In biochemistry courses (including this one), the basics of steady-state enzyme kinetics are most often taught for the specific case of the Briggs-Haldane mechanism:

\[
E + S \xrightarrow{k_i} E \cdot S \xrightarrow{k_i} E + P
\]

This can sometimes lead to confusion about which kinetic parameters and equations relate to experimental results, which relate to theoretical mechanisms in general, and which are specific to the Briggs-Haldane mechanism in particular. The following is an attempt to unconfuse you on those points:

**Empirical kinetic parameters**

In principle, one can make on *any* enzyme a series of measurements that give the initial rate \( v \) of the reaction as a function of a substrate concentration (at some total enzyme concentration \( E_0 \) at fixed temperature, pH, etc...). Such an experiment allows you to determine these empirical steady-state kinetic parameters:

- \( V_{\text{max}} \), the asymptotic rate in the limit of high substrate concentration \([S]\),
- \( K_M \), the substrate concentration at which the rate is \( V_{\text{max}}/2 \), and
- \( k_{\text{cat}} = \frac{V_{\text{max}}}{E_0} \)

For enzymes with more than one substrate (e.g., hexokinase) each substrate has its own \( K_M \). \( K_M \), \( k_{\text{cat}} \), and \( V_{\text{max}} \) are meaningful (and useful!) experimentally determined quantities even if the mechanism of the enzyme is not known.

**Hyperbolic rate-vs.-substrate-concentration curves**

In most cases (but not all\(^2\)), the measured \( v \) will be found to have a hyperbolic dependence on \([S]\). In such cases, the best way to determine the kinetic parameters is to fit the data with the Michaelis-Menten equation:

\[v = \frac{V_{\text{max}} [S]}{K_M + [S]}\]

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\(^1\)Some or all of the kinetic parameters cannot be defined or measured in certain rare cases in which the rate curve does not increase monotonically or cannot be shown to saturate.

\(^2\)As an example, mechanisms in which two molecules of the same substrate must bind before any covalent chemistry happens, \( E + S \times E\mathbb{C} + S \times E\mathbb{C}_2 \times E\mathbb{C} \times \mathbb{G} \) do not in general have hyperbolic \( v \) vs. \([S]\) curves.
\[
v = \frac{V_{\text{max}} [S]}{K_M + [S]} = V_{\text{max}} \frac{1}{1 + \left(\frac{[S]}{K_M}\right)}
\]

As can be seen from the rightmost expression above, this is a rectangular hyperbola of the form \((\text{constant}) \times \left\{ax / (1 + ax)\right\}\) which has maximum rate \(V_{\text{max}}\) at half-maximum rate equal to \(K_M\), and initial slope \(V_{\text{max}} / K_M\).

If the curve is indeed hyperbolic, one can make certain basic interpretations of the kinetic parameters that are independent of what the actual mechanism is. These are:

- \(k_{\text{cat}}\) is the effective first-order rate constant for the overall enzymatic reaction under conditions when substrate concentration(s) are sufficiently high that substrate-binding steps are not rate limiting. Those are the conditions under which the enzyme behaves like it is doing a simple first-order reaction: the reaction rate is proportional to \(E_0\) and independent of \([S]\). \(k_{\text{cat}}\) must be less than or equal to the sum of all first order rate constants in the mechanism. \(k_{\text{cat}}\) will in general be some mathematical function of many or all rate constants in the mechanism with the exception of the second-order rate constants for substrate-binding steps.

- \(k_{\text{cat}} / K_M\) is the effective second-order rate constant for the reaction between the enzyme and a substrate when the concentration of that substrate is sufficiently low that its binding limits the rate of the overall enzymatic reaction. Those are the conditions under which the enzyme behaves like it is doing a simple second-order reaction with the substrate: the reaction rate is proportional to \(E_0\) and proportional to \([S]\). At least one of the second-order rate constants for binding of a substrate must be greater than \(k_{\text{cat}} / K_M\) for that substrate.

- One basic interpretation that CANNOT be made without knowing the mechanism: \(K_M\) is NOT necessarily the equilibrium constant for dissociation \((K_D)\) of substrate from enzyme. Even for a simple mechanism like Briggs-Haldane, \(K_D = K_M\) only when the rate constants in the mechanism satisfy certain conditions (see below).

**Briggs-Haldane mechanism**

For the Briggs-Haldane mechanism (given at the top of p. 1), we have the rate equation

\[
v = \frac{\partial P}{\partial t} = k_2 [E \cdot S].
\]
To determine \([E\cdot S]\), we make the steady-state approximation that this concentration does not change with time:

\[0 = \frac{\partial([E\cdot S])}{\partial t} = k_1[E][S] - (k_2 + k_{-1})[E\cdot S].\]

Defining the total enzyme concentration \(E_0 / [E] + [E\cdot S]\) gives

\[ [E\cdot S] = \frac{E_0[S]}{\left(\frac{k_{-1} + k_1}{k_1}\right) + [S]} = \frac{E_0}{1 + \left(\frac{k_1}{k_{-1} + k_1}\right)[S]}, \]

demonstrating that \([E\cdot S]\) has a hyperbolic dependence on \([S]\). Substituting this into the rate equation, we get

\[ v = \frac{k_2 E_0[S]}{\left(\frac{k_{-1} + k_1}{k_1}\right) + [S]} . \]

By comparing this with the Michaelis-Menten equation, we find that for this particular mechanism,

- \(k_{\text{cat}} = k_2,\)
- \(K_M = (k_{-1} + k_2) / k_1,\) and
- \(k_{\text{cat}}/K_M = k_2 k_1 / (k_{-1} + k_2).\)

Note that \(K_M \cdot K_D\) (the dissociation equilibrium constant for substrate binding, \(k_{-1} / k_1\)) ONLY IF \(k_{-1} \gg k_2\).