Enzyme Chemistry

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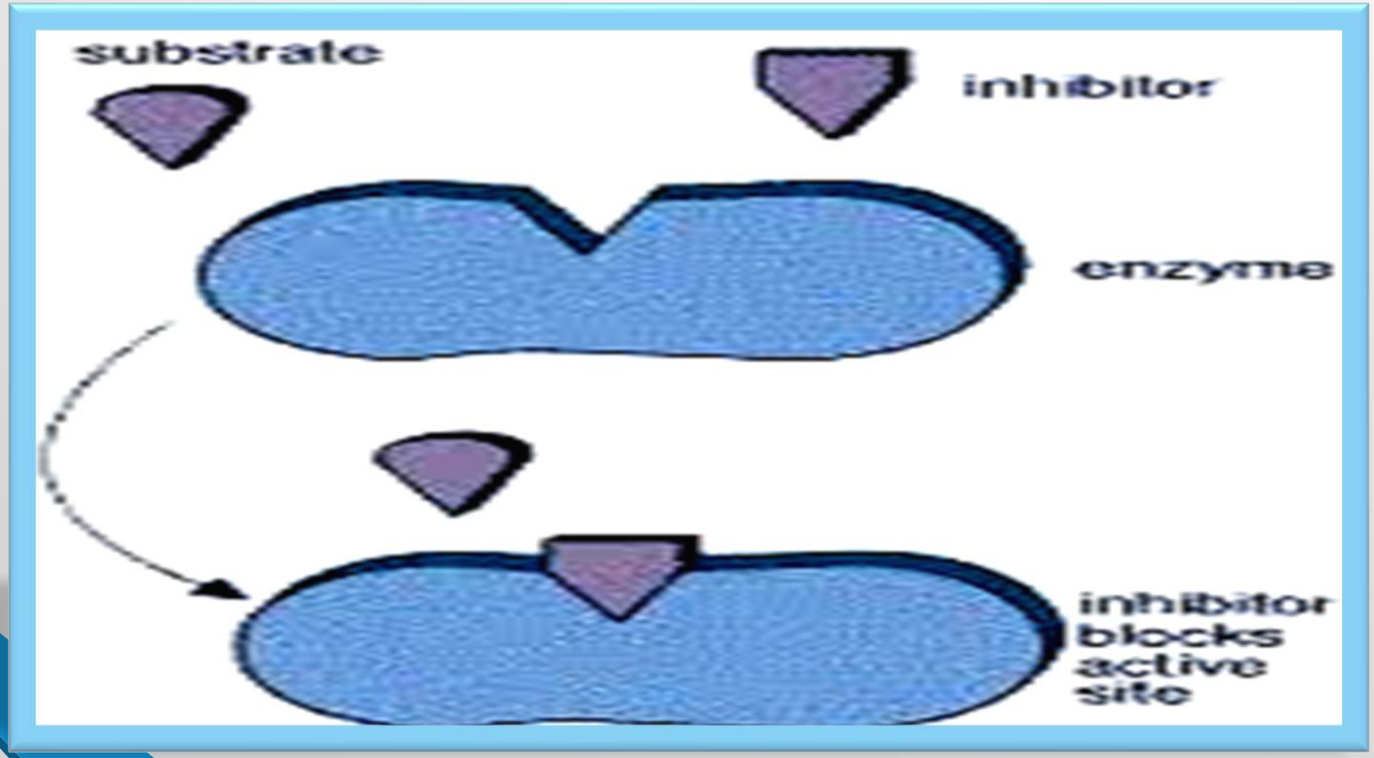
INHIBITION

INHIBITION

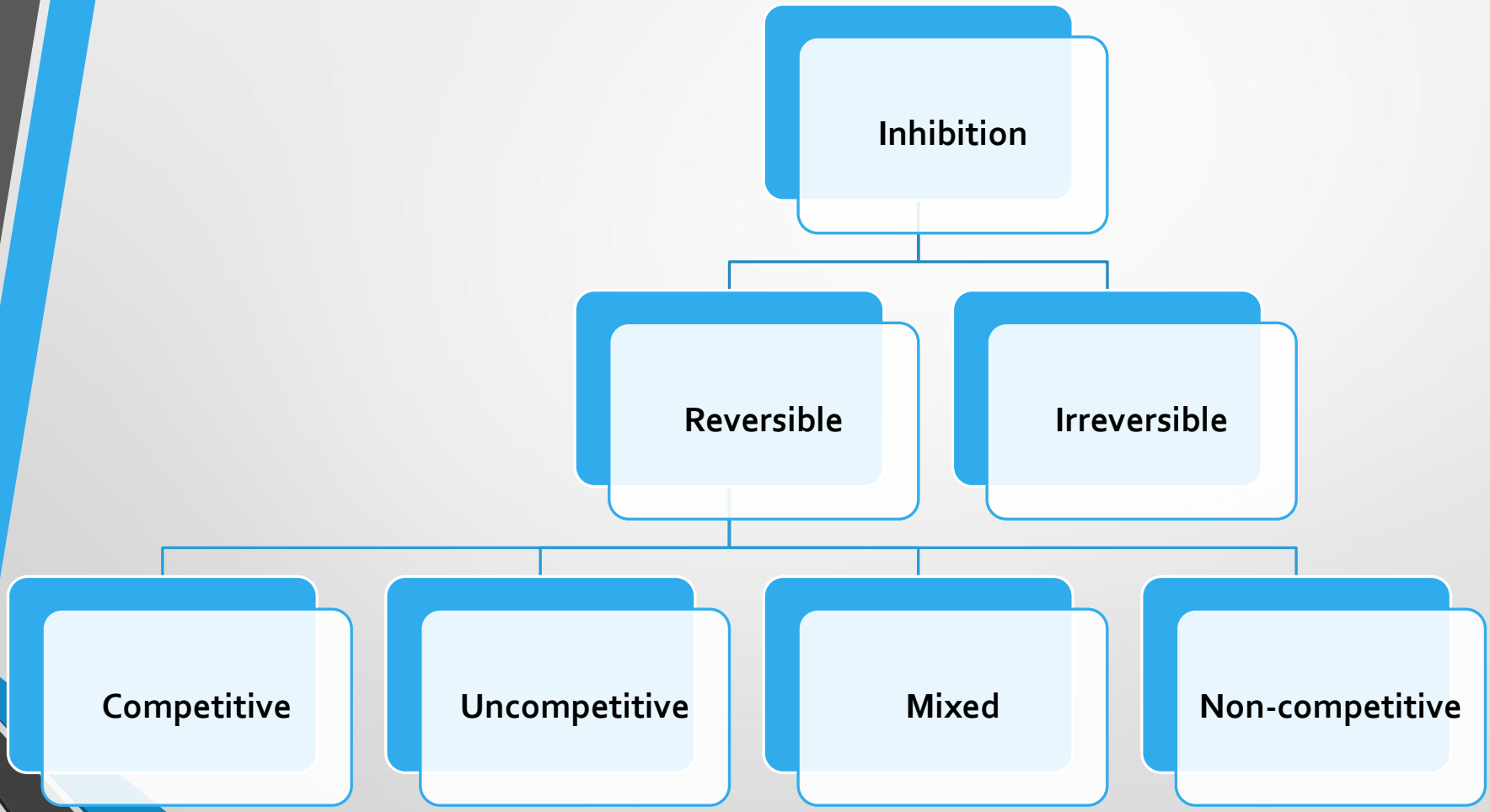
- The prevention of an enzyme process as a result of interaction of inhibitors with the enzyme.

INHIBITORS:

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called an inhibitor.



TYPES OF INHIBITION



REVERSIBLE INHIBITION

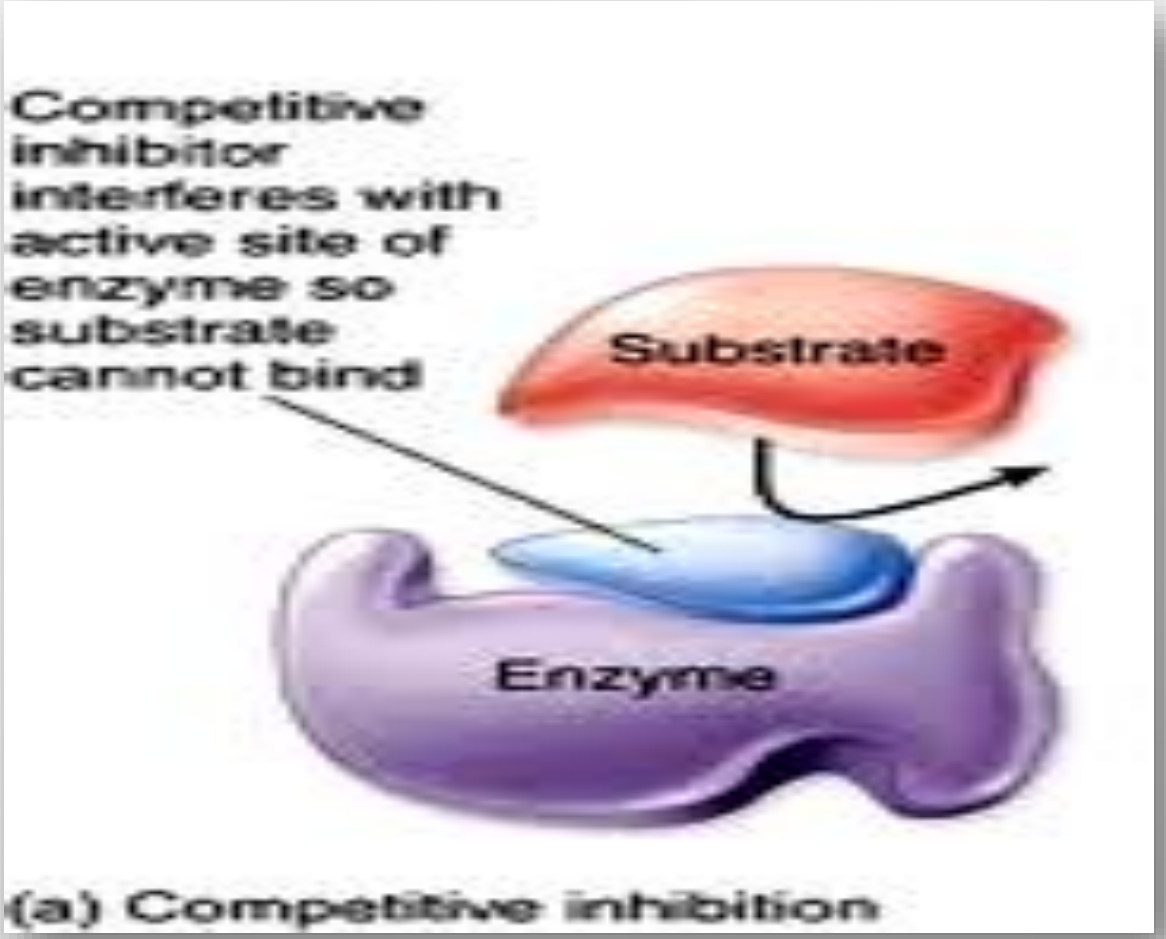
- It is an inhibition of enzyme activity in which the inhibiting molecular entity can associate and dissociate from the protein's binding site.

TYPES OF REVERSIBLE INHIBITION

- There are four types:

- Competitive inhibition.
- Uncompetitive inhibition.
- Mixed inhibition.
- Non-competitive inhibition.

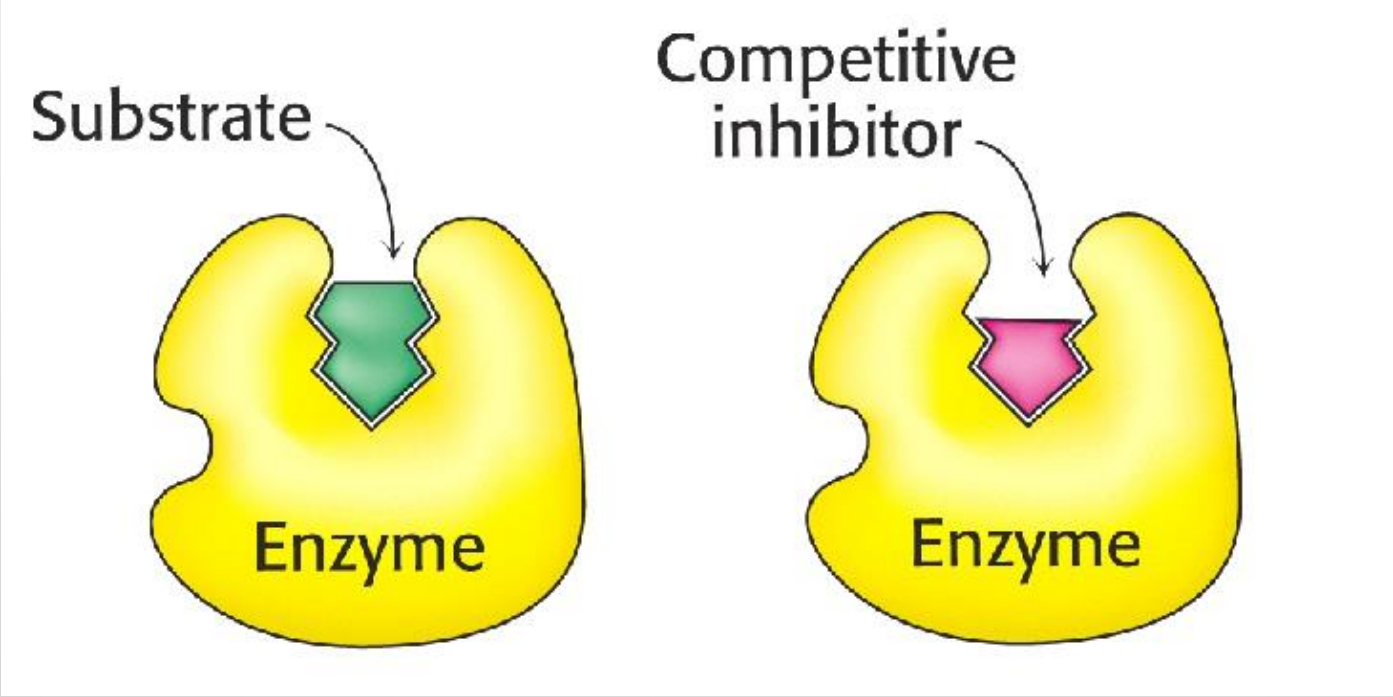
COMPETITIVE INHIBITION



- In this type of inhibition, the inhibitors compete with the substrate for the active site. Formation of E.S complex is reduced while a new E.I complex is formed.

Competitive inhibitors

- resemble the substrates (similar shape of molecule)
- bind to the active sites, but the complex is non-reactive
- they compete with normal substrates for the active sites



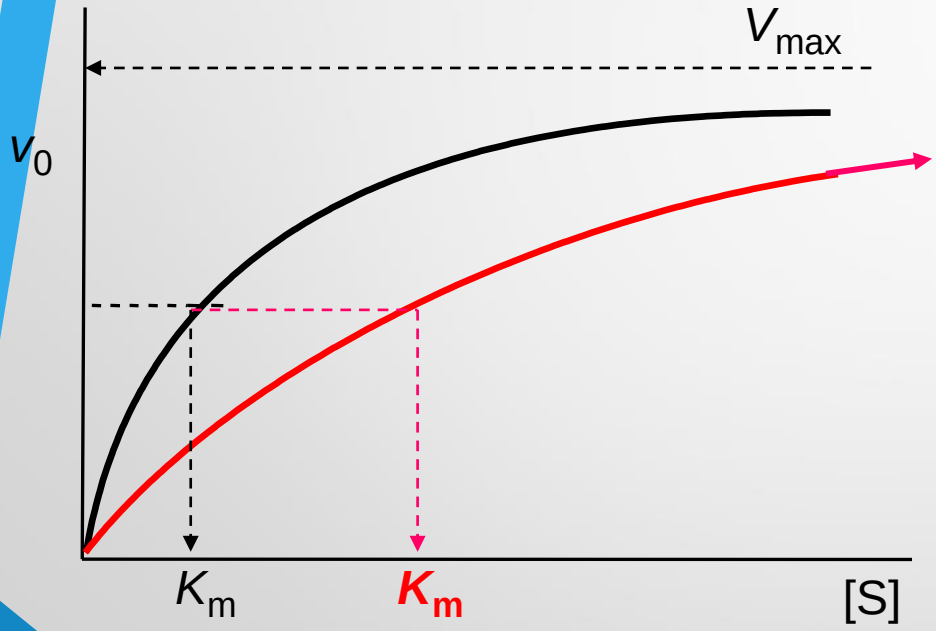
EXAMPLES OF COMPETITIVE INHIBITION

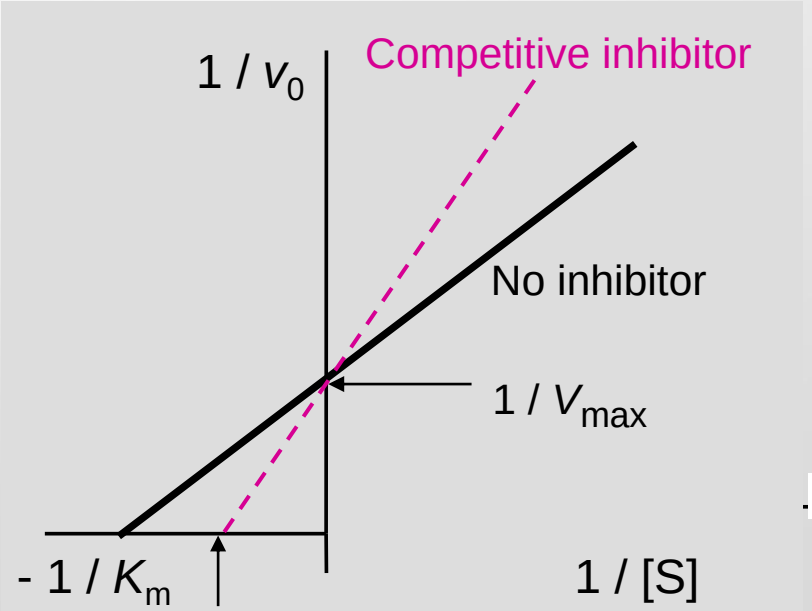
Statin Drug As Example Of Competitive Inhibition:

- Statin drugs such as *lipitor* compete with HMG-CoA(substrate) and inhibit the active site of *HMG CoA-REDUCTASE* (that bring about the catalysis of cholesterol synthesis).

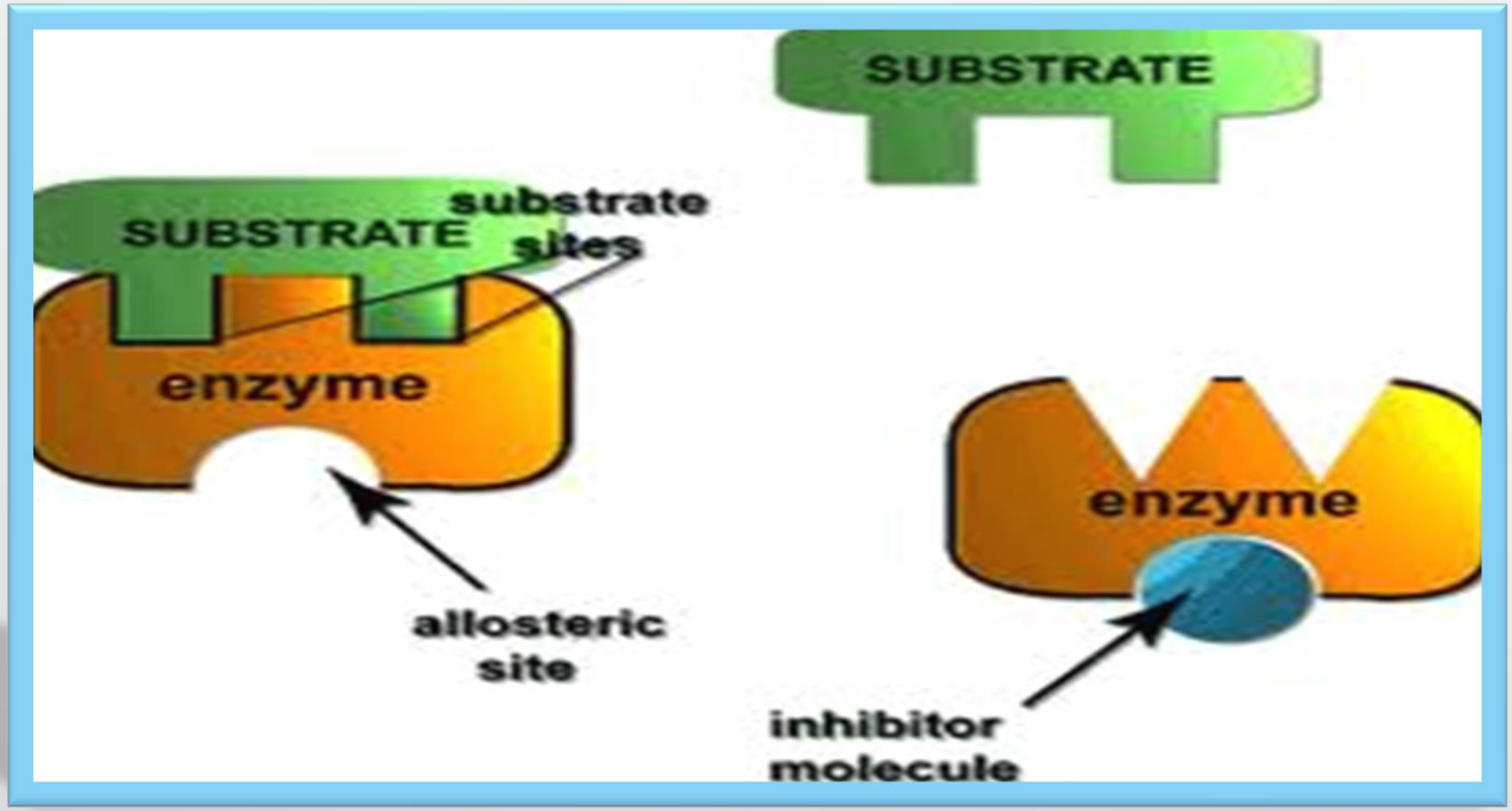
Competitive inhibitors increase K_m without any change in V_{max}

The V_{max} can be reached even in the presence of inhibitor, but at much higher concentrations of [S] that have to overcome the competing inhibitor concentration.





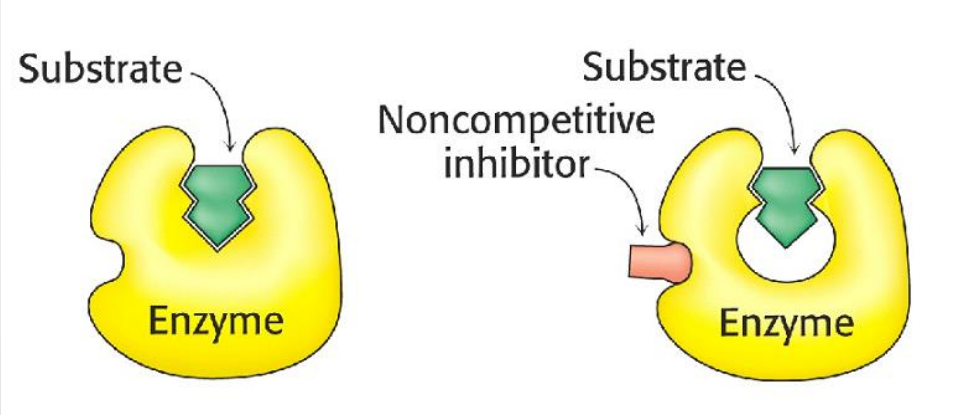
UNCOMPETITIVE INHIBITION



- In this type of inhibition, inhibitor does not compete with the substrate for the active site of enzyme instead it binds to another site known as ***allosteric*** **site.**

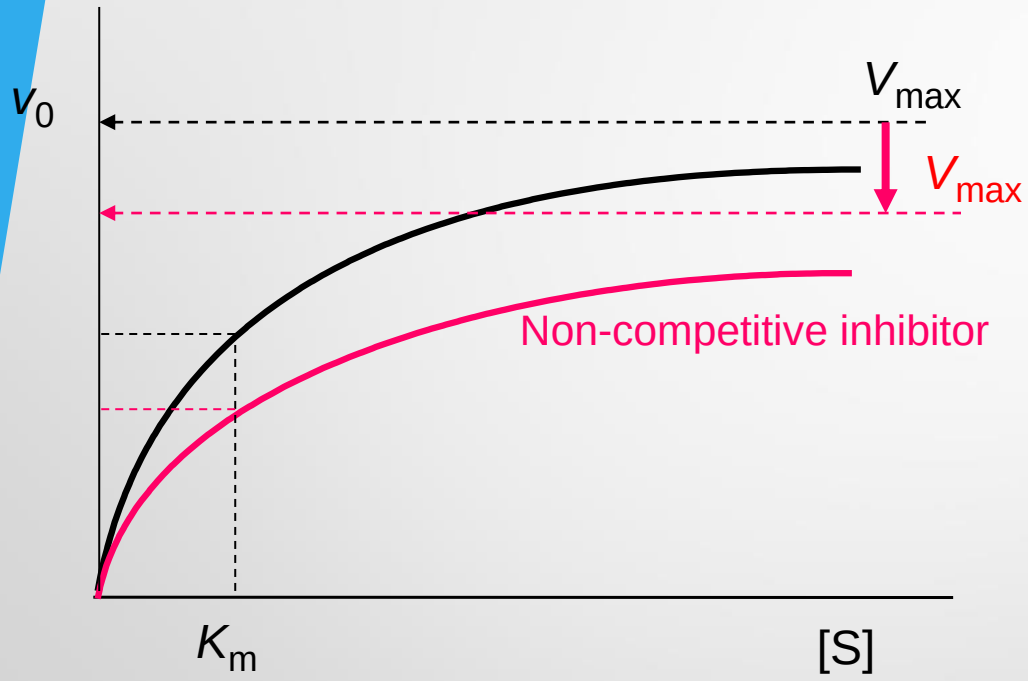
Non-competitive inhibition

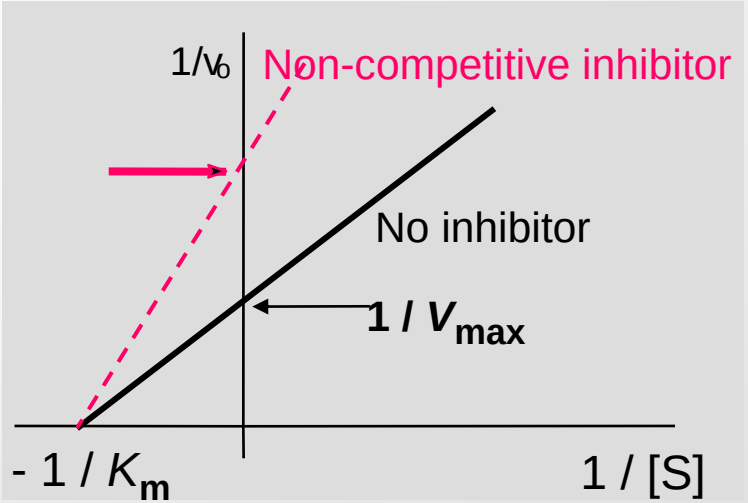
Non-competitive inhibitors bind to both free enzyme and enzyme-substrate complex, but in contrast to competitive inhibitors, not in the active site (the structure of inhibitor is distinct from that of substrate).



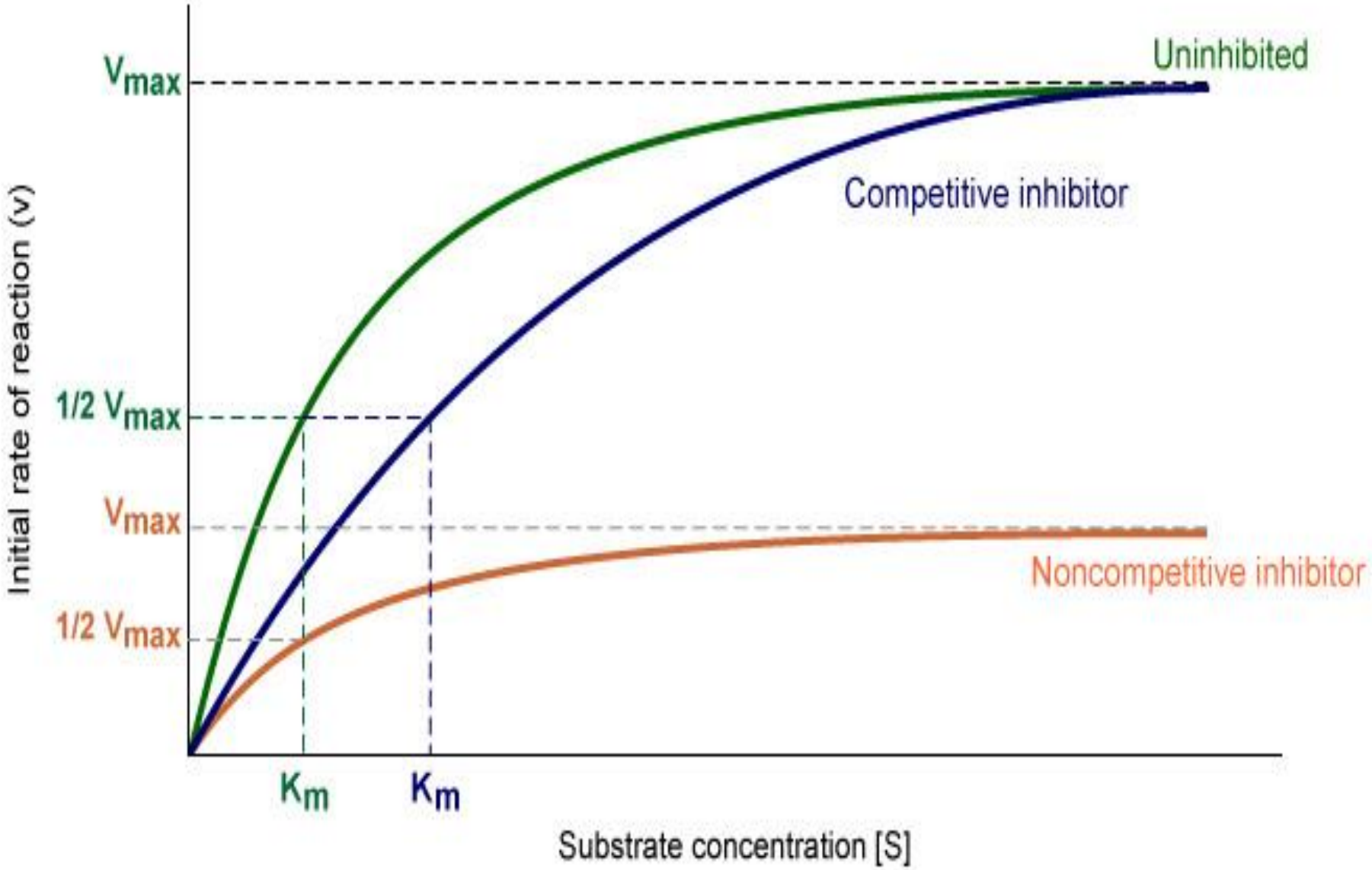
Non-competitive inhibition cannot be overcome by increasing the substrate concentration. The non-inhibited remaining molecules of the enzyme behave like a more diluted solution of the enzyme.

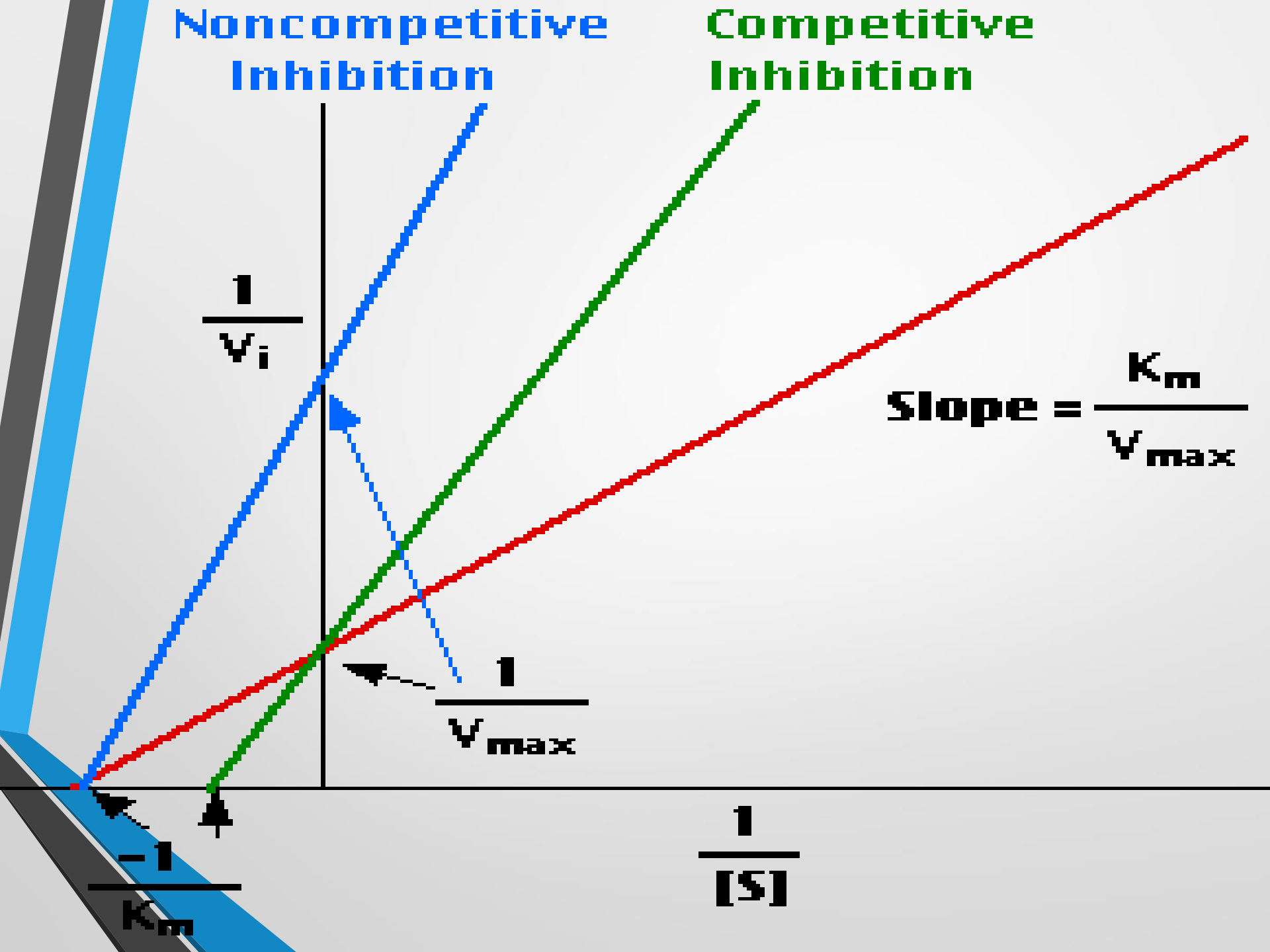
Non-competitive inhibitors decrease V_{max} without any change in K_m



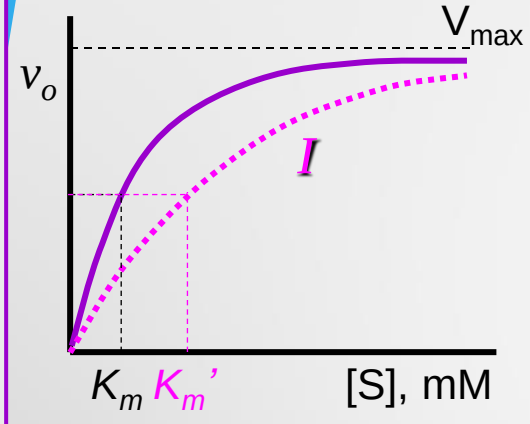
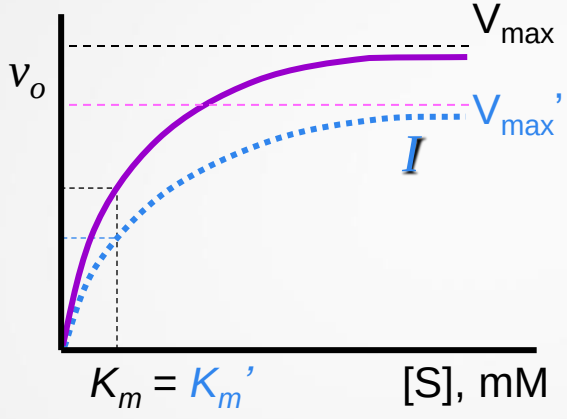
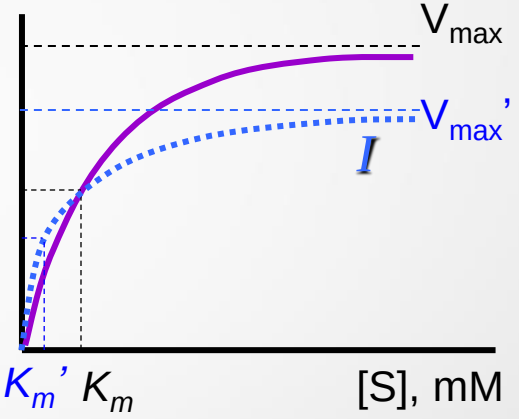
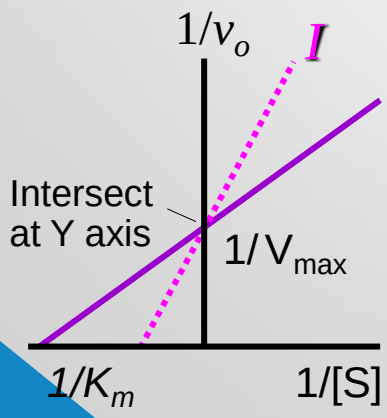
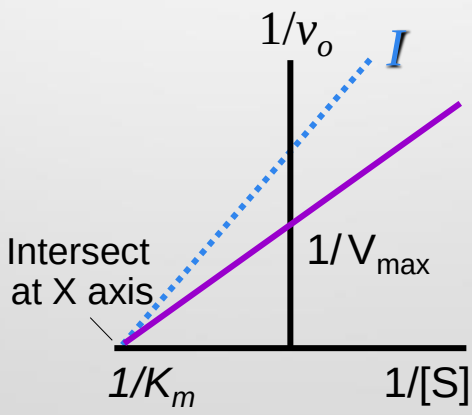
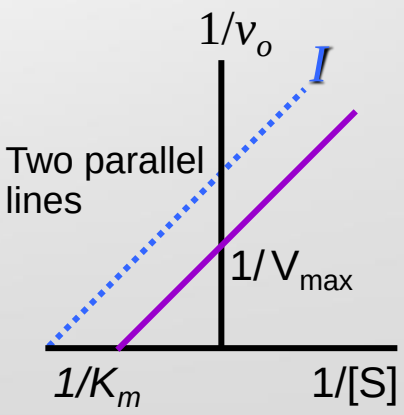


The Effects of Inhibition on Enzyme Kinetics



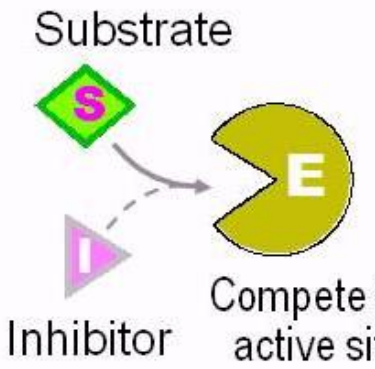
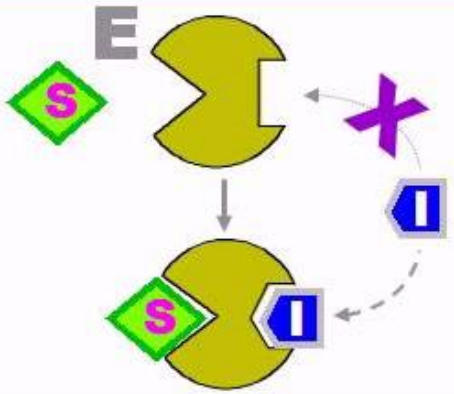

Noncompetitive Inhibition
Competitive Inhibition
1/Vi
Slope = Km/Vmax
1/Vmax
-1/Km
1/[S]

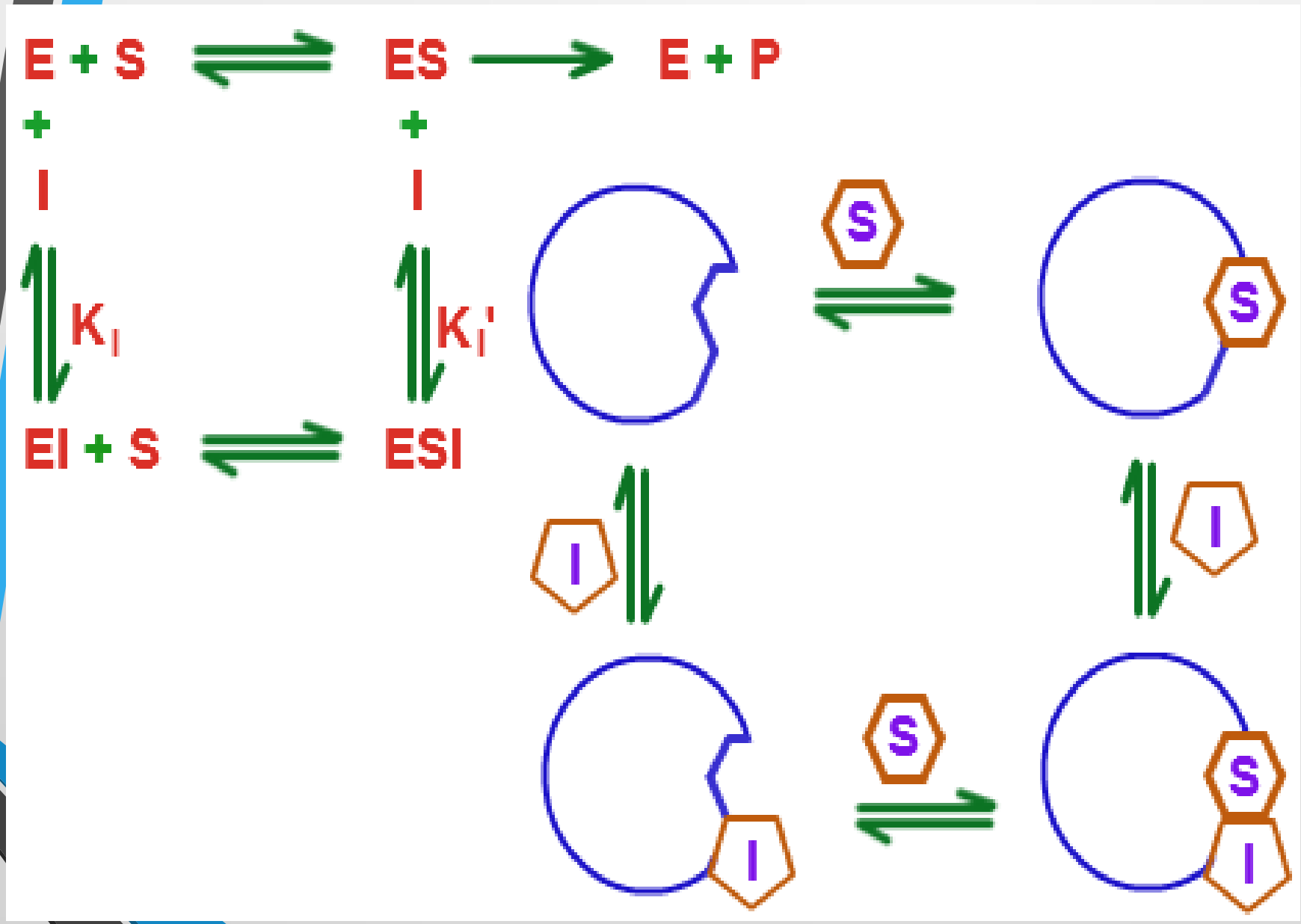
Enzyme Inhibition (Plots)

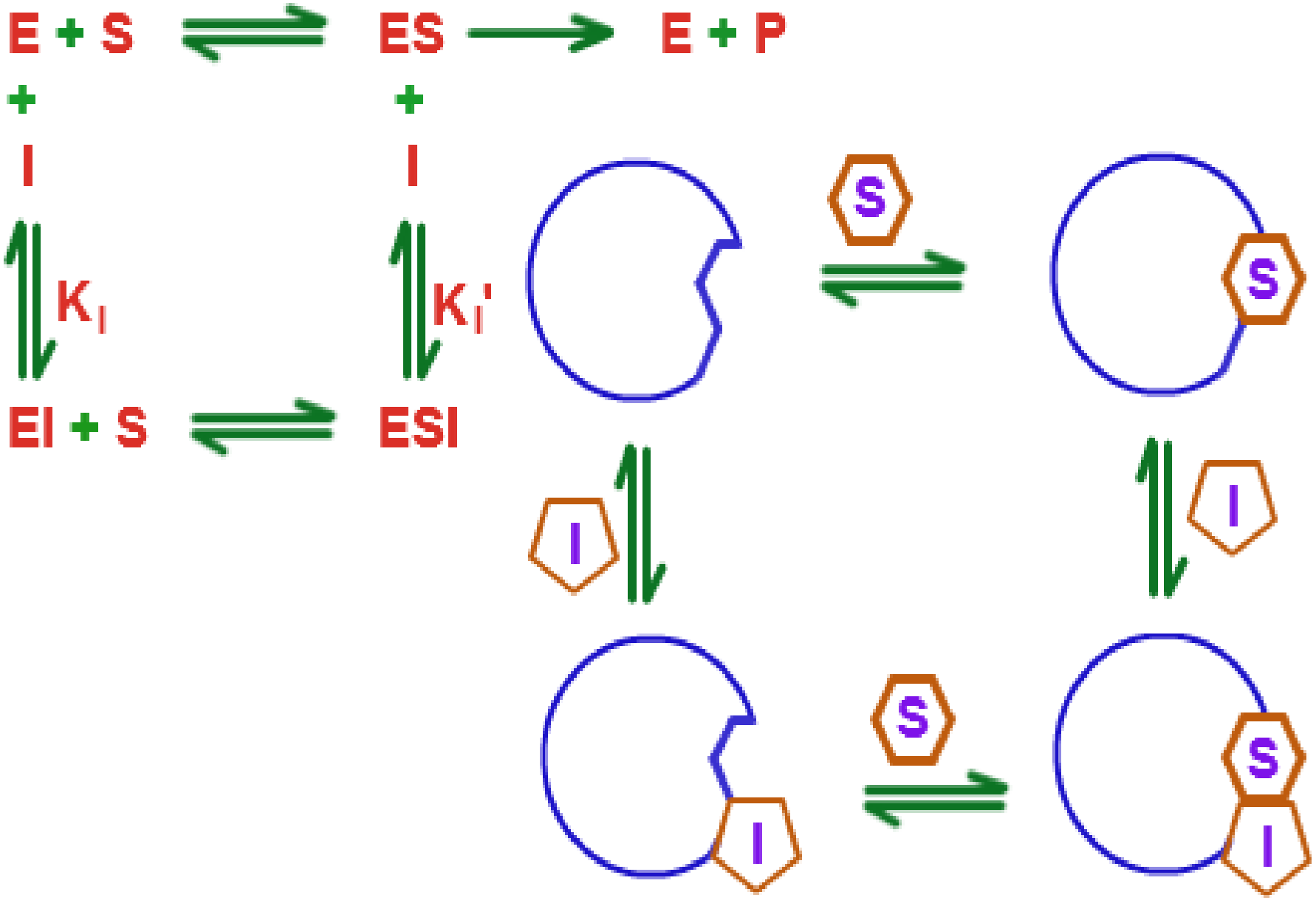
	Competitive	Non-competitive	Uncompetitive
Direct Plots			
	V_{max} unchanged K_m increased	V_{max} decreased K_m unchanged	Both V_{max} & K_m decreased
Double Reciprocal			

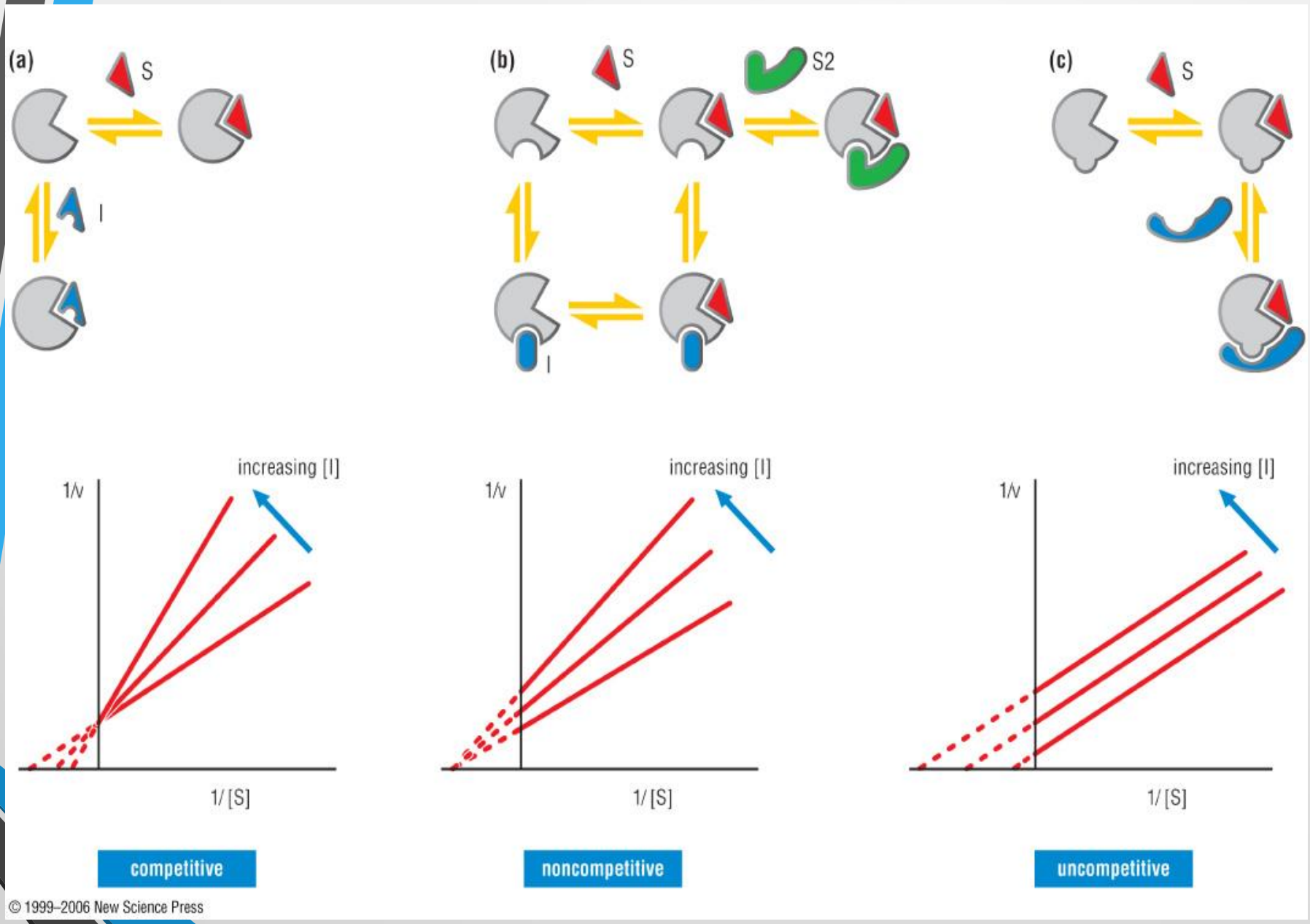
Juang RH (2004) BCbasics

Enzyme Inhibition (Mechanism)

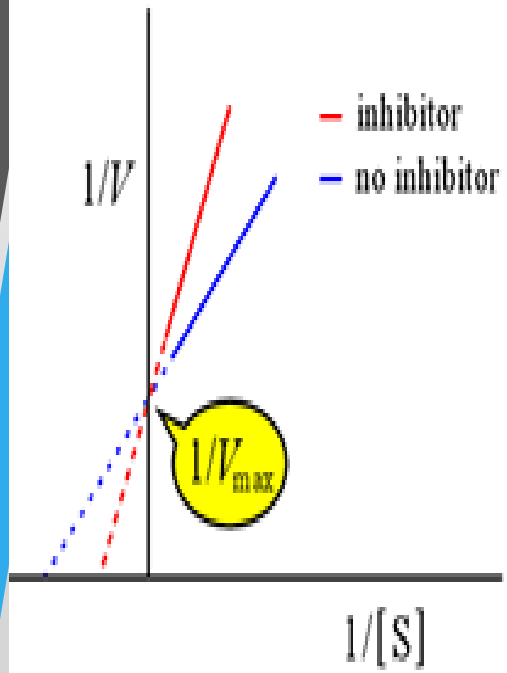
	Competitive	Non-competitive	Uncompetitive
Cartoon Guide			
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\updownarrow$ EI	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ $+$ I I $\updownarrow$ $\downarrow\uparrow$ $EI + S \rightarrow EIS$	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\updownarrow$ 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*].


E + S
ES
E + P
+
I
+
I
K_I
K_I'
EI + S
ESI
S
S
I
I
S
S
I


E + S
ES
E + P
+
+
I
I
K_I
K_I'
EI + S
ESI
S
S
I
I
S
S
I



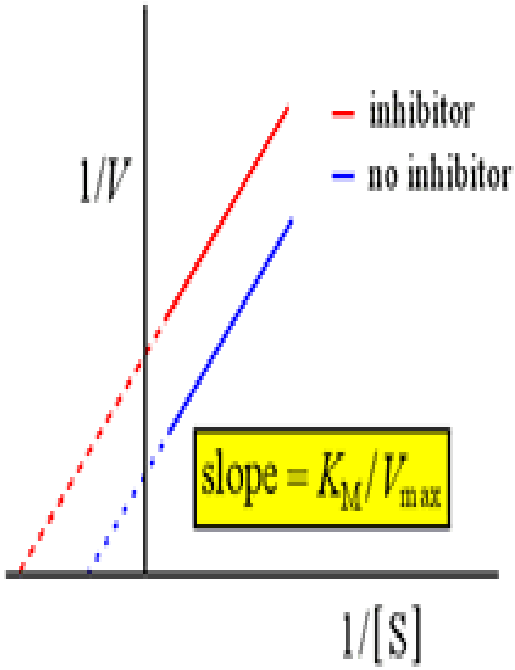
The Lineweaver-Burk plots for inhibition



Competitive inhibition

K_M increased

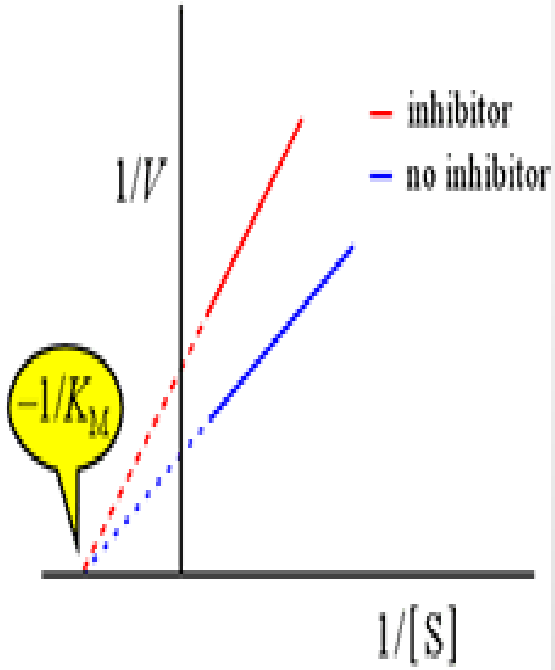
V_{max} unaffected



Uncompetitive inhibition

K_M reduced

V_{max} reduced



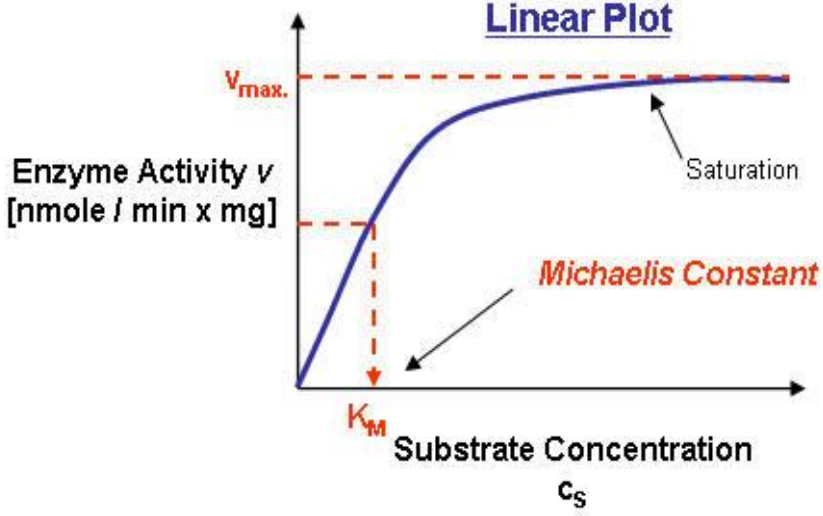
Noncompetitive inhibition

K_M unaffected

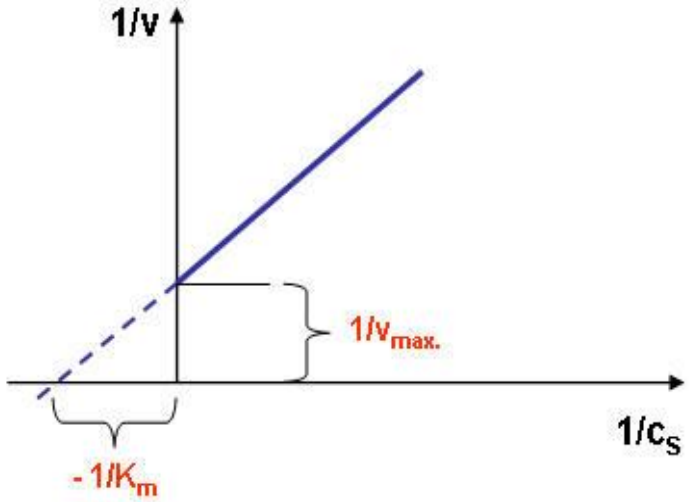
V_{max} reduced

Experimental Result

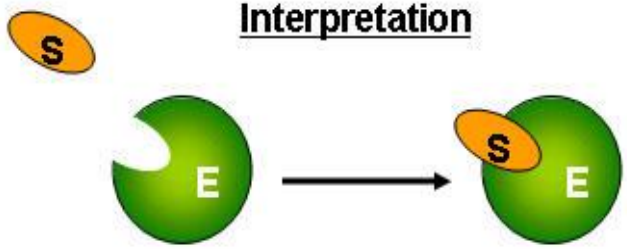
Linear Plot



Lineweaver-Burk Plot



Interpretation



Mathematical Description

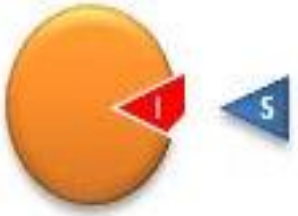
Michaelis- Menten Equation

$$v(c_S) = \frac{v_{max} \times c_S}{K_M + c_S}$$

Lineweaver-Burk Equation

$$\frac{1}{v} = \frac{K_M}{v_{max}} \times \frac{1}{c_S} + \frac{1}{v_{max}}$$

→ The Michaelis-Menten kinetic describes the most simple, "ideal" situation of enzyme catalyzed chemical reactions



competitive

mixed

$K_{IS} < K_{II}$

noncompetitive

$K_{IS} = K_{II}$

mixed

$K_{II} < K_{IS}$

uncompetitive

$$v = \frac{V_M S}{K_M\left(1 + \frac{I}{K_{IS}}\right) + S}$$

$$v = \frac{V_M S}{K_M\left(1 + \frac{I}{K_{IS}}\right) + S\left(1 + \frac{I}{K_{II}}\right)}$$

$$v = \frac{\frac{V_M}{\left(1 + \frac{I}{K_{II}}\right)} S}{\frac{K_M}{\left(1 + \frac{I}{K_{II}}\right)} + S}$$

$$v = \frac{\frac{V_M}{\left(1 + \frac{I}{K_{II}}\right)} S}{K_M \frac{\left(1 + \frac{I}{K_{IS}}\right)}{\left(1 + \frac{I}{K_{II}}\right)} + S}$$

EXAMPLES OF UNCOMPETITIVE INHIBITION

- Drugs to treat cases of poisoning by methanol or ethylene glycol act as uncompetitive inhibitors.
- Tetramethylene sulfoxide and 3- butylthiolene 1-oxide are uncompetitive inhibitors of liver alcohaldehydrogenase.

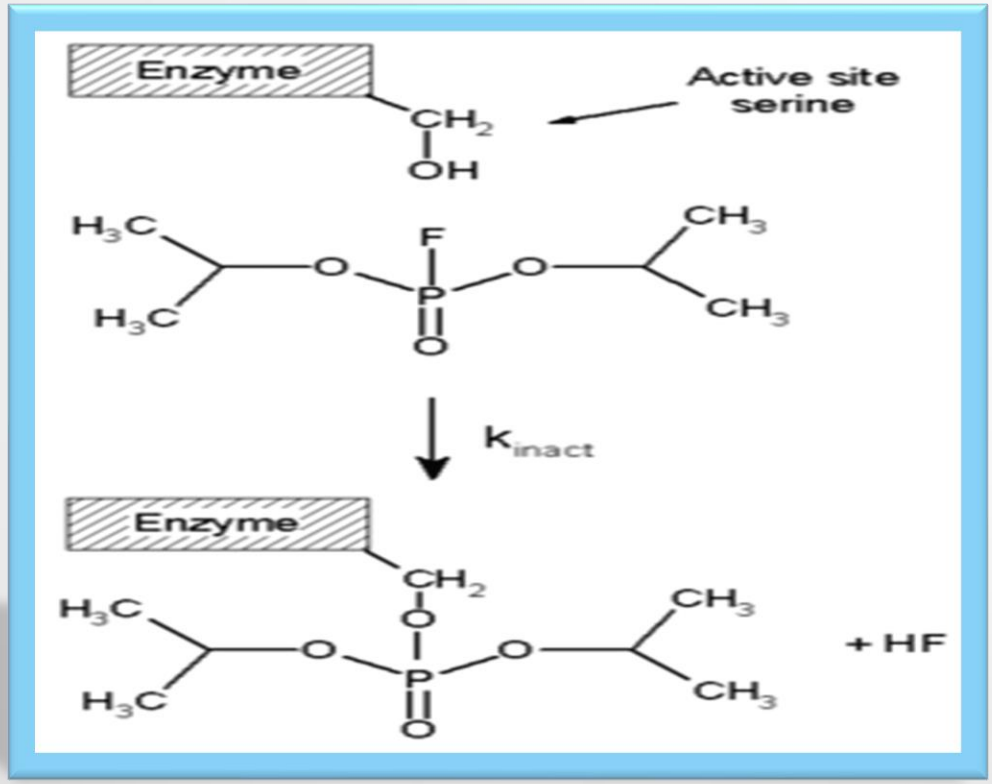
Mixed inhibition

- In this type of inhibition both E.I and E.S.I complexes are formed.
- Both complexes are catalytically inactive.

NON COMPETITIVE INHIBITION

- It is a special case of inhibition.
- In this inhibitor has the same affinity for either enzyme E or the E.S complex.

IRREVERSIBLE INHIBITION



- This type of inhibition involves the *covalent attachment* of the inhibitor to the enzyme.
- The *catalytic activity* of enzyme is completely lost.
- It can only be restored only by synthesizing molecules.

EXAMPLES OF IRREVERSIBLE INHIBITION

- ***Aspirin*** **which targets and covalently modifies a key enzyme involved in inflammation is an irreversible inhibitor.**

- **SUICIDE INHIBITION :**

➢ **It is an unusual type of irreversible inhibition where the enzyme converts the inhibitor into a reactive form in its active site.**

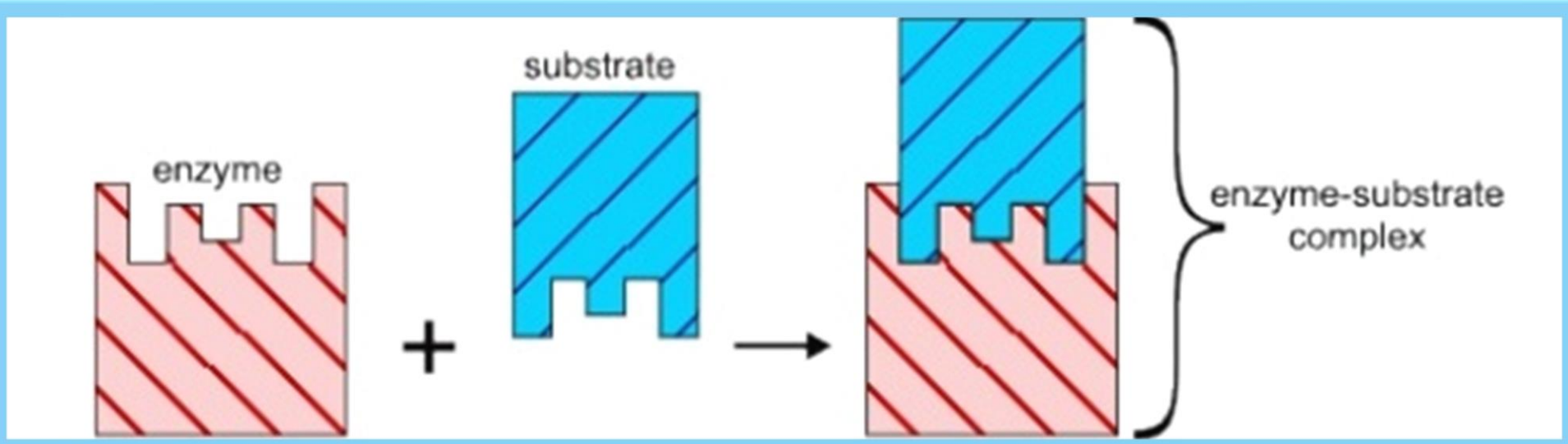
ENZYME SPECIFICITY

ENZYME SPECIFICITY

- Enzymes are highly specific in nature, interacting with one or few substrates and catalyzing only one type of chemical reaction.
- Substrate specificity is due to complete fitting of active site and substrate .

Example:

➢ Oxydoreductase do not catalyze hydrolase reactions and hydrolase do not catalyze reaction involving oxidation and reduction.



TYPES OF ENZYME SPECIFICITY

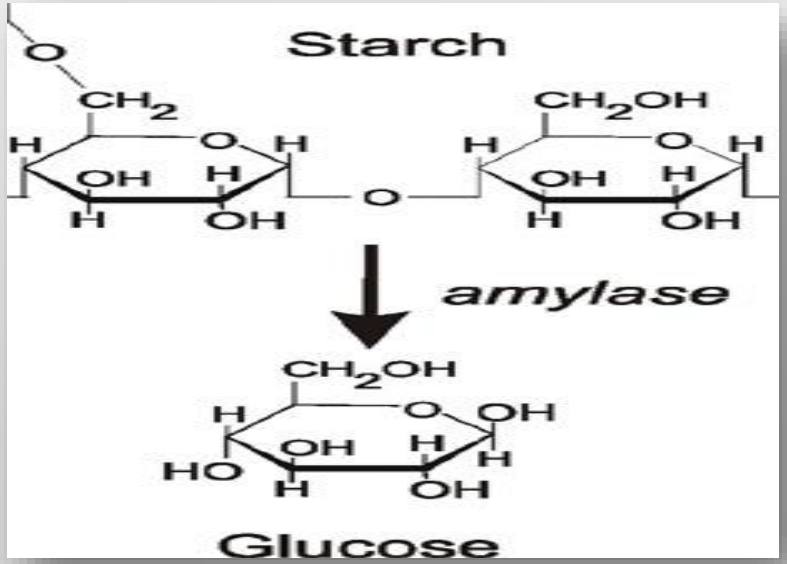
- **Enzymes show different degrees of specificity:**
  - **Bond specificity.**
  - **Group specificity.**
  - **Absolute specificity.**
  - **Optical or stereo-specificity.**
  - **Dual specificity.**

BOND SPECIFICITY

- In this type, enzyme acts on substrates that are similar in structure and contain the same type of bond.

Example :

- ***Amylase*** which acts on α-1-4 glycosidic ,bond in starch dextrin and glycogen, shows bond specificity.

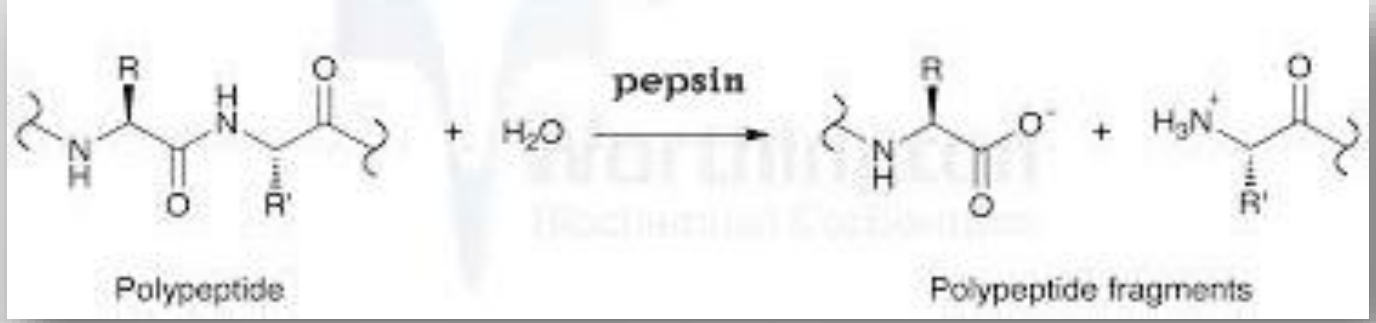


GROUP SPECIFICITY

- **In this type of specificity, the enzyme is specific not only to the type of bond but also to the structure surrounding it.**

Example:

- ***Pepsin*** **is an endopeptidase enzyme, that hydrolyzes central peptide bonds in which the amino group belongs to aromatic amino acids e. g phenyl alanine, tyrosine and tryptophan.**

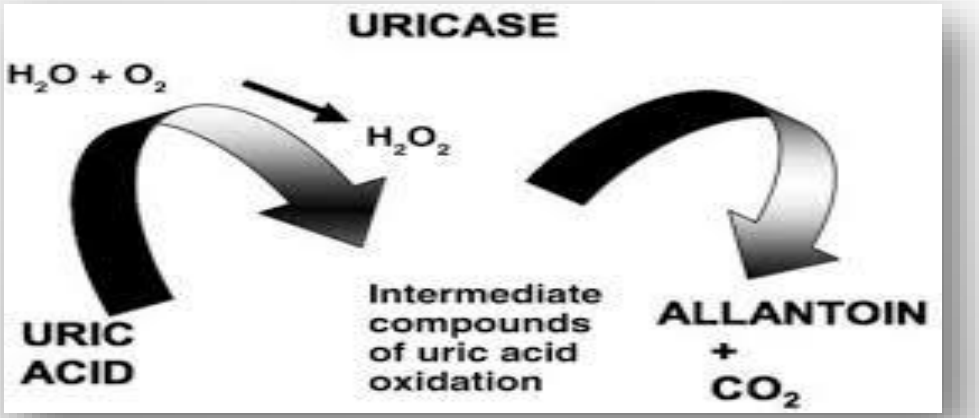


SUBSTRATE SPECIFICITY

- In this type of specificity ,the enzymes acts only on one substrate

  Example :

➢ ***Uricase*** ,which acts only on uric acid, shows substrate specificity.



➢ ***Maltase*** , which acts only on maltose, shows substrate specificity.

$$C_{12}H_{22}O_{11}(aq) + H_2O(l) \xrightarrow{\text{Maltase}} 2C_6H_{12}O_6(aq)$$

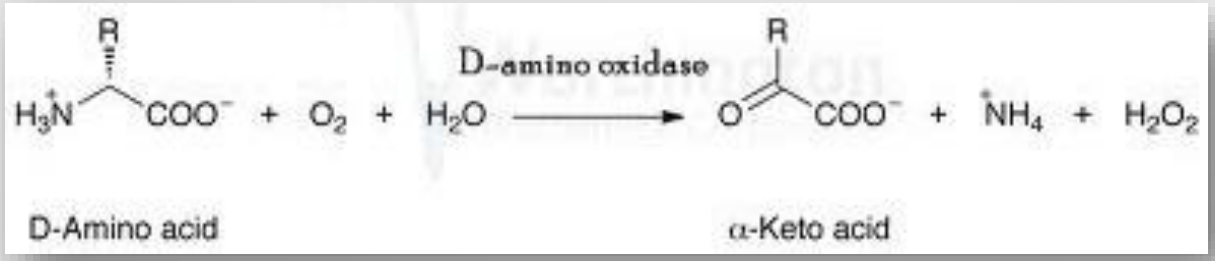
Maltose — Glucose

OPTICAL / STEREO-SPECIFICITY

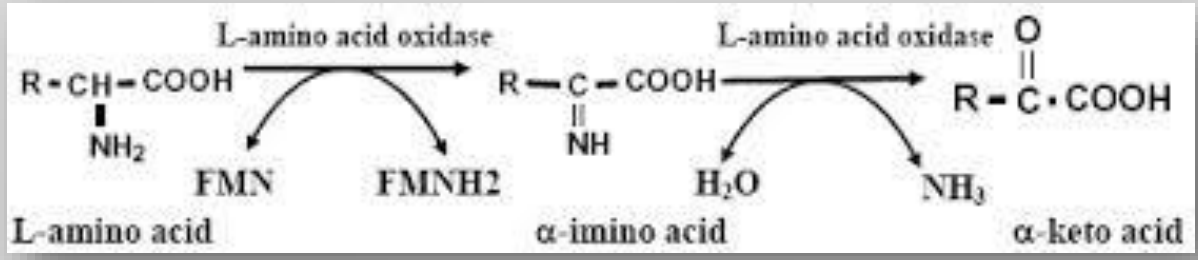
- **In this type of specificity , the enzyme is not specific to substrate but also to its optical configuration**

Example:

- **D amino acid oxidase acts only on D amino acids.**



- **L amino acid oxidase acts only on L amino acids.**



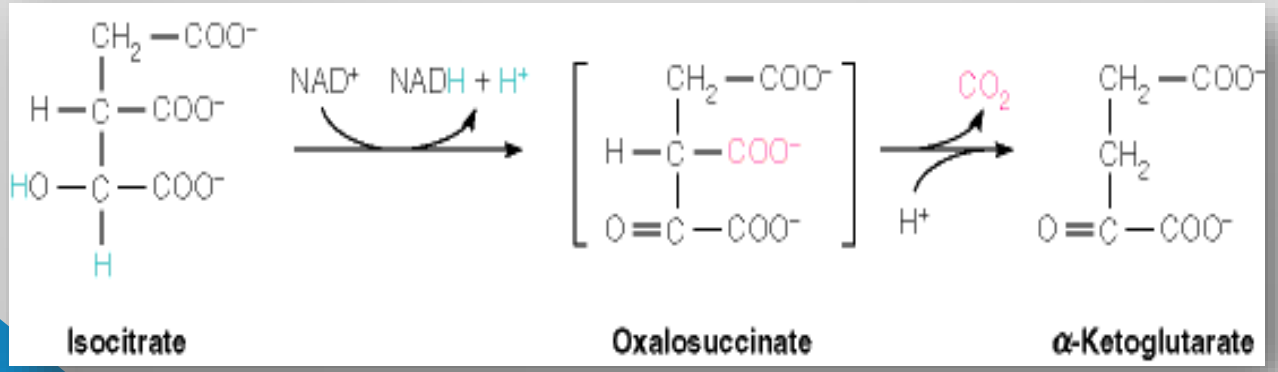
DUAL SPECIFICITY

- **There are two types of dual specificity.**

➢ **The enzyme may act on one substrate by two different reaction types.**

Example:

- ***Isocitrate dehydrogenase*** **enzyme acts on isocitrate (one substrate) by oxidation followed by decarboxylation(two different reaction types) .**

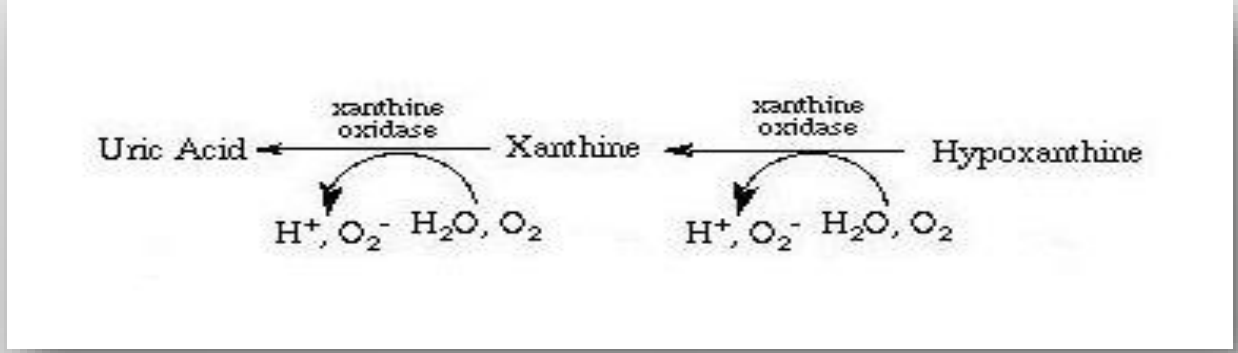


DUAL SPECIFICITY

- The enzyme may act on two substrates by one reaction type

Example:

- ***Xanthine*** oxidase enzyme acts on xanthine and hypoxanthine(two substrates) by oxidation (one reaction type



1. Define the following:
    a. Enzymes
    b. Apoenzyme
    c. Coenzyme
    d. Holoenzyme
    e. Metalloenzyme
    f. Regulatory enzyme
    g. Active site of the enzyme
    h. Allosteric site of the enzyme
    i. Substrate
2. Discuss the helpers (cofactors) of enzymes.

3. Enumerate the six major classes of enzymes.
4. Discuss the characteristics of enzymes.
5. Explain the models of enzyme-substrate complex.
6. Explain enzyme kinetics.
   a. Factors that affect enzyme activity or reaction velocity.
   b. Ways of expressing enzyme activity.
7. Discuss the operation and plots used to illustrate enzyme kinetics.
   a. Michaelis-Menten kinetics
   b. Lineweaver-Burke Double Reciprocal Plot
   c. Michaelis constant and its significance
   d. Kinetic order of reactions

8. Discuss enzyme inhibition and its effect on reaction velocity.
    a. Reversible
    b. Irreversible
9. Discuss the different ways of regulating enzyme activity.
10. Explain the factors affecting enzyme activity.
11. Clarify uses and clinical application of enzymes.