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FACULTY OF ENGINEERING &TECHNOLOGY
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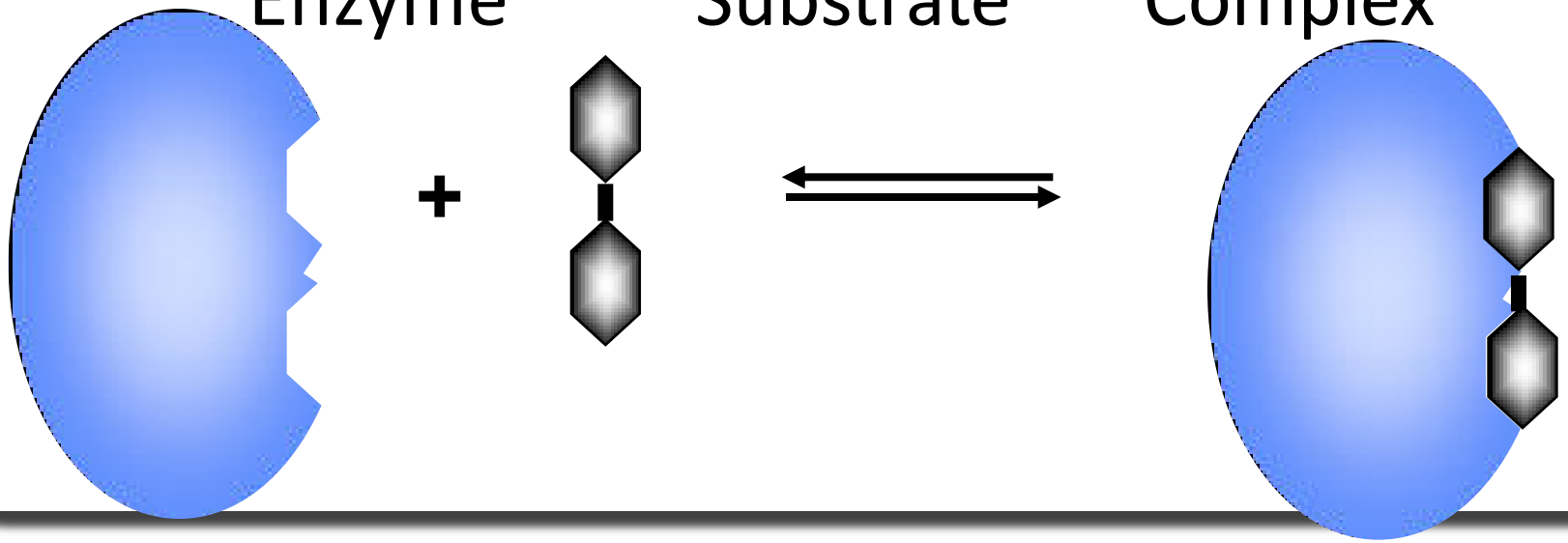
Enzyme Kinetics

Enzyme-substrate complex

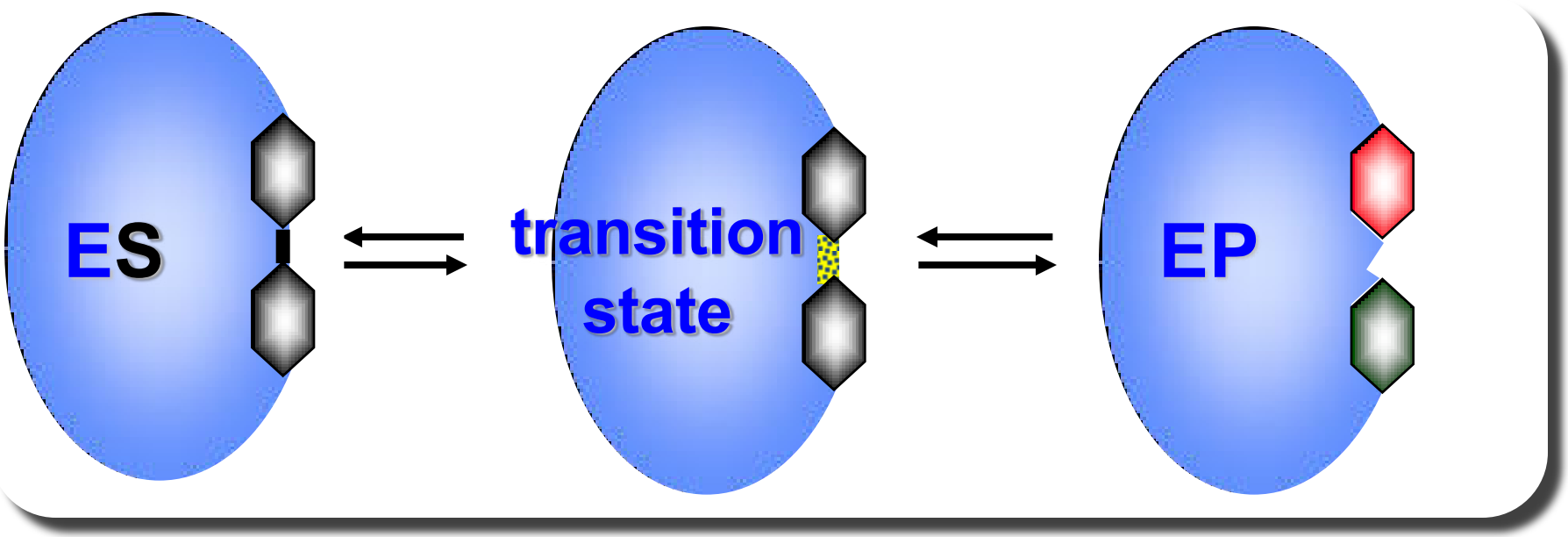
- Step 1:
- Enzyme and substrate combine to form complex
- E + S ⇌ ES
- Enzyme Substrate Complex



Enzyme-product complex

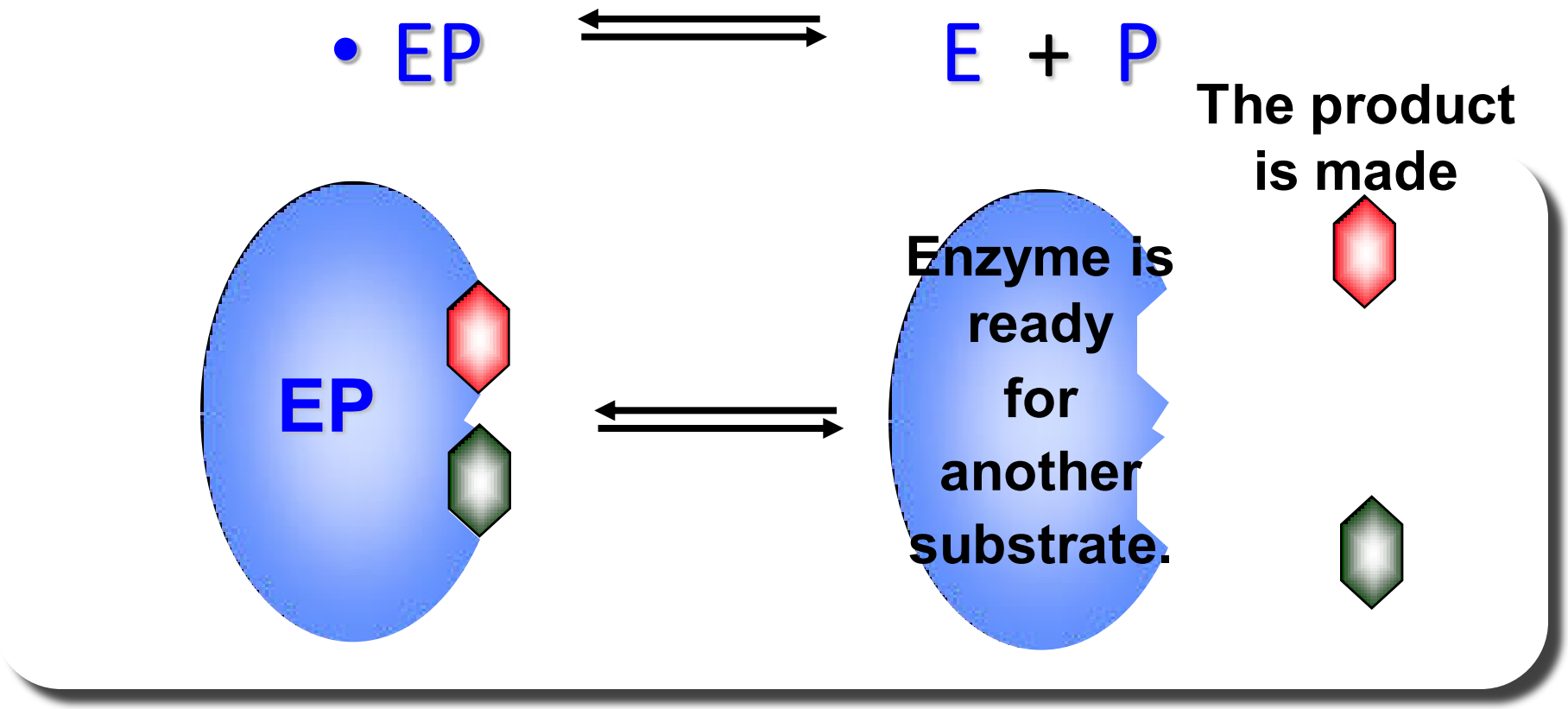
- Step 2:
- An enzyme-product complex is formed.

- ES ⇌ EP



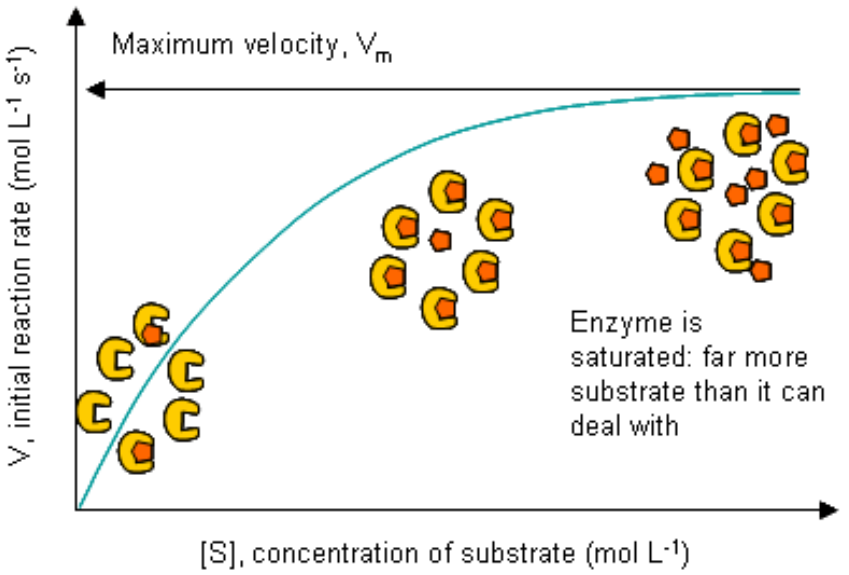
Product

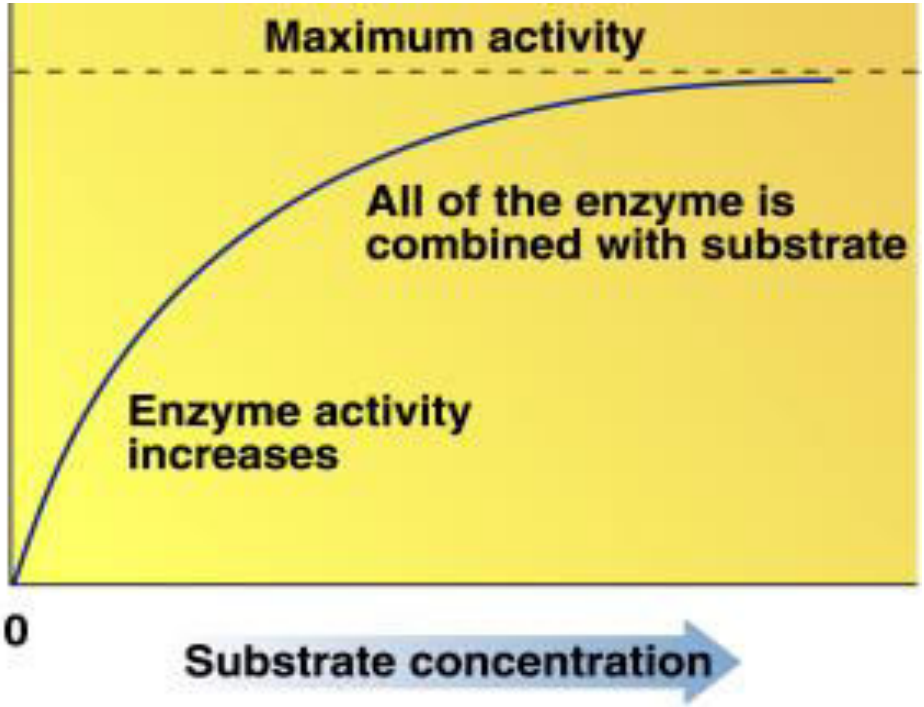
- The enzyme and product separate



Substrate Concentration and Reaction Rate

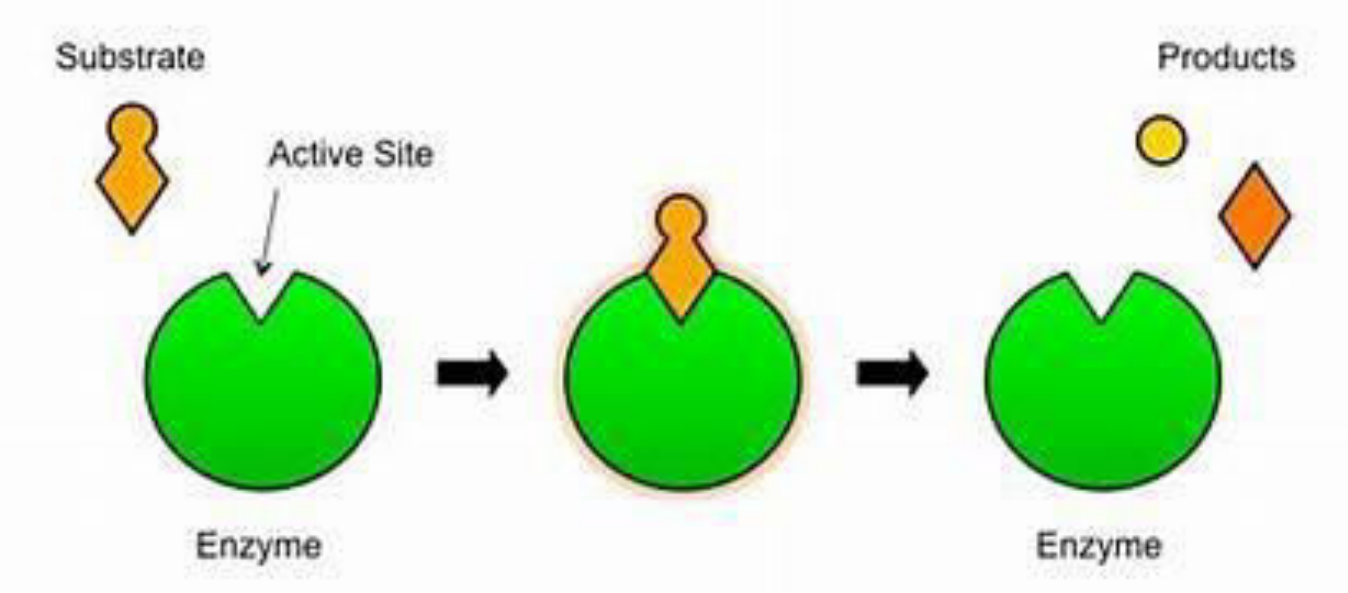
- **The rate of reaction increases as substrate concentration increases (at constant enzyme concentration)**
- **Maximum activity occurs when the enzyme is saturated (when all enzymes are binding substrate)**





Active site of an Enzyme

In biology, the **active site** is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyse a reaction of that substrate (catalytic site). Although the active site is small relative to the whole volume of the enzyme (it only occupies 10~20% of the total volume),[1] it is the most important part of the enzyme as it directly catalyzes the chemical reaction. It usually consists of three to four amino acids, while other amino acids within the protein are required to maintain the protein tertiary structure of the enzyme.[2]



Unit of Enzyme Activity

- Enzyme units are never expressed in terms of their concentration (as mg or µg) but are expressed as activity.
- To maintain uniformity world over enzyme activity as units is expressed according to the I.U.B system

Enzyme Activity

Classical units:

Unit of enzyme activity:

μmol substrate transformed/min = unit

Specific activity:

μmol substrate/min-mg E = unit/mg E

Molecular activity:

μmol substrate/min- μmol E = units/μmol E

Activity Units

-The catalytic activity of enzymes is exhibited only under specific conditions, such as pH, ionic strength, buffer type, presence of cofactors and suitable temperature. Therefore, the rate of substrate conversion or product formation can be measured in a test system designed to follow the enzyme activity.

-The International System of Units (SI) designation is $mol.s^{-1}$ and its recommended designation is the "katal" (kat*).

Decimal units are formed in the usual way, e. g.: $\mu kat = 10^{-6}\,kat = \mu mol \cdot s^{-1}$

-Concentration of enzymatic activity is given as $\mu kat\ l^{-1}$.

The following activity units are derived from this:

a) The *specific catalytic activity*, i.e. the activity of the enzyme preparation in relation to the protein concentration.

b) The *molar catalytic activity*. This can be determined when the pure enzyme with a known molecular weight is available.
-It is expressed as "katal per mol of enzyme" (kat mol–1). When the enzyme has only one active site or center per molecule, the molar catalytic activity equals the "turnover number", which is defined as the number of substrate molecules converted per unit time by each active site of the enzyme molecule.

Enzymatic activity

Reaction rate is expressed as a **change in concentration per unit time** (mol/L / *s*).

For enzyme-catalyzed reaction: **substrate turnover per unit time is commonly used:**

- Unit: **katal (kat) = mol of substrate / s**

μkat and nkat are used in medicine

- International unit: **IU = μmol of substrate / min**

1 kat = 6 x 10^7 U

Specific Activity of Enzymes

Definition:
Units of enzyme activity per mg protein

1 Unit = amount of enzyme that will convert one µmole of substrate to product in one minute at a given pH (optimum value) and temperature (usually 25°C or 37°C).

Specific activity is used as an estimate of enzyme purity.

Specific Activity of Enzymes

Units per mg of protein

Example

5 mg of protein were in an extract that catalyzed the change of 100 micromoles of substrate to product in 10 minutes. What is the specific activity of the enzyme?

Answer: **2 units per mg of protein**

Turnover Number of an Enzyme

Turnover number, also termed k_{cat} is defined as the maximum number of chemical conversions of substrate molecules per second that a single catalytic site will execute for a given enzyme concentration.

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

For example, carbonic anhydrase has a turnover number of 400,000 to 600,000 s^{-1}, which means that each carbonic anhydrase enzyme molecule can produce up to 600,000 molecules of product (bicarbonate ions) per second.

Calculation of Turnover Number

Example calculation

- An enzyme (1.84 □gm, MW 36800), in presence of excess substrate catalyzes at a rate of 4.2 □mol substrate/min. Calculate the TON. (□mol S/min/□mol E)

- $$\square\text{mol E:} = \frac{1.84\ \square\text{gm}}{36800\ \square\text{gm}/\square\text{mol}} = 5 \times 10^{-5}\ \square\text{mol E}$$

- $$\text{TON} = \frac{V_{max}}{E_t} = \frac{4.2\ \square\text{mol S/min}}{5 \times 10^{-5}\ \square\text{mol E}} = 84000\ \text{min}^{-1}$$

Turnover Number and Km for some typical Enzyme

Enzyme	Function	k_{cat} = Turnover Number*	K_M**
Catalase	Conversion of H_2O_2 to H_20 and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

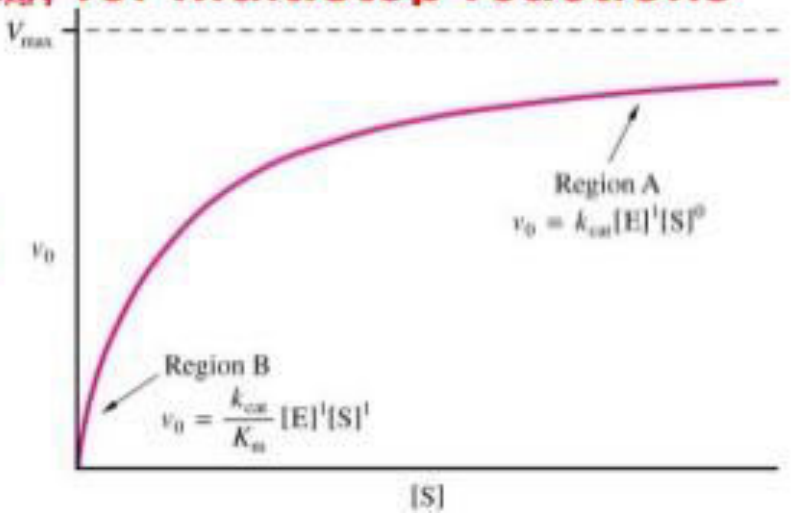
*The definition of turnover number is the moles of substrate converted to product per mole of enzyme per second. The unites are sec^{-1}.

**The units of KM are millimolar.

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

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

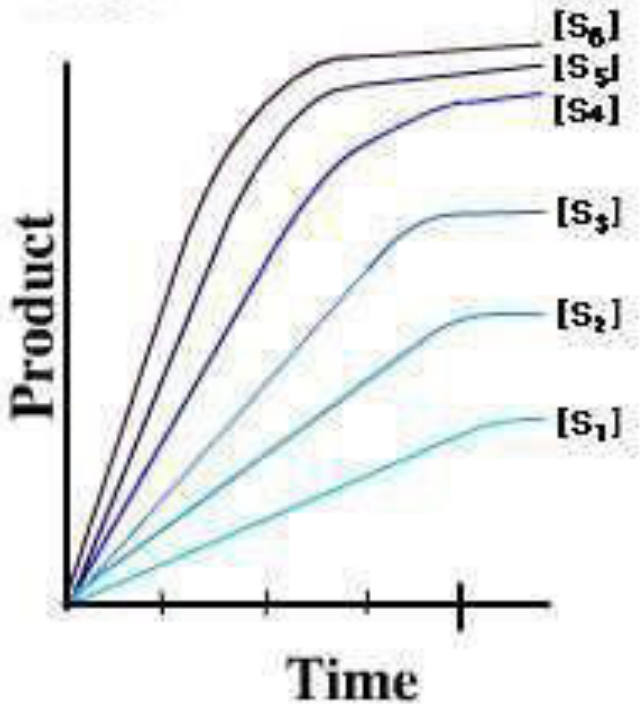


ENZYMES-Activity Measurements

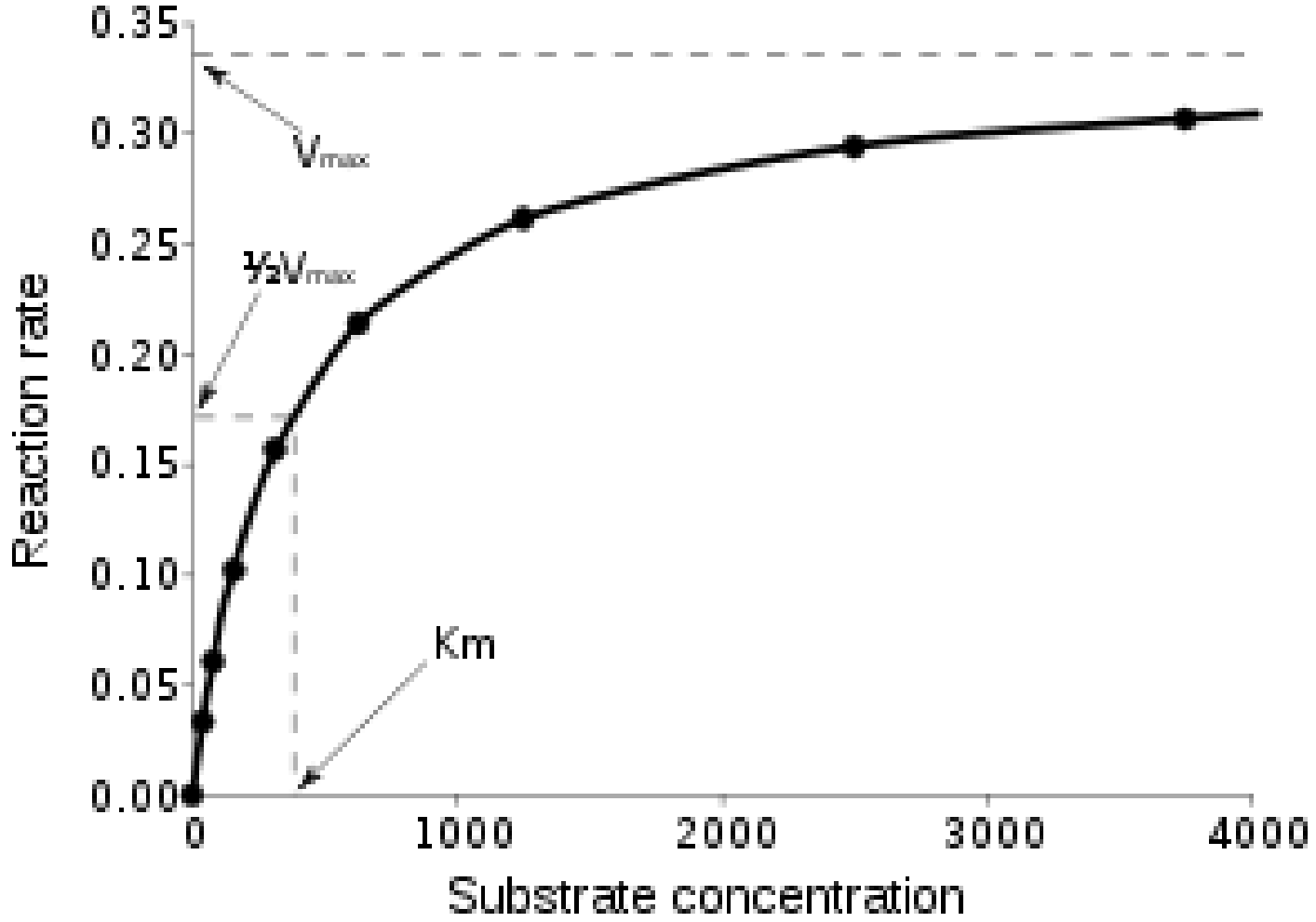
Enzyme and substrate form a complex

$$E + S \underset{k2}{\overset{k1}{\rightleftharpoons}} S \xrightarrow{k3} E + P$$

This graph represents the initial velocity of six samples with varying substrate concentrations.



Hyperbolic Saturation Kinetics for an enzyme (relation between the substrate concentration (S) and velocity (*v) of reaction*



Michaelis-Menten Equation

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

V0 = Velocity of reaction

Vmax = Maximum velocity of reaction

[S] = Substrate concentration

Km = Michaelis constant

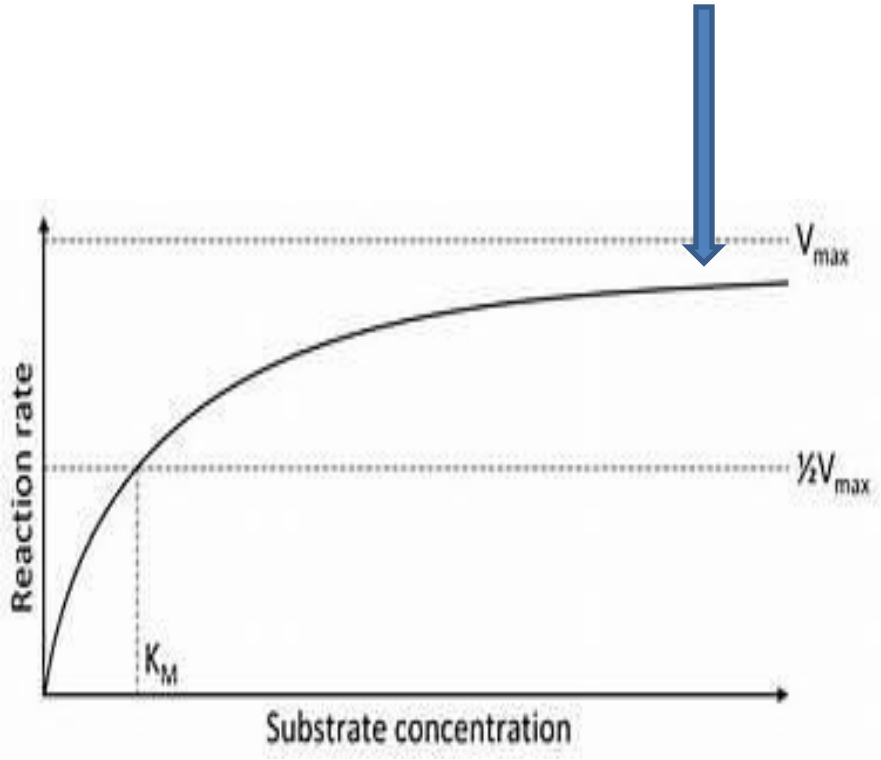
Km and Vmax

- **Km**
  - **Is a ratio of rate constants =(k2 + k3)/k1**
  - **Is equal to [S] when initial rate(v) is equal to ½ Vmax**
  - **Is a property of ES complex; does not depend on the concentration of E or S**
- **Vmax**
  - **Maximum velocity at a fixed [E]**
  - **Directly proportional to the [E]**

Problem of V_{max} in M-M kinetics

Leonor Michaelis
1875–1949

Maud Menten
1879–1960



V_{max} is approached but never achieved

Lineweaver- Burk Double-Reciprocal Plot

Michaelis-Menten equation $\Rightarrow$ $$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

Dean Burk

invert

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

Hans Lineweaver

factor

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

and simplify

Double-Reciprocal or Lineweaver-Burk equation $\Rightarrow$

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

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

This equation resembles the equation of a straight line

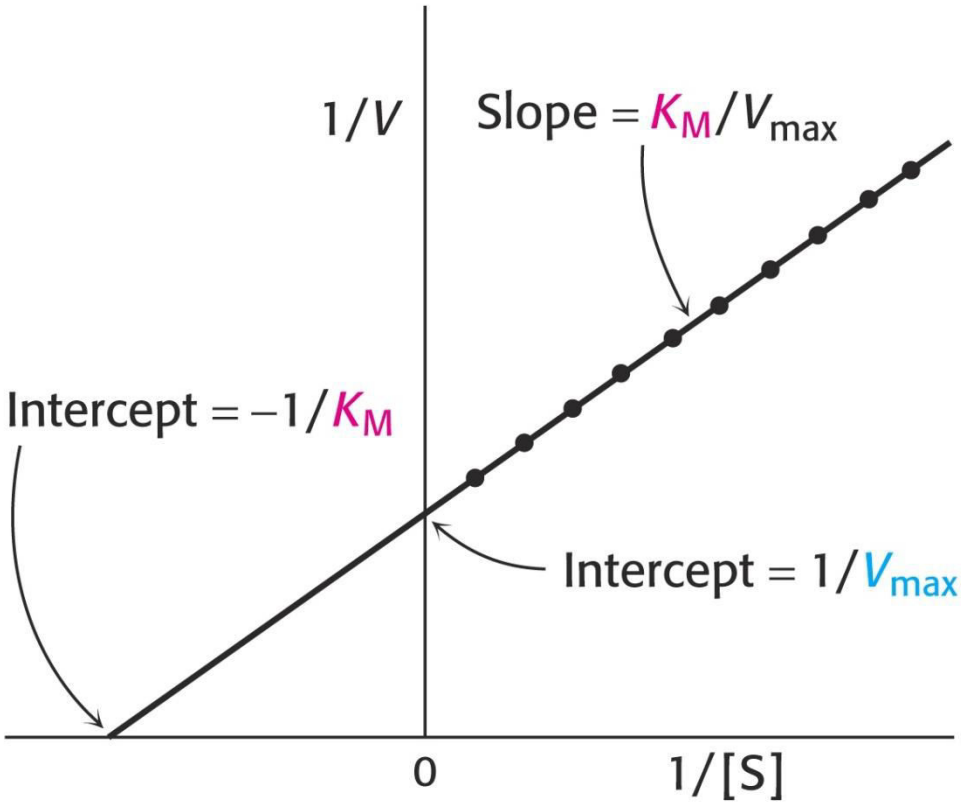
$$y = mx + c$$

where

$y = 1/v_0$ $\qquad$ $m = Km/Vmax$

$x = 1/[S]$ $\qquad$ $x = 1/Vmax$

Lineweaver-Burk Double reciprocal Plot



From the same data set now, K_m and V_{max} can be obtained accurately as intercept values

Why study inhibition of Enzyme activity ?

- **At least two reasons to do this:**

We can use inhibition as a probe for understanding the kinetics and properties of enzymes in their uninhibited state

Many, perhaps most, drugs are inhibitors of specific enzymes.

The concept of inhibition

- **An enzyme is a biological catalyst that alters the rate of a reaction without itself becoming altered by its participation in the reaction.**

- **The ability of an enzyme (particularly a proteinaceous enzyme) to catalyze a reaction can be altered by binding small molecules to it**

  - **sometimes at its active site**
  - **sometimes at a site distant from the active site.**

Inhibitors and Accelerators

Usually these alterations involve a reduction in the enzyme's ability to accelerate the reaction (inhibitors)

less commonly

alterations that give rise to an increase in the enzyme's ability to accelerate a reaction (accelerators)

Why more inhibitors than accelerators?

- **Natural selection: if there were small molecules that can facilitate the enzyme's propensity to speed up a reaction, nature probably would have found a way to incorporate those facilitators into the enzyme over the billions of years that the enzyme has been available.**

- **Most enzymes are already fairly close to optimal in their properties; we can readily mess them up with effectors, but it's more of a challenge to find ways to make enzymes better at their jobs.**

Types of inhibitors

- **Irreversible**
  - **Inhibitor binds without possibility of release**
  - **Usually covalently attached**
  - **Each inhibition event effectively removes a molecule of enzyme from availability**

- **Reversible**
  - **Usually attached by weak noncovalent interactions (ionic or van der Waals)**
  - **Several kinds**
  - **Classifications somewhat superseded by detailed structure-based knowledge of mechanisms, but not entirely**

Types of reversible inhibition

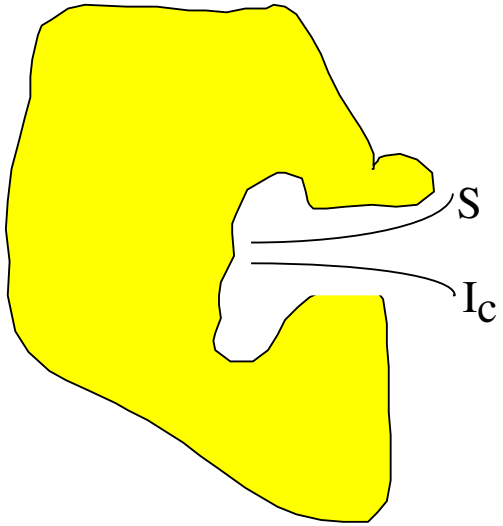
- **Competitive**
  - Inhibitor binds at **active site**
  - Prevents binding of substrate
- **Noncompetitive**
  - Inhibitor binds **distant** from active site
  - Interferes with turnover
- **Uncompetitive (rare?)**
  - Inhibitor binds to **ES complex**
  - Removes ES, interferes with turnover
- **Mixed**
  (usually Competitive + Noncompetitive)

How to distinguish?

- **Reversible vs irreversible**
  - **dialyze an enzyme-inhibitor complex against a buffer free of inhibitor**
  - **if turnover or binding still not improved , it's irreversible**

- **Competitive vs. other reversible:**
  - **Kinetics**
  - **Structural studies if feasible**

Competitive inhibition



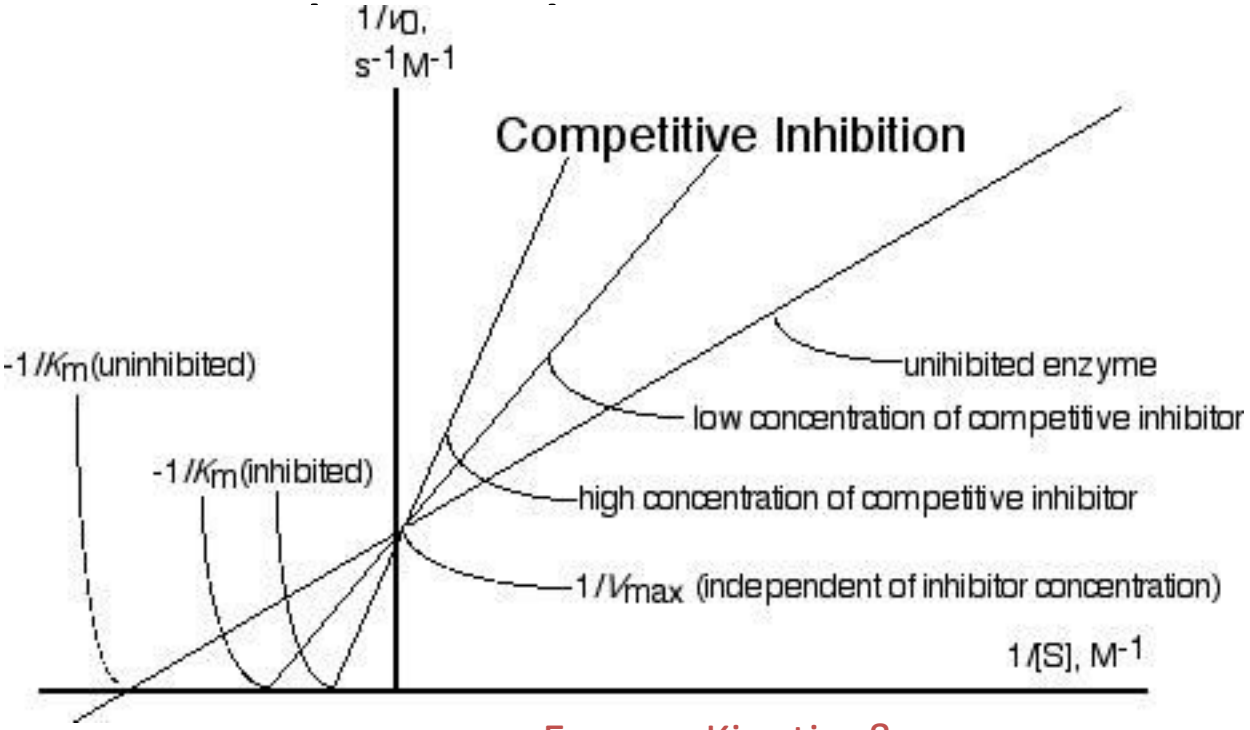
- **Increase substrate concentration:**
  Rate will increase as the inhibitor will be out-competed by increased numbers of substrate molecules.

- **How many substrate molecules it will take to overwhelm the inhibitor depends on how strongly the enzyme is attracted to the substrate as compared to the attraction of the enzyme for the inhibitor. However, once the substrate is bound, the inhibitor does *not* influence how quickly the enzyme turns the substrate over.**

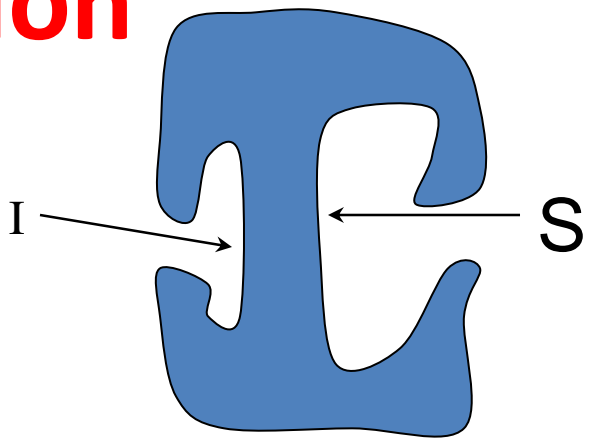
Kinetics of competition

- **Competitive inhibitor hinders binding of substrate but not reaction velocity:**
- **Affects K_m of the enzyme, not V_{max}**
- **Which way does it affect it?**
  - **K_m = amount of substrate that needs to be present to run the reaction velocity up to half its saturation velocity.**
  - **Competitive inhibitor requires us to shove more substrate into the reaction in order to achieve that half-maximal velocity.**
  - **So: competitive inhibitor *increases* K_m**

L-B: competitive inhibitor

- K_m goes up so -1/ K_m moves toward origin
- 

Noncompetitive inhibition

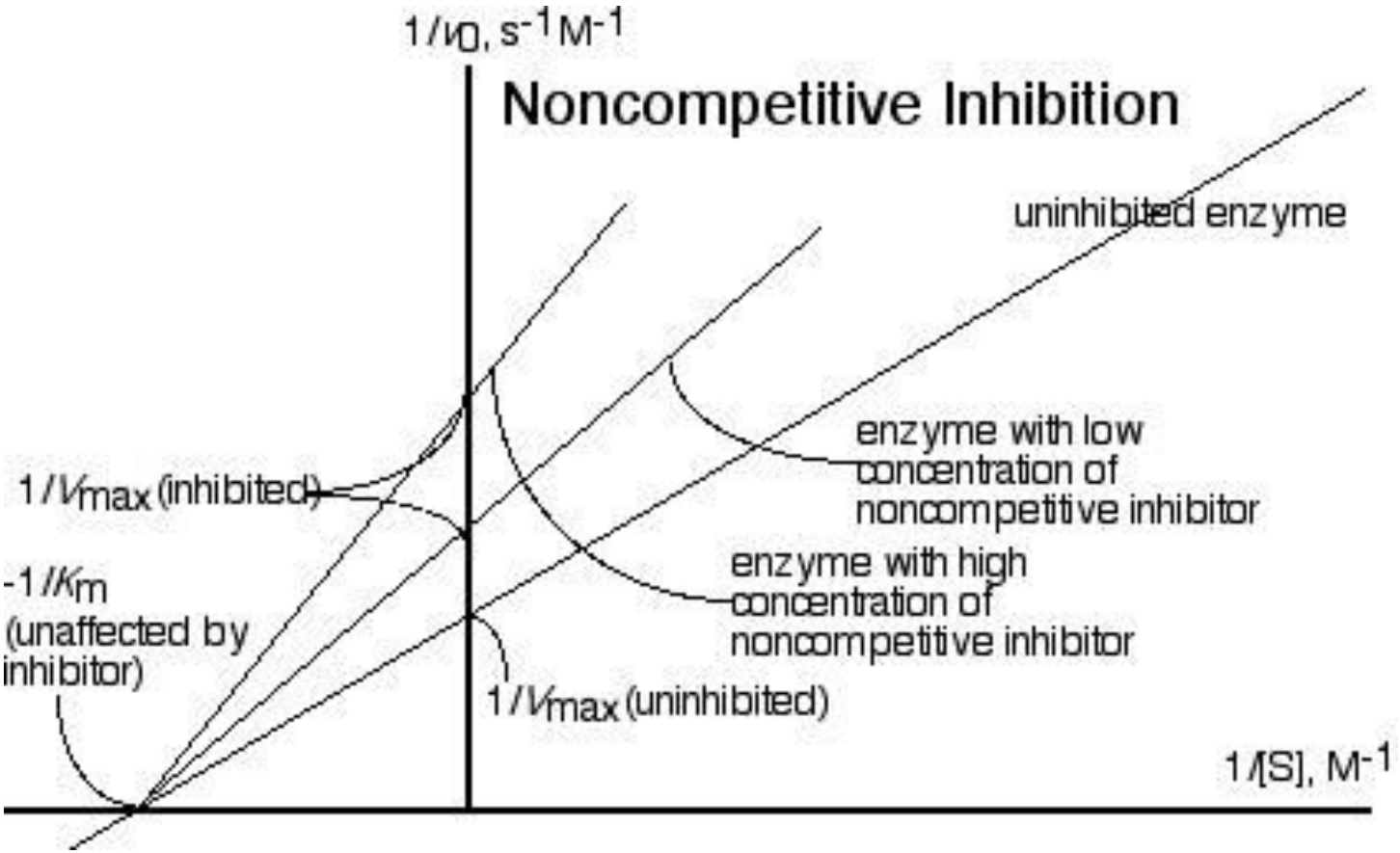


- **Noncompetitive inhibitor has no influence on how available the binding site for substrate is, so it does not affect K_m at all**

- **However, it has a profound inhibitory influence on the speed of the reaction, i.e. turnover. So it reduces V_{max} and has no influence on K_m.**

L-B for non-competitives

- Decrease in $V_{max} \Rightarrow 1/V_{max}$ is increased
- X-intercept unaffected



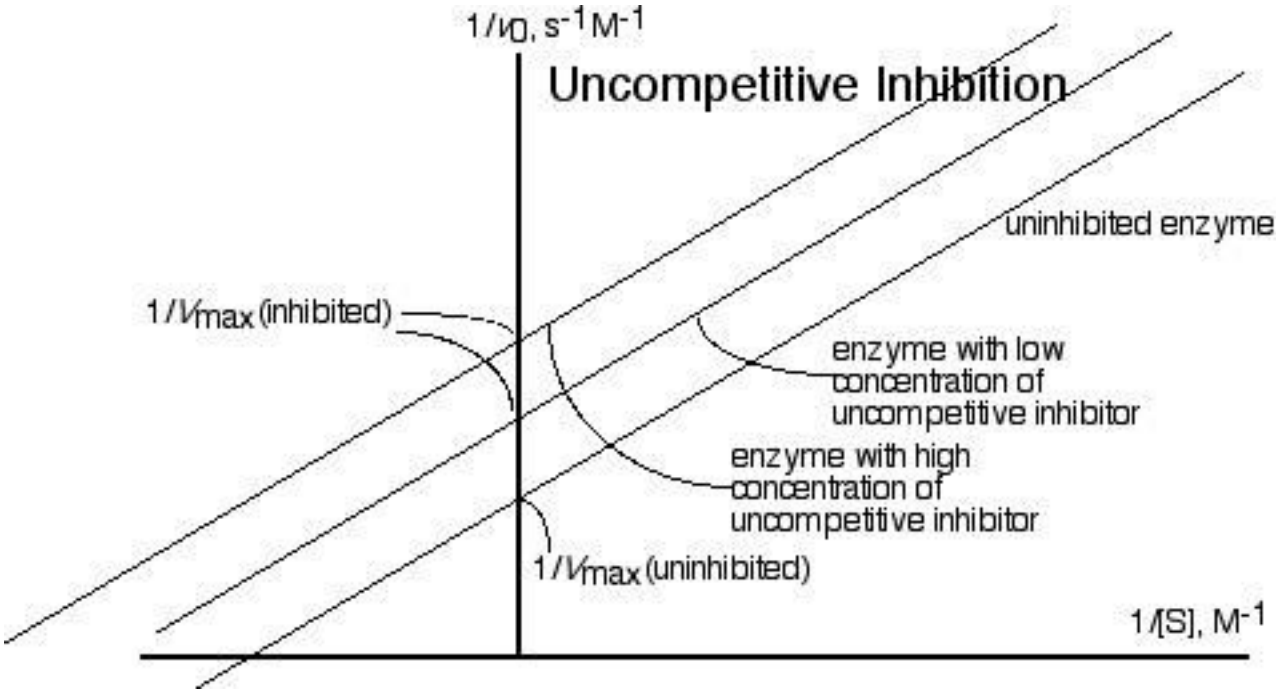
Uncompetitive inhibition

- Inhibitor binds only if ES has already formed
- It creates a ternary ESI complex
- This removes ES, so by LeChatlier's Principle it actually drives the original reaction (E + S $\rightarrow$ ES) to the right; so it decreases K_m
- *But* it interferes with turnover so V_{max} goes down

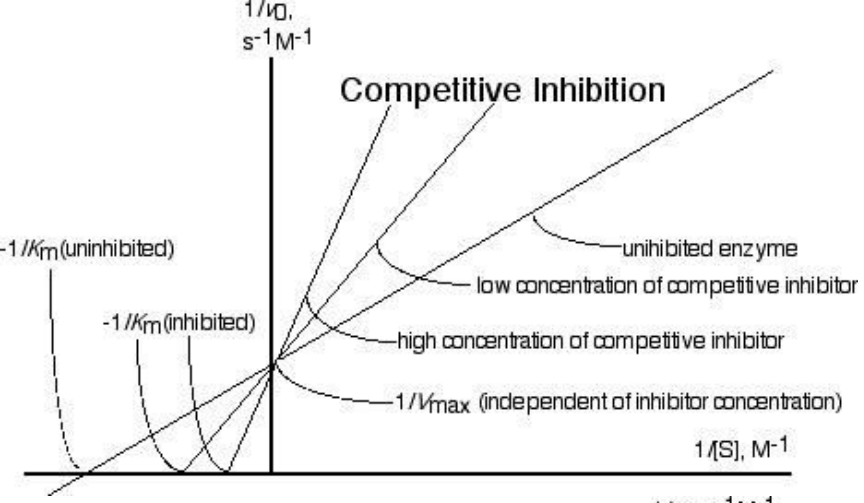
- If K_m and V_{max} decrease at the same rate, then it's classical uncompetitive inhibition.

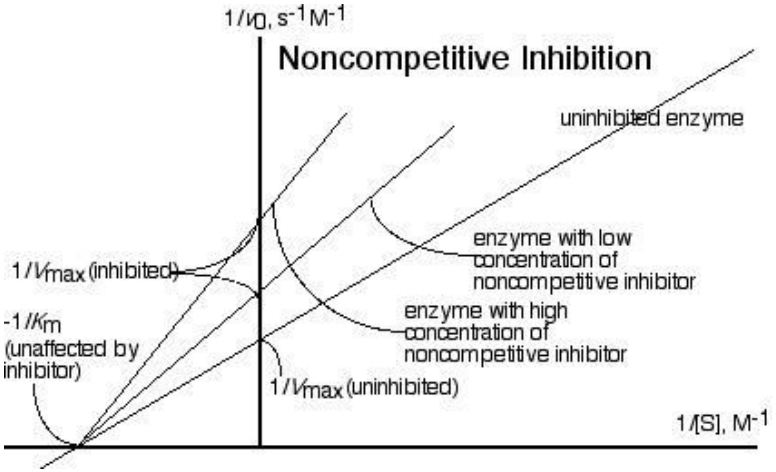
L-B for uncompetitives

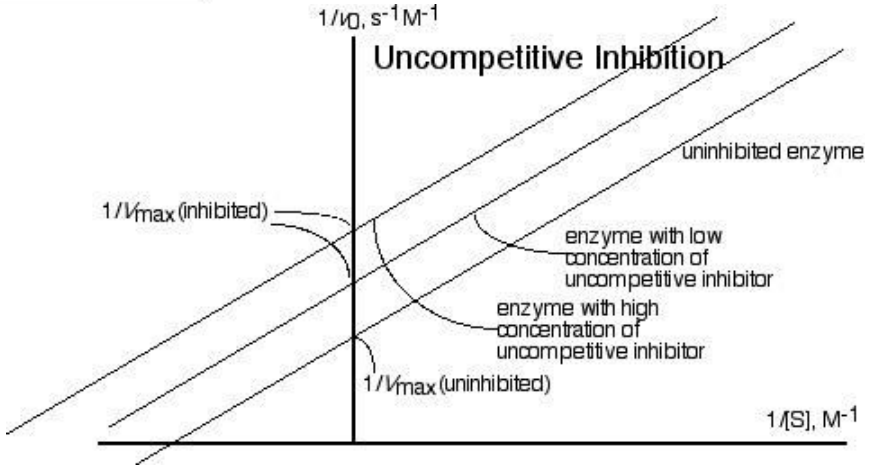
- K_m moves toward origin
- V_{max} moves away from the origin
- Slope ($\propto K_m/V_{max}$) is unchanged



The three reversible inhibitions

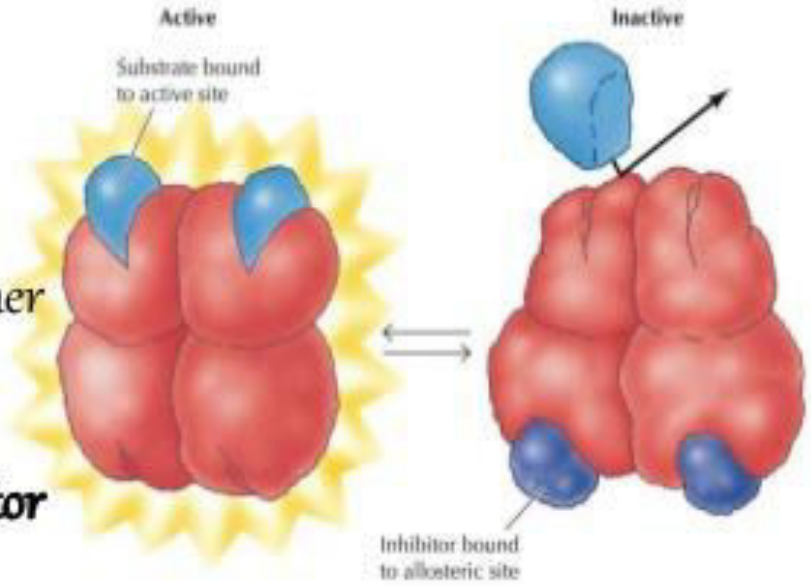




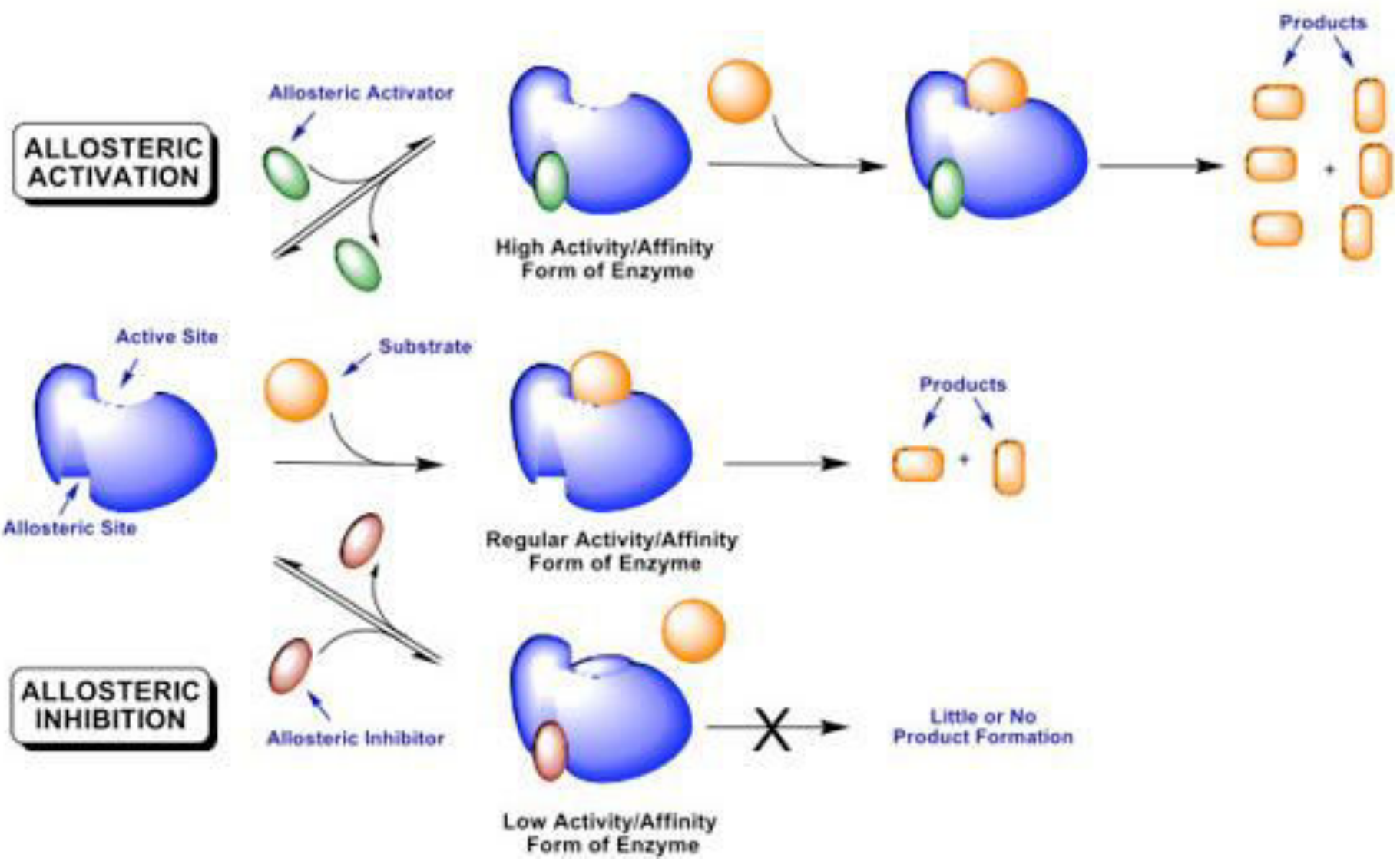


Allosteric Enzymes

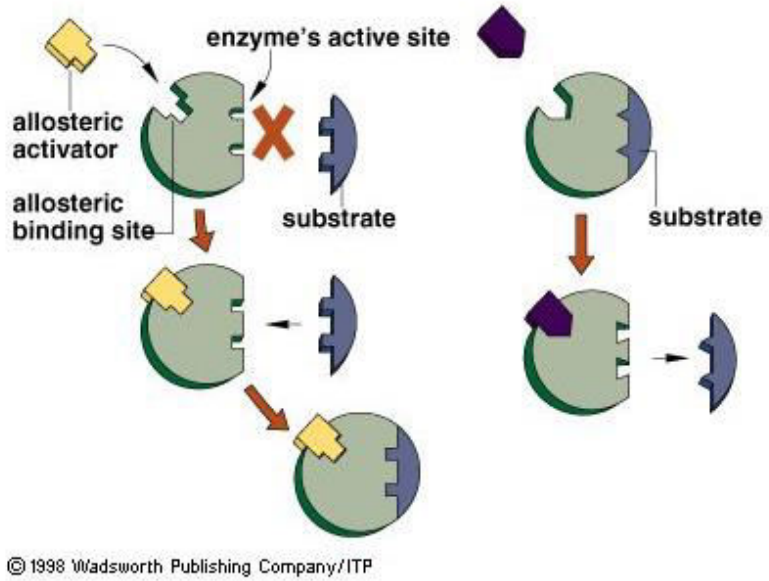
- **Allosteric enzymes** are those having **"other shapes"** or conformations induced by the binding of modulators.
- These enzymes have **two receptor sites.**
- One site fits the **substrate** like other enzymes.
- The other site fits an **inhibitor or activator molecule.**
- Allosteric enzymes are very important in **feedback regulation.**

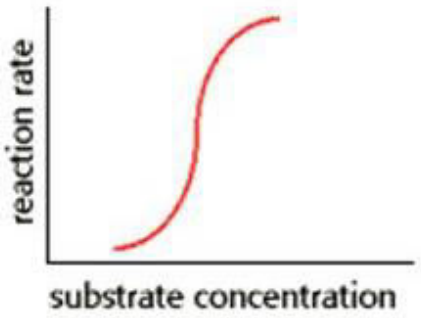


Allosteric Modulation of Enzyme



Kinetics of Allosteric Enzyme



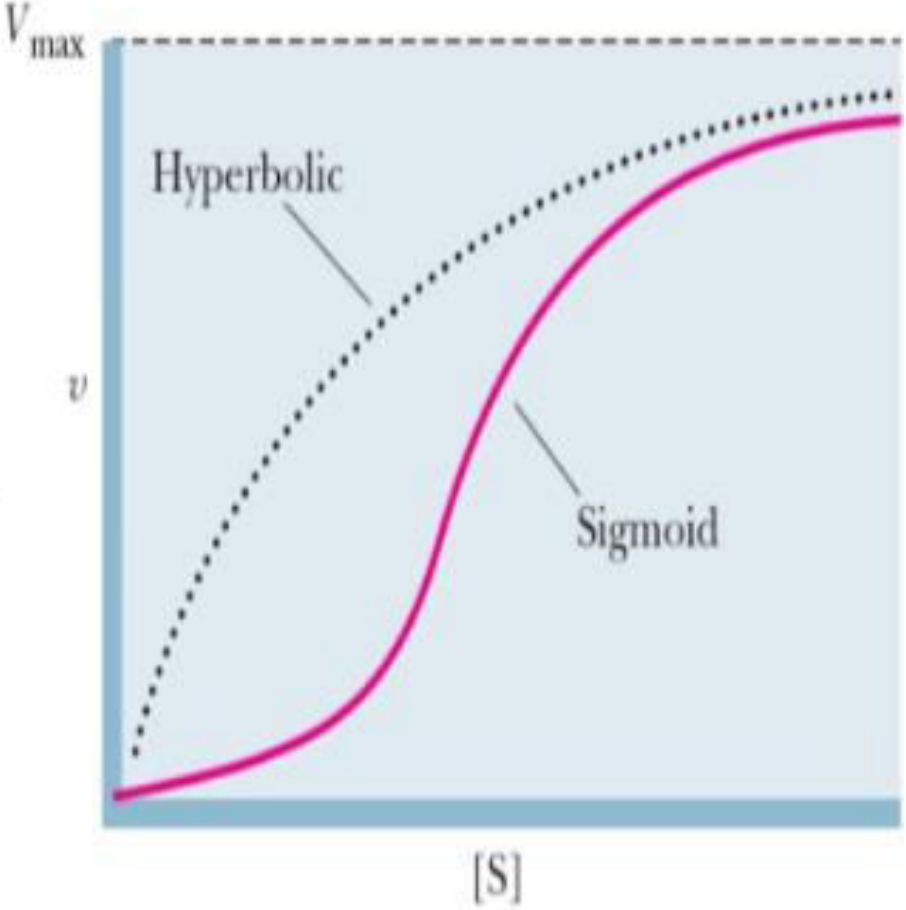


Allosteric enzymes can be regulated between very low and very high reaction rates with only small changes in substrate concentration.

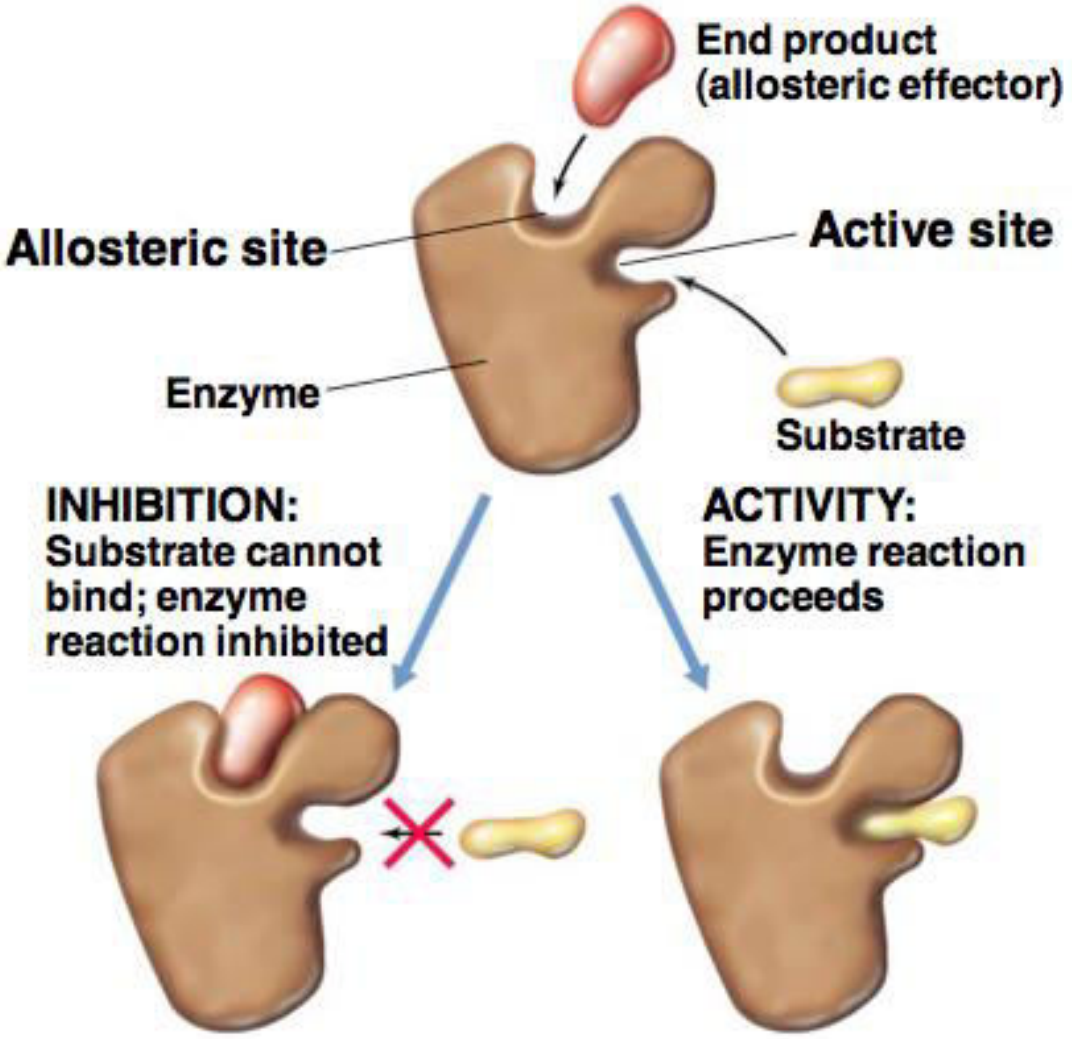
Allosteric enzymes are used by cells to regulate metabolic pathways where the concentration of cellular substrates fluctuate over narrow concentration ranges.

Kinetics of Allosteric Enzyme

- Allosteric enzymes show **relationships** between V_o **and [S]** that ***differ*** from normal **Michaelis-Menten behavior.**
- They exhibit **saturation** with the **substrate** when [S] is sufficiently **high.**
- When V_o is plotted against [S] a **sigmoid saturation curve** results.
- The symbol $[S]_{0.5}$ **or** $K_{0.5}$ is used to represent the substrate concentration giving half maximal velocity.



Endproduct Allosteric Inhibition



Properties of Allosteric Enzyme

- Allosteric enzyme have one or more allosteric sites
- Allosteric sites are binding sites distinct from an enzyme active site or substrate binding site
- Molecule that bind to allosteric sites are called effector or modulator
- Effector may be positive or negative, this effector regulate the enzyme activity. The enzyme activity is increased when a positive allosteric effector binds at the allosteric site known as activator site. On the other hand, negative allosteric effector bind at the allosteric site called inhibitor site and inhibit the enzyme activity.
- Binding to allosteric sites alter the activity of the enzyme, this is called cooperative binding. Allosteric enzymes display sigmoidal plot of V_0 vs [S].

Thank You