

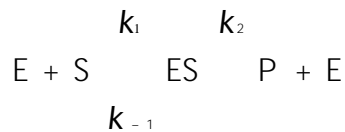
## Enzyme Kinetics II

Michaelis-Menten Enzyme kinetics

Don't forget the two assumptions - They both lead to the same equation, the Michaelis-Menten equation.

What is this awe inspiring equation? The Michaelis-Menten kinetic model explains several aspects of the behavior of many enzymes. Each enzyme has a  $K_m$  value that is characteristic of that enzyme under certain conditions.

- Graphical model of the representation of the M&M eq. -  
 Reaction velocity (V) vs concentration of substrate [S]  
 - as [S] increases, velocity increases and eventually levels off =  $V_{max}$   
 1st order vs zero order rates of reaction - back to the two assumptions  
 There are two important values for each enzyme that are described by the M&M equation;  $V_{max}$  and  $K_m$  (Michaelis-Menten constant)  
 Graphically, these are shown as  $1/2 V_{max} = K_m$  can not reach real  $V_{max}$  so....
- Mathematical model of the representation of the M&M eq. -  
 For the reaction:



1) The Michaelis constant  $K_m$  is:

$$K_m = \frac{K_{-1} + K_2}{K_1}$$

Think of what this means in terms of the equilibrium.

Large vs. a small  $K_m$

2) When investigating the initial rate ( $V_o$ ) the Michaelis-Menten equation is:

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

Graphical representation is a hyperbola. Think of the difference between  $O_2$  binding of myoglobin and hemoglobin.

- When  $[S] \ll K_m$ , the velocity is dependent on  $[S]$
  - When  $[S] \gg K_m$ , the initial velocity is independent of  $[S]$
  - When  $[S] = K_m$ , then  $V_o = 1/2 V_{max}$
  - Prove this mathematically and graphically.
- $K_m$  is a measure of the affinity of the enzyme for its substrate and also informs about the rate of a reaction. The binding constant is approximated by  $K_m$
- Rules for using the M&M equation:
- The reaction must be first order and  $[S] \gg E$  (two assumptions)

**Turnover Number** -  $k_{cat}$  - the direct measure of the catalytic production of product. The larger the  $k_{cat}$  is, the more rapid the catalytic events at the enzyme's active site must be. The number of times a binding and reaction event "turns over"

- When the  $[S] \ll K_m$  so that most of the enzyme is in the free state  $[E]_t = [E]_{free}$  then  $V = k_{cat} / K_m [E][S]$
- This is a second order rate constant between the substrate and the free enzyme. This is a good measure of efficiency and specificity.
- When the  $k_{cat}/K_m$  is near very high, the fastest the enzyme can catalyze a reaction is the diffusion rate of a molecule!  
 $10^8 - 10^9 / M \cdot sec$

Lineweaver-Burk (double reciprocal plot)

- $V_{max}$  and  $K_m$  are not likely to be determined by increasing  $[S]$
- Instead the  $[S]$  vs.  $V_o$  data are transformed to a plot of their reciprocal of each value.
- $1/[S]$  vs.  $1/V_o$

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

And this can be simplified to:

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

This is the equation for a straight line

$$Y = mX + b$$

$$Y = 1/V_o \text{ and } X = 1 / [S]$$

So What?

- $K_m$  - relates to affinity ;  $V_{max}$  relates to efficiency
- $K_m$  tell how much substrate to use in an assay
- If more than one enzyme share the same substrate,  $K_M$  also will determine how to decide which pathway the substrate will take

$V_{max}$  tells about pathways

- Rate limiting enzyme in pathway
- $K_m$  and  $V_{max}$  can be used to determine effectiveness of inhibitors and activators for enzyme studies and clinical applications