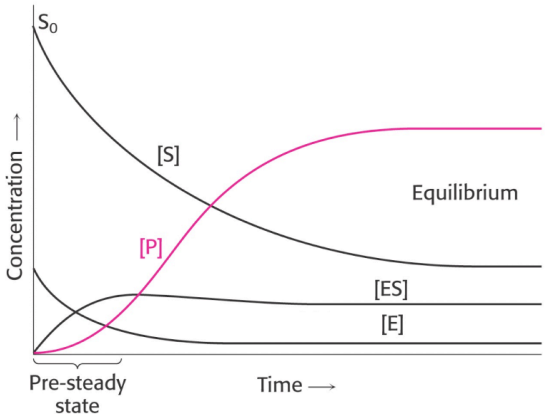
Bioanalytical chemistry

Concept review: Enzyme kinetics

Required reading: **Sections 2.5 to 2.5.3 and 2.7, 2.8** of
Mikkelsen and Cortón, *Bioanalytical Chemistry*

Enzyme kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_2} E + P$$



Rate of formation of $ES = k_1[E][S]$

Rate of breakdown of $ES = (k_{-1} + k_2)[ES]$

Under steady state conditions, [ES] is constant:

$$k_1[E][S] = (k_{-1} + k_2)[ES]$$

$$[ES] = \frac{k_1[E][S]}{(k_{-1} + k_2)}$$

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

$$V_{max} = k_2[E_{total}]$$

$$V_o = k_2[ES]$$

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

$$K_m = \frac{(k_{-1} + k_2)}{k_1}$$

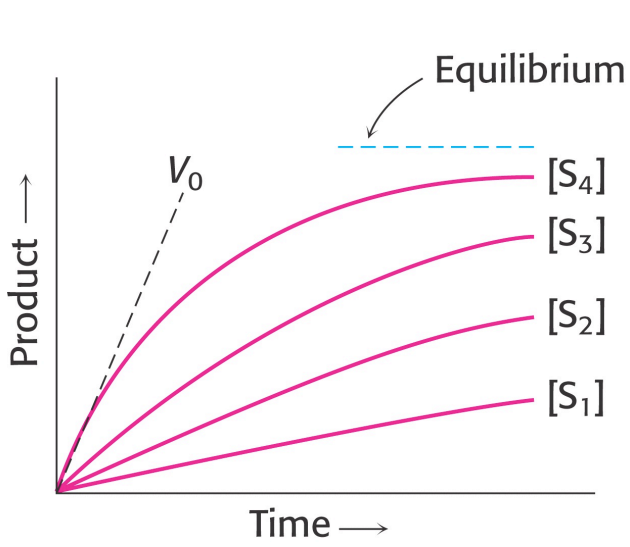
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

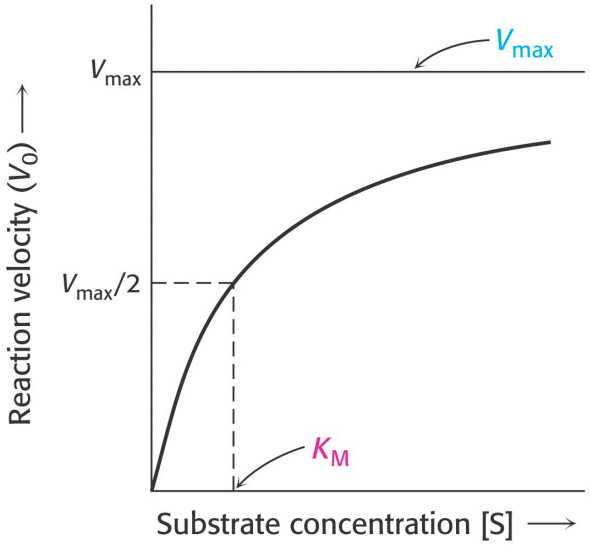
Michaelis-Menten equation

- An enzyme E combines with substrate S to form an ES complex, with a rate constant k1.
- The ES complex has two possible fates. It can dissociate to E and S, with a rate constant k-1, or it can proceed to form product P, with a rate constant k2.
- We assume that almost none of the product reverts to the initial substrate, a condition that holds in the initial stage of a reaction before the concentration of product is appreciable.
- Solving for the initial velocity Vo provides an equation known as the Michaelis-Menten equation.
- *Question: In the handout notes you write Vmax=Kcat*[Etot], but I think Vmax=K2*[Etot], and so is that in the lecture note. Does the Kcat equal with K2 here?*
- *Answer: Correct. kcat and k2 are just two different ways of writing the same thing (Note: 'cat' and '2' should be subscript).*

Graph of initial velocity vs [S]

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

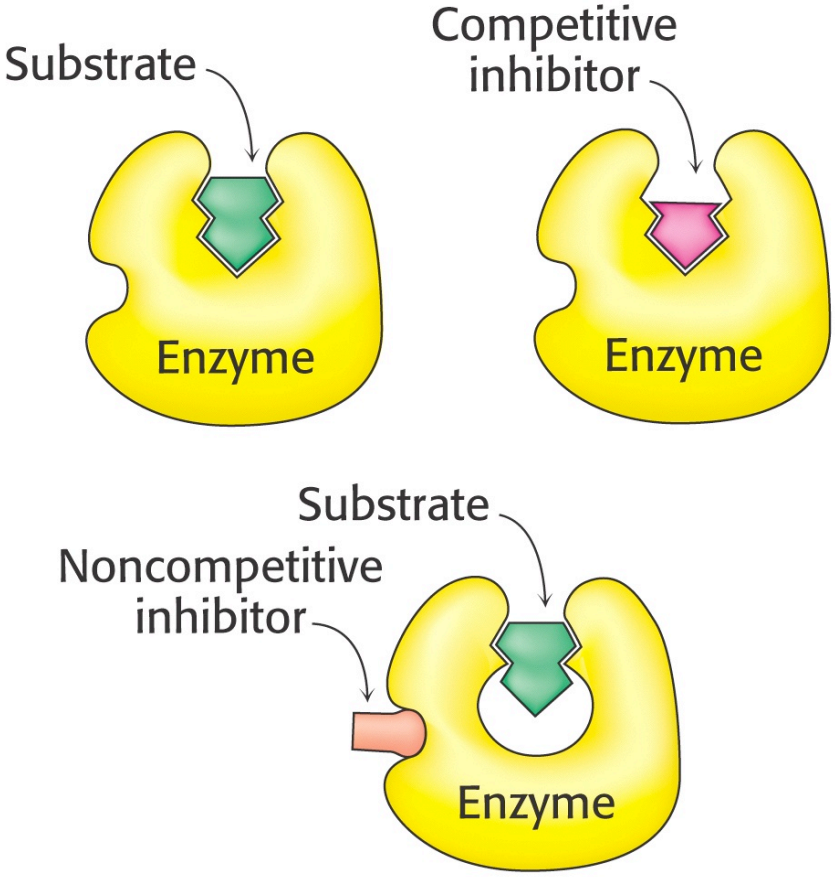




- The initial velocity of the enzyme reaction is the value that is normally measured. Under these conditions the amount of product is negligible and thus the rate of the reverse reaction is insignificant.
- A direct plot of Vo vs. [S] is hyperbolic. The Km and Vmax can be extracted from this curve using curve-fitting software
- It is informative to look at how the equation simplifies under the following circumstances
- [S] = Km
- [S] >> Km
- [S] << Km

- Though not discussed here in detail, you should appreciate that Inhibitors are molecules that bind to enzymes and decrease the rate of the reaction.

Competitive and Noncompetitive inhibitors

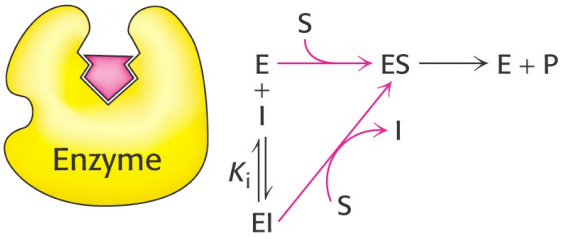


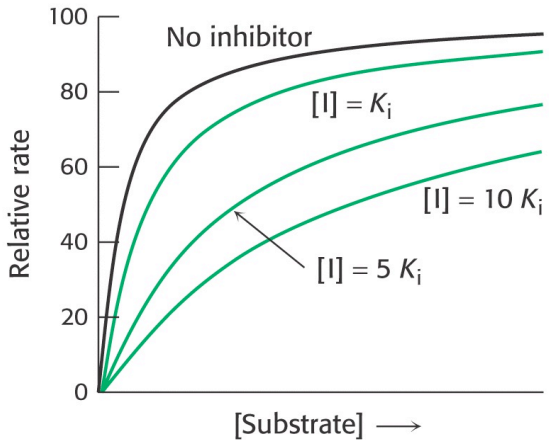
- A competitive inhibitor 'competes' for the same binding site on the enzyme as the substrate. A consequence is that when [S] >> Km the inhibitor will have no effect on the enzyme rate. This type of inhibitor does not change the Vmax but does change the apparent Km.
- A noncompetitive inhibitor binds at a different site on the free enzyme and changes the shape of the active site such that the reaction proceeds at a reduced rate. No matter how much substrate is present, the inhibitor will still be able to bind to the enzyme. Accordingly, this type of inhibitor changes the Vmax but not the Km value.
- There is also so-called uncompetitive inhibition where the inhibitor binds to a different site on the enzyme-substrate complex. In this type of inhibition, both Vmax and Km are changed.

Inhibitor effects on reaction rate

Competitive Inhibitor

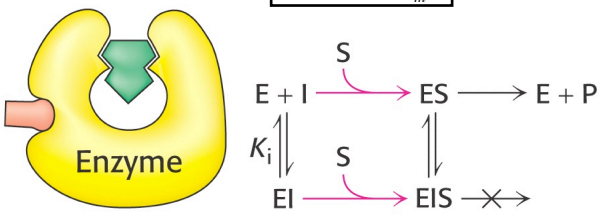
$$V_o = \frac{V_{max}[S]}{[S] + K_m\left(1 + \frac{[I]}{K_i}\right)}$$

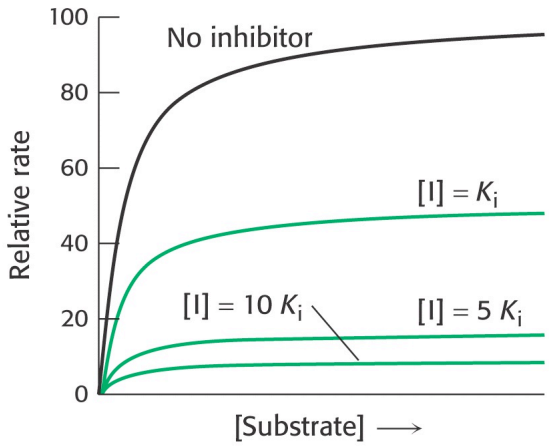




Noncompetitive Inhibitor

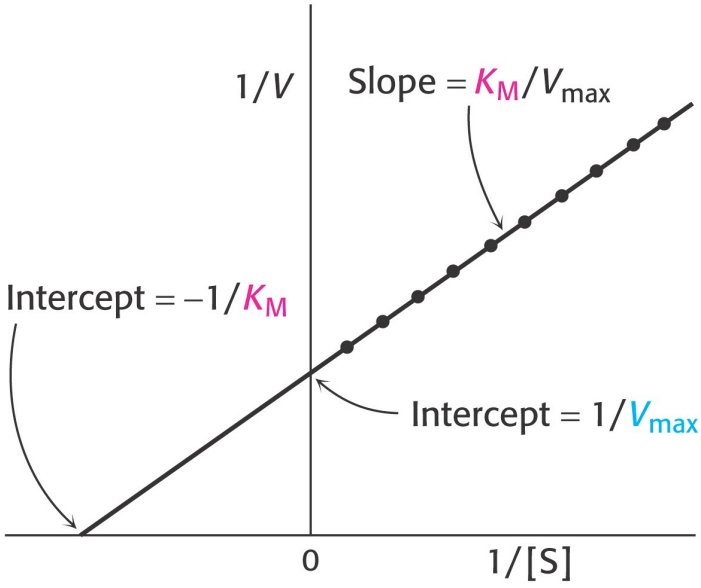
$$V_o = \frac{\left(\frac{V_{max}}{1 + \frac{[I]}{K_i}}\right)[S]}{[S] + K_m}$$



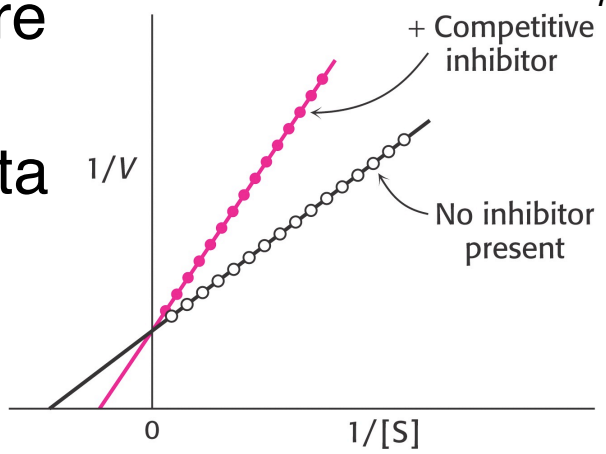


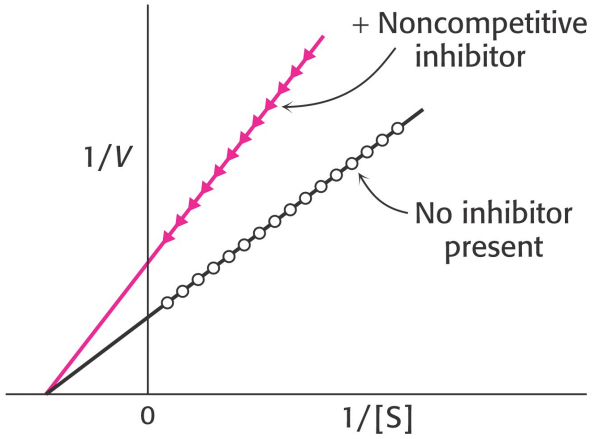
- The Ki is the concentration of inhibitor that gives a 50% decrease in the initial rate.
- In each of these graphs, initial velocity is plotted against substrate at different concentrations of inhibitor. To prove this to yourself set [I] = Ki and [S] << Km and compare to the Michaelis-Menten equation with no inhibitor.
- In both cases the Vo should be half of what it is in the absence of inhibitor

Double reciprocal plots are commonly used for the presentation of kinetic data



$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$





- By rearranging the Michaelis-Menten equation you can get an equation of the form y = mx + b where y = 1/Vo, x = 1/[S], m = Km/Vmax, and b = 1/Vmax
- In the enzyme literature you will still see kinetic data often represented as double reciprocal plots. However, this practice is slowly being fazed out because computers can fit the direct plots (V vs. [S]) directly.
- The advantage of double reciprocal plots is that they are linear and many researchers can understand them at a glance.