## Supplemental Study 2 - Familiarize yourself with Michaelis-Menton Enzyme Kinetics

Go to mycourses and download the Michaelis-Menton Enzyme Kinetics spreadsheet model that I have written for you. Enzyme kinetics is described to you on page 162 (panel 3-3) in your textbook. It is also nicely summarized in the Wikipedia article below. Go over all parameters of the model from the list below and experiment with changing it on the most left hand column of the spreadsheet. Be sure you can describe the effects each parameter on the system of diff EQs and the Michaelis-Menton curve.

1. initial substrate concentration
2. initial enzyme concentration
3. rate constant of association of E and S to ES (this is kf)
4. rate constant of dissociation of ES to E and S (this is kr)
5. rate constant of catalyzed reaction ES to E and P (this is kcat)

Important: use this tool as you read the book to convince yourself of the properties of enzyme-coupled reactions they describe. Answer the following questions.

1. How does change in binding affinity or rate of association (kf) of enzyme and substrate affect Km and how does Km change the Michaelis-Menton curve?

2. Can you distinguish an efficient enzyme from an inefficient enzyme from the shape of this curve?

3. Did you notice that the E concentration over time approaches its initial concentration as the substrate runs out? What does this imply about enzymes in general?

4. Use the model to compare properties of two real enzymes…you will need to look up values for the rate constants kf, kr and Kcat or else infer them from Km.

5. Can you think of how to design a mathematical change to the spreadsheet model that would account for activation of the enzyme by another molecule (i.e. this would model an allosteric enzyme)

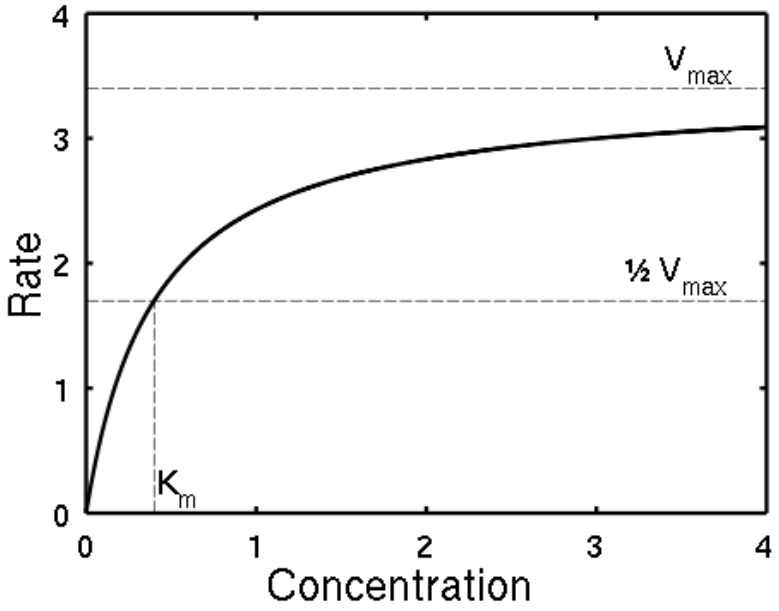
FROM WIKIPEDIA

In biochemistry, **Michaelis–Menten kinetics** is one of the simplest and best-known models of enzyme kinetics. It is named after American biochemist Leonor Michaelis and Canadian physician Maud Menten. The model takes the form of an equation describing the rate of enzymatic reactions, by relating reaction rate$V$ to [$S$], the concentration of a substrate S. Its formula is given by

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

Here, $V_{\max}$ represents the maximum rate achieved by the system, at maximum (saturating) substrate concentrations. The Michaelis constant $K_m$ is the substrate concentration at which the reaction rate is

half of $V_{max}$. Biochemical reactions involving a single substrate are often assumed to follow Michaelis–Menten kinetics, without regard to the model's underlying assumptions




MODEL

In 1903, French physical chemist Victor Henri found that enzyme reactions were initiated by a bond between the enzyme and the substrate.[1] His work was taken up by American biochemist Leonor Michaelis and Canadian physician Maud Menten who investigated the kinetics of an one of the most simple enzymatic reaction mechanisms, invertase, that catalyzes the hydrolysis of sucrose into glucose and fructose.[2] In 1913, they proposed a mathematical model of the reaction.[3] It involves an enzyme E binding to a substrate S to form a complex ES, which in turn is converted into a product P and the enzyme. This may be represented schematically as

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

where$k_f$, $k_r$ and $k_{cat}$ denote the rate constants,[4] and the double arrows between S and ES represent the fact that enzyme-substrate binding is a reversible process.

Under certain assumptions – such as the enzyme concentration being much less than the substrate concentration – the rate of product formation is given by

$$v = V_{\max}\frac{[S]}{K_m + [S]} = k_{\mathrm{cat}}[E]_0\frac{[S]}{K_m + [S]}.$$

The reaction rate increases with increasing substrate concentration [$S$], asymptotically approaching its maximum rate $V_{max}$, attained when all enzyme is bound to substrate. It also follows that $V_{max} = k_{cat}[E]_0$, where $[E]_0$ is the enzyme concentration. $k_{cat}$, the turnover number, is maximum number of substrate molecules converted to product per enzyme molecule per second.

The Michaelis constant $K_m$ is the substrate concentration at which the reaction rate is at half-maximum, and is a measure of the substrate's affinity for the enzyme. A small $K_m$ indicates high affinity, meaning that the rate will approach $V_{max}$ more quickly.[5]

The model is used in a variety of biochemical situations other than enzyme-substrate interaction, including antigen-antibody binding, DNA-DNA hybridization and protein-protein interaction.[5][6] It can be used to characterise a generic biochemical reaction, in the same way that the Langmuir equation can be used to model generic adsorption of biomolecular species.[6]

APPLICATION

Parameter values vary wildly between enzymes:[7]

| **Enzyme** | **$K_m$ (M)** | **$k_{cat}$ (1/s)** | **$k_{cat}$ / $K_m$ (1/M.s)** |
|---|---|---|---|
| Chymotrypsin | $1.5 \times 10^{-2}$ | 0.14 | 9.3 |
| Pepsin | $3.0 \times 10^{-4}$ | 0.50 | $1.7 \times 10^{3}$ |
| Tyrosyl-tRNAsynthetase | $9.0 \times 10^{-4}$ | 7.6 | $8.4 \times 10^{3}$ |
| Ribonuclease | $7.9 \times 10^{-3}$ | $7.9 \times 10^{2}$ | $1.0 \times 10^{5}$ |
| Carbonic anhydrase | $2.6 \times 10^{-2}$ | $4.0 \times 10^{5}$ | $1.5 \times 10^{7}$ |
| Fumarase | $5.0 \times 10^{-6}$ | $8.0 \times 10^{2}$ | $1.6 \times 10^{8}$ |

The constant $k_{cat}$ / $K_m$is a measure of how efficiently an enzyme converts a substrate into product. It has a theoretical upper limit of $10^8 – 10^{10}$ /M.s; enzymes working close to this, such as fumarase, are termed superefficient.[8]

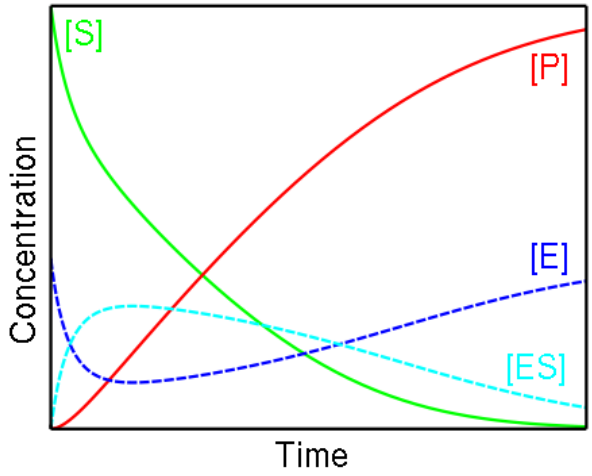
Michaelis-Menten kinetics have also been applied to a variety of spheres outside of biochemical reactions,[4] including alveolar clearance of dusts,[9] the richness of species pools,[10], clearance of blood alcohol,[11] and bacterial phage infection.[12]

DERIVATION

Applying the law of mass action, which states that the rate of a reaction is proportional to the product of the concentrations of the reactants, gives a system of four non-linear ordinary differential equations that define the rate of change of reactants with time *t*:[13]

$$\begin{aligned} d[S]/dt &= -k_f[E][S] + k_r[ES] \\ d[E]/dt &= -k_f[E][S] + k_r[ES] + k_{cat}[ES] \\ d[ES]/dt &= +k_f[E][S] - k_r[ES] - k_{cat}[ES] \\ d[P]/dt &= +k_{cat}[ES] \end{aligned}$$

In this mechanism, the enzyme E is a catalyst, which only facilitates the reaction, so its total concentration, free plus combined, $[E] + [ES] = [E]_0$ is a constant. This conservation law can also be obtained by adding the second and third equations above.[13][14]




**Equilibrium approximation**

In their original analysis, Michaelis and Menten assumed that the substrate is in instantaneous chemical equilibrium with the complex, and thus $k_f[E][S] = k_r[ES]$.[3][14] Combining this relationship with the enzyme conservation law, the concentration of complex is[14]

$$[ES] = \frac{[E]_0[S]}{K_d + [S]}$$

where $K_d = k_r / k_f$ is the dissociation constant for the enzyme-substrate complex. Hence the velocity *v* of the reaction – the rate at which P is formed – is[14]

$$v = d[P]/dt = \frac{V_{\max}[S]}{K_d + [S]}$$

where $V_{max} = k_{cat}[E]_0$ is the maximum reaction velocity.