Kinetics Calculations

Kinetics is all about measuring rates. In chemistry, a **reaction rate** is defined as change in concentration over change in time. In enzyme kinetics, things are no different; we are concerned with the change in concentration of products or subtrates as an ezyme reaction progresses.

Since we cannot see the actual molecules reacting inside an enzyme, we must observe them indirectly, using an **enzyme assay**. This assay often involves monitoring the change in color as a reaction progresses.

Initial Velocity & the Beer–Lambert Law

In our case, because we are actually observing the release of *p*-nitrophenol (PNP) from the substrate as the enzyme performs its reaction, the initial rate, or **initial velocity**, that we are determining is $\delta[\text{PNP}]/\delta t$.

We cannot measure this directly, but it can be calculated from the **steepest slope** of absorbance over time at the beginning of a reaction. In our case, we measure the slope of absorbance at 420 nm over minutes of the reaction. Then, we use the Beer–Lambert law, which is $A = \varepsilon l c$, where ε is the extinction coefficient in $\text{mM}^{-1}\ \text{cm}^{-1}$, l is the path length in cm, and c is the concentration in mM. Rearranging the Beer–Lambert law ($[\text{PNP}] = c_{\text{PNP}} = A_{420\ \text{nm}}/\varepsilon_{\text{PNP}} l$), we can calculate the rate of PNP release from the steepest slope[1] as follows:

$$rate = \frac{\delta[\text{PNP}]}{\delta t} = \frac{\delta A_{420\,\text{nm}}/\delta t}{\varepsilon_{\text{PNP}} l} = \frac{slope}{\varepsilon_{\text{PNP}} l}$$

Since absorbance, being the logarithm of the ratio of light transmitted and light received, has no units,[2] using the above equation provides a rate in units of mM PNP/min, if the provided slope is in inverse minutes.

For example, if we measure an intitial steepest slope of $A_{420\ \text{nm}}$ of $1.80\ \text{min}^{-1}$:

$$rate = \frac{1.80\ \text{min}^{-1}}{(18\ \text{mM}^{-1}\cancel{\text{cm}^{-1}})(0.5\ \cancel{\text{mm}})} \cdot \frac{10\ \cancel{\text{mm}}}{\cancel{\text{cm}}} = 2\ \text{mM/min}$$

The Michaelis–Menten Equation

Two scientists, Leonor Michaelis and Maud Menten found an equation that relates the rate of an enzyme reaction, which we just determined above, to the concentration of that enzyme's substrate, and that equation is called the **Michaelis–Menten equation**:

$$rate = \frac{v_{\max}[\text{S}]}{K_{\text{M}} + [\text{S}]}$$

…where [S] is the molar substrate concentration.

v_{max}[3] in the equation is **maximum velocity**. It is the fastest rate that the enzyme can obtain under **saturating conditions**, when we have far more substrate molecules than enzyme molecules.

The constant K_M in the equation is called the **Michaelis constant**. It has units of molar concentration and represents the molarity for which the reaction rate is half of its maximal value. It is an indication of how well an enzyme binds to a substrate, with *lower* values corresponding to tighter binding.

There is another very important value that is not actually given in the Michaelis–Menten equation. The **catalytic rate constant**, k_{cat}, is the maximum number of product molecules released per molecule of enzyme per unit of time. It is also called a "turnover number" because it gives the number of substrate molecules turned over into product by a single enzyme molecule in a given unit of time. k_{cat} is an indication of how good the enzyme is at performing the reaction, with bigger values corresponding to faster enzymes.

Properly, k_{cat} is the rate constant of the chemical reaction of the enzyme–substrate complex reacting to produce product and free enzyme.

$$\mathrm{E} + \mathrm{S} \rightleftharpoons \mathrm{ES} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P}$$

It is a **first-order rate constant**, which—if you remember second-semester General Chemistry—means that the rate of the reaction depends primarily on one thing, in this case, enzyme concentration, *if* the reaction is performed under saturating conditions. Under saturating conditions, we have so much S, that we assume that all of the enzyme is bound up with substrate to make ES.

Just like any other first-order rate constant in chemistry, k_{cat} can be used in a **rate law** to give the rate of a reaction from the concentration of reactant on which that rate constant depends, which again, is enzyme. In this case, at saturating conditions, that rate law would be:

$$rate = k_{cat}[\mathrm{E}]$$

The maximum rate of the reaction will happen at the initial moments of the reaction, when the ES concentration is greatest, and [ES] effectively is equal to the initial [E]. So, at saturating conditions:

$$v_{max} = k_{cat}[\mathrm{E}]_0$$

k_{cat} is traditionally calculated from v_{max}[3] by dividing that maximum rate by the initial enzyme concentration: $k_{cat} = v_{max}/[\mathrm{E}]_0$.

k_{cat} and K_M values can be determined a number of ways. From either plots of rate *vs.* substrate concentration or plots of observed rate constants (k_{obs}, see below) *vs.* substrate concentration, K_M can be found by finding the value on the *x* axis where rate or k_{obs} are at half their maximum value, respectively.

Alternatively, one can use the Lineweaver–Burk method, which is described in the next section.

Before we explain that, however, there is a third value of importance in enzyme kinetics, the enzyme's **specificty constant**. This is simply k_{cat}/K_M. The specificity constant is an indicator of how efficient the enzyme is. Enzymes with high k_{cat}/K_M are efficient at what they do; They have a good balance of binding substrates and turning them over quickly.

Lineweaver–Burk Method

To be written...

While this website displays Lineweaver–Burk plots for reference, the actual kinetic constants are calculated using curve-fitting software, from plots of k_{obs} *vs.* substrate concentration, which provide more accurate values. This process is described below.

Other Advanced Kinetics Terms & Methods

Activity & Specific Activity

There are two other terms used by enzyme kineticists related to enzyme assays that can be defined here: "enzyme activity" and "specific activity".

The **activity** of an enzyme is a measure of the *number of molecules* of substrate consumed or product released over time, (rather than *concentration* over time). Enzyme activity is usually reported in **enzyme units** (U), which are equivalent to µmol/min.

In our case, it is simply calculated by multiplying the rate of PNP production times the volume (*V*) of the reaction mixture and adjusting for a change in units.

$$activity = rate \cdot V_{\text{total}}$$

For example, if our assay volume is 100 µL and we have a rate of 200 mM/min:

$$activity = \frac{2\ \cancel{\text{mmol}}/\cancel{\text{L}}}{\text{min}} \cdot 0.0001\ \cancel{\text{L}} \cdot \frac{1{,}000\ \mu\text{mol}}{\cancel{\text{mmol}}} = 0.2\ \mu\text{mol/min} = 0.2\ \text{U}$$

Activity depends on the amount of enzyme present, and so **specific activity** is calculated, which is a ratio of the enzyme's activity over its mass, usually expressed in units of U/mg. So, we need to know the mass of the enzyme for each specific experiment, which we can find if we know the concentration (in mass per volume, *not* molar) and the volume of enzyme added to the reaction mixture.

$$specific\ activity = \frac{activity}{m_{\text{BglB}}} = \frac{activity}{c_{\text{BglB}} V_{\text{BglB}}}$$

For example, if we have 25 µL of a 1.00 mg/mL enzyme solution, then:

$$specific\ activity = \frac{0.2\ \text{U}}{(1.00\ \text{mg}/\cancel{\text{mL}})(0.025\ \cancel{\text{mL}})} = 8\ \text{U/mg}$$

If we know the molar mass of our enzyme, we can use the specific activity to determine the turnover numbers of enzymes, including k_{cat}, as described next.

Alternative Ways to Determine Turnover Numbers & Rate Constants

The **observed rate constant**, k_{obs}, is the ratio of the rate over the initial enzyme concentraion, *rate*/$[E]_0$.

We must be careful, though, not to mix up the initial molar enzyme concentration, $[E]_0$, in the reaction mixture with the molar concentration of our enzyme solution *before* we have added it to our reaction mixture. If we want to calculate k_{obs} directly from enzyme rate, we need to adjust for the change in concentration upon mixing. (Do you remember the equation $M_1V_1 = M_2V_2$?) For example, if we use the example of the stock enzyme solution from before with a concentration of 1 mg/mL, if its molar mass is 50 kDa, then this corresponds to a molar concentration of 0.02 mM. If we add 25 µL to our reaction mixture to have a total reaction volume of 100 µL, then our $[E]_0$ would be 0.005 mM. So:

$$k_{obs} = \frac{rate}{[\mathrm{BglB}]_0} = \frac{2\ \cancel{\mathrm{mM}}/\mathrm{min}}{(0.005\ \cancel{\mathrm{mM/L}})} = 400\ \mathrm{min}^{-1}$$

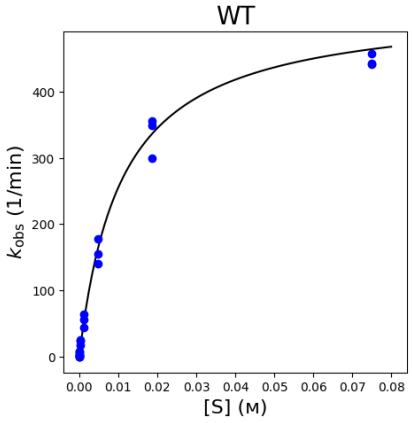
One can also calculate k_{obs} from activities in the following manner, by dividing the activity by the number of enzyme molecules present:

$$k_{obs} = \frac{activity}{n_{\mathrm{BglB}}} = \frac{0.2\ \cancel{\mu\mathrm{mol}}/\mathrm{min}}{(0.02\ \cancel{\mathrm{mmol}}/\cancel{\mathrm{L}})(0.025\ \cancel{\mathrm{mL}})} \cdot \frac{\cancel{\mathrm{mmol}}}{1{,}000\ \cancel{\mu\mathrm{mol}}} \cdot \frac{1{,}000\ \cancel{\mathrm{mL}}}{\cancel{\mathrm{L}}} = 400\ \mathrm{min}^{-1}$$

Alternatively, k_{obs} can be calculated by multiplying the specific activity times the molar mass of the enzyme and converting units. For example, if our specific activity is 8 U/mg:

$$k_{obs} = specific\ activity \cdot MM = 8\ \mathrm{U/mg} \cdot 50\ \mathrm{kDa} = \frac{8\ \cancel{\mu\mathrm{mol}}/\mathrm{min}}{\cancel{\mathrm{mg}}} \cdot \frac{50{,}000\ \mathrm{g}}{\cancel{\mathrm{mol}}} \cdot \frac{\cancel{\mathrm{mol}}}{10^6\ \cancel{\mu\mathrm{mol}}} \cdot \frac{10^3\ \cancel{\mathrm{mg}}}{\mathrm{g}}$$
$$= 400\ \mathrm{min}^{-1}$$

What is nice about this value is that k_{obs}, like the catalytic rate constant k_{cat}, is a turnover number. The units of any turnover number are inverse time. Like k_{cat}, it *actually* is an expression of the number of product molecules released (or substrate molecules consumed) per molecule of enzyme per unit of time, but the units of number of product molecules and number of enzyme molecules cancel out.



The catalytic rate constant, k_{cat}, which we defined above, is, again, the *maximum* number of product molecules released per molecule of enzyme per unit of time. It is the *maximum* turnover number possible for an enzyme. Thus, it is simply the *maximum* k_{obs} value determined. To find k_{cat} with this method, we simply find the maximum k_{obs} value from a plot of k_{obs} *vs.* substrate concentration.

For example, in the plot shown here, the maximum k_{obs} might be estimated as close to 500 min^{-1}. So, the k_{cat} is 500 min^{-1}.

Half that value is 250 min^{-1}, and the value of [S] at that k_{obs} value is approximately 0.01 M. So the K_M in this example can be estimated to be 0.01 M.

Curve fitting algorithms are used to find the actual constants, by fitting the following equation to the curve:

$$k_{obs} = \frac{k_{cat}[S]}{K_M + [S]}$$

Notice how similar this equation is to the Michaelis–Menten equation shown above! You can get the Michaelis–Menten equation back by multiplying both k_{cat} and k_{obs} by $[E]_0$.

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[1] Slope here is the initial slope of a curve of absorbance over time during the initial rate component of saturating conditions. Some instruments confusingly report this slope as "Max V" because it is the steepest slope (velocity) of any line fit to the data during measurement.

[2] Many scientists wrongly use a synonym for absorbance (*A*), optical density (OD), as if it were a unit, for example recording an absorbance of "0.500 OD" instead of simply 0.500. This problem is enhanced by the fact that many instruments provide values in mOD, that is "milliOD", so a measurement reported by such a machine with an absorbance of "500 mOD" is in fact an absorbance of 0.500.

[3] v_{max} is used here (for maximum velocity) instead of the more common V_{max} to avoid confusion with volume. This is also a completely different value from "Max V" reported by some instruments. For an explanation of "Max V" see footnote 1.


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