i. $n = 1$?

      ii. $n = 4$?

(Answer this by plotting the elasticity as a function of substrate concentration as discussed above.)

(c) When a modifier is added to Eq. 2 it becomes

$$v = \frac{V_m \frac{s}{K_s}\left(1 - \frac{\Gamma}{K_{eq}}\right)\left(\frac{s}{s_{0.5}} + \frac{p}{p_{0.5}}\right)^{n-1}}{\frac{1+\left(\frac{x}{x_{0.5}}\right)^n}{1+\alpha\left(\frac{x}{x_{0.5}}\right)^n} + \left(\frac{s}{s_{0.5}} + \frac{p}{p_{0.5}}\right)^n}. \tag{3}$$

i. Set $s_{0.5} = 1$, $p_{0.5} = 10^4$, $x_{0.5} = 1$, $V_m = 2$, $K_{eq} = 10^4$, $s = 1$ and $p = 1$ and plot the rate as a function of modifier concentration for

A. $\alpha = 10^{-4}$,

B. $\alpha = 10^4$.

What types of modifiers do these values of α represent (activator/inhibitor)?

3. The CSV files in the download section of the website provide experimental datasets for a kinetic characterisation of an enzyme reaction that produces NADH, where the substrate concentration was varied and the reaction followed in a spectrophotometric assay. The substrate concentration is given in the filename. Each dataset contains two columns, the first one is the time (s) and the second one is the [NADH] (mM) at each particular time-point. No product was initially present (i.e. these are initial rates).

(a) ListPlot a few tables to ensure that the data appears linear (within experimental error).

(b) Use FindFit on every table to fit a line and obtain its gradient (i.e. the rate of the reaction).

(c) For every table you now know the initial substrate concentration and the associated rate. Make a new table which consists of these concentration values and associated rates. ListPlot this new table.

(d) Use FindFit to fit the forward rate in Eq. 1 to this data and obtain values for V_m and K_s. What are the units of these parameters? How well do they compare to the exact values $V_m = 1.23$ and $K_s = 12$?

(e) Plot your rate equation on the same axes as the ListPlot in (c). Is this a good fit?