Systems Biology Tutorial 3: Enzyme-catalysed reaction kinetics

A skeleton Jupyter notebook that gives you pointers for answering this tutorial is available from the download section of the course website.

1. Consider the **Michaelis-Menten equation**

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

with $K_s = 1$, $K_p = 10$, $V_m = 2$, $K_{eq} = 100$ and $p = 0$.

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

   (b) Plot the rate as a function of substrate concentration ($0 \le s \le 10$) and vary the following parameters:

   $0 \le p \le 10000$
   Plot the graphs for $p = 0, 1, 10, 100$ and 1000 on the same set of axes. What happens at low substrate concentrations when the product concentration is high? Why?

   $0.1 \le K_s \le 10$
   Plot the graphs for $K_s = 0.1, 0.5, 1, 5$ and 10 on the same set of axes (set $p = 0$). What happens to the rate as K_s increases? What property of the reaction are you varying and does the result agree with your intuition?

   $0 \le V_m \le 10$
   Plot the graphs for $V_m = 0, 2, 4, 6, 8$ and 10 on the same set of axes (set $p = 0$). 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 ($0 \le s \le 50$).

      i. Plot $\frac{s}{v} \cdot \frac{\partial v}{\partial s}$ as a function of s. To calculate the derivative ${\partial v}/{\partial s}$, use the class `numdifftools.Derivative` (read the documentation/docstring to see how this class must be instantiated and used, set the 'step' value to 0.0001).

      ii. Perturbation analysis (use the code as given in the skeleton notebook):
      - calculate the rate at a chosen substrate concentration (call this v_{wild});
      - increase the substrate concentration by 1% (multiply by 1.01). Calculate the new rate (call this v_{up});
      - decrease the substrate concentration by 1% (multiply by 0.99). Calculate the new rate (call this v_{down});
      - calculate the elasticity at the specific chosen substrate concentration by using

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

      - 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**

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

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

(a) Plot the rate as a function of substrate concentration ($0 \leq s \leq 5$) and vary the Hill coefficient (plot the graphs for $h = 1, 2, 3$ and 4 on the same set of axes).

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

i. $h = 1$?

ii. $h = 4$?

(Answer this by plotting the elasticity as a function of substrate concentration for $0 \leq s \leq 5$ as in Question 1(c)i above, for both values of h.)

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

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

Set $s_{0.5} = 1$, $p_{0.5} = 10^4$, $x_{0.5} = 1$, $V_m = 2$, $K_{eq} = 10^4$, $h = 4$, $s = 1$ and $p = 1$ and plot the rate as a function of modifier concentration x ($0 \leq x \leq 5$) for

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

ii. $\alpha = 10^4$.

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

3. **Fitting to data:** 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) Using the `pd.read_csv()` function, load these data into pandas dataframes (a separate dataframe for each substrate concentration). Plot the data (NADH concentration *vs.* time) for the 8 mM and 20 mM datasets to ensure that the data appears linear (within experimental error).

(b) Do a linear regression (`sp.stats.linregress`) on each dataset to fit a line and obtain its slope (i.e. the rate of the reaction). The skeleton notebook gives some pointers on how to do this.

(c) For every dataset you now know the initial substrate concentration and the associated rate (slope). Make a new array for all the substrate concentrations, and collate these concentrations and the calculated rates from Question (3b) into a new pandas dataframe. Plot the rates against the substrate concentrations.

(d) Use `lmfit.Model()` as in Tutorial 2 to fit the forward rate in Eq. 1 to these 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 graph in Question (3c). Is this a good fit?