Chemistry and Biochemistry Department
College of Medicine /AL-Mustansiriyah University

Enzymes Lectures / Lecture 4/ 2024-2025

Enzymes Kinetic

Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzymes

In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of the enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Rate limiting step

The rate limiting step in any reaction is its slowest step. In enzymatic reactions, the conversion of the enzyme-substrate complex to the product is normally rate limiting. The rate of this step (and therefore the entire enzymatic reaction) is directly proportional to the concentration of ES

The concentration of ES changes as the reaction progresses

Therefore, the rate of product formation also changes over time. When the reaction reaches equilibrium (steady state) the concentration of ES (and therefore the rate) remains relatively constant.

Reaction Kinetics

When an enzyme is added to a lot of substrate, the reaction that follows occurs in three stages with distinct kinetics:

Pre-steady state

Steady state (equilibrium)

Post-steady

The pre-steady state phase is very short as equilibrium is reached within microseconds.

If you measure the rate in the first few seconds of a reaction (V_0) you are actually measuring the steady state.

This is the rate used in Michaelis-Menton Kinetics.

Phase	Concentration of ES	Rate of product formation
Pre-steady state	**Burst of ES complexes form**	**Slow** as must first wait for ES to form. Speeds up as ES forms.
Steady state (equilibrium)	**ES remains constant. It is formed as quickly as it breaks down.**	**Constant rate of formation, Faster than pre-steady state.**
Post-steady state	**Substrate depletes so fewer ES complexes can form**	**Slow as there are fewer ES complexes. Slows down as substrate runs out.**

Michaelis-Menton Kinetics

Two terms that are important within Michaelis-Menton Kinetics are:

Vmax – the maximum rate of reaction when all enzyme active sites are saturated with substrate

Km – the substrate concentration that gives half maximal velocity.

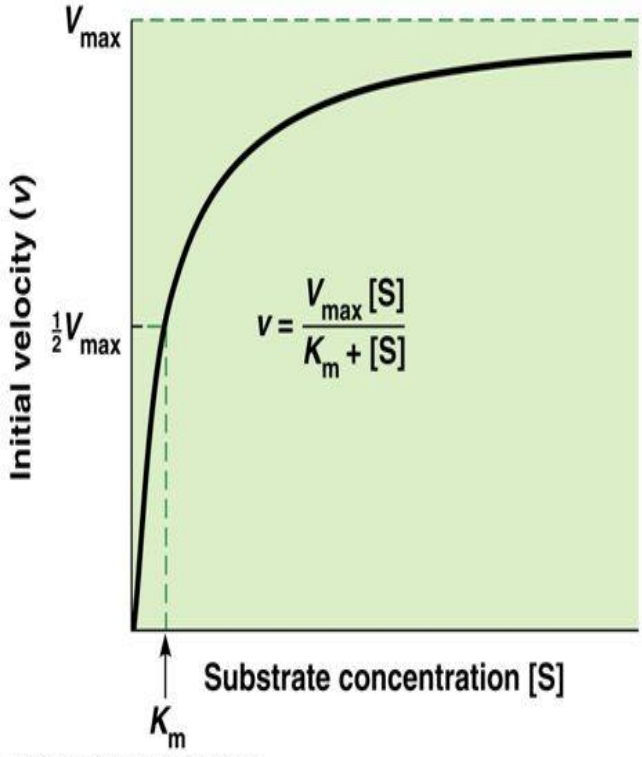
Km is a measure of the affinity an enzyme has for its substrate, as a lower Km means that less of the substrate is required to reach half of Vmax .

The Relationship Between Reaction Velocity and Substrate Concentration

$K_{cat} = V_{max}/[E_t]$

$[E_t]. K_{cat}=V_{max}$

$[E_t]$ = enzyme concentration (Molar [M])



Turnover number (K catalysis): K_{cat}

K_{cat} = (cat=catalysis) rate at which substrate molecules are converted to product by a single enzyme molecule operating at V_{max}

units: time $(sec)^{-1}$

Michaelis-Menten Kinetics: Michaelis-Menten Equation

- Combining Eq[4.8] and Eq[4.9], we have:

$$v = k_{+2}\{[S][E]_0\}/\{K_M + [S]\} \qquad [4.10]$$

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

- The maximum reaction velocity (V_{max}) occurs when the enzyme is fully saturated with substrate—ie when [ES] is no longer-rate-limiting and approaches $[E]_0$:

$$[ES] \rightarrow [E]_0$$

- Thus, under such steady-state conditions, the reaction reaches V_{max} solely determined by the product of k_{+2} and $[E]_0$:

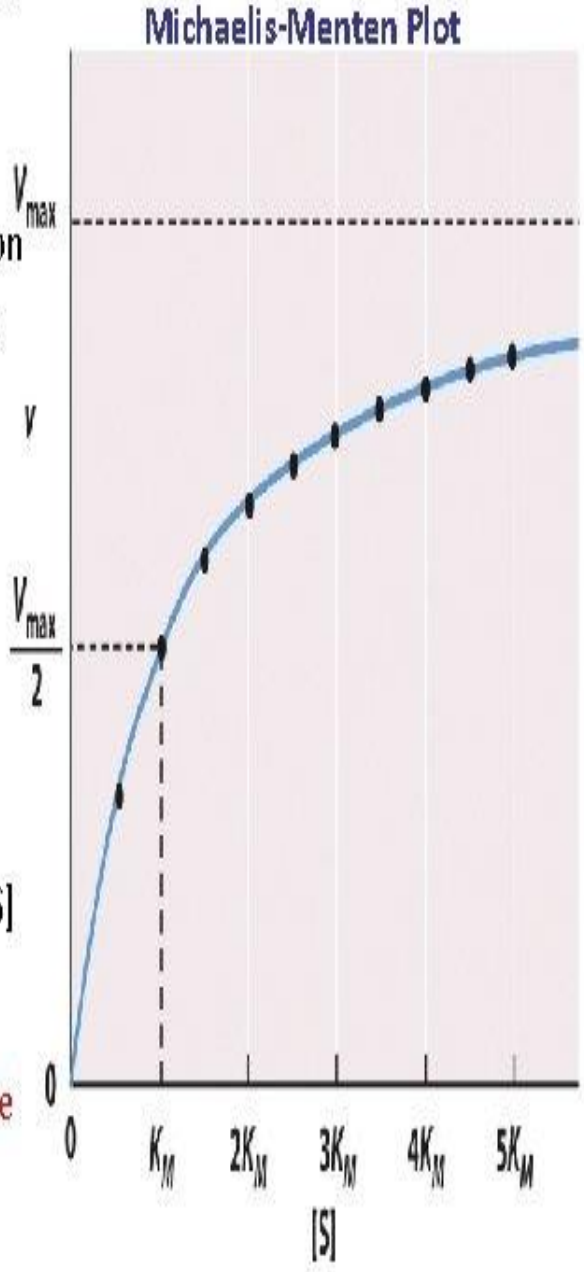
$$V_{max} = d[P]/dt = k_{+2}[E]_0 \qquad [4.11]$$

- Now, combining Eq[4.10] and Eq[4.11], we obtain:

$$v = \{V_{max}[S]\}/\{K_M + [S]\} \qquad [4.12]$$

- Eq[4.12] is the Michaelis-Menten equation that describes a hyperbola characteristic of substrates catalyzed by simple enzymes—ie a plot of v versus [S] —or the Michaelis-Menten Plot!

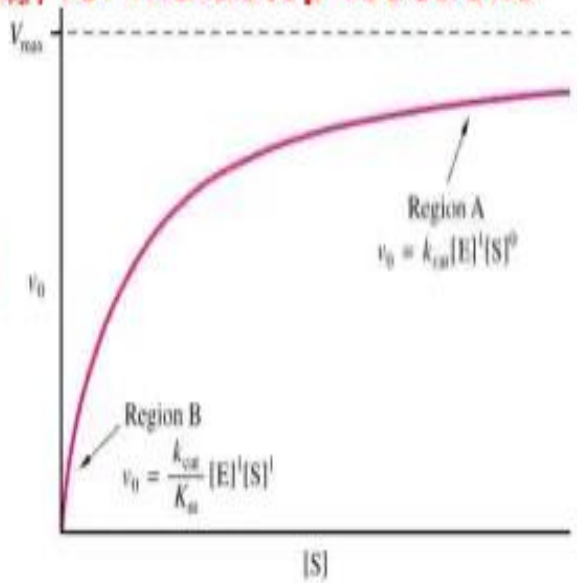
- Can we determine the values of K_M and V_{max} from the Michaelis-Menten plot? If so, should we adapt this approach?



What does k_{cat} mean?

1. k_{cat} is the 1st order rate constant describing ES → E+P
2. Also known as the turnover number because it describes the number of reactions that a molecule of enzyme can catalyze per second under optimal condition.
3. Most enzyme have k_{cat} values between 10^2 and 10^3 s^{-1}
4. For simple reactions $k_2 = k_{cat}$, for multistep reactions k_{cat} = rate limiting step

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



This equation concerns the steady state of an enzymatic reaction with one substrate, and is given by:

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

It describes how the initial rate of reaction, V0, is affected by the initial substrate concentration, [S]0
It only looks at the start of the reaction.

This allows it to ignore the reverse reaction where substrate is formed from product. This is because at the start of the reaction there is no product present to become substrate.

The plot of rate against concentration has the shape of a rectangular hyperbola. However, a more useful representation of Michaelis–Menten kinetics is a graph called a Lineweaver–Burk plot.

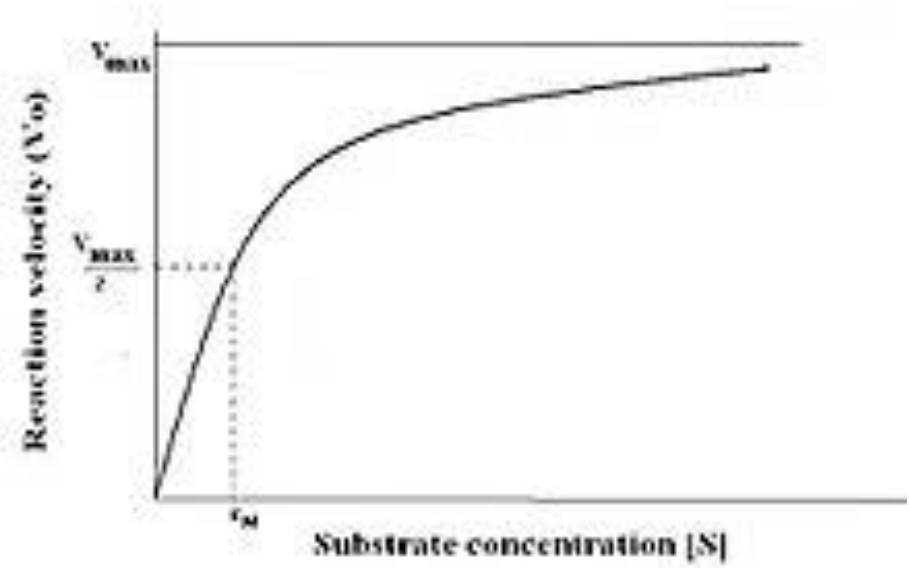
The equation used to generate this plot is given by

$$\frac{1}{V} = \frac{K_m}{V_{max}}\frac{1}{S} + \frac{1}{V_{max}}$$

This allows an easier interpretation of various quantities from the graph, such as the presence of an inhibitor.
The Michaelis-Menten equation arises from the general equation for an enzymatic reaction: E + S ↔ ES ↔ E + P, Thus, the enzyme combines with the substrate in order to form the ES complex, which in turn converts to product while preserving the enzyme
The rate of the forward reaction from E + S to ES may be termed k1, and the reverse reaction as k-1.
Likewise, for the reaction from the ES complex to E and P, the forward reaction rate is k2, and the reverse is k-2. Therefore, the ES complex may dissolve back into the enzyme and substrate, or move forward to form product.

At initial reaction time, when t ≈ 0, little product formation occurs, therefore the backward reaction rate of k-2 may be neglected. The new reaction becomes:

$$E + S \leftrightarrow ES \longrightarrow E + P$$



Assuming steady state, the following rate equations may be written as

Rate of formation of ES = k1[E][S]

Rate of breakdown of ES = (k-1 + k2) [ES]

and set equal to each other (Note that the brackets represent concentrations). Therefore

k1[E][S] = (k-1 + k2) [ES]

Rearranging terms,

[E][S]/[ES] = (k-1 + k2)/k1

The fraction [E][S]/[ES] has been coined Km, or the Michaelis constant

According to Michaelis-Menten's kinetics equations, at low concentrations of substrate, [S], the concentration is almost negligible in the denominator as KM >> [S], so the equation is essentially
V0 = Vmax [S]/KM
which resembles a first order reaction
At High substrate concentrations, [S] >> KM, and thus the term [S]/([S] + KM) becomes essentially one and the initial velocity approached Vmax, which resembles zero order reaction .
The Michaelis-Menten equation is In this equation
Vo is the initial velocity of the reaction
Vmax is the maximal rate of the reaction
[Substrate] is the concentration of the substrate

$$V_0 = V_{max}\left(\frac{[\text{Substrate}]}{[\text{Substrate}] + K_m}\right)$$

Km is the Michaelis-Menten constant which shows the concentration of the substrate when the reaction velocity is equal to one half of the maximal velocity for the reaction

It can also be thought of as a measure of how well a substrate complexes with a given enzyme, otherwise known as its binding affinity An equation with a low Km value indicates a large binding affinity, as the reaction will approach Vmax more rapidly. An equation with a high Km indicates that the enzyme does not bind as efficiently with the substrate, and Vmax will only be reached if the substrate concentration is high enough to saturate the enzyme.

As the concentration of substrates increases at constant enzyme concentration, the active sites on the protein will be occupied as the reaction is proceeding.

When all the active sites have been occupied, the reaction is complete, which means that the enzyme is at its maximum capacity and increasing the concentration of substrate will not increase the rate of turnover.

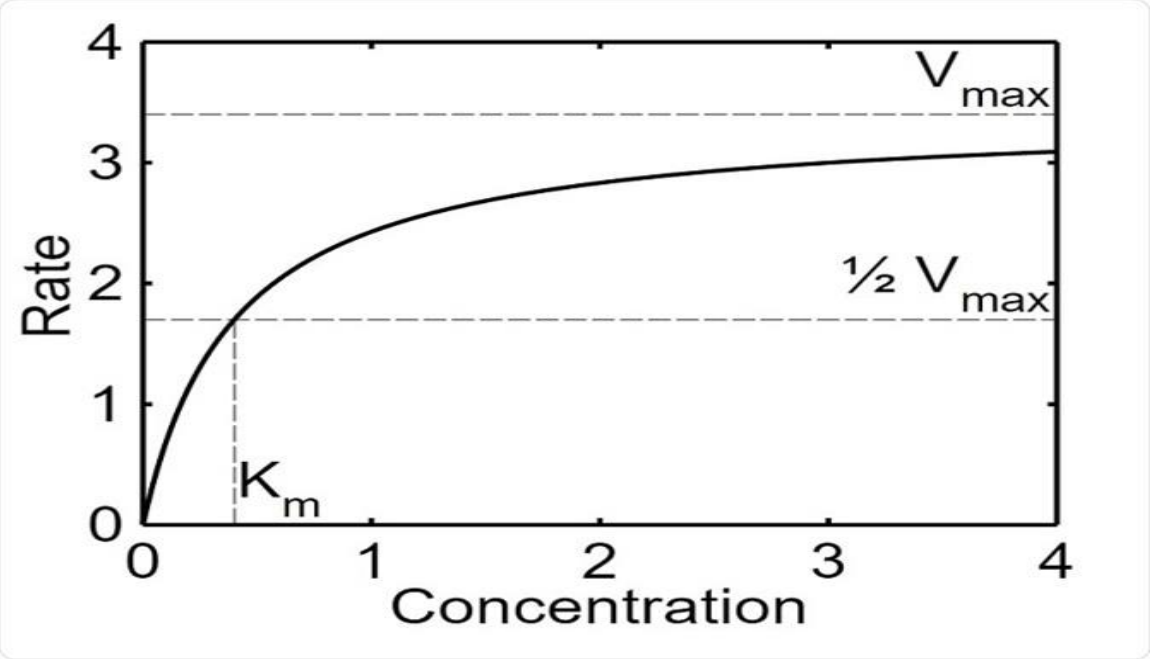
Vmax is equal to the product of the catalyst rate constant (kcat) and the concentration of the enzyme.

The Michaelis-Menten equation can then be rewritten as V= Kcat [Enzyme] [S] / (Km + [S])
Kcat is equal to K2, and it measures the number of substrate molecules "turned over" by enzyme per second.

The unit of Kcat is in 1/sec. The reciprocal of Kcat is then the time required by an enzyme to "turn over" a substrate molecule. The higher the Kcat is, the more substrates get turned over in one second.
Km is the concentration of substrates when the reaction reaches half of Vmax.
A small Km indicates high affinity since it means the reaction can reach half of Vmax in a small number of substrate concentration. This small Km will approach Vmax more quickly than high Km value
The turnover number of an enzyme, is the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.

In enzyme kinetics, we are interested to know how many maximum molecules of substrate can be converted into product per catalytic site of a given concentration of enzyme per unit time. kcat=Vmax / Et(1) with kcat = Turnover number, Vmax= Maximum rate of reaction when all the enzyme catalytic sites are saturated with substrate and
ET =Total enzyme concentration or concentration of total enzyme catalytic sites.
The units of Turn over number (kcat) are kcat
= (moles of product/sec)/ (moles of enzyme) or sec-1.
Leonor Michaelis and Maud Menten introduced a mathematical illustration to describe the action of enzymes with two constants, V_{max} and $K_{m.}$
The maximal velocity (V_{max}) refers to the point at which the increase the concentration of the substrate does not increase the rate of a reaction catalyzed by an enzyme. This occurs because the substrate molecules saturate the active sites of the enzyme and are not able to

form more complexes with the enzyme. This value is given as a rate (mmol/s), which is the maximum velocity of the reaction when the enzyme is saturated.



Since, Vmax is achieved at infinite substrate concentration, it is impossible to estimate Vmax and hence Km from a hyperbolic plot

Because of this difficulty, the Michaelis–Menten equation was transformed into an equation for a straight line by Lineweaver and Burk.

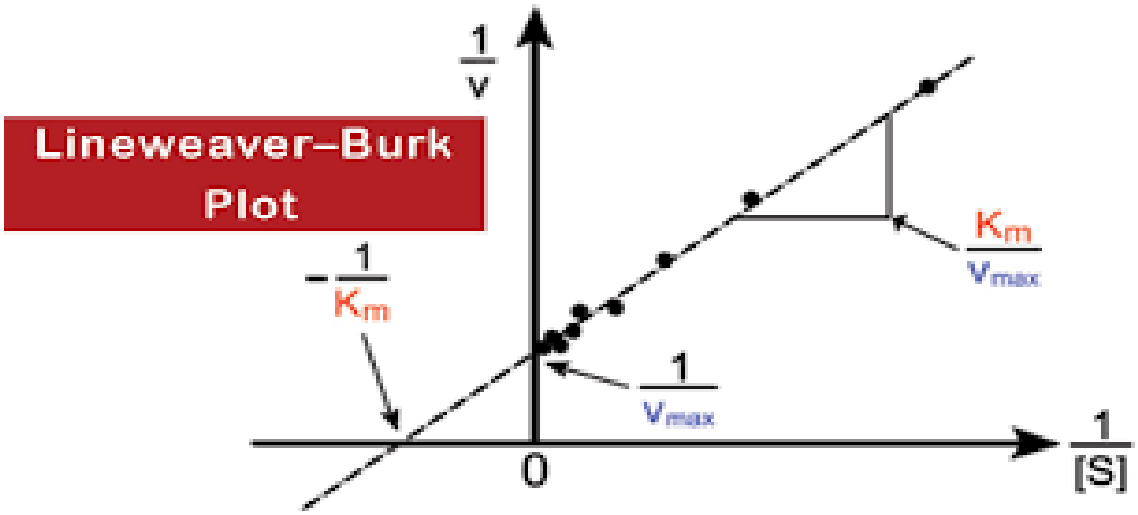
Where V is the reaction velocity (the reaction rate), K_m is the Michaelis–Menten constant, V_{max} is the maximum reaction velocity, and [S] is the substrate concentration.

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

It gives a straight line, with the intercept on the y-axis equal to 1/Vmax, and the intercept on the x-axis equal to Km/Vmax. The slope of the line is equal to Km/Vmax

Vmax and Km can be determined experimentally by measuring V0 at different substrate concentrations. Then a double reciprocal or Lineweaver–Burk plot of 1/V0 against 1/[S] is made.

Lineweaver-Burk) plot is created by plotting the inverse initial velocity ($1/V_0$) as a function of the inverse of the substrate concentration (1/[S]). ... This plot is a useful way to determined different inhibitors such as competitive, uncompetitive, and non competitive.



The Michaelis constant (K_m) is the concentration of the substrate when half of the active binding sites of an enzyme are occupied by the substrate.

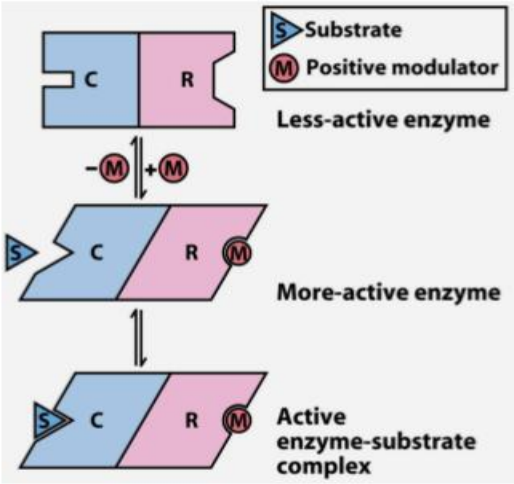
The constant helps to depict the affinity of the enzyme for their substrate

This value is given as the concentration of the substrate (mM) at half of V_{max}

An enzyme with a high K_m has a low affinity for the substrate, and a high concentration of the substrate is needed in order for the enzyme to become saturated. Conversely, an enzyme with a high K_m has a high affinity for the substrate and the enzyme may become saturated even with a low amount of substrate.

Allosteric enzymes

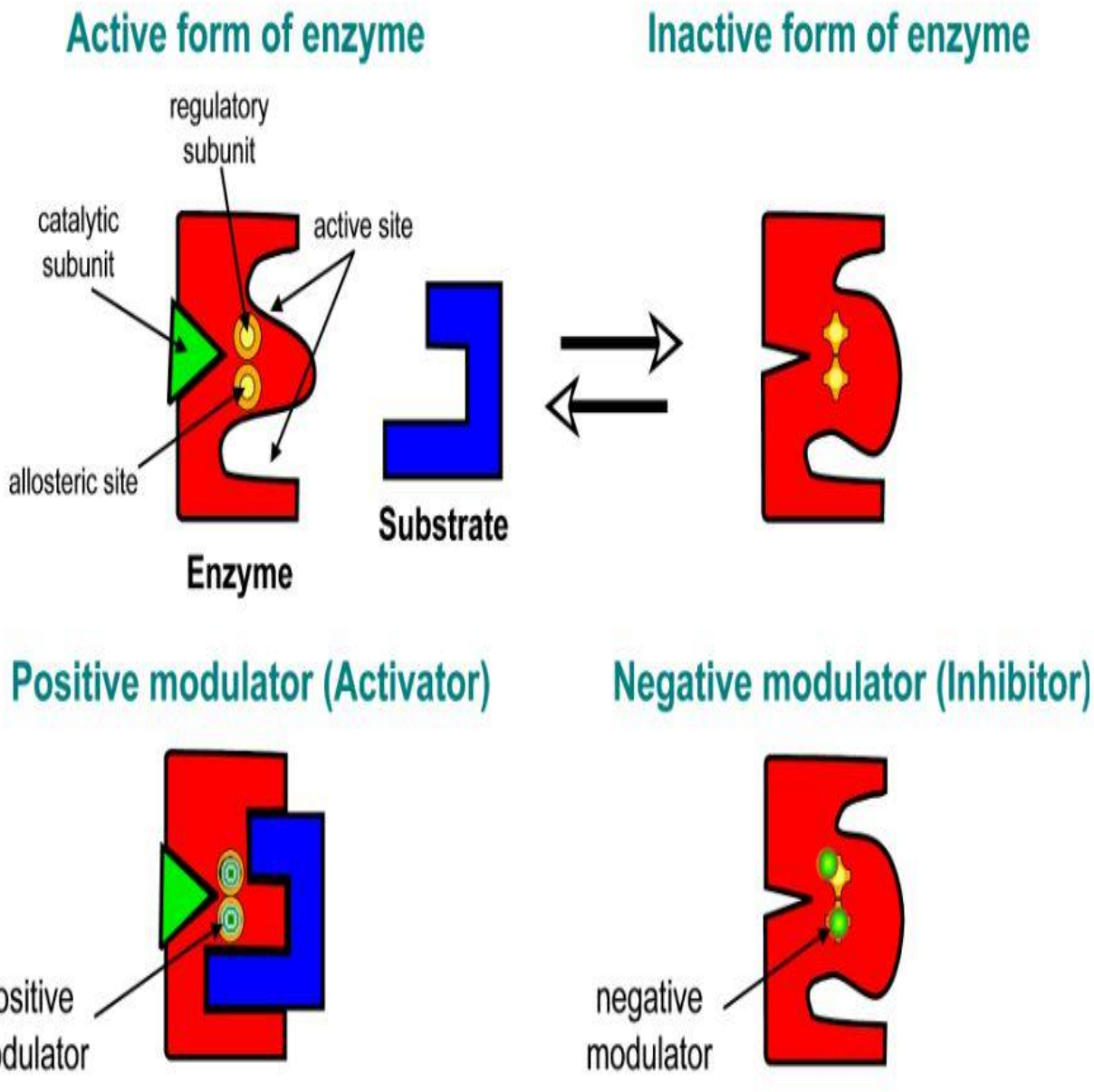
- Allosteric enzymes tend to be multi-sub unit proteins
- The reversible binding of an allosteric modulator (here a positive modulator M) affects the substrate binding site



Allosteric enzymes do not obey Michaelis-Menten kinetics. ... Thus, allosteric enzymes show the sigmodial curve. The plot for reaction velocity, vo, versus the substrate concentration does not exhibit the hyperbolic plot predicted using the Michaelis-Menten equation.

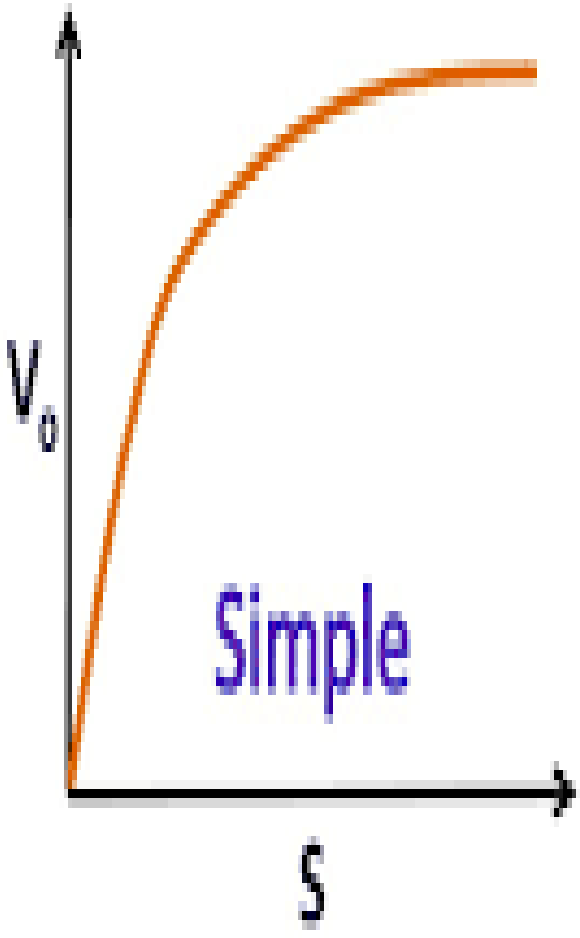
An allosteric enzyme is an enzyme that contains a region to which small, regulatory molecules ("effectors") may bind in addition to and separate from the substrate binding site and thereby affect the catalytic activity.
On binding the effector, the catalytic activity of the enzyme towards the substrate may be enhanced, in which case the effector is an activator, or reduced, in which case it is a de-activator or inhibitor.

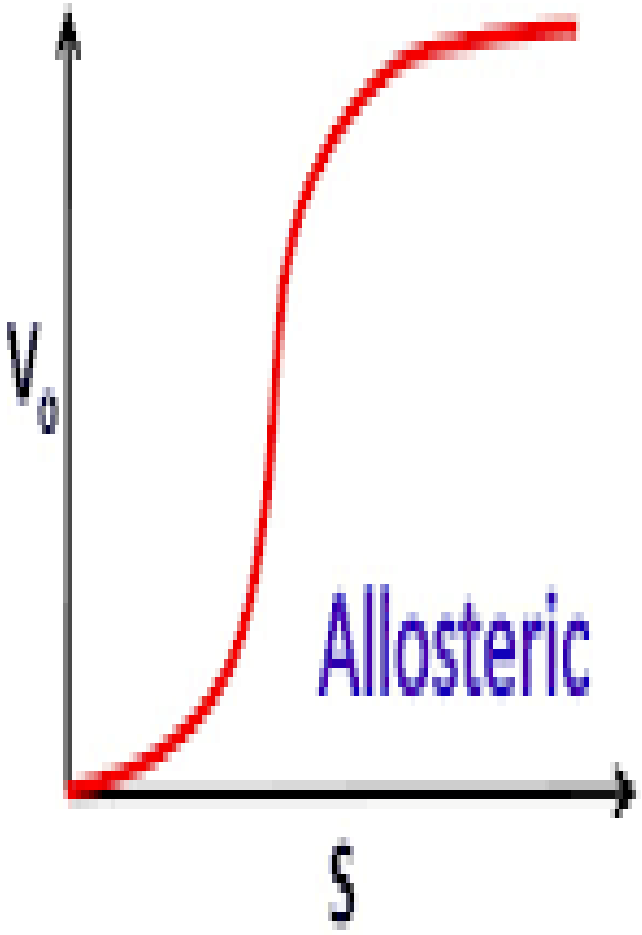
Allosteric Enzymes and Modulators



Quickly understand

Simple vs allosteric Enzymes



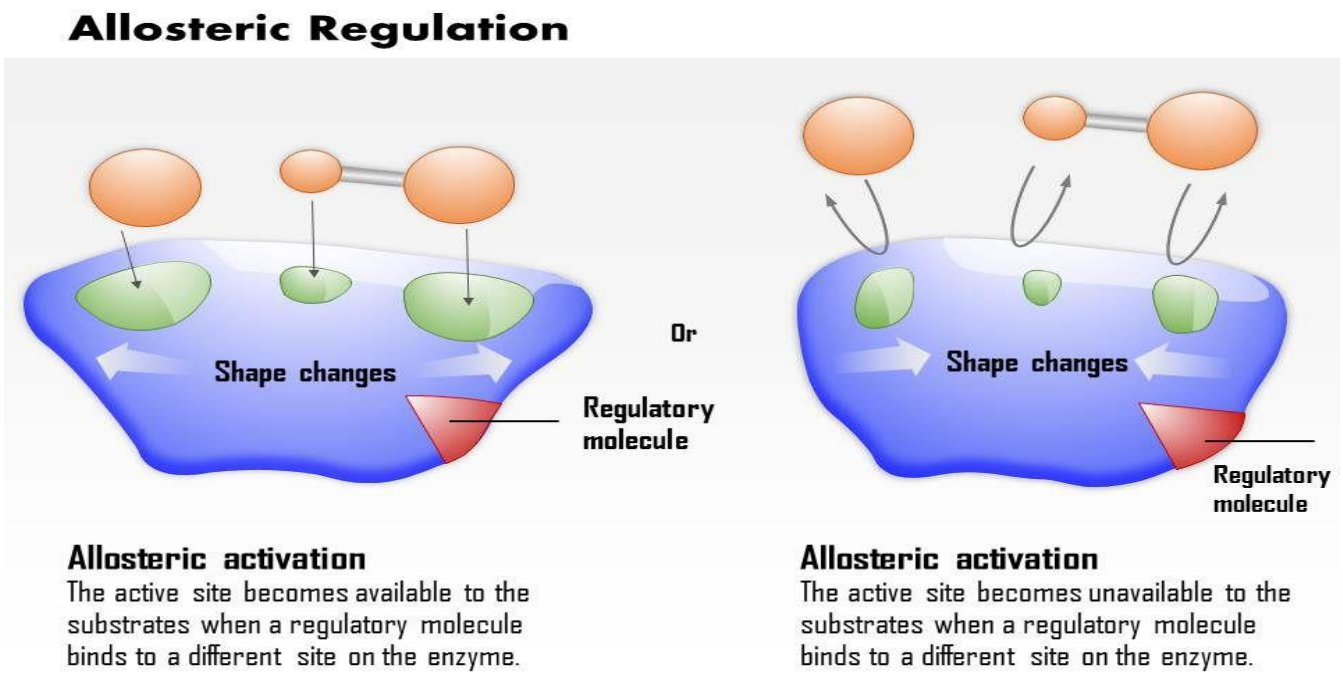


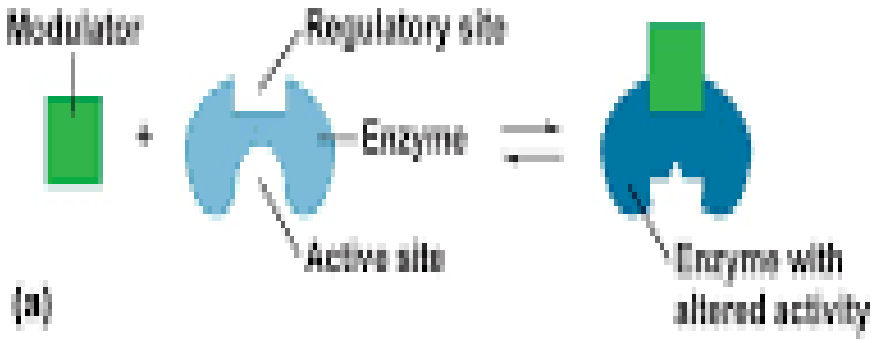
Allosteric Regulation

Allosteric regulations the regulation of activities of an enzyme or a protein caused by the binding of modulators (or effectors) at the site other than the active site of the enzyme or protein. There are two effects of Allosteric regulations and two effectors are may be available for the effects

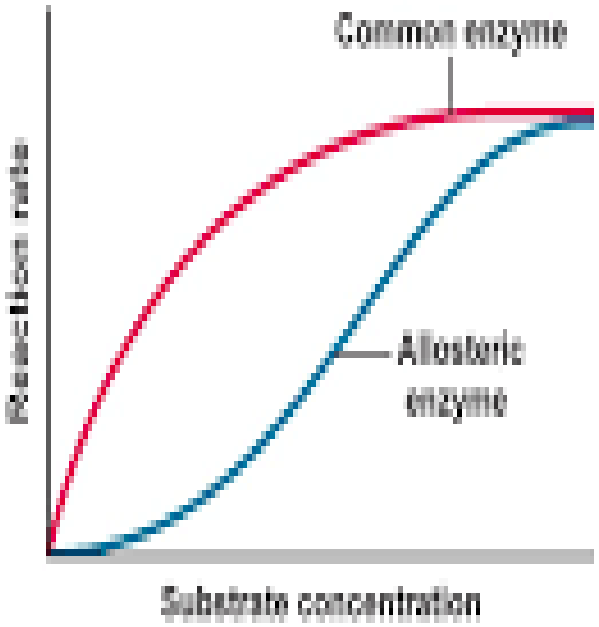
Effectors maybe-feed-forward activators or feedback inhibitors.

Allosteric--derived from the Greek--meaning other shape • Characterized by sigmoidal kinetics (as opposed to Michaelis-Menton kinetics) – show cooperativity – quaternary structure – (note: hemoglobin shows allosteric behavior although it is not an enzyme) • Conformational change usually involves binding small effector molecules that are not substrates- -these bind to an allosteric site on the enzyme that result in rapid changes in enzyme activity

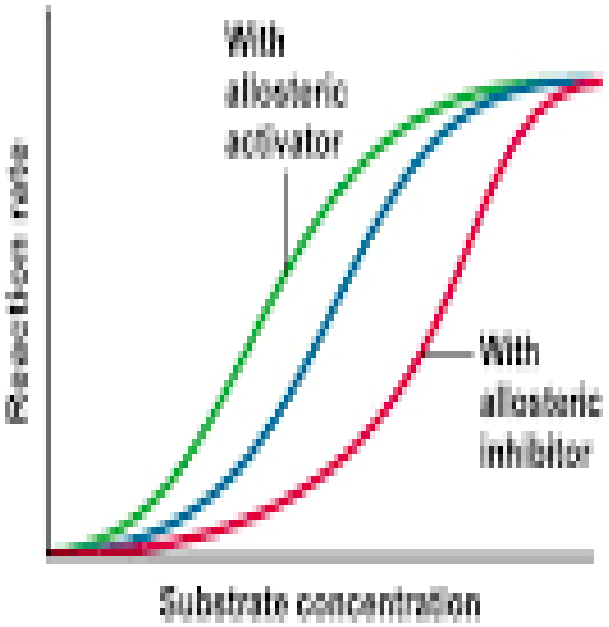




(a)



(b)



(c)

Enzyme Inhibitors

- Specific enzyme inhibitors regulate enzyme activity and help us understand mechanism of enzyme action. (Denaturing agents are not inhibitors)

- Irreversible inhibitors form covalent or very tight permanent bonds with aa at the active site of the enzyme and render it inactive. 3 classes: groupspecific reagents, substrate analogs, suicide inhibitors

- Reversible inhibitors form an EI complex that can be dissociated back to enzyme and free inhibitor. 3 groups based on their mechanism of action: competitive, non-competitive and uncompetitive.

What are enzyme inhibitors?

Substances can interfere with enzyme activity are called **inhibitors**. They can be classed in two ways, depending on their mode of action:

- Inhibitors can be either **competitive** (active site directed) or **non-competitive** (non-active site directed), depending on whether they compete with the substrate for binding at the active site or not.

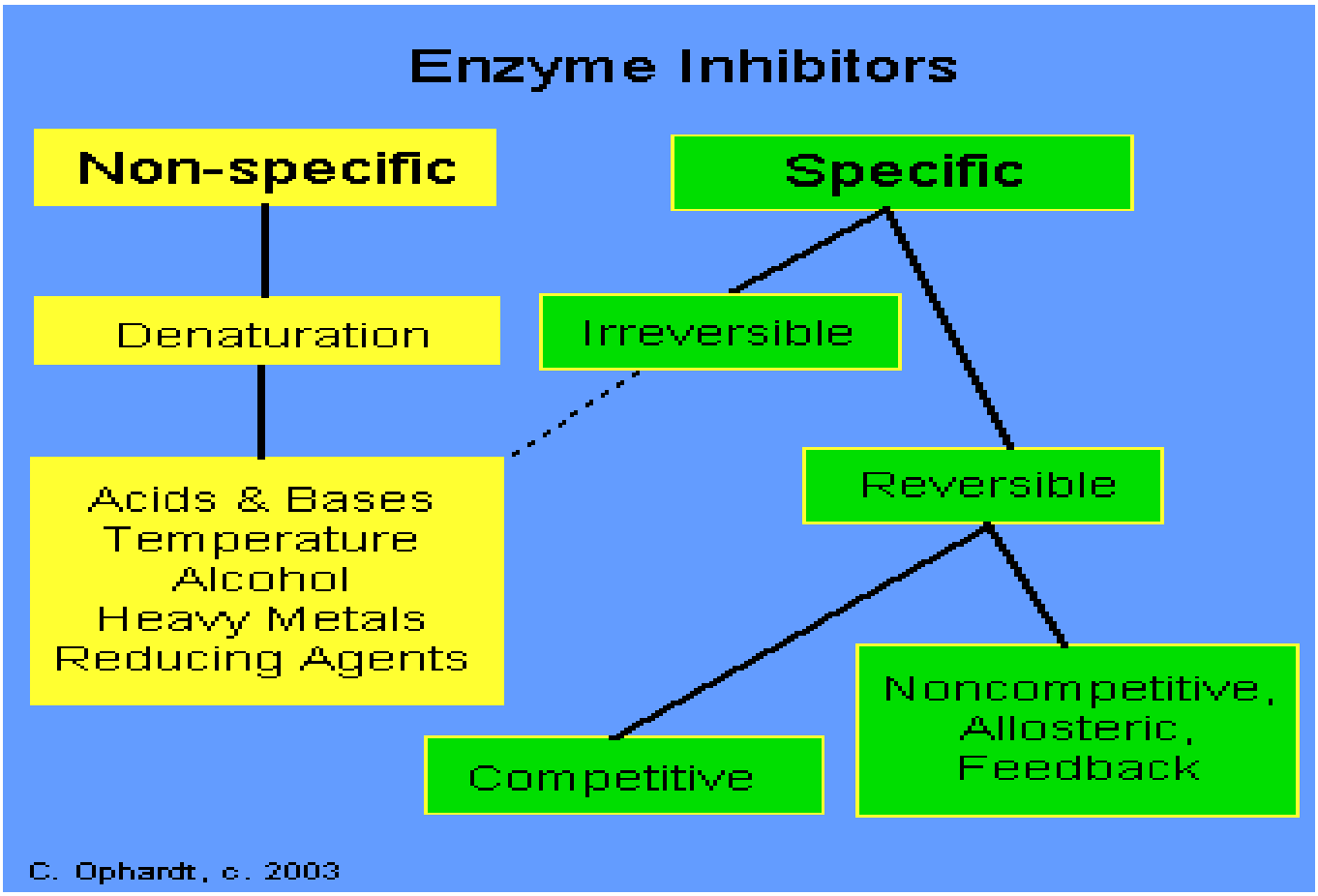
T Blundell & N.Campillo, Wellcome Images

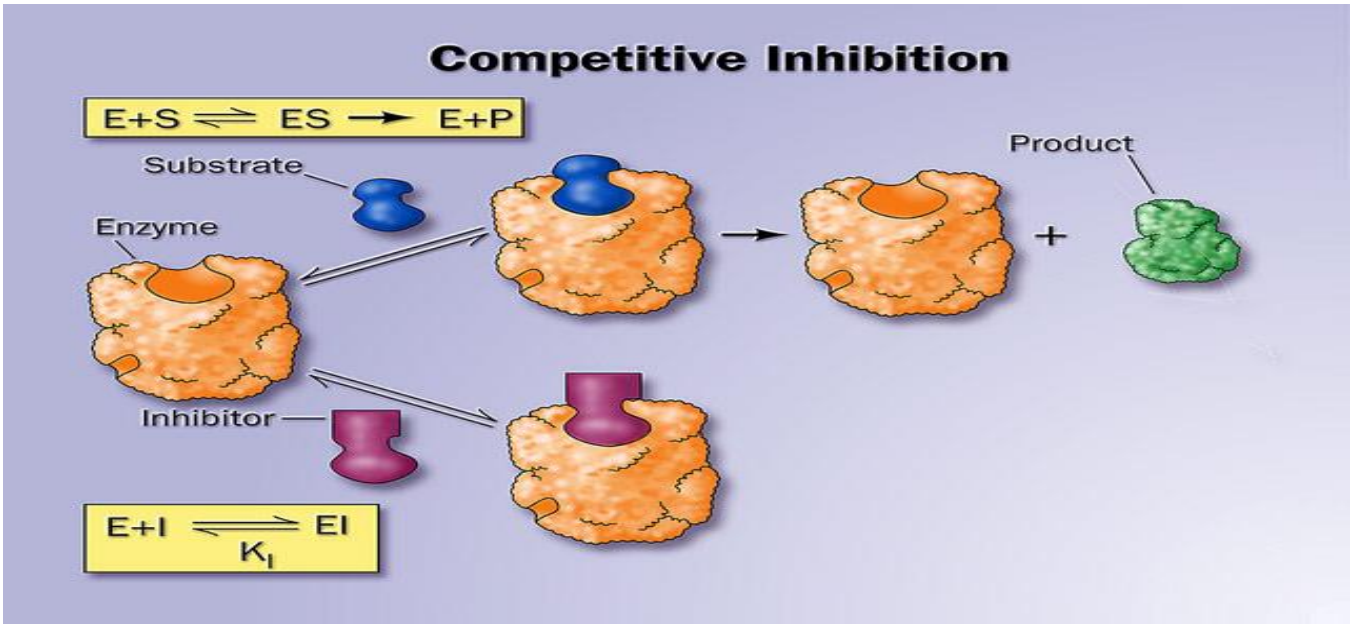
- Inhibitors can be either **reversible** or **irreversible**, depending on whether their inhibitory effect on the enzyme is permanent or not.

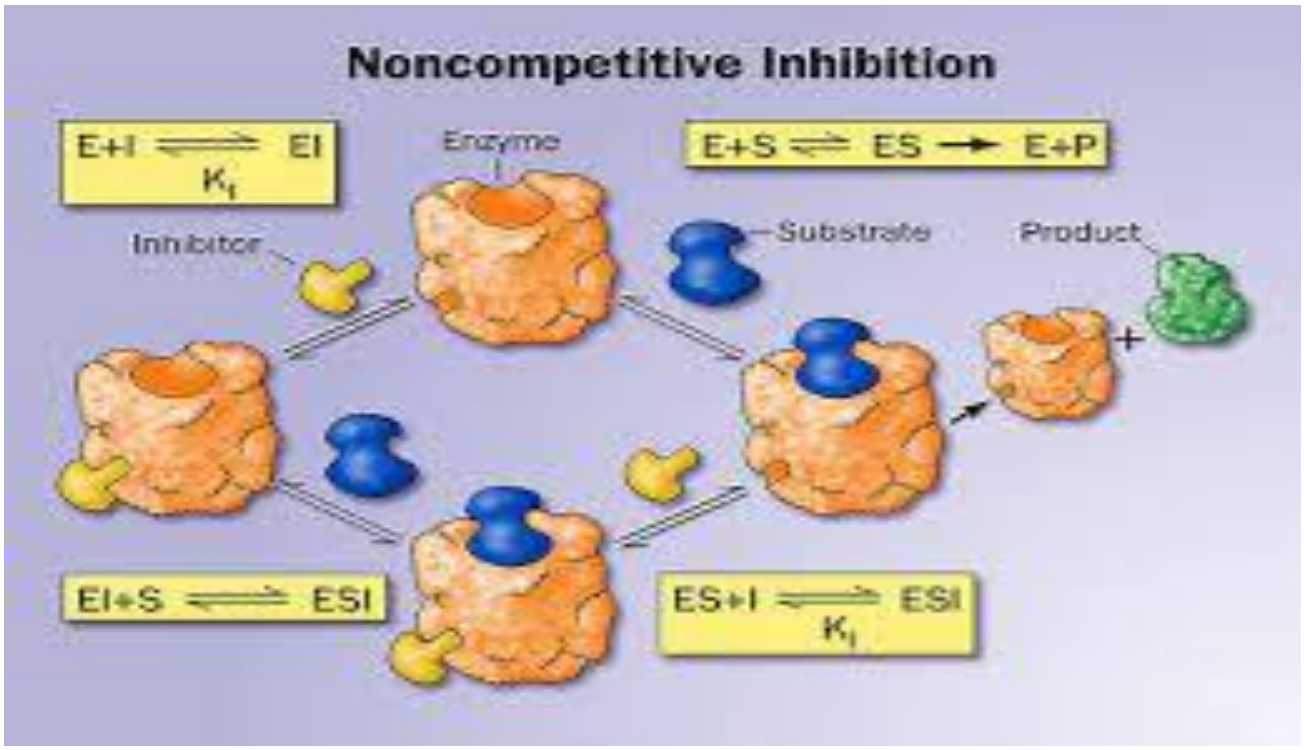
1 of 34 © Boardworks Ltd 2008

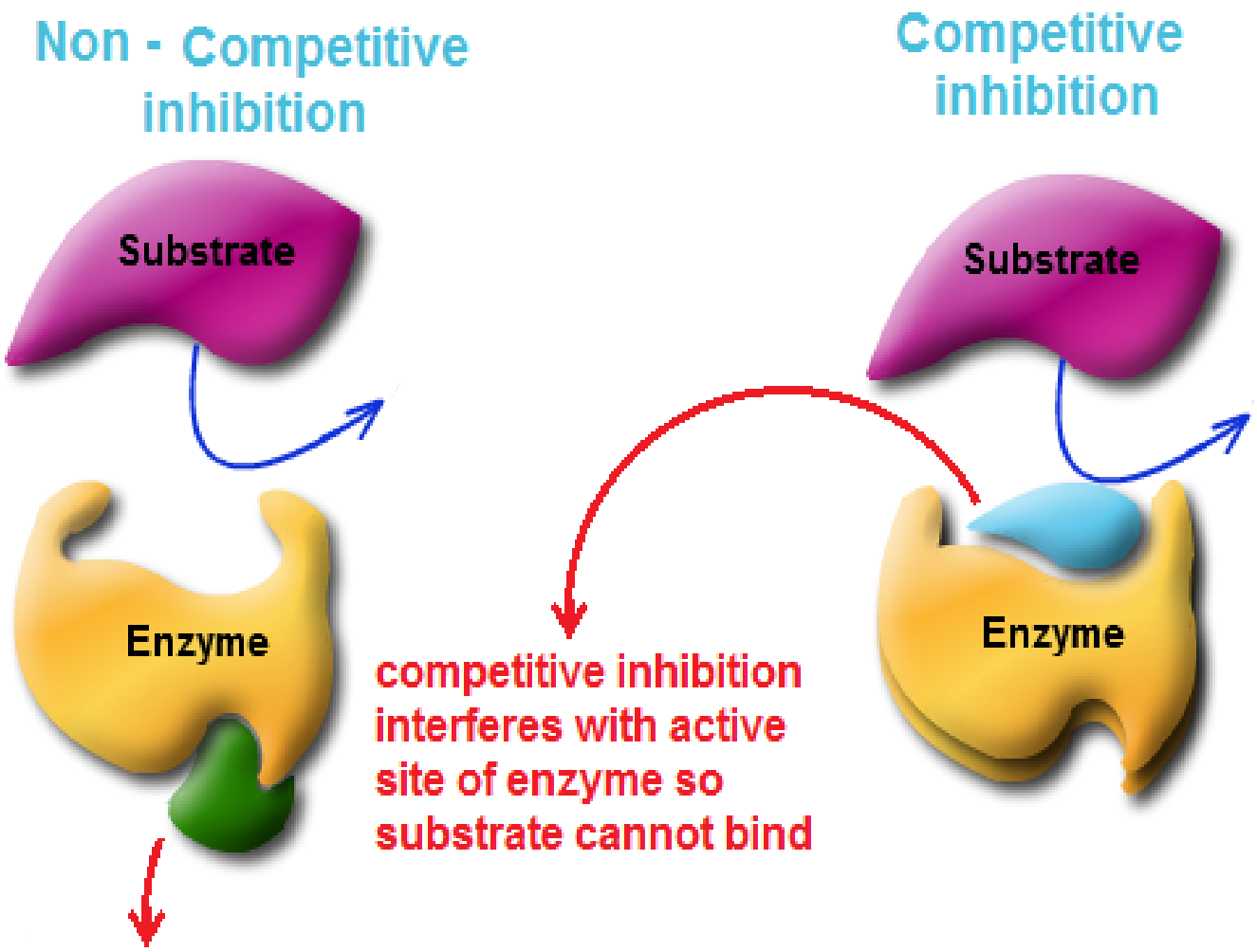
Enzymes are protein and they can be inactivated by the agents that denature them. The chemical substances which inactivate the enzymes are called inhibitors and the process is called as enzymes inhibition .Inhibitors are sometimes referred to as negative modifier. They may be small inorganic ions , or organic substances

An enzyme inhibitor is a molecule that binds to an enzyme and decreases its activity by binding to enzymes' active sites, inhibitors reduce the compatibility of substrate and enzyme and this leads to the inhibition of Enzyme-Substrate complexes' formation, preventing the catalyzation of reactions and decreasing (at times to zero) the amount of product produced by a reaction
It can be said that as the concentration of enzyme inhibitors increases, the rate of enzyme activity decreases, and thus, the amount of product produced is inversely proportional to the concentration of inhibitor molecules




Competitive Inhibition
E+S ⇌ ES → E+P
Substrate
Enzyme
Product
+
Inhibitor
E+I ⇌ EI
K_I


Noncompetitive Inhibition
E+I ⇌ EI
K_I
Enzyme
E+S ⇌ ES → E+P
Inhibitor
Substrate
Product
+
EI+S ⇌ ESI
ES+I ⇌ ESI
K_I

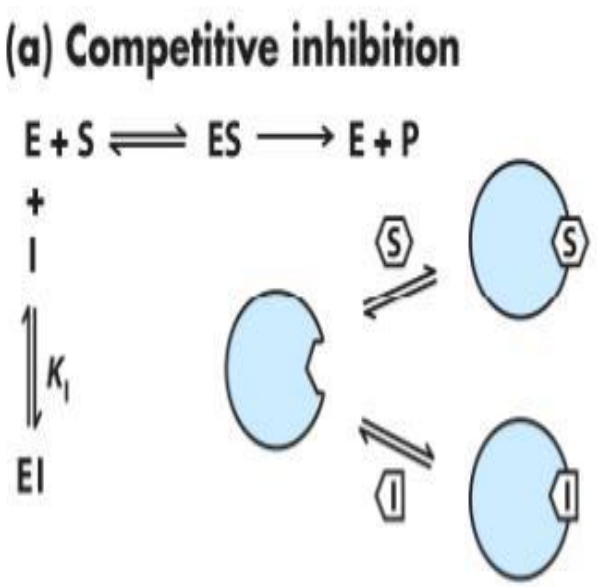

Non - Competitive inhibition
Competitive inhibition
Substrate
Substrate
Enzyme
Enzyme
competitive inhibition interferes with active site of enzyme so substrate cannot bind
non-competitive inhibition change shape enzyme so it cannot bind to substrate.

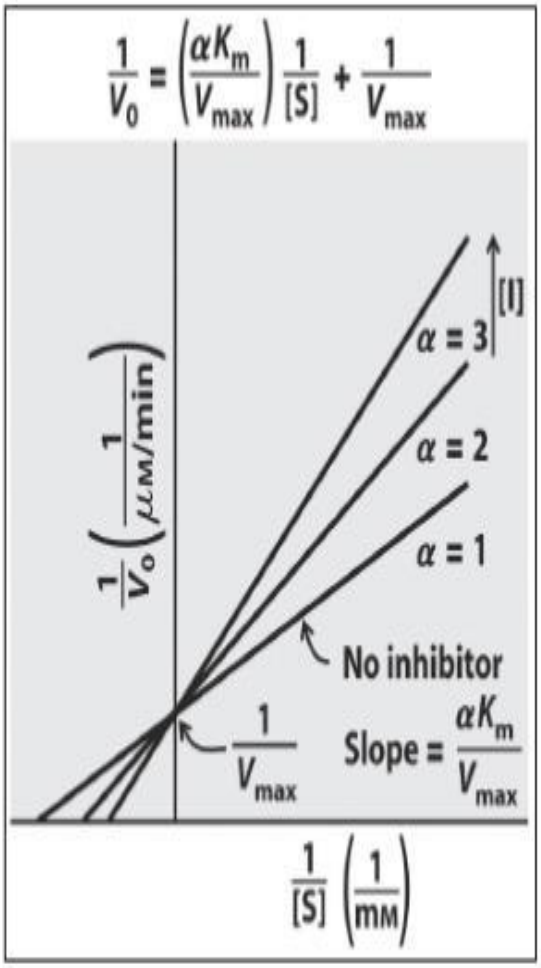
Competitive inhibitors

- Compete with substrate for binding to enzyme
- E + S = ES or E + I = EI . Both S and I cannot bind enzyme at the same time
- In presence of I, the equilibrium of E + S = ES is shifted to the left causing dissociation of ES.
- This can be reversed / corrected by increasing [S]
- Vmax is not changed, KM is increased by (1 + I/Ki)
- Eg: AZT, antibacterial sulfonamides, the anticancer agent methotrexate etc

Competitive Inhibition

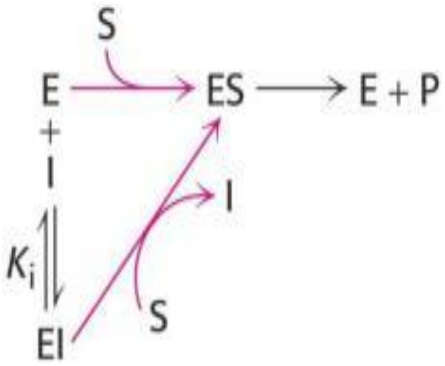
- A competitive inhibitor (I) is a substance that competes with the substrate for the same active site
- αK_m = "apparent" constant when the inhibitor is present





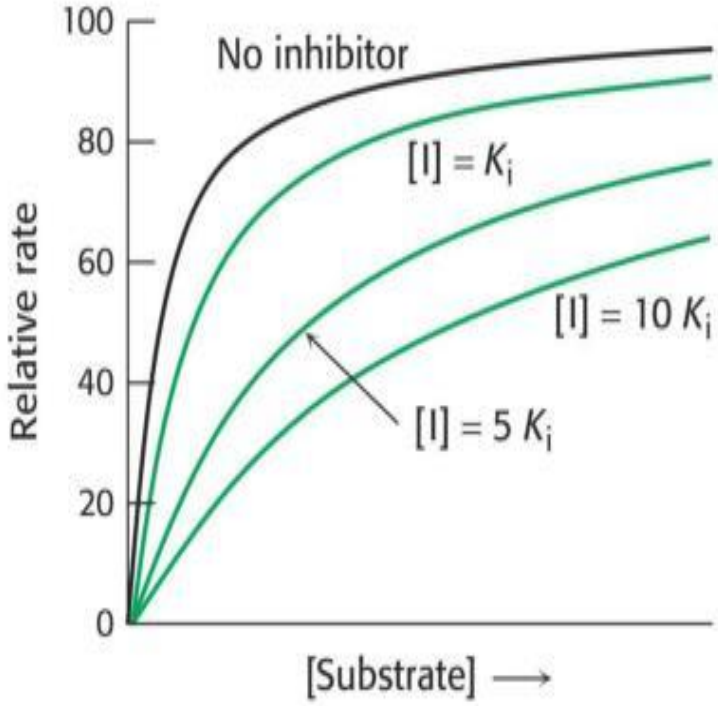
Kinetics of competitive inhibitor

K_i = dissociation constant for inhibitor



Increase [S] to overcome inhibition

V_{max} attainable, K_m is increased



Reversible inhibitors

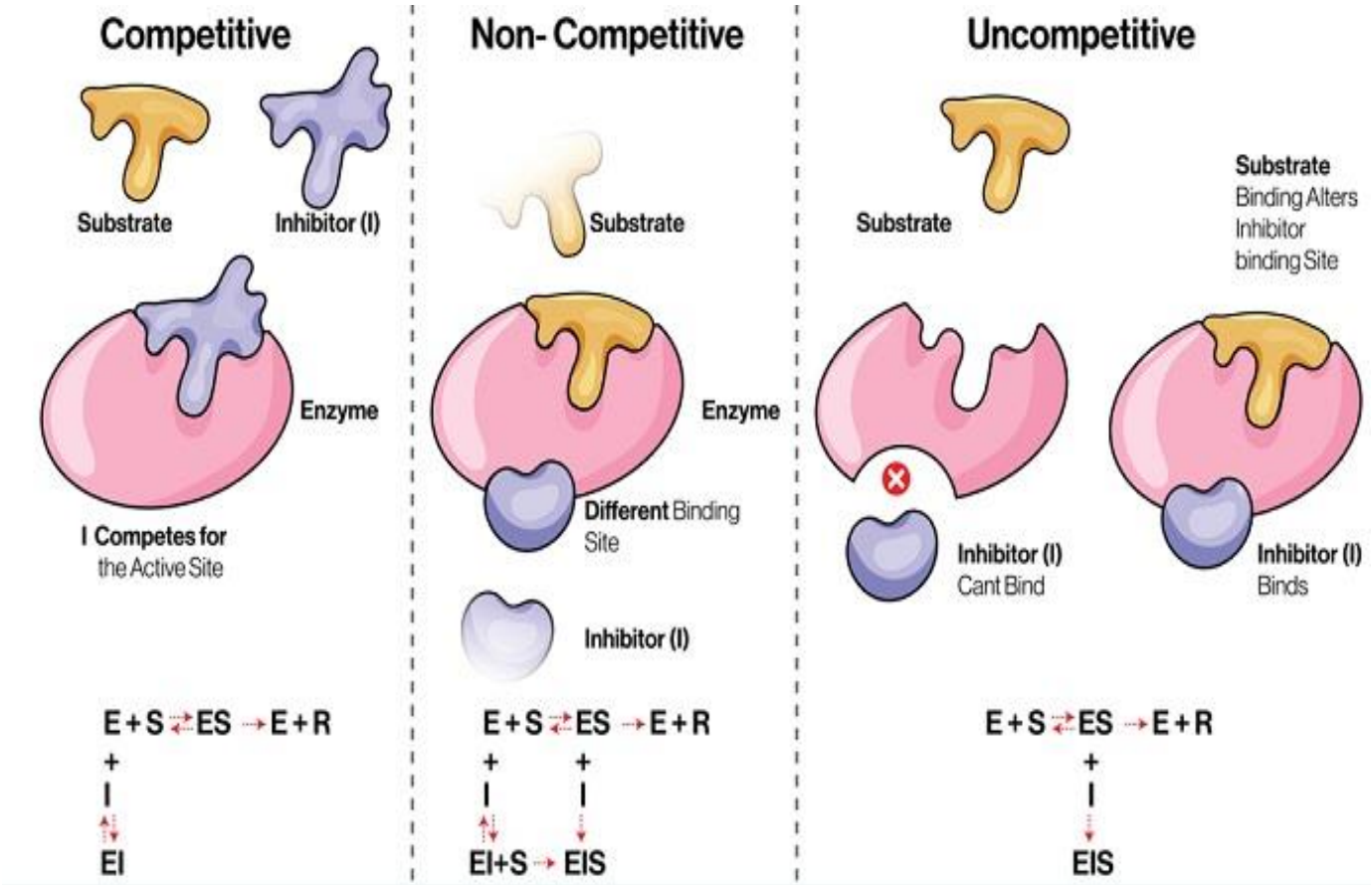
Reversible inhibitors attach to enzymes with non-covalent interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds. Multiple weak bonds between the inhibitor and the active site combine to produce strong and specific binding. In contrast to substrates and irreversible inhibitors, reversible inhibitors generally do not undergo chemical reactions when bound to the enzyme and can be easily removed by dilution or dialysis .

There are four kinds of reversible enzyme inhibitors. They are classified according to the effect of varying the concentration of the enzyme's substrate on the inhibitor

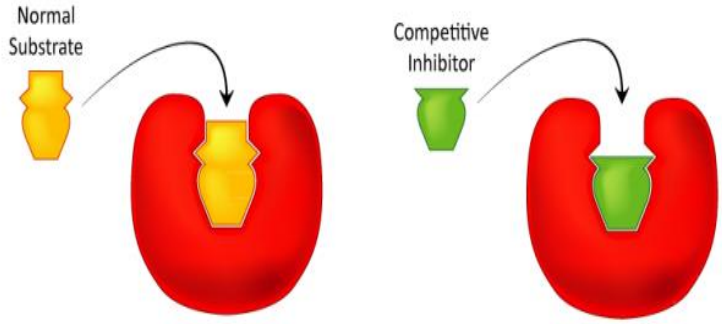
In competitive inhibition, the substrate and inhibitor cannot bind to the enzyme at the same time.(Competitive inhibitors are often similar in structure to the real substrate)

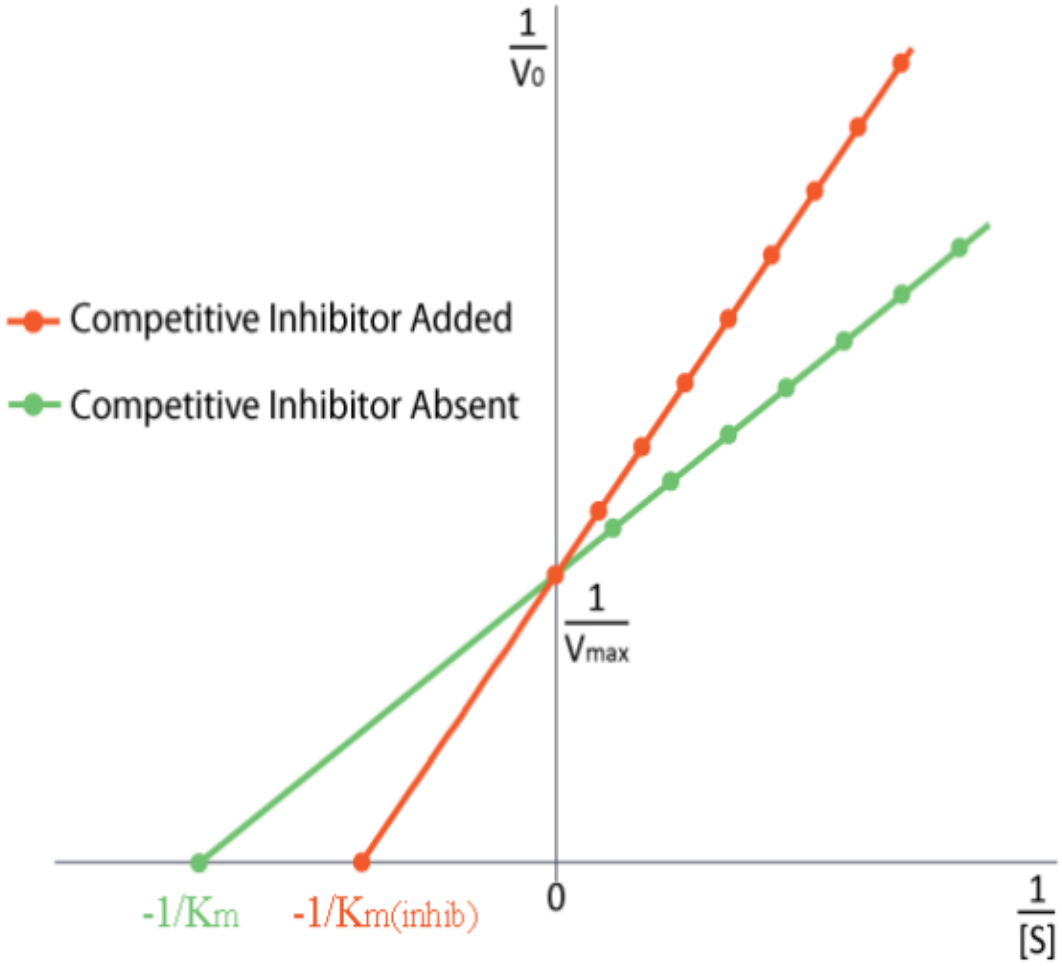
This usually results from the inhibitor having an affinity for the active site of an enzyme where the substrate also binds; the substrate and inhibitor compete for access to the enzyme's active site. This type of inhibition can be overcome by sufficiently high concentrations of substrate (Vmax remains constant), i.e., by out-competing the inhibitor.

However, the apparent Km will increase as it takes a higher concentration of the substrate to reach the Km point, or half the Vmax.



Line-Weaver Burk Plot of competitive inhibition





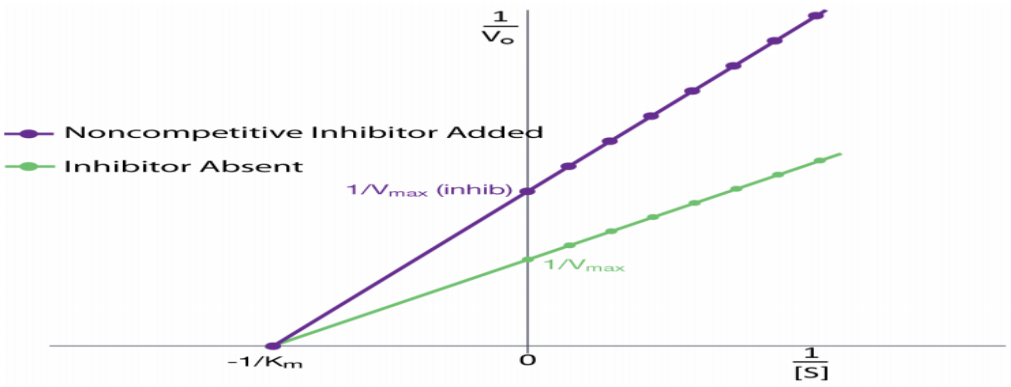
In uncompetitive inhibition

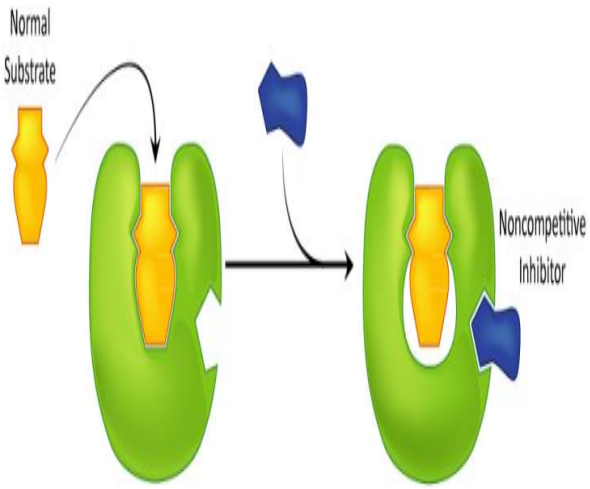
The inhibitor binds only to the substrate-enzyme complex

This type of inhibition causes Vmax to decrease (maximum velocity decreases as a result of removing activated complex) and Km to decrease (due to better binding efficiency as a result of Le Chatelier's principle and the effective elimination of the ES complex thus decreasing the Km which indicates a higher binding affinity).

In non-competitive inhibition, the binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate. As a result, the extent of inhibition depends only on the concentration of the inhibitor. Vmax will decrease due to the inability for the reaction to proceed as efficiently, but Km will remain the same as the actual binding of the substrate, by definition, will still function properly.

Line-Weaver Burk Plot of noncompetitive inhibition.

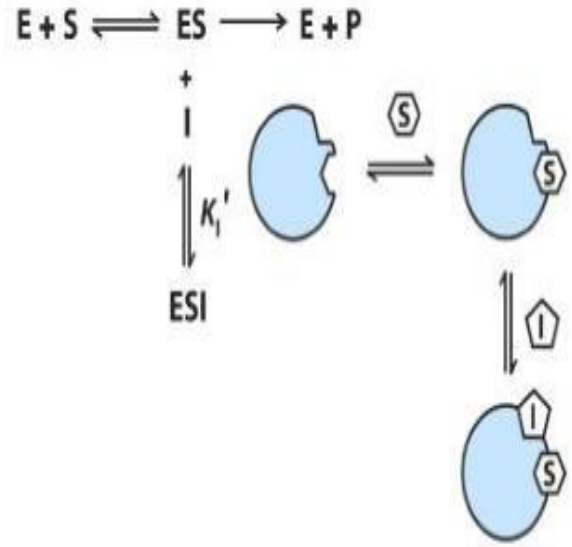


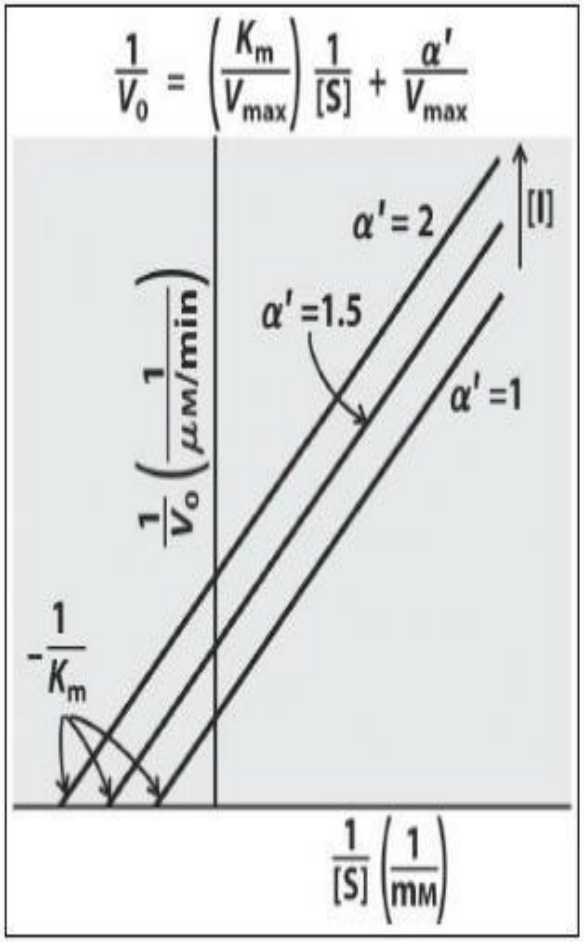


Uncompetitive Inhibition

- An uncompetitive inhibitor (I) binds at a site distinct from the substrate active site

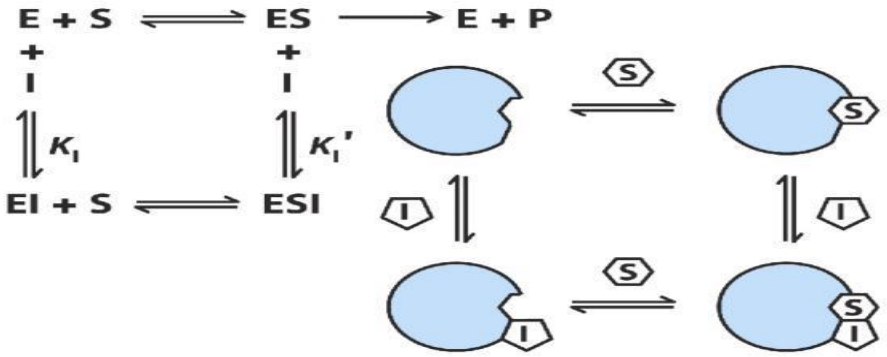
(b) Uncompetitive inhibition





Here the inhibitor does not have any affinity for free enzyme .Inhibitor binds to enzyme –substrate complex; but not the free enzyme . In such cases both Vmax and Km are decreased.

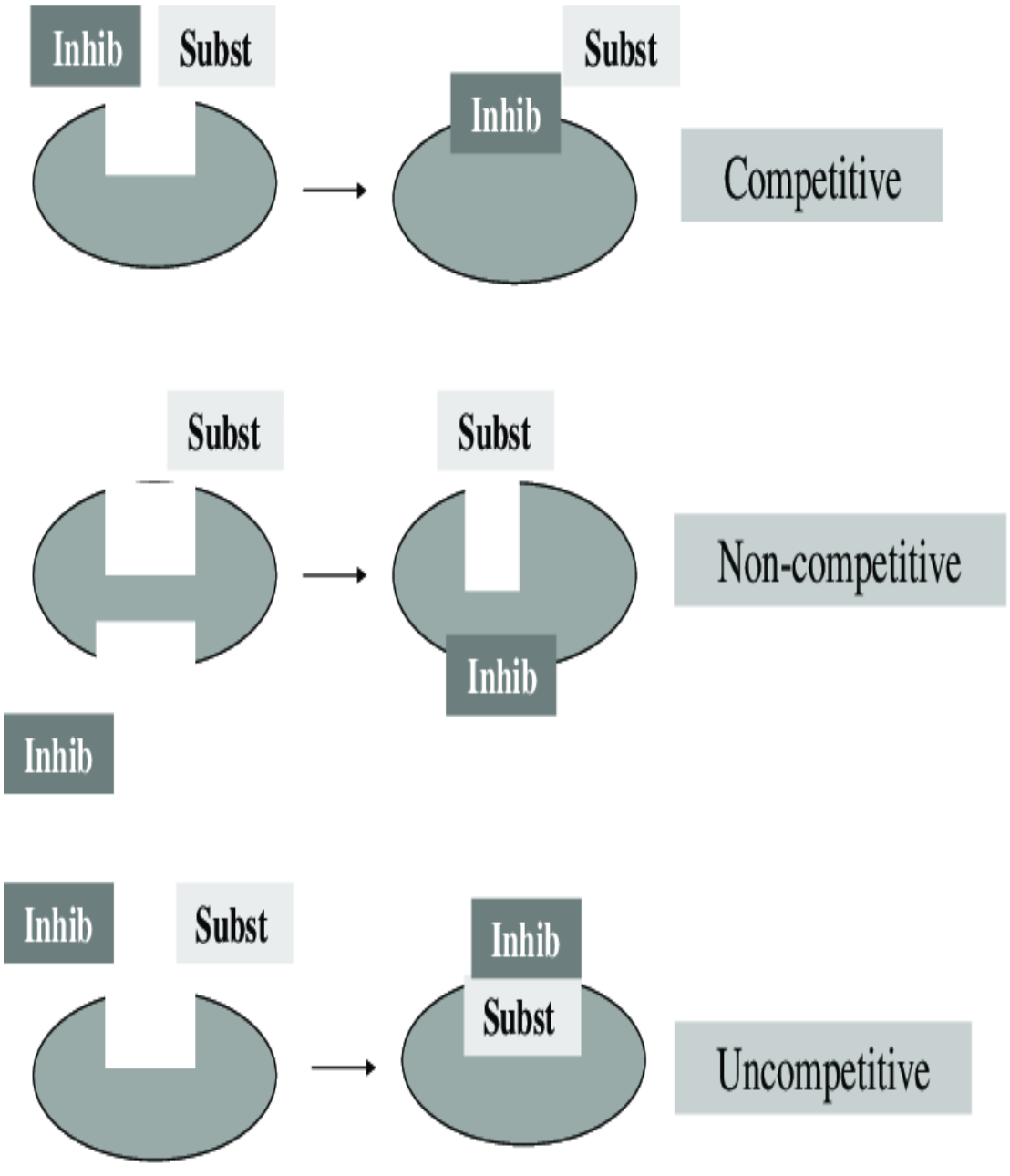
Mixed (Noncompetitive) Inhibition



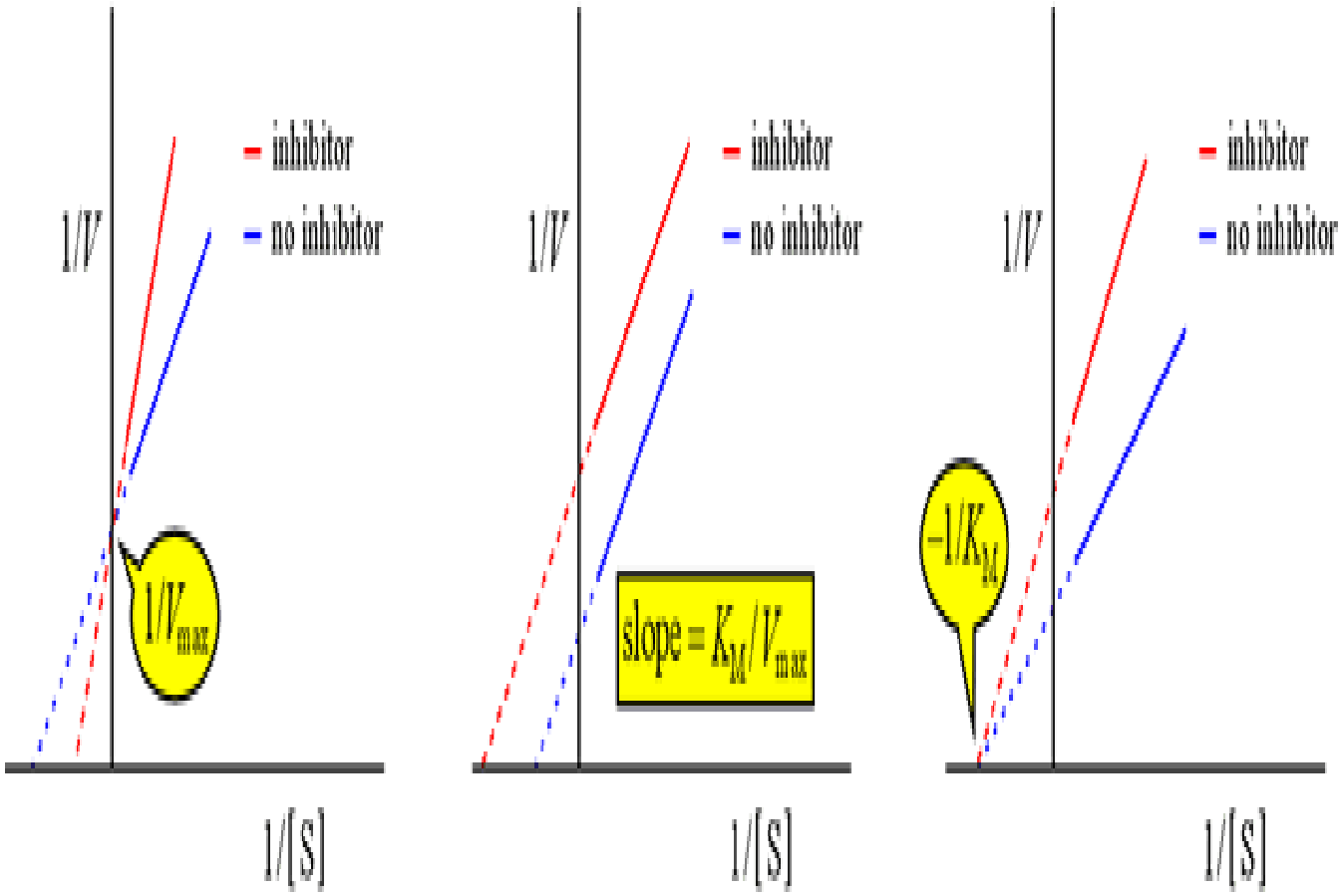
In mixed inhibition, the inhibitor can bind to the enzyme at the same time as the enzyme's substrate. However, the binding of the inhibitor affects the binding of the substrate, and vice versa. This type of inhibition can be reduced, but not overcome by increasing concentrations of substrate

Although it is possible for mixed-type inhibitors to bind in the active site, this type of inhibition generally results from an allosteric effect where the inhibitor binds to a different site on an enzyme. Inhibitor binding to this allosteric site changes the conformation (i.e., tertiary structure or three-dimensional shape) of the enzyme so that the affinity of the substrate for the active site is reduced.

Main Types of Enzyme Inhibition



The Lineweaver-Burk plots for inhibition



Competitive inhibition

K_M increased

V_{max} unaffected

Uncompetitive inhibition

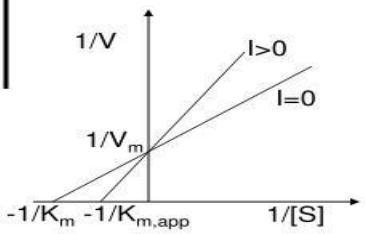
K_M reduced

V_{max} reduced

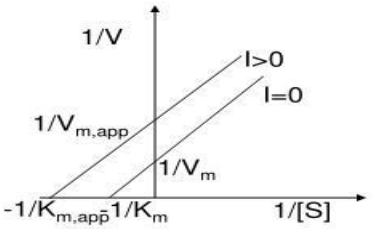
Noncompetitive inhibition

K_M unaffected

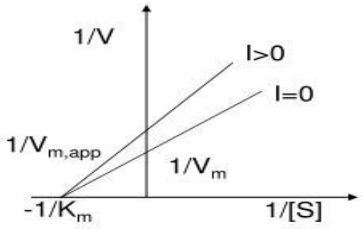
V_{max} reduced



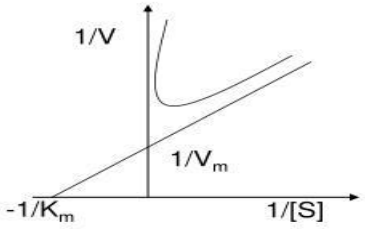
Competitive



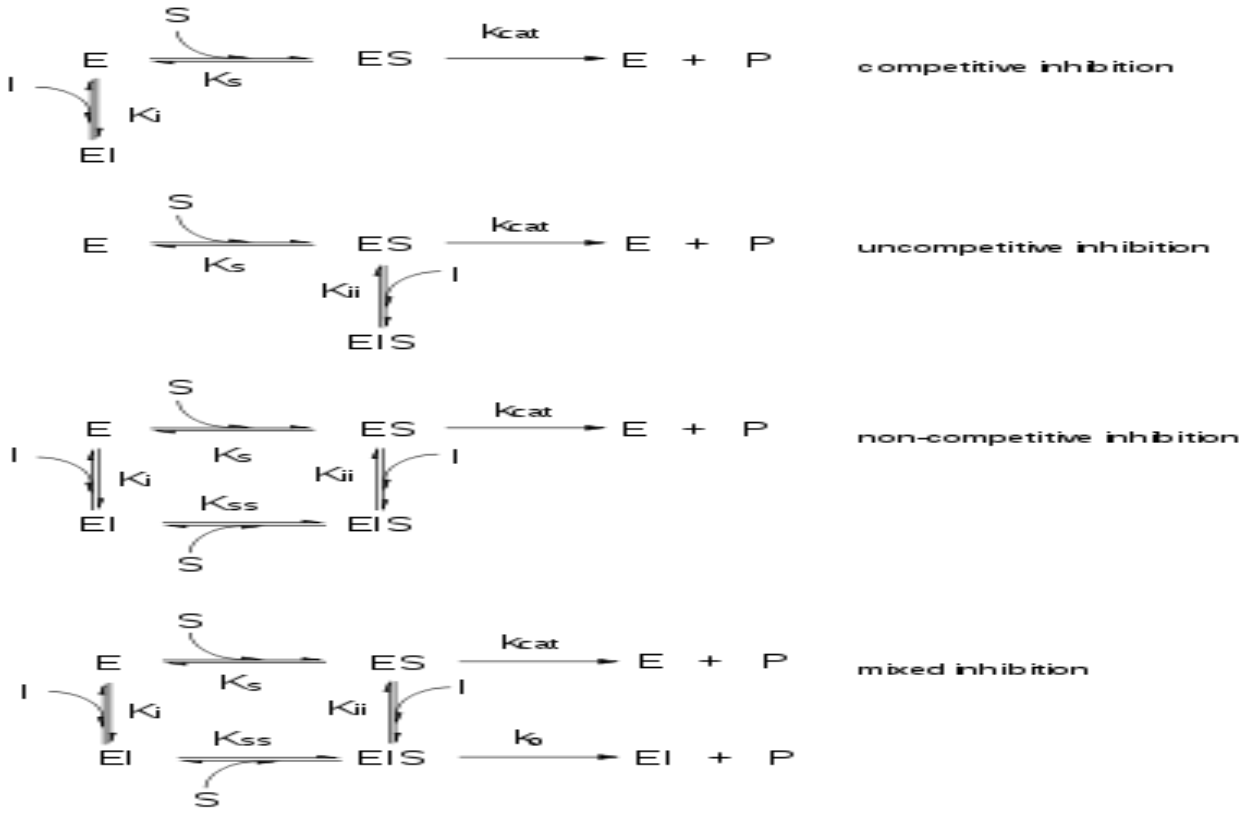
Uncompetitive



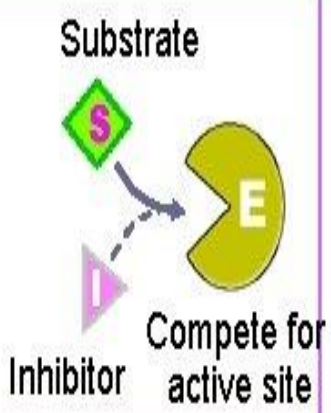
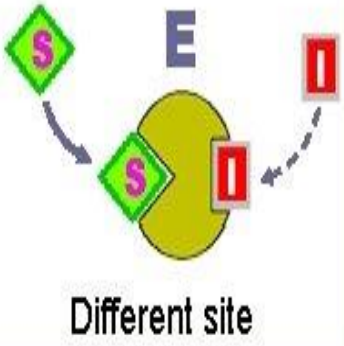
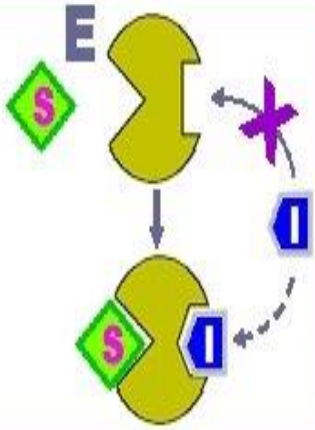
Non-Competitive



Substrate Inhibition


S
E
ES
kcat
E + P
competitive inhibition
Ks
I
Ki
EI
uncompetitive inhibition
Kii
EIS
non-competitive inhibition
Kss
mixed inhibition
ko

Enzyme Inhibition (Mechanism)

	Competitive	Non-competitive	Uncompetitive
Cartoon Guide			
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ + I $\downarrow\uparrow$ EI	$E + S \rightleftarrows ES \rightarrow E + P$ + + I I $\downarrow\uparrow$ $\downarrow\uparrow$ $EI + S \rightarrow EIS$	$E + S \rightleftarrows ES \rightarrow E + P$ + I $\downarrow\uparrow$ EIS
	[*I*] binds to free [E] only, and competes with [S]; increasing [S] overcomes Inhibition by [*I*].	[*I*] binds to free [E] or [ES] complex; Increasing [S] can not overcome [*I*] inhibition.	[*I*] binds to [ES] complex only, increasing [S] favors the inhibition by [*I*].