Assignment 1: Enzyme Kinetics and Inhibitors

Computational Biology 2020-2021

Deadline 8 April 2021

Law of Mass Action

Biochemical kinetics is based on the mass action law, introduced by Guldberg and Wagge in the 19th century. It states that the reaction rate is proportional to the probability of a collision of the reactants. This probability is in turn proportional to the concentration of reactants to the power of the molecularity, i.e., the number in which they enter the specific reaction. For a simple reaction of molecules A and B, with k_+ and k_- the so-called kinetic or rate constants, which is defined as:

$$A + B \underset{v_-}{\overset{v_+}{\rightleftharpoons}} 2P$$

the net reaction rate equals the forward rate minus the backward rate. The forward rate is a function of the concentrations of A and B times the forward kinetic rate constant k_+. The backward rate is a function of concentration P times the backward kinetic rate constant k_- :

$$v = v_+ - v_- = k_+[A][B] - k_-[P]^2$$

where v is the net rate, v_+ the rate of the forward reaction and v_- the rate of the backward reaction. The dynamics (of the concentration) for this system are described by the following ODEs:

$$\frac{dA}{dt} = \frac{dB}{dt} = -v \ , \ \frac{dP}{dt} = 2v$$

The general mass action law for a reaction with substrate S_i and product concentration P_j reads:

$$v = v_+ - v_- = k_+ \prod_i S_i^{m_i} - k_- \prod_j P_j^{m_j}$$

where m_i and m_j denote the respective molecularities of S_i and P_j in this reaction.

The rate constants are related to the equilibrium constant in the following way:

$$K_{eq} = \frac{k_+}{k_-} = \frac{\prod_i S_i^{m_i}}{\prod_j P_j^{m_j}}$$

Enzyme Kinetics

Consider a simple enzyme catalysed reaction substrate S to form a product P is catalyzed by an enzyme E, as shown in the equation below. The S binds to E to form an enzyme-substrate complex ES, after which S is conversed to P in an irreversible reaction. (Note that most enzymatic reactions involve two or more substrates)

$$S + E \underset{k_-}{\overset{k_+}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} P + E$$

In these systems we assume that $[S] \gg [E]$ and that the amount of enzyme molecules (E_{total}) is constant over time. These enzyme molecules appear as free enzymes E or bounded in complexes such as ES. We can define E_{total} the sum of all these states. In our simple example this means that we define E_{total} as the equation below.

$$E_{total} = [E] + [ES]$$

The dissociation and association rates of [ES] are usually higher than the production rate of P, making the latter the rate-determining step. This means that we can write the overall rate as equation below, where k_{cat} is the catalytic constant.

$$v = k_{cat}[ES]$$

Michaelis-Menten Equation

The rate of the overall reaction can be given by the well-known Michaelis-Menten equation, as shown below. It shows how the reaction rate v changes for different concentrations of substrate $[S]$ for a constant amount of enzyme E_{total}, where V_{max} is the maximum velocity and K_m the Michaelis constant.

$$v = V_{max}\frac{[S]}{K_m + [S]} \ , \ K_m = \frac{k_- + k_{cat}}{k_+}$$

The equation has the shape of a rectangular hyperbola with V_{max} as asymptotic value (Figure 1). Half of the maximum velocity is achieved when substrate concentration is equal to the Michaelis constant K_m.

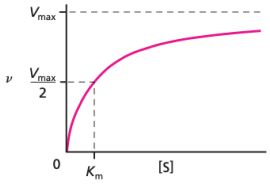

Figure 1: Michaelis-Menten Equation, from Principles of biochemistry (5th ed, H. Robert et. al.)

Derivation Michaelis-Menten Equation

The derivation of the Michaelis-Menten equation starts with the rate-determining step, as shown below.

$$v = k_{cat}[ES] \tag{1}$$

We assume steady-state to determine the [ES], which is now a constant value due to the steady-state, which means that the association rate is equal to the sum of the dissociation rate and the production rate of P.

$$\frac{d[ES]}{dt} = 0$$

$$(k_- + k_{cat})[ES] = k_+[E][S]$$

Rearranging the constant gives us the Michaelis constant.

$$\frac{(k_- + k_{cat})}{k_+}[ES] = [E][S]$$

$$K_m[ES] = [E][S] \tag{2}$$

We use the equation for E_{total} to replace $[E]$ by $(E_{total} - [ES])$ and to reduce the of number variables.

$$K_m[ES] = (E_{total} - [ES])[S]$$

Now bring $[ES]$ to the left and rewrite the equation in such a way that we obtain a value for $[ES]$.

$$[ES](K_m + [S]) = E_{total}[S]$$

$$[ES] = E_{total}\frac{[S]}{K_m + [S]}$$

If we now use this equation for $[ES]$ and put it in equation (1), we obtain the following result:

$$v = k_{cat}[ES] = k_{cat}E_{total}\frac{[S]}{K_m + [S]}$$

Since we assumed $[S] \gg [E]$, we can replace $k_{cat} * E_{total}$ by V_{max}, leaving us with the Michaelis-Menten equation.

$$v = V_{max}\frac{[S]}{K_m + [S]}$$

Question 1: Michaelis-Menten Equation (20p)

(a) Analyse the Michaelis-Menten Equation. What is the meaning of K_m and V_{max}?

(b) Why can we assume $V_{max} = E_{total} * k_2$?

(c) We already noted that $[S] \gg [E]$. Now assume that we are in the unlikely event where $[E] \gg [S]$. Does the assumption $V_{max} = E_{total} * k_2$ still stand? Explain this.

(d) Plot the Michaelis-Menten Equation for $K_m = 1$ and $V_{max} = 25$ and describe it's behaviour.

(e) Change K_m to 0.5. Describe what you see.

(f) Change V_{max} to 15. Describe what you see.

(g) What would happen to V_{max} and K_m if we would add twice as much enzyme to our experiment?

Question 2: Lineweaver-Burk (30p)

The Lineweaver-Burk plot is a handy tool for determining the Michaelis-Menten parameters from data, especially in those times where smart fitting-tools did not yet exist. In this question we will analyse this method step by step.

This question uses the data in MeasuredData.csv, which is a generated data list of measured substrate concentrations [S] and reaction rates [V].

(a) Plot the data and retrieve the Michaelis-Menten parameters. Have a look into the curve_fit-function of scipy.

(b) Now we create the Lineweaver-Burk plot. Instead of plotting the values of S and V, The Lineweaver-Burk plot plots $\frac{1}{V}$ against $\frac{1}{S}$. Describe your plot.

(c) The Lineweaver-Burk plot shows a linear relation between $\frac{1}{V}$ and $\frac{1}{S}$. Rewrite the Michaelis-Menten equation and obtain the terms of a and b in the equation below. (Hint: Start with $(V)^{-1} = (V_{max} * \frac{S}{K_m + S})^{-1}$).

$$\frac{1}{V} = a + b\frac{1}{S}$$

(d) The Michaelis-Menten parameters can be determined by the intersections with the vertical and horizontal axis. Use the equation obtained in (c) to show that the location of these intersections are $\{0, \frac{1}{V_{max}}\}$ and $\{-\frac{1}{K_m}, 0\}$.

(e) Retrieve the Michaelis-Menten parameters from your Lineweaver-Burk plot. Do they correspond with the parameters obtained in (a)?

(f) What are the advantages of obtaining the values of the parameters using the Lineweaver-Burk plot? Can you also think of some disadvantages?

Question 3: Inhibitors (30p)

Inhibitors are small compounds that can reversibly bind to the enzyme and change its activity. They can play important roles regulating metabolic reaction, but can also act as a poison. There are three types of reversible inhibitors: Competitive inhibitors, Uncompetitive inhibitors and Noncompetitive inhibitors. The equilibrium between E and I and the enzyme complex EI is characterized by the Inhibition Constant K_i.

Competitive inhibitors

The competitive inhibitor binds to the free enzyme only, so I is a direct competitor for S.

$$E + I \rightleftharpoons EI \text{ with } K_i = \frac{[I][E]}{[EI]}$$

Uncompetitive inhibitors

The uncompetitive inhibitor binds to the enzyme-substrate complex and prevents the conversion from substrate to product.

$$ES + I \rightleftharpoons ESI \text{ with } K_i = \frac{[I][ES]}{[ESI]}$$

Noncompetitive inhibitors

The noncompetitive inhibitor binds to both the free enzyme and the enzyme-substrate complex. S can still bind to the enzyme-inhibitor complex, but the inhibitor makes the conversion from substrate to product impossible.

$$E + I \rightleftharpoons EI \text{ and } ES + I \rightleftharpoons ESI \text{ with } K_i = \frac{[I][E]}{[EI]} = \frac{[I][ES]}{[ESI]}$$

In the following questions we will analyse the effect of these three inhibitors on the Michealis-Menten equation.

(a) All three inhibitors form a complex with the enzyme, with either E, ES or both. Use the definition of K_i to derive the following equations for E_{total}. (Hint: How do we define E_{total}?)

Competitive inhibitor: $E_{total} = [ES] + (1 + \frac{[I]}{K_i})[E]$
Uncompetitive inhibitor: $E_{total} = (1 + \frac{[I]}{K_i})[ES] + [E]$
Noncompetitive inhibitor: $E_{total} = (1 + \frac{[I]}{K_i})([ES] + [E])$

(b) Derive the following Michaelis-Menten equations for all three inhibitors. Follow the steps of the derivation of the Michaelis-Menten equation in the introduction (start with equation 2), but use the E_{total} as obtained in (a).

Competitive inhibitor: $v_c = V_{max}\frac{[S]}{(1+\frac{[I]}{K_i})K_m+[S]}$
Uncompetitive inhibitor: $v_u = V_{max}\frac{[S]}{K_m+(1+\frac{[I]}{K_i})[S]}$
Noncompetitive inhibitor: $v_n = V_{max}\frac{[S]}{(1+\frac{[I]}{K_i})(K_m+[S])}$

(c) Predict the influence of [I] and K_i on V_{max} and K_m, based on the equations obtained in (b).

(d) Plot the function for the competitive inhibitor for different values of I and Ki together with the standard Michaelis-Menten equation using $V_{max} = 12$ and $K_m = 1$. Describe what you see and explain your result on a molecular level. Does it correspond to the predictions you made in (c)? Do the same for the other two inhibitors.

(e) The datasets DataI2.csv, DataI5.csv and DataI8.csv are measurements of S and V for different concentrations I, respectively $[I] = 2, [I] = 5$ and $[I] = 8$. Find out what kind of inhibitor [I] is using the Lineweaver-Burk plot. Elaborate on your decision.