

Enzymes

Enzymes are characterized by:

Catalytic Power - rates are 10^6 - 10^{12} greater than corresponding uncatalyzed reactions

Specificity - highly specific for substrates

Regulation - achieved in many ways including changes in amount of enzyme synthesized; covalent modification; interaction with metabolic inhibitors and activators; compartmentation; etc.

Terminology

Commonly named by adding the suffix -ase to the name of the substrate or a phrase describing the catalytic reaction.

Urease - catalyzes the hydrolysis of urea

Alcohol dehydrogenase - catalyzes the oxidation of alcohols to aldehydes

Enzymes catalyze reactions in association with other molecules called cofactors:

Metal ions - Ca^{2+} or Zn^{2+}

Coenzyme - not covalently bound to the enzyme; most derived from vitamins (NAD⁺)

Prosthetic group - permanently associated with their protein; heme group of hemoglobin

HOLOENZYME - Active enzyme-cofactor complex

APOENZYME - Enzymatically inactive protein from which the cofactor has been removed

How do enzymes act as catalysts?

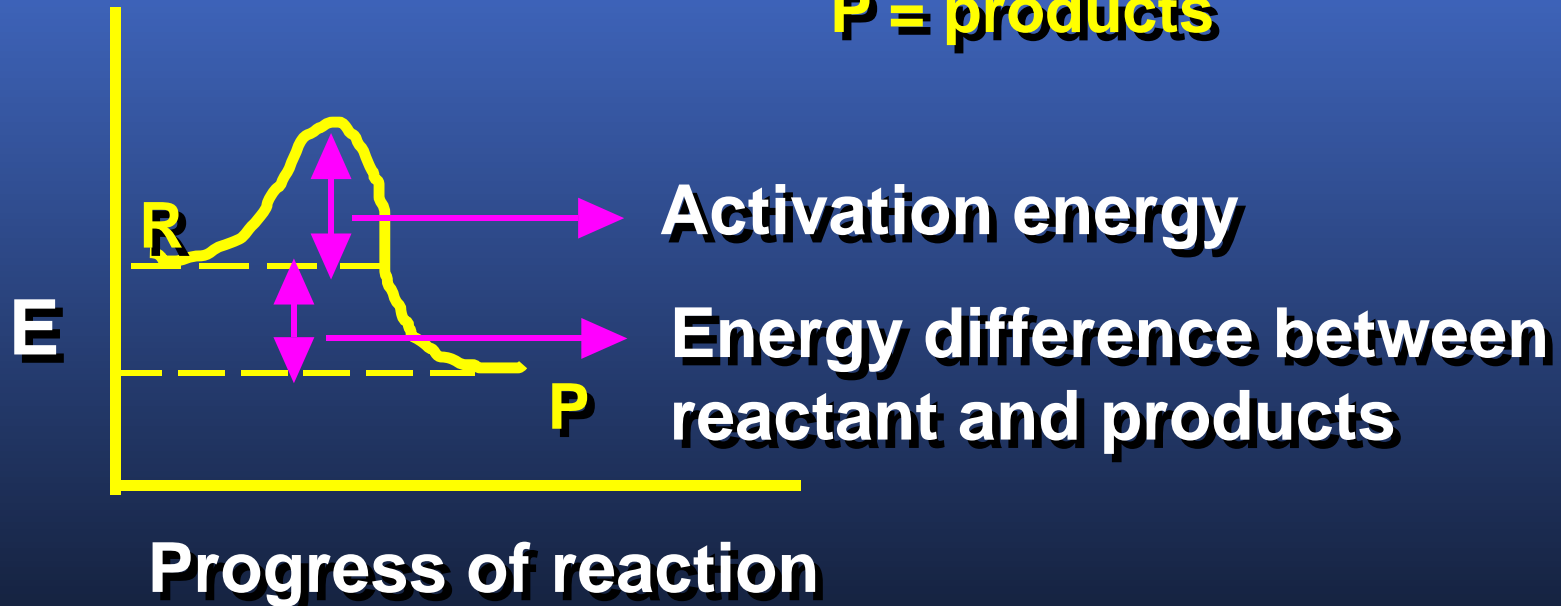
Rate of a reaction depends on how effectively reactants collide to form a transition state. The colliding substances must be in the correct orientation and must possess sufficient energy to approach the physical configuration of the atoms and bonds of the product.

The energy required to reach the transition state from the ground state is the energy of activation.

How do enzymes act as catalysts?

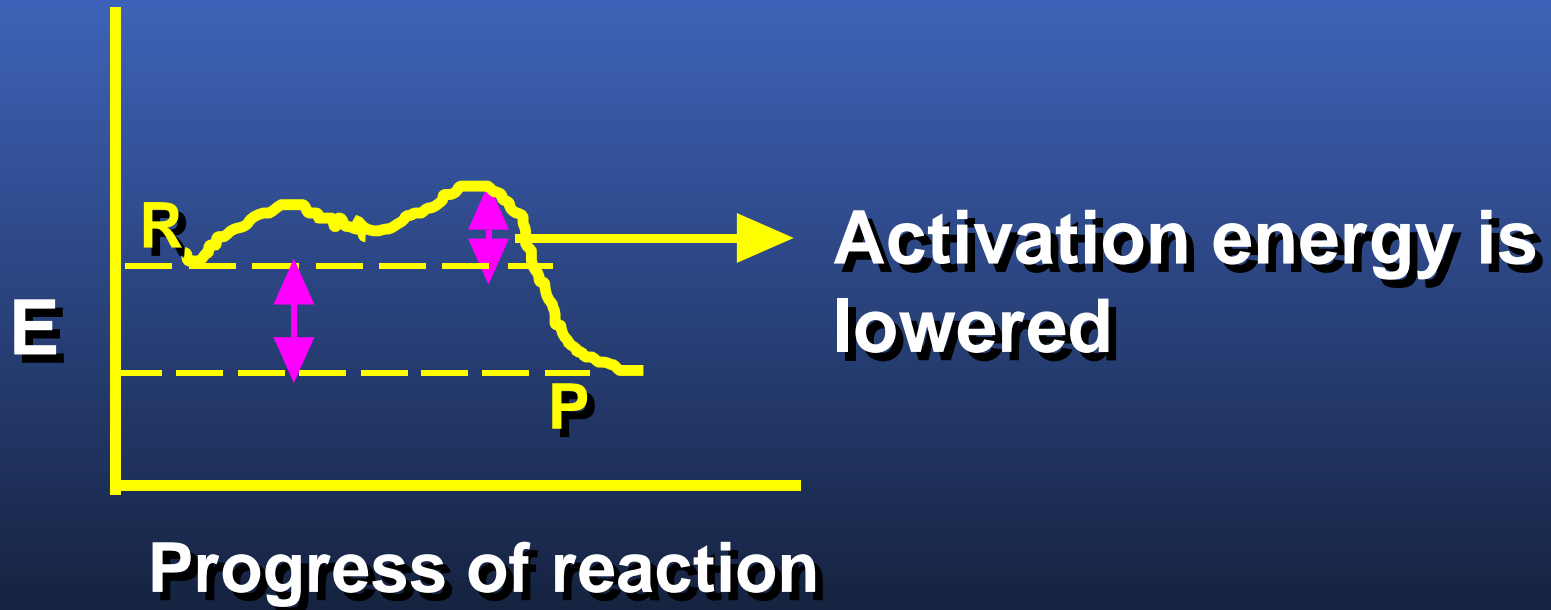
R = reactants

P = products



Uncatalyzed Reaction

Enzymes work by lowering the overall activation energy:



Catalyzed Reaction

Enzymes lower activation energy by orienting the reacting molecules so that the most favorable collisions can occur.

Enzymes cause substrates to come together in favorable orientations in an enzyme-substrate [ES] complex where substrates are bound to a specific region of the enzyme called the active site.

Active Site:

Three-dimensional entity formed by groups that come from different parts of the linear sequence of amino acids.

A cleft or crevice in the tertiary structure.

Water is excluded unless a reactant.

Non-polar nature enhances the binding of substrates.

Lock and Key Theory - Emil Fisher (1894)

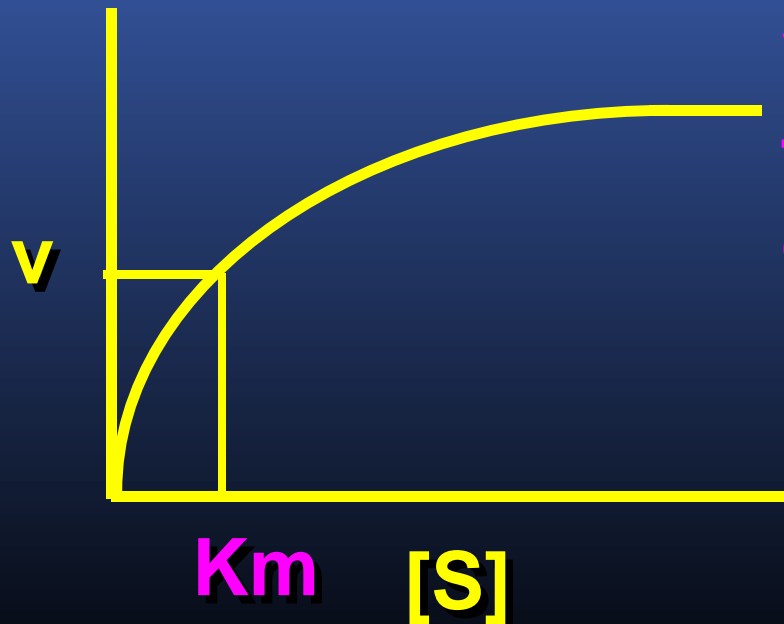
proposed that the active site of an enzyme alone is complementary in shape to that of the substrate. The enzyme is the lock and the substrate the key that fits it.

Induced Fit Model - Daniel Koshland (1958).

Enzymes change their conformation after binding the substrate. The active site has a shape complementary to the substrate only after the substrate is bound.

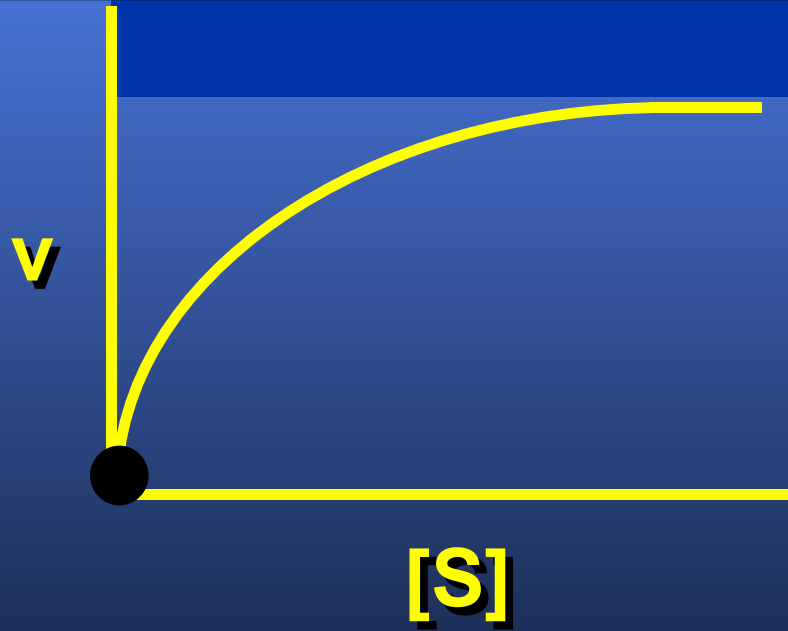
Kinetic Properties of Enzymes:

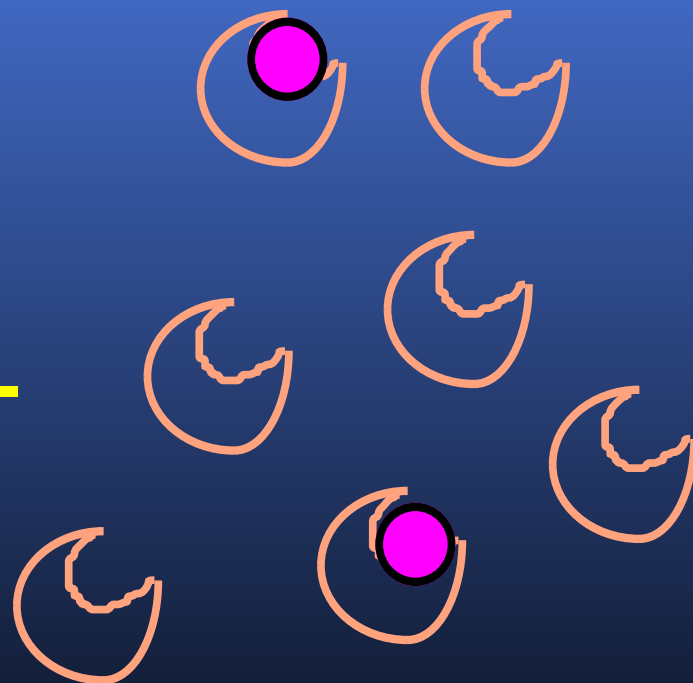
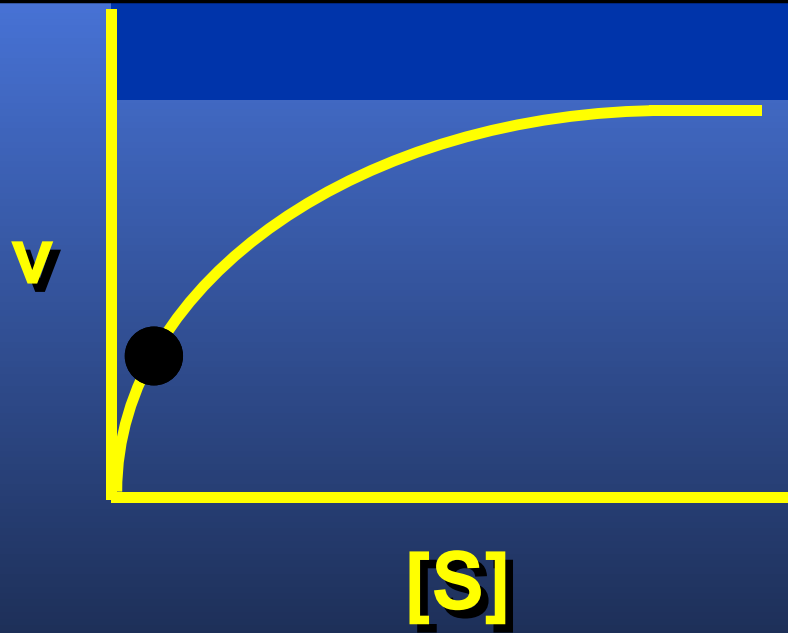
For many enzymes, the rate of catalysis (v) varies with the substrate concentration $[S]$ as follows:

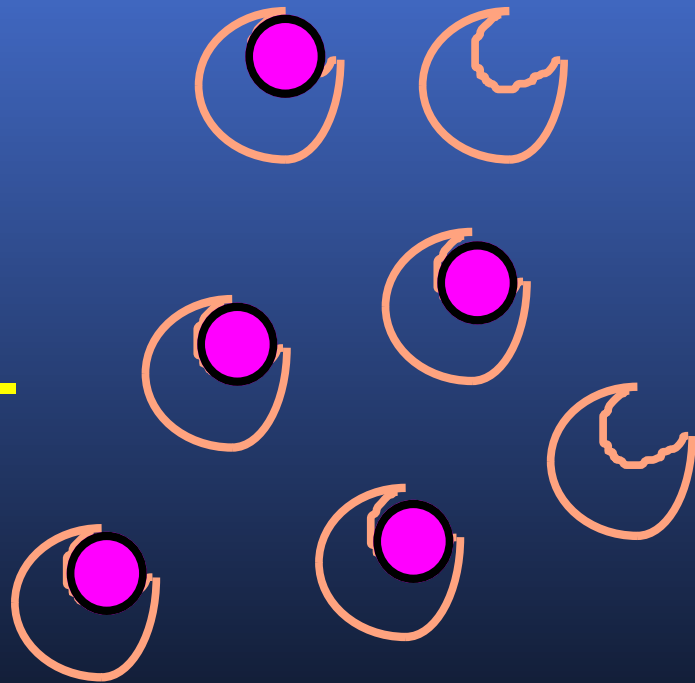
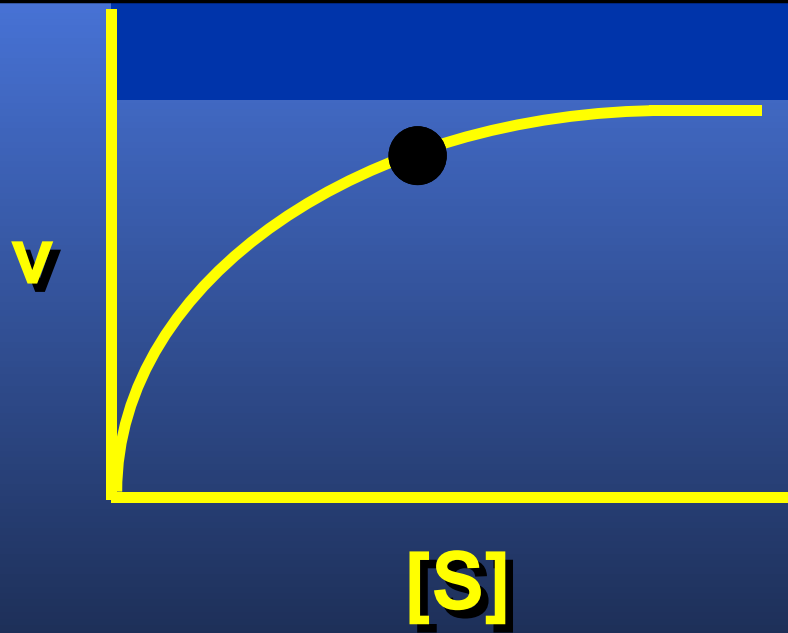


V_{max} (evidence for formation of an ES complex)

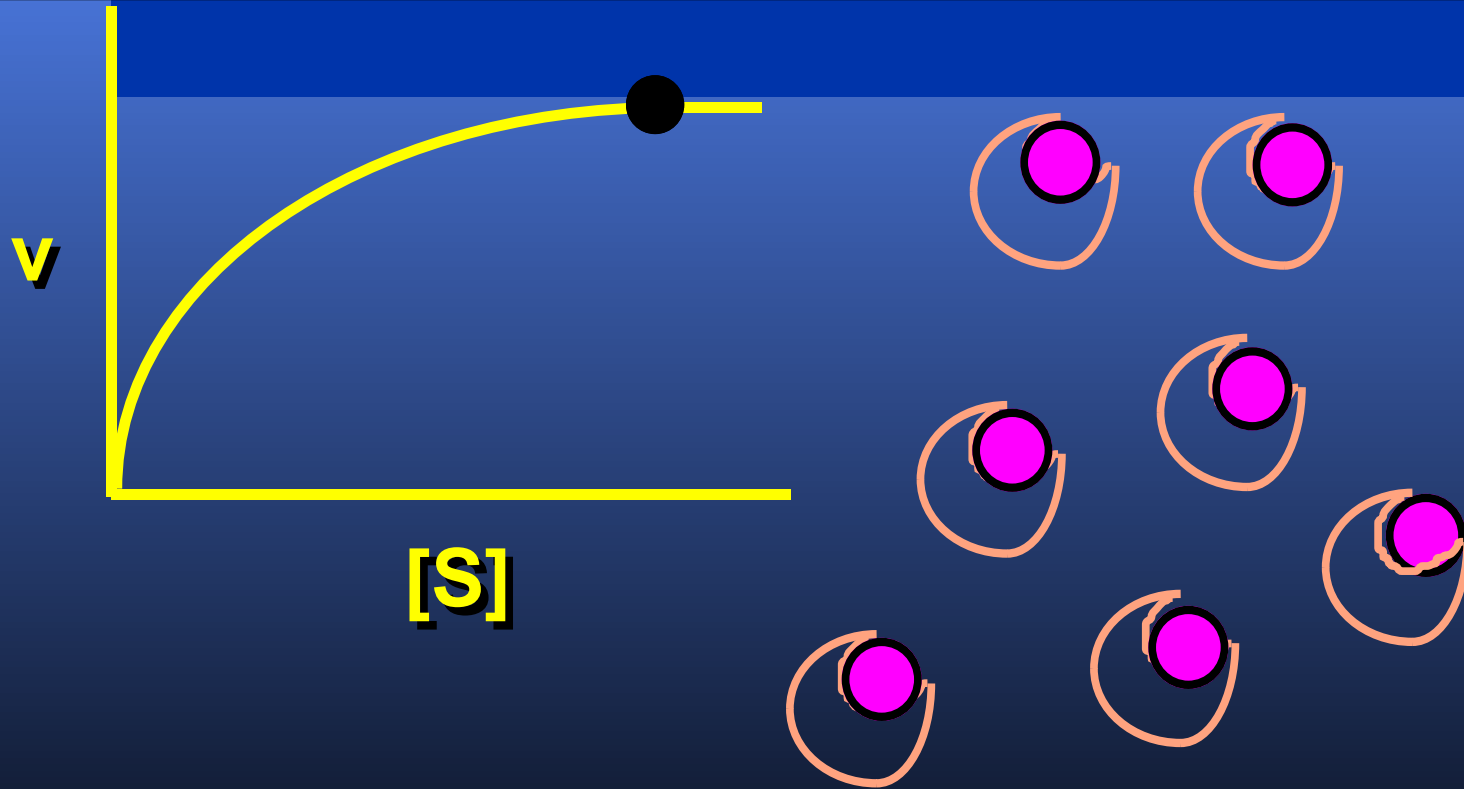
K_m = Substrate concentration at which the velocity is $1/2 V_{max}$.



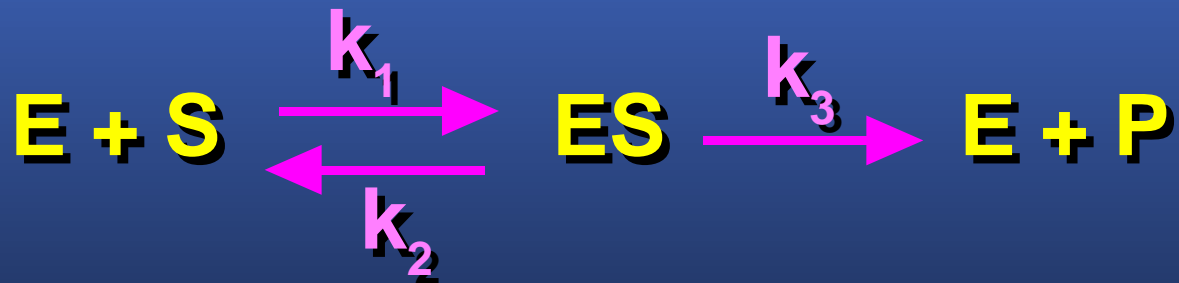




When all active sites are filled, the rate is at a maximum (V_{max}).



Leonor Michaelis and Maud Menten (1913) proposed a model to account for the behavior shown in the V vs. [s] curve:



One assumption is that P cannot revert back to S (there is no k_4).

Michaelis and Menten derived an equation that relates the rate of catalysis (v) to the concentration of enzyme and substrate and the rates of the individual steps in the kinetic pathway:

Starting point is that the rate at which product is formed is: $V = k_3[ES]$.

Expressing [ES] in terms of known quantities:

$$\text{rate of formation of [ES]} = k_1 [E] [S]$$

$$\text{rate of breakdown of [ES]} = (k_2 + k_3) [ES]$$



Michaelis-Menten kinetics apply to catalytic rates occurring under steady-state conditions where the concentration of ES stays the same while the concentrations of reactants and products changes. This happens when the rate of formation of ES is equal to the rate of breakdown of ES:

$$k_1 [E] [S] = (k_2 + k_3) [ES]$$

Rearrange equation:

$$[ES] = \frac{k_1 [E] [S]}{(k_2 + k_3)} = \frac{[E] [S]}{\frac{(k_2 + k_3)}{k_1}}$$

By definition $\frac{(k_2 + k_3)}{k_1}$ is a constant termed the Michaelis constant, K_m .

$$[ES] = \frac{k_1 [E] [S]}{(k_2 + k_3)} = \frac{[E] [S]}{\frac{(k_2 + k_3)}{k_1}}$$

$$[ES] = \frac{k_1 [E] [S]}{(k_2 + k_3)} = [E] [S] / K_m$$

$$[ES] = [E] [S] / K_m$$

$$[ES] = [E] [S] K_m$$

Another assumption - [S] is in vast excess (so that the rate is proportional to [ES]) and [E] is very small compared to [S]; thus

$$[ES] = [E] [S] / K_m$$

$$[E] = [E_{\text{total}}] - [ES]; \quad \text{substitute:}$$

$$[ES] = \frac{([E_t] - [ES]) [S]}{K_m}$$

Solve for ES:

$$[ES] = \frac{[Et][S] - [ES][S]}{K_m} = \frac{[Et][S]}{K_m} - \frac{[ES][S]}{K_m}$$

Add $\frac{[ES][S]}{K_m}$ to both sides:

$$\frac{[ES]}{1} + \frac{[ES][S]}{K_m} = \frac{[Et][S]}{K_m}$$

Factor out [ES]:

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

Divide by $\frac{1 + [S]}{K_m}$

$$[ES] = \frac{[Et][S]}{K_m}$$

substitute $\frac{K_m}{K_m}$ for 1 \longrightarrow $\frac{1 + [S]}{K_m}$

Rearrange to:

$$[ES] = \frac{[Et][S]}{[S] + K_m}$$

Substitute this for [ES] in $V = k_3[ES]$

$$V = \frac{k_3[Et][S]}{[S] + K_m}$$

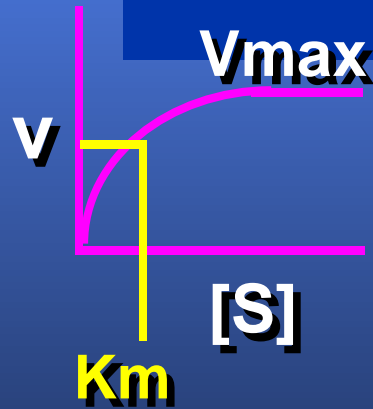
$$V = \frac{k_3 [Et] [S]}{[S] + K_m}$$

V_{max} is attained when all enzyme sites are saturated with substrate (S >>>> K_m) so that $\frac{[S]}{[S] + K_m} = 1$; so that $V_{max} = k_3 [Et]$

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

The Michaelis-Menten Equation!!!

The M-M equation accounts for the kinetic data in the v vs. S curve:



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

When $[S]$ is low ($\lll K_m$) then

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

When $[S]$ is $\ggg K_m$ then $V = V_{\max}$

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

The meaning of K_m is evident from the M-M equation when $[S] = K_m$; then $V = \frac{V_{\max} (K_m)}{2K_m}$

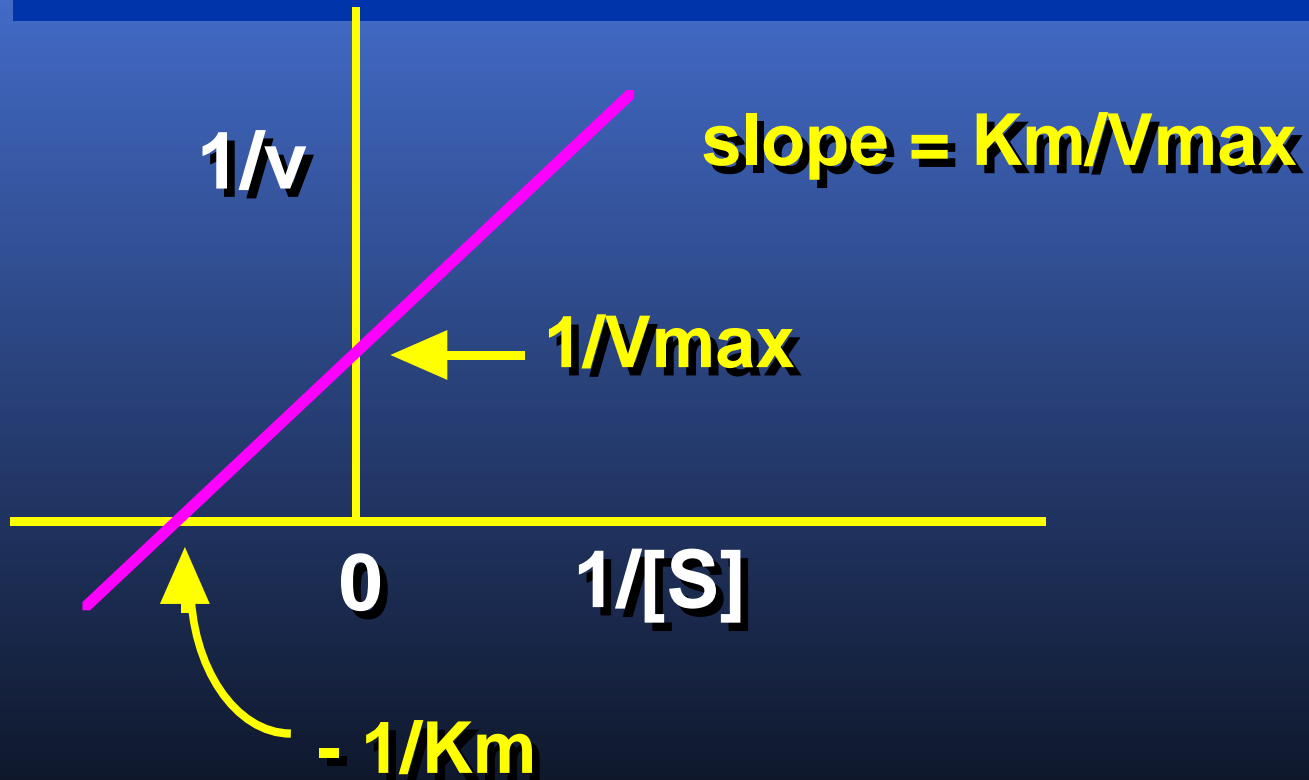
or $V = \frac{V_{\max}}{2}$ and K_m is thus equal to the substrate concentration at which the velocity is 1/2 maximal.

For convenience sake the M-M equation can be converted into a form that gives a straight line:

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

$$y = b + m X$$

A plot of $1/v$ vs. $1/[S]$ yields a
Lineweaver-Burke plot:



Significance of K_m and V_{max} :

K_m has two meanings: 1) concentration of substrate at which 1/2 of active sites are filled; 2) is a ratio of rate constants $\frac{k_2 + k_3}{k_1}$

If $k_2 \gg k_3$; then $K_m = k_2/k_1$ and is equal to the dissociation constant for the ES complex.

Turnover number:

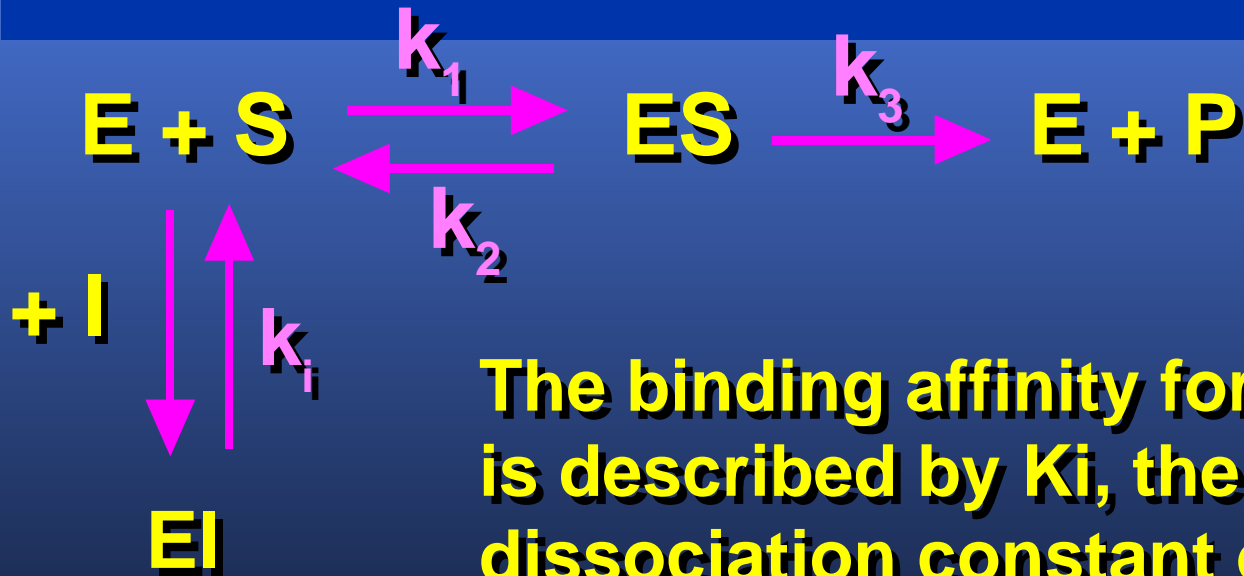
The number of substrate molecules converted to product per unit time when the enzyme is fully saturated with substrate. Equal to k_3 and is termed k_{cat} . V_{max} reveals the turnover number if the concentration of active sites (E_t) is known because $V_{max} = k_3[E_t]$ (or, $V_{max} = k_{cat} [E_t]$). When all of the enzyme sites are occupied by S, then $ES = E_t$.

Enzyme Inhibition

Small molecules or ions can inhibit

Irreversible inhibitors dissociate very slowly, if at all because they are tightly bound.

Reversible inhibition is characterized by rapid dissociation of the enzyme inhibitor (EI) complex.



The binding affinity for I is described by K_i , the dissociation constant of the enzyme-inhibitor complex.

Three types of reversible inhibition:

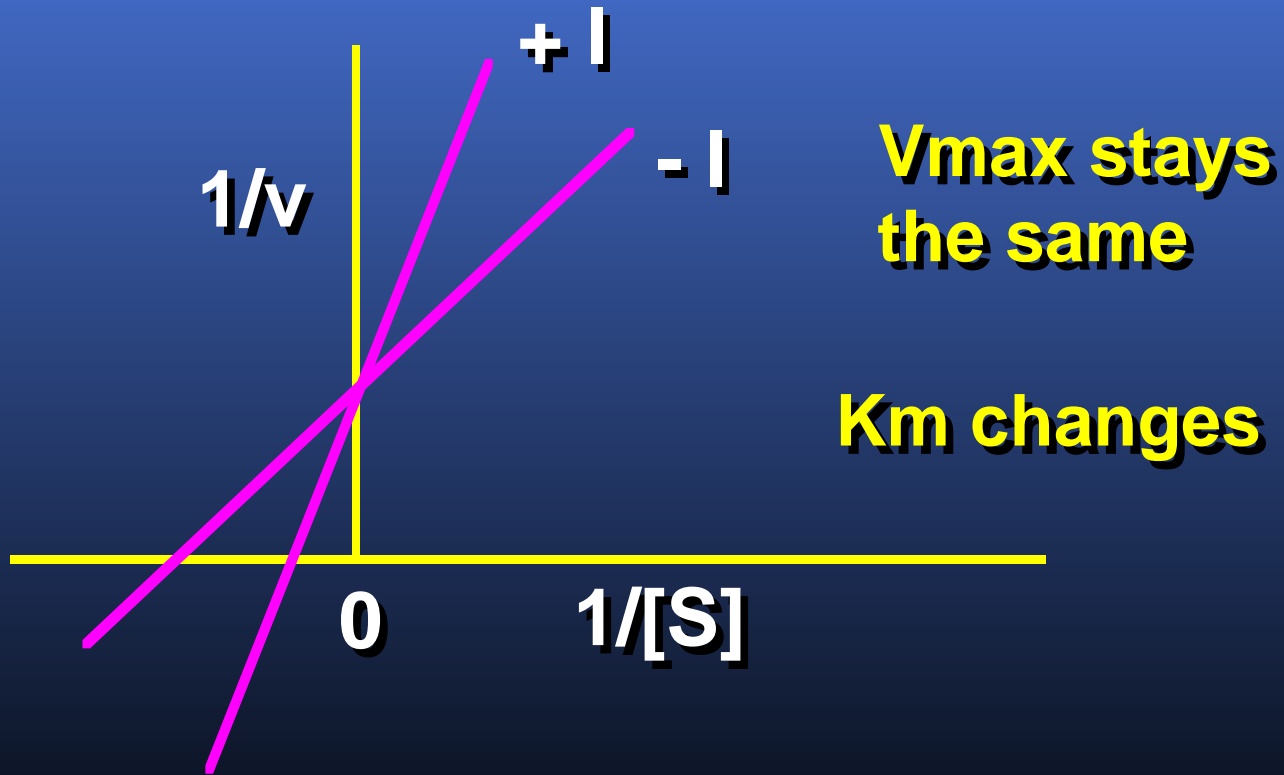
1. Competitive Inhibition

The enzyme binds either substrate or inhibitor, but NOT both.

Rate is diminished by reducing the proportion of enzyme molecules bound to substrate.

Can be overcome by high substrate concentrations, and thus V_{max} is unaffected.

Competitive Inhibition



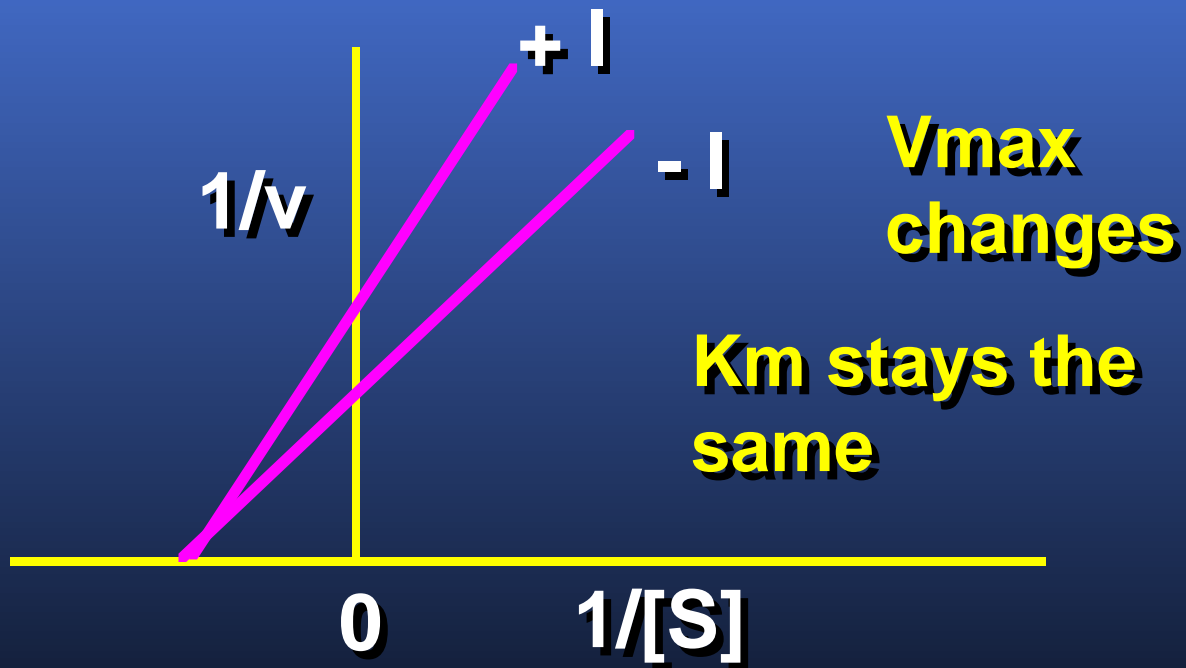
2. Non-competitive Inhibition

The substrate and inhibitor can bind simultaneously to the enzyme (i.e., their binding sites do not overlap).

Non-competitive inhibitors decrease the turnover number of an enzyme.

Since the turnover number is dependent upon V_{max} , non-competitive inhibitors affect V_{max} .

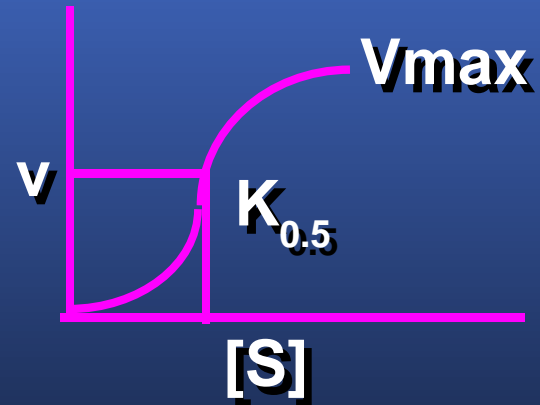
Non-competitive Inhibition



Allosteric Enzymes

Most are multisubunit enzymes.

Show sigmoidal plots of V vs. S .



The binding of substrates or modulators to one active site affects properties of the other active sites.