# Systems Biology Tutorial 3: Enzyme-catalyzed reactions

1. **Michaelis-Menten equation**

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

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

(a) Plot the rate as a function of substrate concentration $(0 \leq s \leq 10)$ for three different product concentrations ($p = 0, p = 100$ and $p = 1000$). Use one set of axes to show the three curves.

i. What happens at low substrate concentrations when the product concentration is high and why?

(b) Plot the rate as a function of substrate concentration $(0 \leq s \leq 10)$ for the three values of $K_s = 0.1$, $K_s = 1$ and $K_s = 10$ (and set $p = 0$). Use one set of axes to show the three curves.

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

(c) Plot the rate as a function of substrate concentration $(0 \leq s \leq 10)$ for the three values of $V_m = 0.1$, $V_m = 1$ and $V_m = 10$ (and set $p = 0$). Use one set of axes to show the three curves.

i. Explain the behaviour of the rate as $V_m$ increases.

(d) 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 $(0 < s \leq 50)$.

- Derive the elasticity as the normalised derivative of the rate with respect to $s$, i.e. $\varepsilon_s^v$. Use `D[v,s]*s/v`.
- Plot this expression (using the parameter values above and set $p = 0$) as a function of $s$ and look for a maximum.

2. **Reversible Hill equation**

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

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 $(0 \leq s \leq 5)$ for the three values of the Hill coefficient: $h = 1$, $h = 2$ and $h = 4$. Use one set of axes to show the three curves.

i. At what substrate concentration is the enzyme most sensitive to a change in substrate concentration for $h = 4$?

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

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

i. Set $h = 4$, $s_{0.5} = 1$, $p_{0.5} = 10^4$, $x_{0.5} = 1$, $V_m = 2$, $K_{eq} = 10^4$, $s = 1$ 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 $\alpha$ 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. Each dataset contains two columns, the first one is the time (seconds) and the second one is the corresponding [NADH] (mM). The substrate concentrations are provided in the filenames. No product was initially present (i.e. these are initial rates).

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

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

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

   (d) Use `NonlinearModelFit` to fit the forward MM 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 $K_s = 4$?

   (e) `Plot` your rate equation and `Show` it on the same axes as the `ListPlot` in 3c.