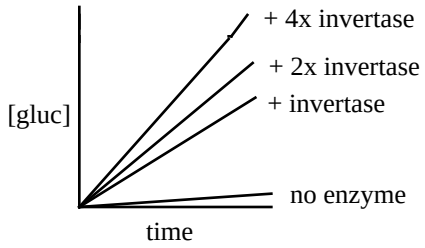
Michaelis-Menten Kinetics

I. Intro to enzymes Enzymology began with the observation that when the kite bird chowed down and subsequently regurgitated food for its kitelets, "what went down wasn't what came up." It was hypothesized that some factor in the bird's stomach juice was responsible for liquifying the worms, possibly by hydrolyzing their proteins. Hence the discovery of **enzymes**.

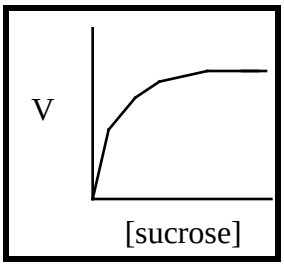
The function of enzymes is to speed up reactions, as demonstrated by the action of invertase on the rate of the reaction:

sucrose ----> glucose + fructose

Plotting the appearance of glucose (as measured by a change in the solution's rotation of light) with respect to time shows that the reaction rate (the slope) is about 10^8 times greater in the presence of invertase:



Plotting the reaction rate as a function of substrate concentration for a fixed concentration of invertase gives a **hyperbolic curve**:



The curve starts off **linear at low substrate concentrations**, similar to the linear plot seen with a chemical catalyst, and approaches a **constant maximum rate at high concentrations**, when all of the enzyme is substrate-bound and thus adding more substrate can't make the reaction go any faster.
(How will the curve look in the absence of invertase?)

II. Michaelis-Menten kinetics In general, an enzyme-catalyzed reaction is represented by the equation:

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

in which E = enzyme, S = substrate, P = product, and k's are rate constants.

Measurable quantities in this equation are:

A. the rate of product appearance, that is, the rate of ES ----> E + P, or

$$\frac{dP}{dt} = v = k_2 [ES]$$

B. the substrate concentration, [S] , and

C. the total enzyme concentration (known since you put it in)

Michaelis-Menten kinetics are based on the assumptions that:

A . the forward reaction rate (k_2) for ES ----> E + P is much greater than the reverse reaction rate, so the reverse reaction is negligible, and

B. formation and decay of ES is in **steady state,** thus

$$\frac{d[ES]}{dt} = 0 = \underbrace{k_1[E][S]}_{\text{formation}} - \underbrace{k_2[ES] - k_{-1}[ES]}_{\text{decay}}$$

This equation rearranges to:

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

or, defining the Michaelis constant:

$$\frac{k_2 + k_{-1}}{k_1} = K_M$$

it can be rewritten:

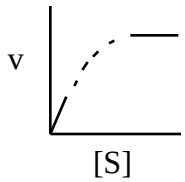
$$[E][S] = K_M[ES]$$

So the rate for formation of P, $v = k_2[ES]$, can be rewritten as:

$$\mathbf{v = [E]\,[S]\,(k_2/K_M)}$$

In this equation, [S] can be measured; the only unknown variable is [E].

To replace [E] with measurable quantities, recall the v vs [S] graph, which shows distinct behaviors at low and high [S]:



At low [S], it can be assumed that $[E] = [E_{tot}]$ (a constant) since very little of the enzyme is bound to substrate. Hence **v is proportional to [S] for low [S]**.

At high [S], when most E is substrate-bound, $[ES] = [E_{tot}]$. Hence **v is constant for high [S]**, as shown by the zero slope.

In general, $[E_{tot}] = [E] + [ES]$

$$= [ES]\,K_M \,/\, [S] + [ES]$$

$$= [ES]\left(1 + \frac{K_M}{[S]}\right)$$

$$v = k_2 [ES] = \frac{k_2 [E_{tot}]}{1 + \frac{K_M}{[S]}}$$

And from this comes the **Michaelis-Menten equation**:

$$v = \frac{k_2 [E_{tot}] [S]}{K_M + [S]} \qquad (V_{max} = k_2 [E_{tot}])$$

Note that, as seen in the graph,

A. The equation defines a rectangular hyperbola.

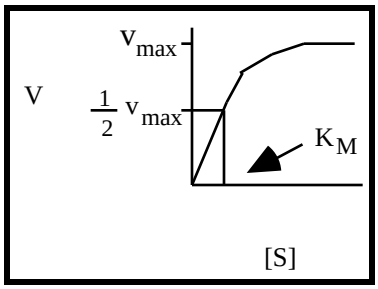
B. At low [S], where $K_M >> [S]$, v is proportional to [S].

C. At high [S], where $K_M << [S]$, $v = k_2[E_{tot}]$, a constant.

So what exactly IS K_M?

- have seen that K_M is composed of kinetic constants

- **K_M is also the [S] at which the reaction rate is half the max:**



So what does this say about the enzyme's affinity for substrate?

K_M contains a dissociative element. This can be seen by writing

$K_S = [E][S]/[ES] = k_{-1}/k_1$ ---- the dissociation constant

and rewriting K_M as $K_S + k_2/k_1$ which is the sum of a dissociative and a kinetic element. The higher K_S, the higher K_M, and the lower the affinity for substrate.

K_M is also part of the second order **enzyme specificity constant, k_2/K_M**. **k_2/K_M** defines the probability of free E interacting with free S**.**

Hence, the limit for k_2/K_M is the collision rate, 10^9 $M^{-1}s^{-1}$.

Lineweaver-Burke double reciprocal plot

A useful way to rewrite the Michaelis-Menten equation is in terms of reciprocals. This is done by starting with the form given previously,

$$v = \frac{k_2 [E_{tot}][S]}{K_M + [S]}$$

Since $k_2[E_{tot}] = V_{max}$,

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

Taking the reciprocal gives:

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{1}{V_{max}}\left(\frac{K_M}{[S]}\right)$$

The advantage of this form is that its graph is a line with $-1/K_M$ as the x-intercept and $1/V_{max}$ as the y-intercept: