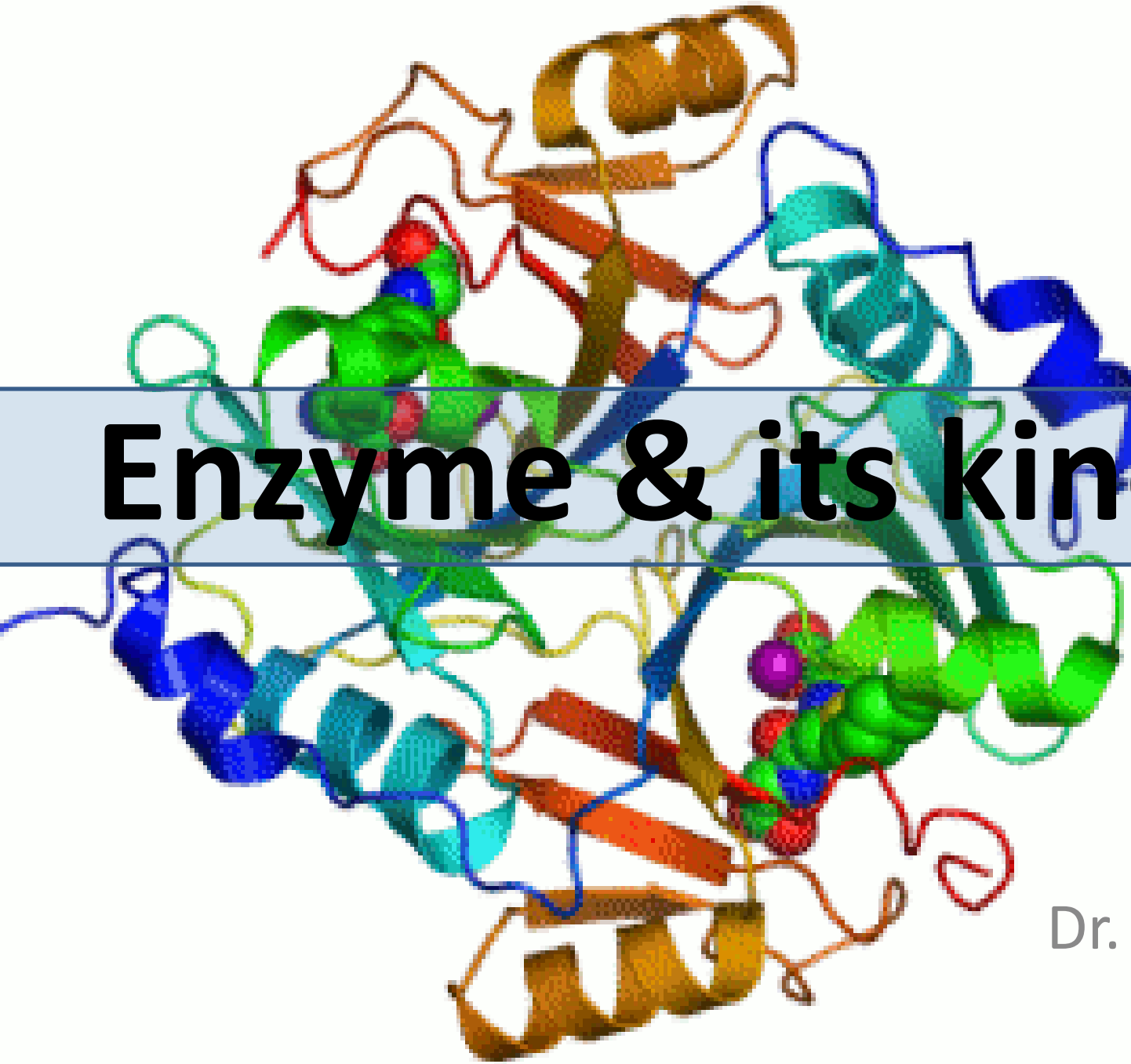


Enzyme & its kinetics

Dr. S.K. Halder

Introduction

- A cell must be capable to perform multitude of chemical changes in order to stay alive, grow and reproduce.
- Cells have capability to digest complex nutrients in the surroundings in smalls which subsequently enter the cell and utilized for energy generation and cellular component.
- Cell produces array of organic catalysts which help to breakdown the complex nutrient, named enzymes, are generally proteins.
- Enzymes are produced by cells in minute amount which capable to speeding up the reactions associated with life processes.
- Any impairment of enzyme activity is reflected by some changes in cell, or even death. There are no life with out enzyme.
- Enzymes catalyze thermodynamically possible reaction both inside and outside the cells.
- They catalyzed a particular reaction or change of either a single specific substance or a group of substances.
- Though enzymes are synthesized by living cells, they remain reactive in cell free extract.
- Enzymes required for catalyzing covalent bonds.

Enzyme Kinetics

Kinetics is the study of the rate and mechanism of a reaction.

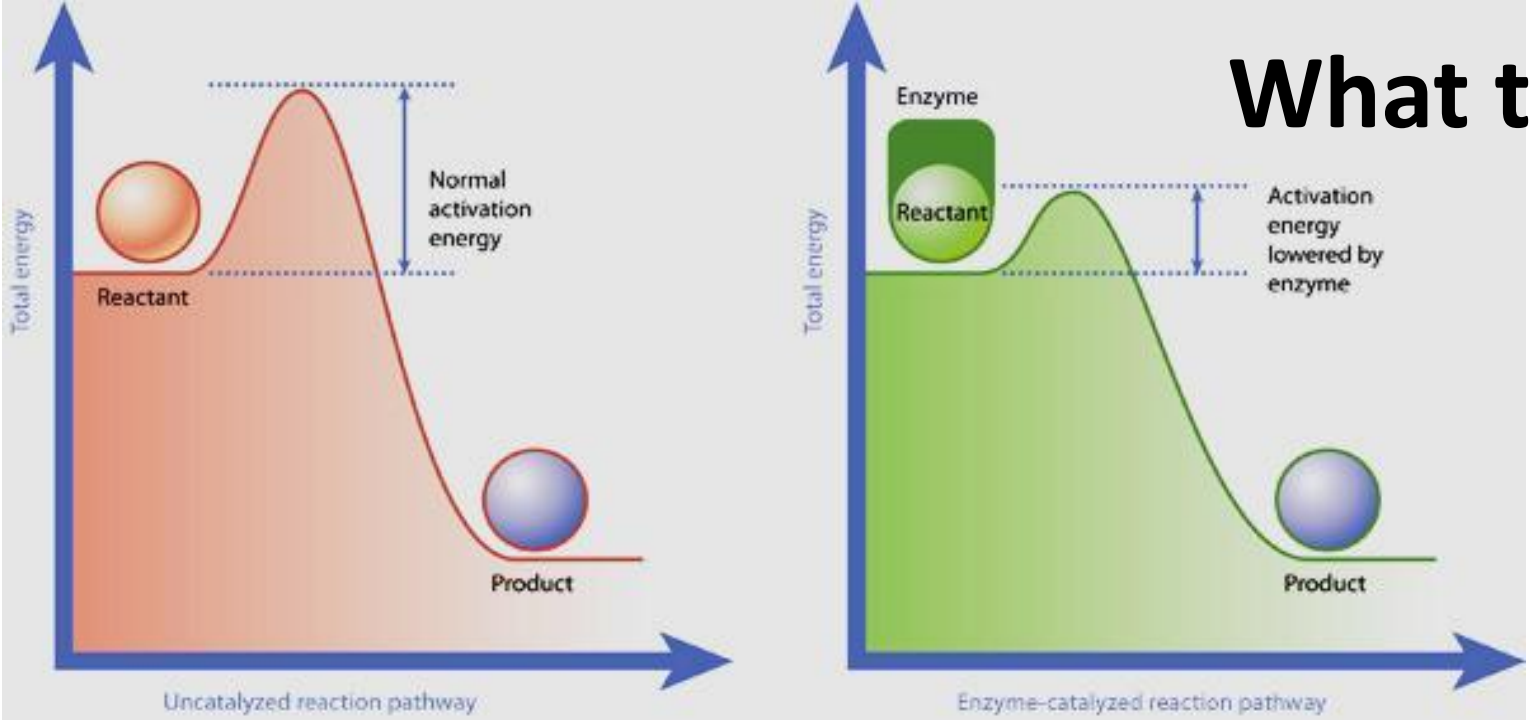
- Study of the rates of enzyme-catalyzed reactions

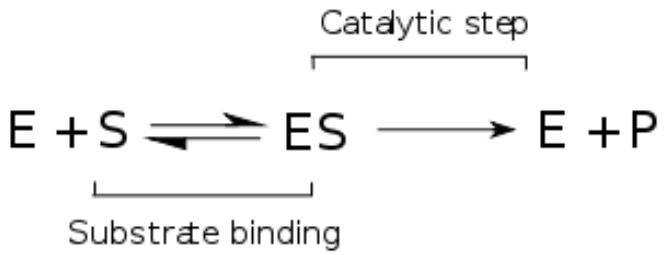
  Rates are usually measured in terms of how many moles of reactant or product are changed per time period.

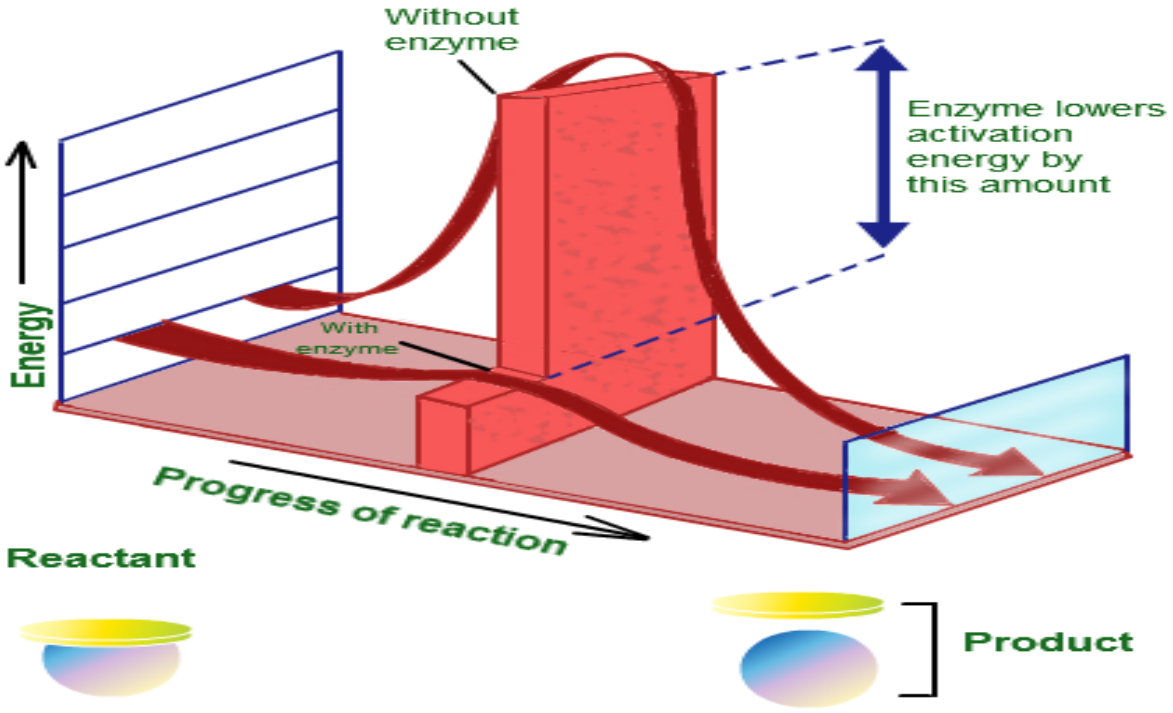
- Provides information on enzyme specificities and mechanisms

  A mechanism is a detailed step-by-step description of how a reaction occurs at the molecular level.

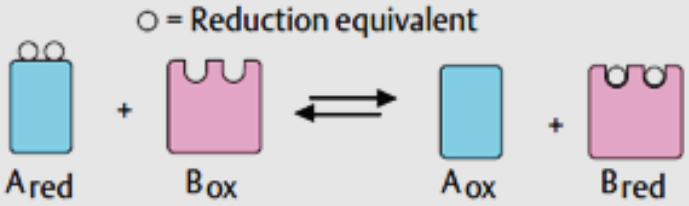
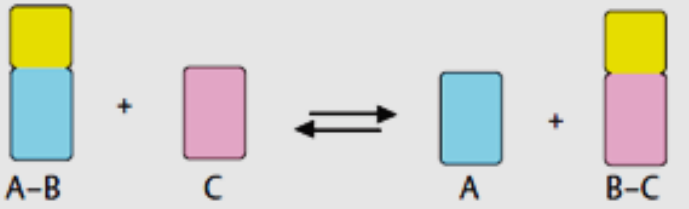
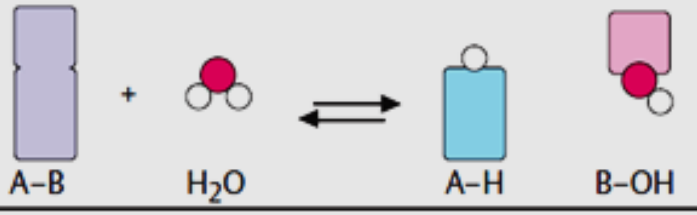
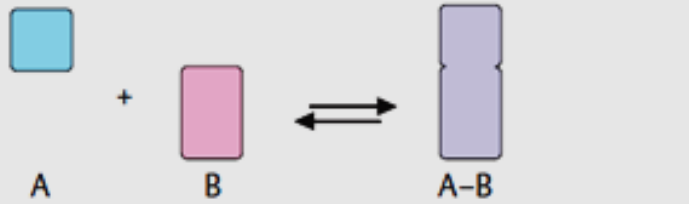
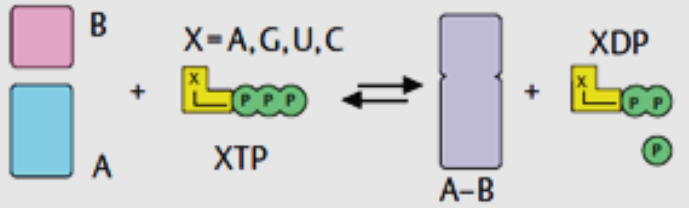
What they do?


Total energy
Normal activation energy
Reactant
Product
Uncatalyzed reaction pathway
Enzyme
Reactant
Activation energy lowered by enzyme
Total energy
Product
Enzyme-catalyzed reaction pathway


Catalytic step
E + S
ES
E + P
Substrate binding

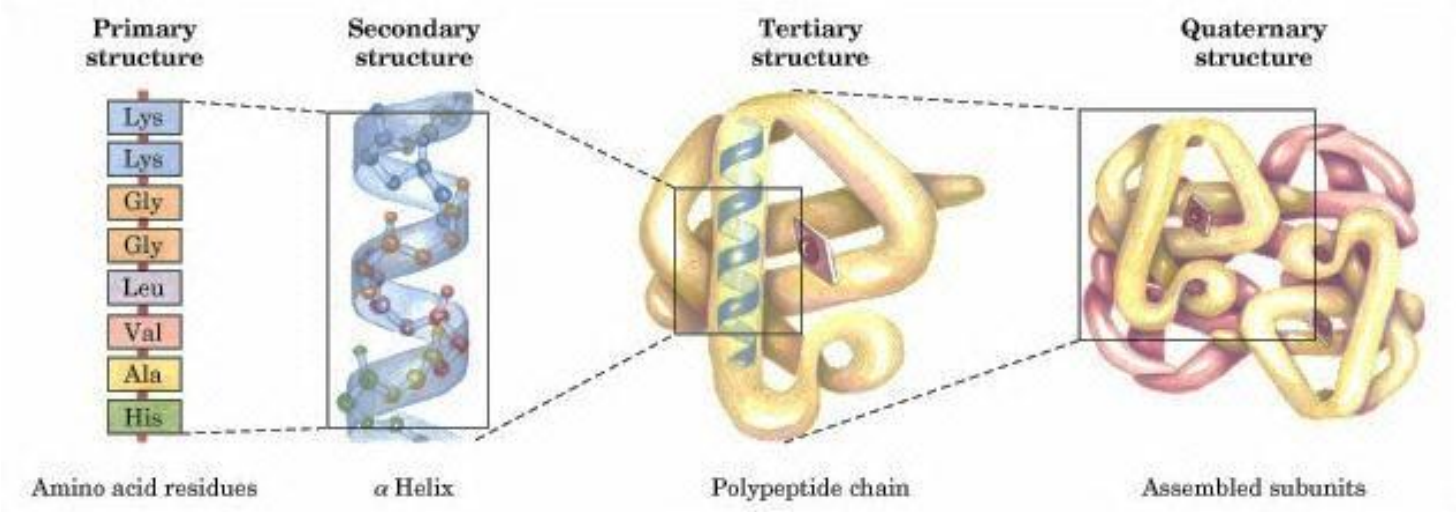

Without enzyme
Enzyme lowers activation energy by this amount
With enzyme
Energy
Progress of reaction
Reactant
Product

Classification

Class	Reaction type	Important subclasses
1 Oxidoreductases		Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases		C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases		Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")		C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases		Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")		C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

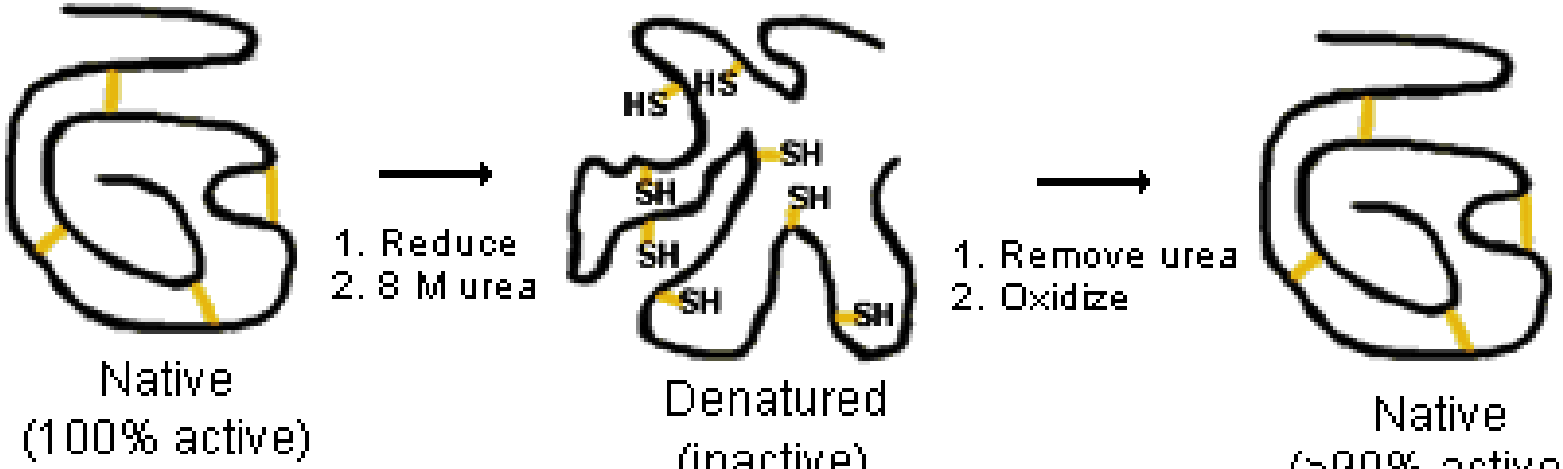
3D structure

- Proteins are polymers – specifically polypeptides -sequences formed from various L-α-amino acids.
- By convention, a chain under 40 residues is often identified as a peptide, rather than a protein.
- To be able to perform their biological function, proteins fold into one or more specific spatial conformations, driven by a number of non-covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals forces, and hydrophobic packing.
- To understand the functions of proteins at a molecular level, it is often necessary to determine their three-dimensional structure by X-ray crystallography, NMR spectroscopy, and dual polarisation interferometry

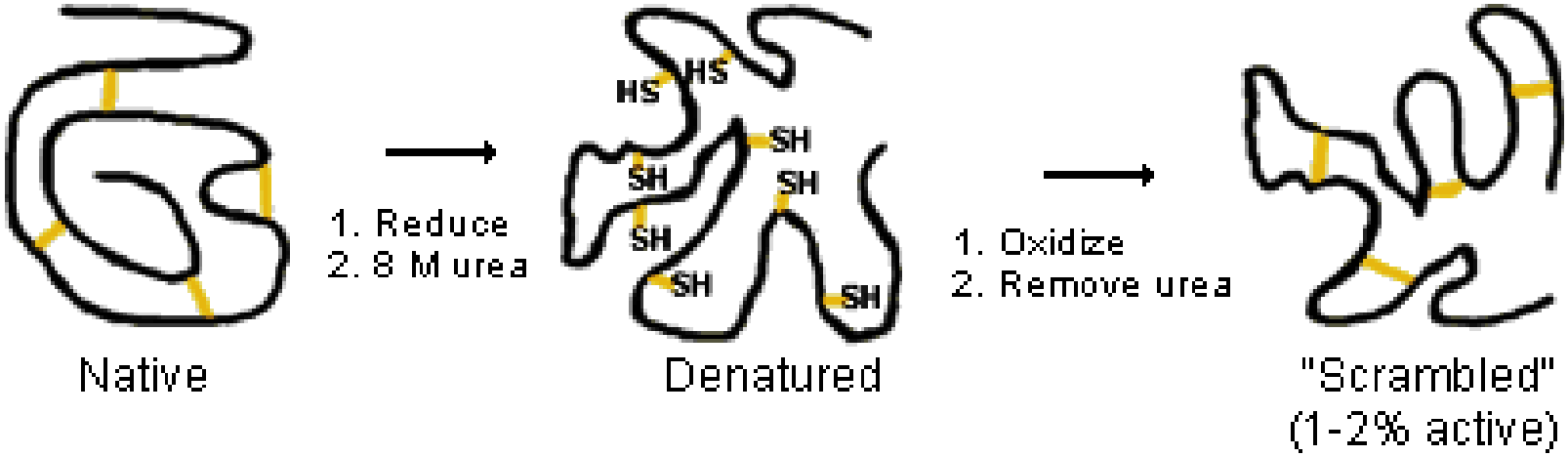


Enzyme activity depends on 3D conformation !!!

The Observation:



The Control:



Environmental factors

- *The activity of an Enzyme is affected by its environmental conditions.*
- *Changing these, alter the rate of reaction.*
- *In nature, organisms adjust the conditions of their enzymes to ensure an optimum rate of reaction.*
- *When necessary, or they may have enzymes which are adapted to function well in extreme conditions where they live, termed extremophilic organisms/enzymes.*

Factors affecting enzyme activity

- Enzyme concentration
- Substrate concentration
- pH
- Temperature
- Ionic state
- Salts
- Inhibitors
- Activators
- Chelating agent
- Products

Effect of temperature

- Increasing temperature increases the Kinetic Energy that molecules possess. In a fluid, this means that there are more random collisions between molecules per unit time.
- Since enzymes catalyse reactions by randomly colliding with Substrate molecules, increasing temperature increases the rate of reaction, forming more product.
- However, increasing temperature also increases the Vibrational Energy that molecules have, specifically in this case enzyme molecules, which puts strain on the bonds that hold them together.
- As temperature increases, more bonds, especially the weaker Hydrogen and Ionic bonds, will break as a result of this strain. Breaking bonds within the enzyme will cause the Active Site to change shape.
- This change in shape means that the Active Site is less Complementary to the shape of the Substrate, so that it is less likely to catalyse the reaction. Eventually, the enzyme will become Denatured and will no longer function.
- As temperature increases, active sites of more enzyme molecules will be less Complementary to the shape of their Substrate, and more enzymes will be Denatured. This will decrease the rate of reaction.
- In summary, as temperature increases, initially the rate of reaction will increase, because of increased Kinetic Energy. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease.

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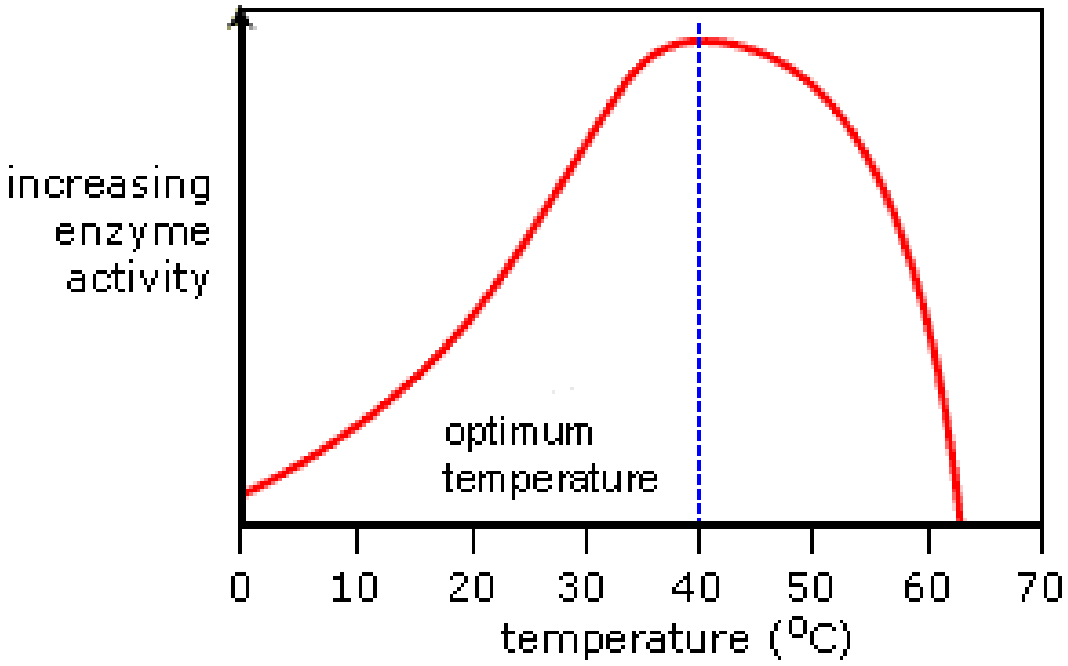
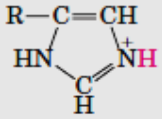
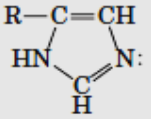
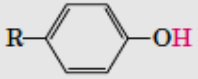
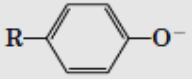
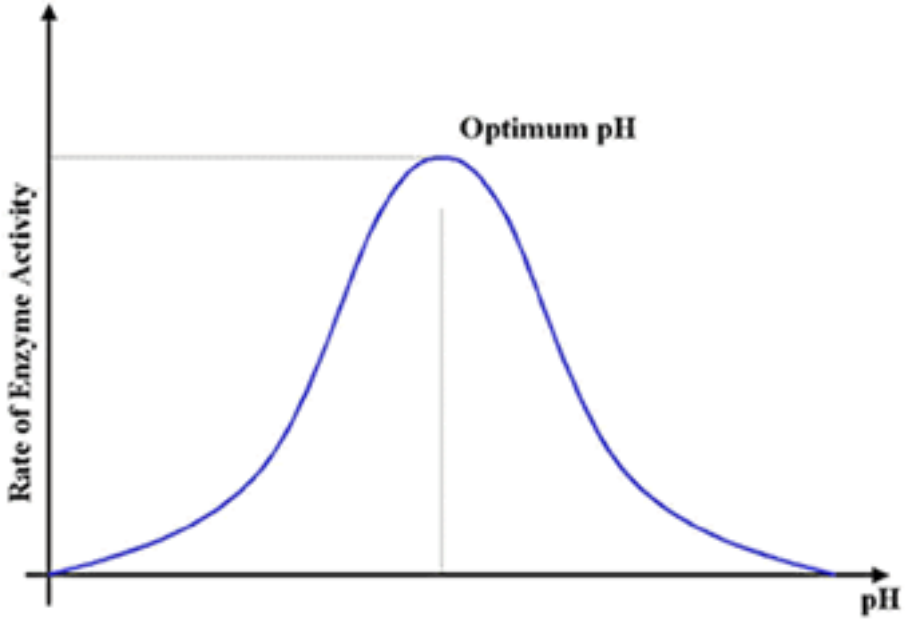


Effect of pH

- H+ and OH- Ions are charged and therefore interfere with Hydrogen and Ionic bonds that hold together an enzyme, since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme, and importantly, its Active Site.
- Different enzymes have different Optimum pH. This is the pH value at which the bonds within them are influenced by H+ and OH- Ions in such a way that the shape of their Active Site is the most Complementary to the shape of their Substrate. At the Optimum pH, the rate of reaction is at an optimum.
- Any change in pH above or below the Optimum will quickly cause a decrease in the rate of reaction, since more of the enzyme molecules will have Active Sites whose shapes are not (or at least are less) Complementary to the shape of their Substrate.

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg	R—⁺NH(H)—H	R—NH₂
Cys	R—SH	R—S⁻
His		
Ser	R—OH	R—O⁻
Tyr		



Effect of substrate concentration

- Increasing Substrate Concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.
- However, after a certain concentration, any increase will have no effect on the rate of reaction, since Substrate Concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.

Effect of enzyme concentration

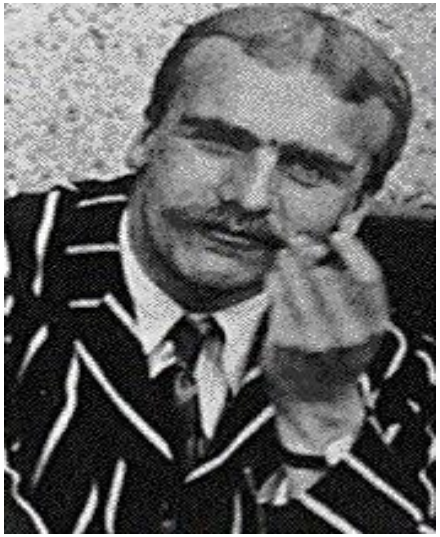
- Increasing Enzyme Concentration will increase the rate of reaction, as more enzymes will be colliding with substrate molecules.
- However, this too will only have an effect up to a certain concentration, where the Enzyme Concentration is no longer the limiting factor.

The Legends

Leonor Michaelis

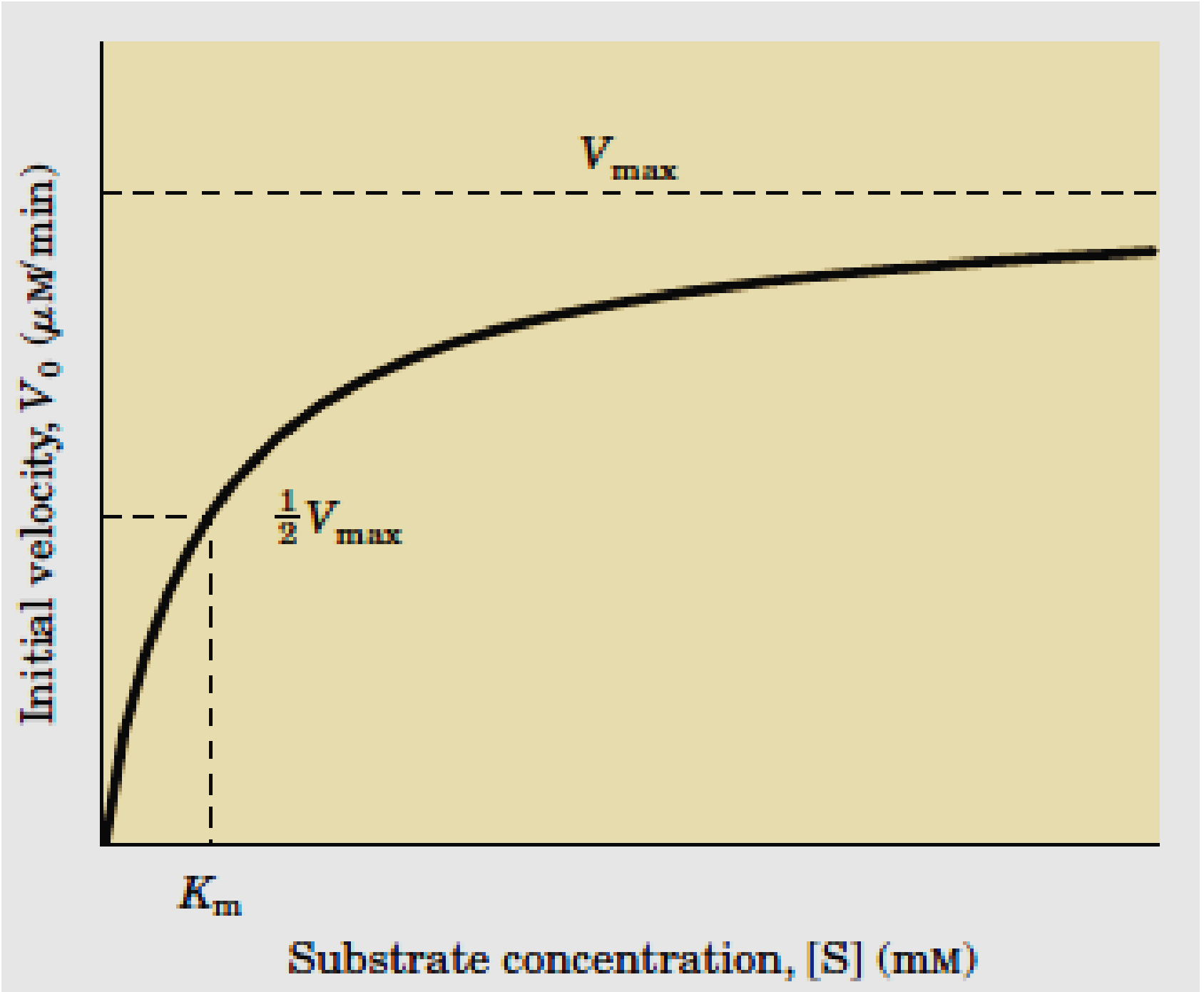
Maud Menten

George Edward Briggs



J. B. S. Haldane

- A key factor affecting the rate of a reaction catalyzed by an enzyme is the concentration of substrate, [S].
- Studying the effects of substrate concentration is complicated by the fact that [S] changes during the course of an in vitro reaction as substrate is converted to product.
- One simplifying approach in kinetics experiments is to measure the initial rate (or initial velocity), designated V_0, when [S] is much greater than the concentration of enzyme, [E].
- In the initial stage of reaction, V_0 can then be explored as a function of [S]….the reaction is 1^{st} order.
- At higher substrate concentrations, V_0 increases by smaller and smaller amounts in response to increases in [S].
- Finally, a point is reached beyond which increases in V_0 is no longer happened with [S] increases. This plateau-like V_0 region is close to the maximum velocity, Vmax ….the reaction is zero order.


V_{max}
$\frac{1}{2}V_{max}$
K_m
Initial velocity, V_0 (μM/min)
Substrate concentration, [S] (mM)

- The ES complex is the key to understanding this kinetic behavior, just as it was a starting point for our discussion of catalysis.
- The kinetic pattern was proposed by Victor Henri, followed by Wurtz, to propose in 1903 that the combination of an enzyme with its substrate molecule to form an ES complex is a necessary step in enzymatic catalysis.
- **This idea was expanded into a general theory of enzyme action, particularly by Leonor Michaelis and Maud Menten in 1913.**
- They postulated that the enzyme first combines reversibly with its substrate to form an enzyme-substrate complex in a relatively fast reversible step:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES}$$

- The ES complex then breaks down in a slower second step to yield the free enzyme and the reaction product P:

$$\mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

- Because the slower second reaction must limit the rate of the overall reaction, the overall rate must be proportional to the concentration of the species that reacts in the second step, that is, ES.

THE RELATIONSHIP BETWEEN INITIAL VELOCITY AND SUBSTRATE CONCENTRATION

- Consider a single-substrate enzyme-catalyzed reaction where there is just one substrate-binding site per enzyme. The simplest general equation for such a reaction would be:

- $E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} E + P$

- If investigations are restricted to the initial period of the reaction, the product concentration is negligible and the formation of ES from product can be ignored.
- Under these conditions, therefore, the reaction simplifies :

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P$$

- The rate of formation of ES at any time t = k_1[E] [S],
- where [E] is the concentration of free enzyme and [S] the concentration of free substrate at time t.
- Also at time t, the rate of breakdown of ES back to E and S = k_{-1}[ES]
- where [ES] is the concentration of enzyme-substrate complex at this time.
- The Michaelis-Menten assumption was that **an equilibrium** between enzyme, substrate and enzyme-substrate complex was almost instantly set up and maintained, **the breakdown of enzyme-substrate complex to products being too slow to disturb this equilibrium.**

- Using this assumption, therefore:

  $K_1[E][S] = k_{-1}[ES]$

- The constants may then be separated from the variables, giving:

  $[E][S] / [ES] = K_{-1} / K_1 = Ks$

  where K_s is the dissociation constant of ES.

The total concentration of enzyme present $[E_o]$ must be the sum of the concentration of free enzyme [E] and the concentration of bound enzyme [ES]. Therefore, in order to involve total enzyme concentration into the above equation, the following substitution is made:

$[E] = [E_o] - [ES]$

$([E_o] - [ES])[S] / [ES] = Ks$

$Ks\ [ES] = ([E_o] - [ES])[S] = [E_o]\ [S] - [ES]\ [S]$

$[ES]\ [S] + Ks\ [ES] = [E_o]\ [S]$

$[ES]([S] + Ks) = [E_o]\ [S]$

$[ES] = [E_o]\ [S] / [S] + Ks$

[ES] has been isolated in this way because this term governs the rate of formation of products (the overall rate of reaction)

According to the relationship:

$$V_o = k_2\ [ES]$$

If we substitute the expression for [ES] derived above, we obtain:

$$V_o = k_2[E_o]\ [S] / [S] + K_s$$

- Moreover, we know that when the substrate concentration is very high, all the enzyme is present as the enzyme-substrate complex and the limiting initial velocity V_{max} is reached. Under these conditions:

$$V_{max} = k_2[E_o]$$

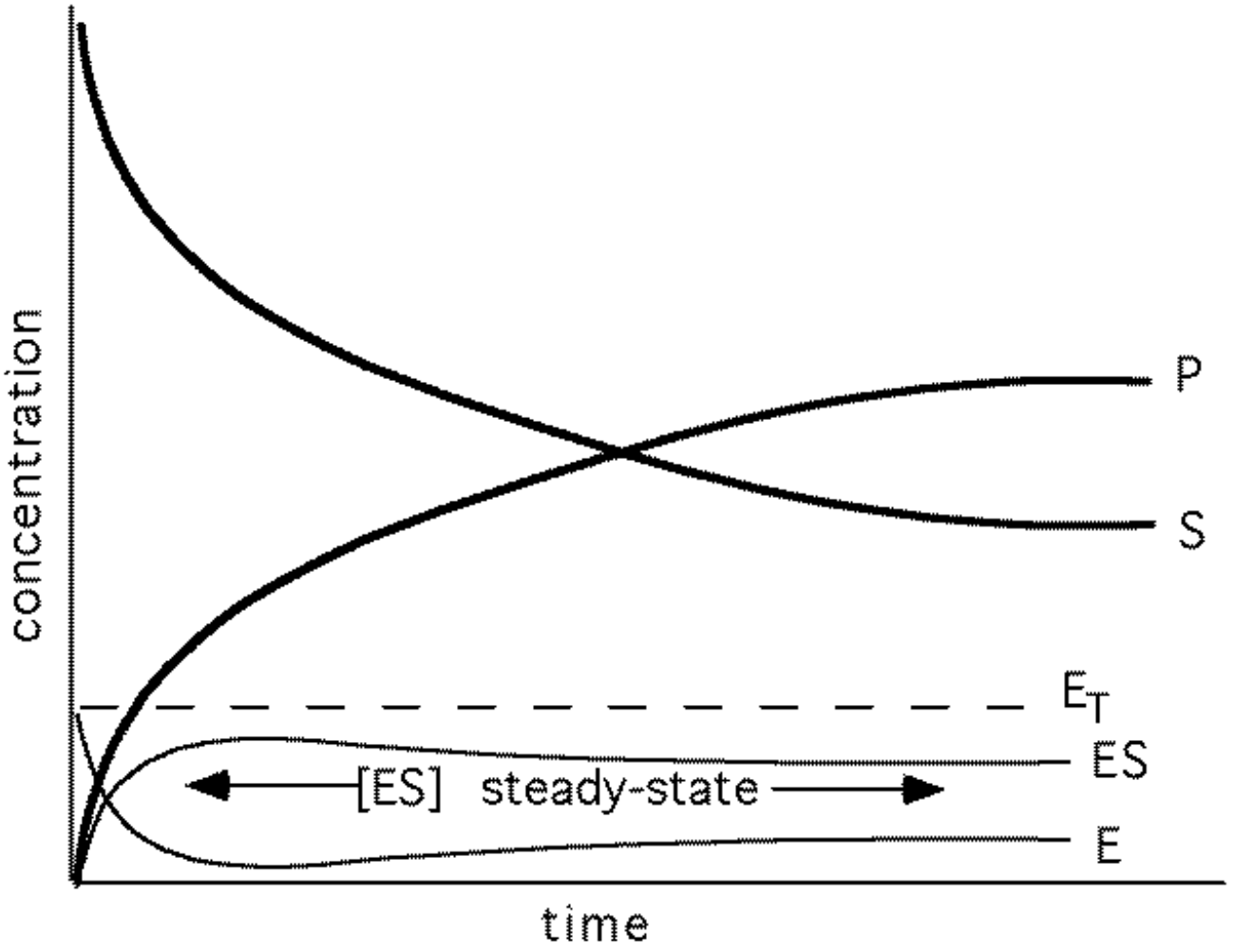
- Therefore, we can substitute V_{max} for k_2 $[E_o]$ in the expression for V_o and get:

$$\mathbf{V_o = V_{max}\,[S] / [S] + K_s}$$

Limitation of M-M equilibrium-assumption

It cannot be generally applicable because many, enzyme-catalyzed reactions are likely to proceed at rates fast enough to disturb such an equilibrium

Steady state kinetics



The Briggs-Haldane modification of the Michaelis-Menten equation

The equation derived by Michaelis and Menten was modified by Briggs and Haldane (1925) who introduced a more generally valid assumption, that of the steady-state.

- $E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} E + P$

- The rate of formation of ES at any time t (within the initial period when the product concentration is negligible) = k_1 [E] [S].
- The rate of breakdown of ES at this time = k_{-1} [ES] + k_2 [ES],
- Using the steady-state assumption:
- $k_1[E][S] = k_{-1}$ [ES] + k_2 [ES] = [ES]($k_{-1} + k_2$)
- [E] [S] / [ES] = ($k_{-1} + k_2$) / k_1 = Km
- where Km is another constant. Substituting [E] = [E_o] - [ES] as before:
- ([E_o] - [ES]) [S] / [ES] = Km
- [ES] = [E_o] [S] / [S] + K m
- $V_o = k_2$[ES]
- $V_o = k_2[E_o]$ [S] / [S] + Km
- Vmax = k_2 [E_o]
- **V_o = Vmax [S] / [S] + Km**

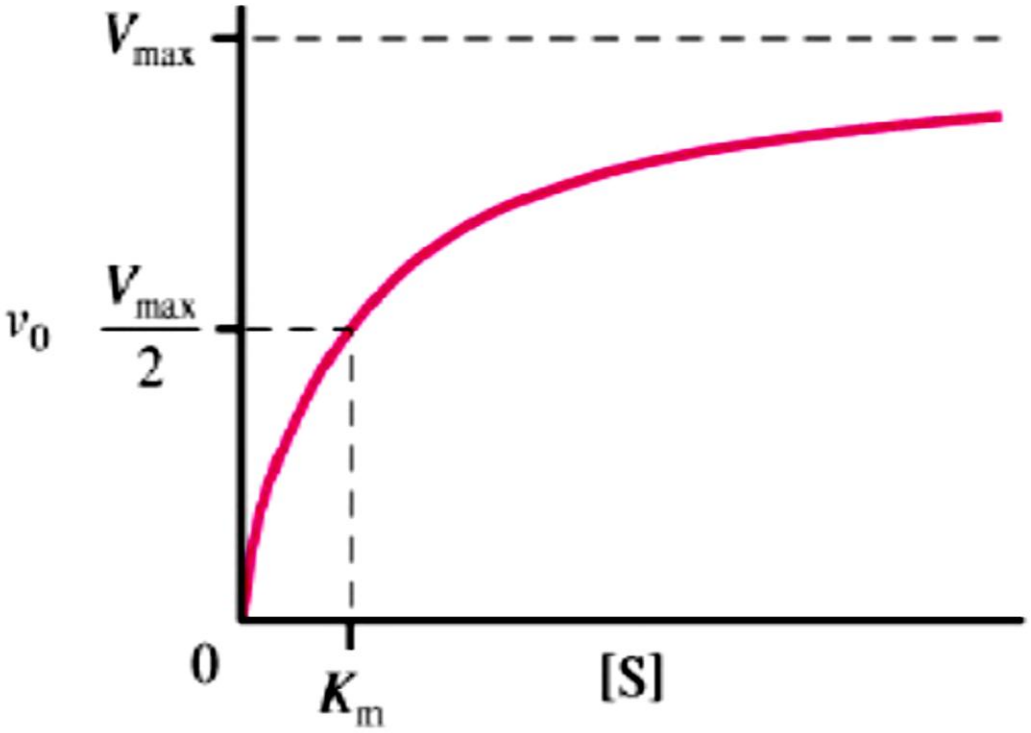
- Finally, since the substrate concentration is usually much greater than the enzyme concentration, [S] = [So], so

  $V_o = V_{max} [S_0] / [S_0] + K_m$ (at constant $[E_o]$)

- **This has the same form as the equation derived by Michaelis and Menten: only the definition of the constant in the denominator has changed.**
- **Hence the equation has, retained the name Michaelis-Menten equation and Km is called the Michaelis constant.**

Understanding *Km*

- Km is the [S] at 1/2 Vmax
- Km is a constant for a given enzyme
- Km is an estimate of the equilibrium constant for S binding to E
- Small Km means tight binding; high Km means weak binding



- K_M gives an idea about affinity
  - ↑K_M ↓substrate affinity
  - ↓K_M ↑substrate affinity

Understanding Vmax

The theoretical maximal velocity indicates that

- V_{max} is a constant for a given enzyme.
- V_{max} is the theoretical maximal rate of the reaction - but it is NEVER achieved.
- To reach V_{max} would require that **ALL** enzyme molecules have tightly bound substrate.
- ***V_{max}* is limited by**
  - How many enzyme molecules are present.
  - Time required for product to be released.

Importance of enzyme kinetics

- It shows the specificity of an enzyme for a particular substrate.
- It gives idea about steady state and equilibrium mechanism.
- It indicates the role of an enzyme in metabolism.
- It provides valuable information for enzymatic reaction mechanism.
- It gives an insight into the role of an enzyme under physiological conditions.
- It can help show how the enzyme activity is controlled and regulated.

Important indices

- The reaction rate increases with increasing substrate concentration [S], asymptotically approaching its maximum rate Vmax, attained when all enzyme is bound to substrate.
- It also follows that $V_{max} = k_{cat}[E]_0$, where $[E]_0$ is the enzyme concentration.
- k_{cat}, the turnover number.
- *In enzymology, turnover number (k_{cat}) is defined as the maximum number of molecules of substrate that an enzyme can convert to product per catalytic site per unit of time (a turnover rate) when the enzyme is saturated with substrate, and can be calculated as follows:*

$$k_{\mathbf{cat}} = \frac{V_{\mathbf{max}}}{[E]_T}$$

As example, carbonic anhydrase has a turnover number of 400,000 to 600,000 per second, which means that each carbonic anhydrase molecule can produce up to 600,000 molecules of product (bicarbonate ions) per second

Catalytic efficiency

- Some times K_{cat} is unsatisfactory.
- It is less useful at low [S] but it reflects the properties of enzyme when it is saturated with substrate.
- K_m is also unsatisfactory because within the cell [S] is low.
- K_m is a useful indicator of the affinity of an enzyme for the substrate.
- K_{cat}, K_m will be the useful parameter of catalytic efficiency.
- **K_{cat} / K_m is a measure of activity, catalytic efficiency**
- **A high K_{cat} / K_m ratio implies an efficient enzyme. This could result from: Large K_{cat}, Small K_m.**

Examples & Importance

Enzyme	*Substrate*	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

- *Comparison of two enzyme*
- *Evaluation/assessment of rate of product formation*
- *To determine the overall rate of a multistep reaction*

Liner transformation of M-M equation

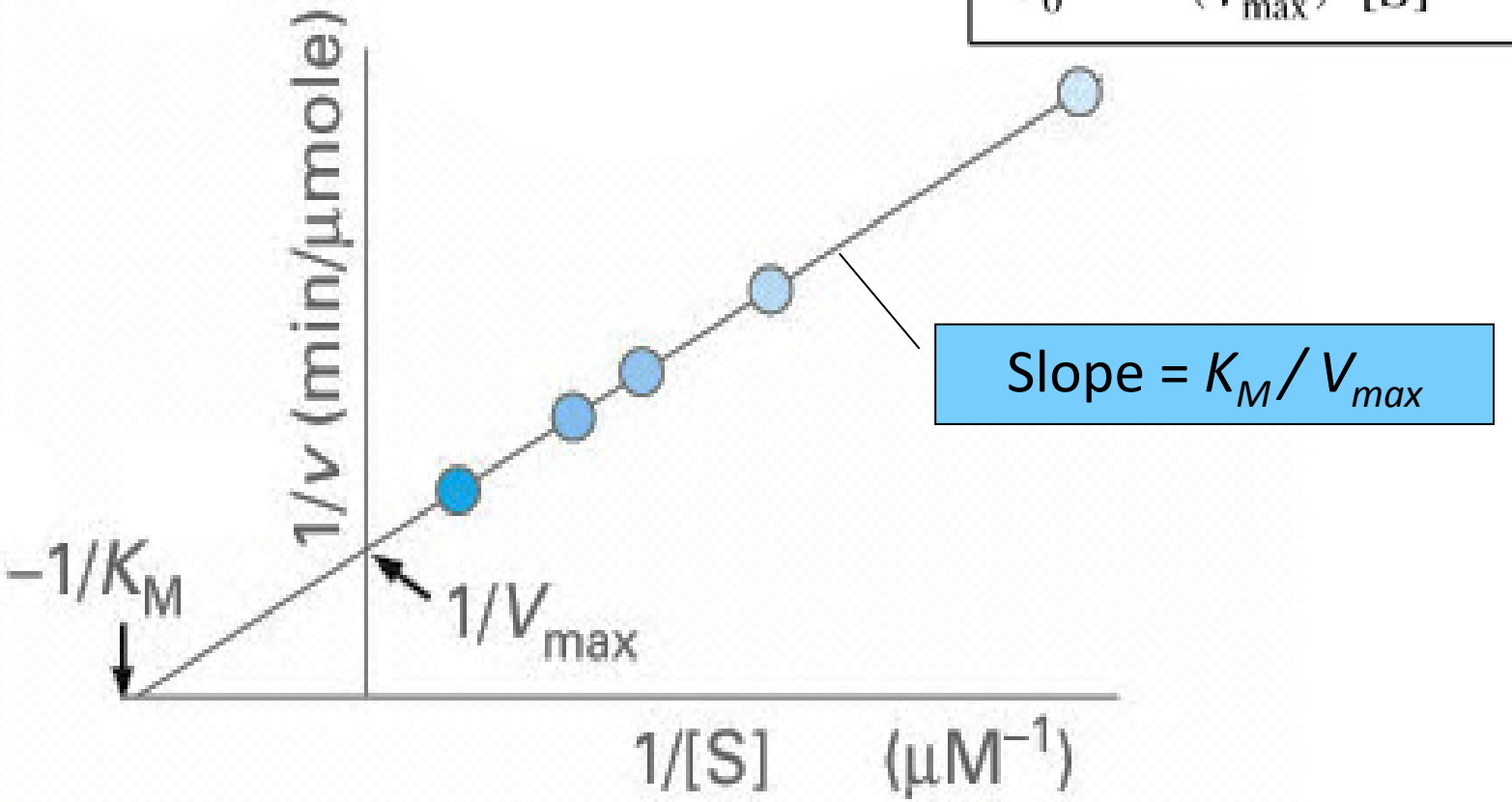
- The graph of the Michaelis-Menten equation, V_0 against $[S_o]$, is unsatisfactory as a means of determining V_{max} and K_m.
- Very low substrate concentration have to use to draw the initial segment of the plot, but V_0 can not be measured precisely in this stage.
- Use of so high [S] required to attain the highest velocity and the plateau of the plot, but it is experimentally difficult.
- Where the points obtained by plotting the experimentally observed initial velocities against the respective substrate concentrations, turn out to be too scattered, the hyperbolic graph is can hardly to be drawn.
- Hence, it is very difficult to use a plot of V_0 against $[S_0]$ to obtain an accurate value of Vmax, and Km.
- The graph, being a curve, cannot be accurately extrapolated upwards from values of V_0 at non-saturating concentrations.

Lineweaver – Burk Plot

$1/V_0 = Km + [S]/Vmax[S] = Km/Vmax[S] + [S]/Vmax\ [S]$

Lineweaver-Burk equation:

$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



Lineweaver – Burk Plot

- Lineweaver and Burk Plot (1934) overcame this problem without making any fresh assumptions. They simply took the Michelis-Menten equation and inverted it.
- This is of the form $y = mx + c$, which is the equation of a straight line graph; a plot of y against x has a slope m and intercept c on the y-axis
- A plot of $1/V_0$ against $1/[S_0]$ systems obeying the Michaelis-Menten equation. The graph, being linear, can be extrapolated even if no experiment has been performed. From the extrapolated graph the values of *Km* and Vmax can be determined.
- Departure from linearity for a particular enzyme-catalyzed reaction indicates that the assumptions inherent in the Michaelis-Menten equation are not valid in this instance.

Criticism of Lineweaver-Burk Plot

The Lineweaver-Burk plot has been criticized on several grounds.

- Firstly, it has least importance because, the extrapolation across the l/V_0 axis to determine the value of — 1/Km sometimes reaches the edge of the graph paper before reaching the $1/[S_0]$ axis, possibly resulting in the graph having to be redrawn with altered axis.
- Secondly, it is said to give undue weight to measurements made at low substrate concentrations, when results are likely to be most inaccurate.

Eadie-Hofstee plot

In biochemistry, an Eadie–Hofstee diagram is a graphical representation of enzyme kinetics in which reaction rate is plotted as a function of the ratio between rate and substrate concentration

invert and multiply with $V_{\max}$:

$$\frac{V_{\max}}{v} = \frac{V_{\max}(K_m + [S])}{V_{\max}[S]} = \frac{K_m + [S]}{[S]}$$

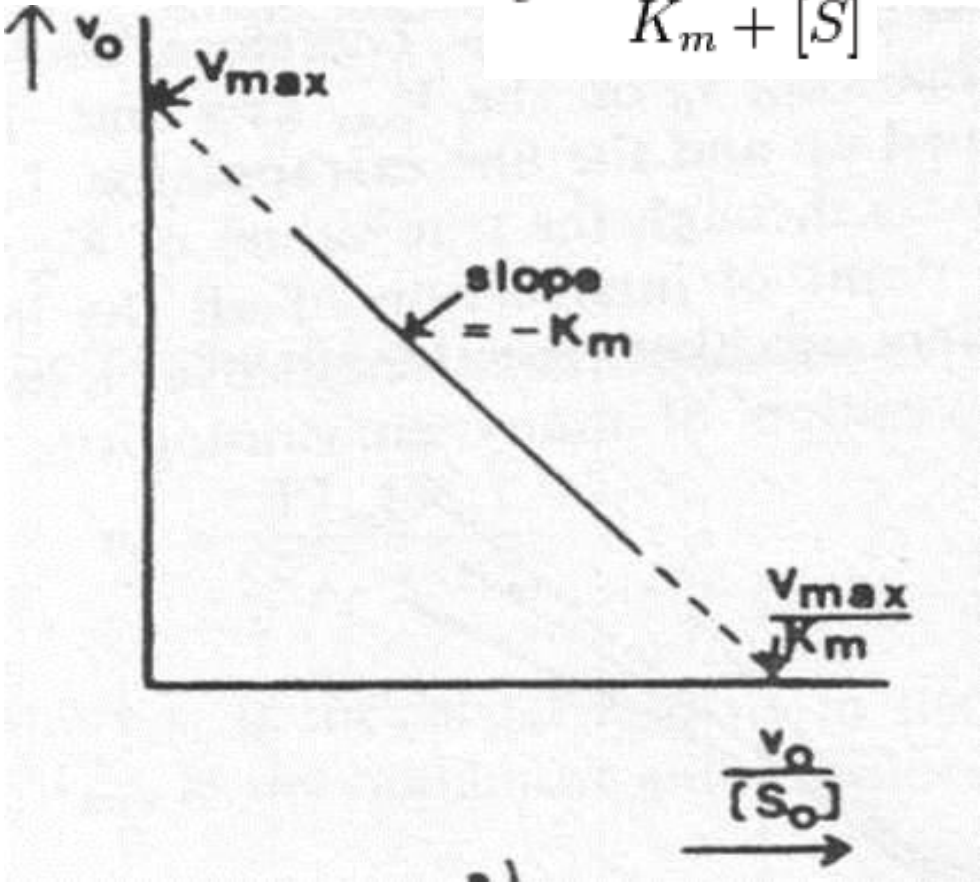
Rearrange:

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

Isolate v:

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

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



Woolf-Hanes plot

In biochemistry, a Hanes–Woolf plot is a graphical representation of enzyme kinetics in which the ratio of the initial substrate concentration [S] to the reaction velocity v is plotted against [S].

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

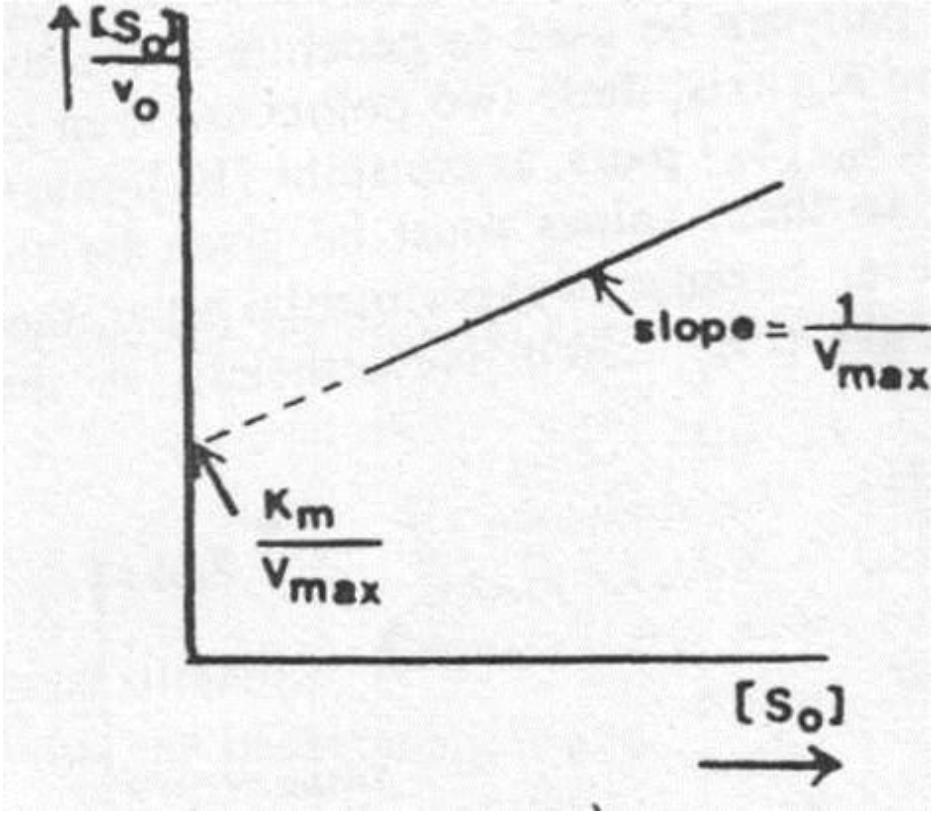
invert and multiply by [S]:

$$\frac{[S]}{v} = \frac{[S](K_m + [S])}{V_{\max[}S]} = \frac{K_m + [S]}{V_{\max}}$$

Rearrange:

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

The **Woolf-Hanes plot** similarly starts with the Lineweaver-Burk equation, which in this instance is multiplied throughout by $[S_0]$



Conclusion of Liner transformation

- Computerized data processing, usually based on the least-squares approach to curve fitting and sometimes incorporating automatic rejection of points outside an arbitrarily set limit, gives a quick and convenient method of obtaining an estimate of *Km* and Vmax.

- However, the results are not entirely reliable, and should never be accepted blindly: it is important to consider the actual graphs obtained, particularly to see if there is any evidence of a departure from linearity.

Enzyme inhibition

- An enzyme inhibitor is a molecule that binds to an enzyme and decreases its activity.
- The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalyzing its reaction.
- Inhibitor binding is either reversible or irreversible.
- Irreversible inhibitors usually react with the enzyme and change it chemically (e.g. via covalent bond formation). These inhibitors modify key amino acid residues needed for enzymatic activity.
- In contrast, reversible inhibitors bind non-covalently and different types of inhibition are produced depending on whether these inhibitors bind to the enzyme, the enzyme-substrate complex, or both.
- Enzyme inhibitors also occur naturally and are involved in the regulation of metabolism. For example, enzymes in a metabolic pathway can be inhibited by downstream products. This type of negative feedback slows the production line when products begin to build up and is an important way to maintain homeostasis in a cell.

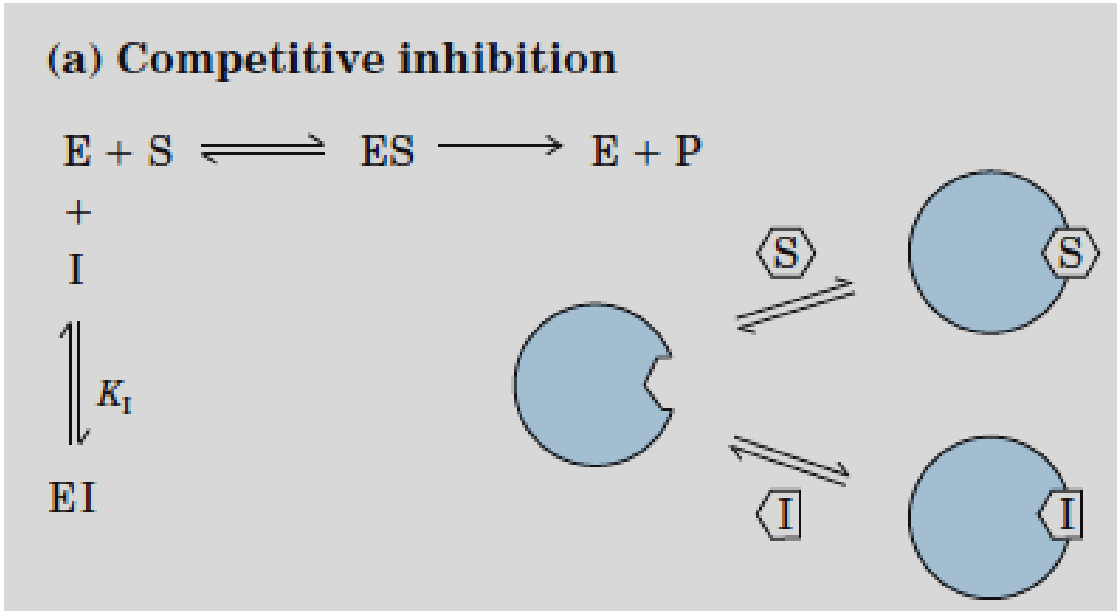
Reversible

- Competitive
- Un-competitive
- Mixed

Irreversible

Competitive inhibition

- Competitive inhibitor competes with the substrate for the active site of enzyme.
- While the inhibitor (I) occupies the active site it prevents binding of the substrate to the enzyme.
- Many competitive inhibitors are compounds that resemble the substrate, i.e. have structural similarity, and combine with the enzyme to form an EI complex, lowered progressively with the rise in inhibitor concentration.
- Combinations of this type will reduce the efficiency of the enzyme.
- The inhibitors binds with enzyme by week and dissociable bond, so that inhibitor can easily eliminated through dialysis.
- Lowering the substrate affinity, rise in Km for the relevant substrate. The Km will be Km(1+[I]/Ki), [I] is molar concentration of the inhibitor. Ki is the association constant for EI formation.
- A high concentration of substrate can overcome the effect of inhibitors and forcing out them. So at high [S], same maximum velocity can be achieved. In other word Vmax not affected by competitive inhibition.



Example:

<u>Succinate dehydrogenase</u>

S: Succinate

I: Malonate

<u>Citrate synthase</u>

S: oxaloacetate and acetyl Co-A

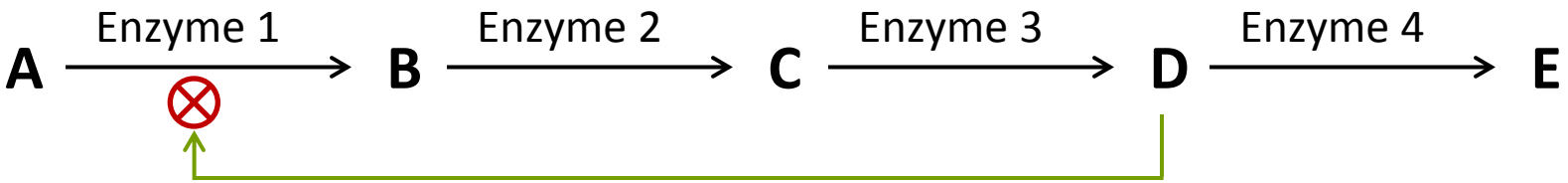
I: citrate and succinyl Co-A

<u>Aconitase</u>

S: cis-aconitate

I: trans-aconitate

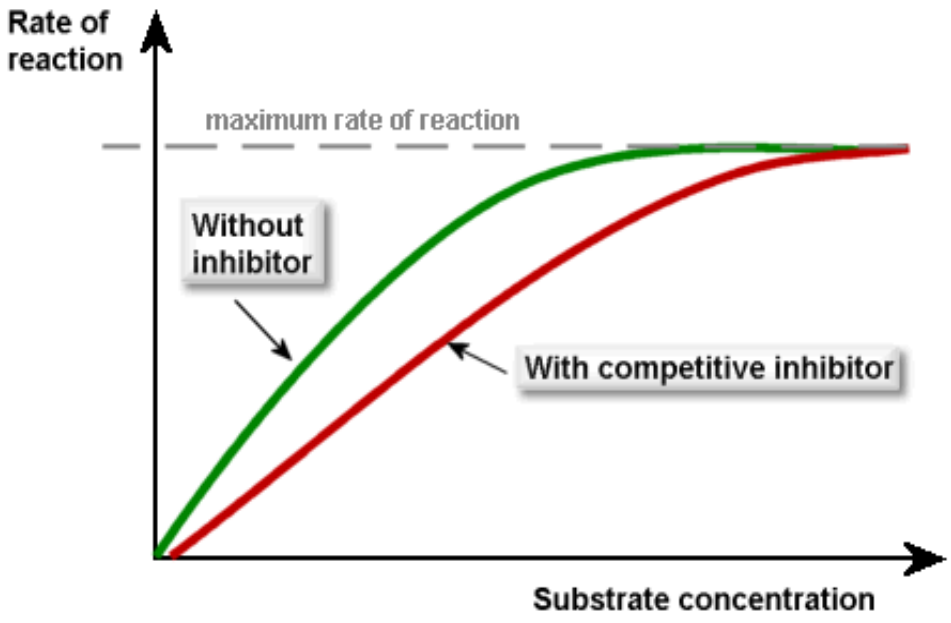
Sometimes, an intermediate/product of a metabolic pathway competitively inhibits an enzyme of an earlier step of that pathway because of its structural similarity with the substrate of that enzyme. This is known as **feedback competitive inhibition,** plays important roles in the in vivo regulation of pathway.

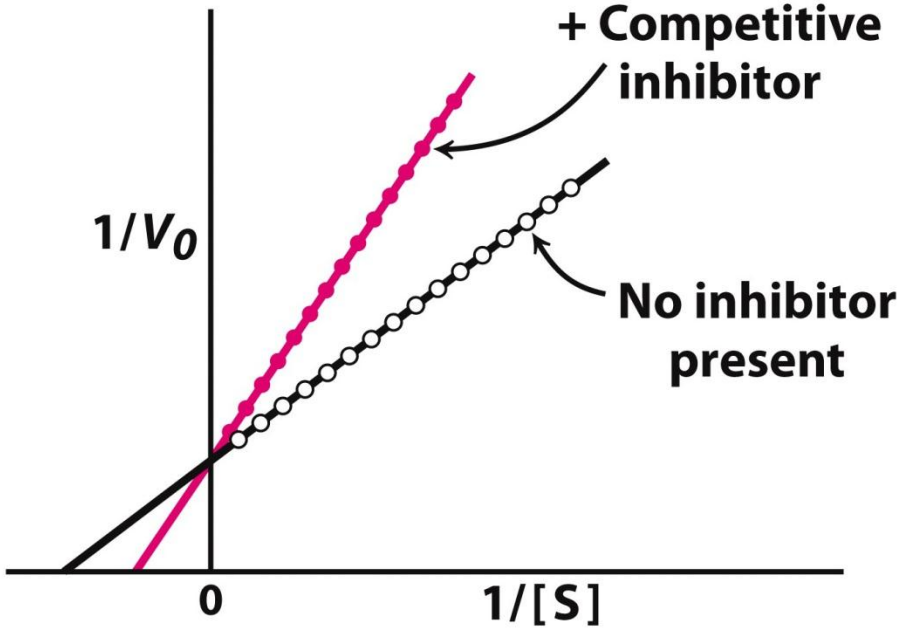


MM LB

$$V_o = \frac{V_{max}\,[S]}{K_m\left(1+\frac{[I]}{K_i}\right)+[S]} \qquad \text{Where Ki} = \frac{[E][I]}{[EI]}$$

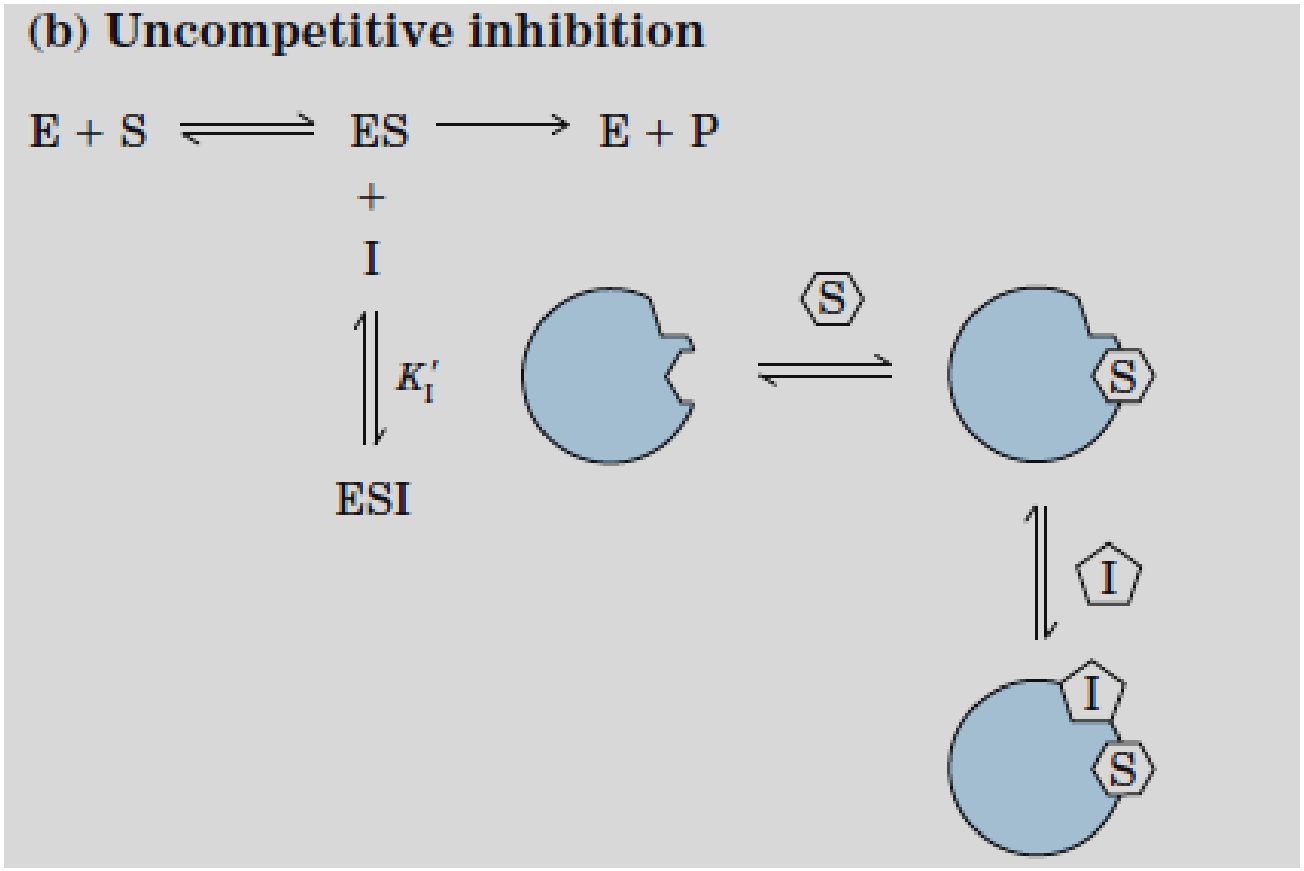
$$\frac{1}{V_o} = \frac{K_m}{V_{max}}\left(1+\frac{[I]}{K_i}\right) \text{ x } \frac{1}{[S]} + \frac{1}{V_{max}}$$





Un-competitive inhibition

- An uncompetitive inhibitor binds at a site distinct from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex.
- Inhibitors are not structurally analogous with the substrate.
- It binds only to ES complex to form ESI, and thereby changes the enzyme conformation to enhance the affinity of the enzyme for the substrate already bound with the enzyme and therefore takes longer time for the substrate or product to leave the active site. This inhibits the release of product from the enzyme, and thereby lowers both V_o and $V_{max.}$ The latter is lowered to $V_{max}/(1+[I]/Ki)$.
- Because the inhibitors enhances the substrate affinity, decreases K_m to $K_m/(1+[I]/Ki)$.
- This type of inhibition is very rare



Alkaline phosphatase extracted from rat intestine is inhibited by L-phenyl alanine

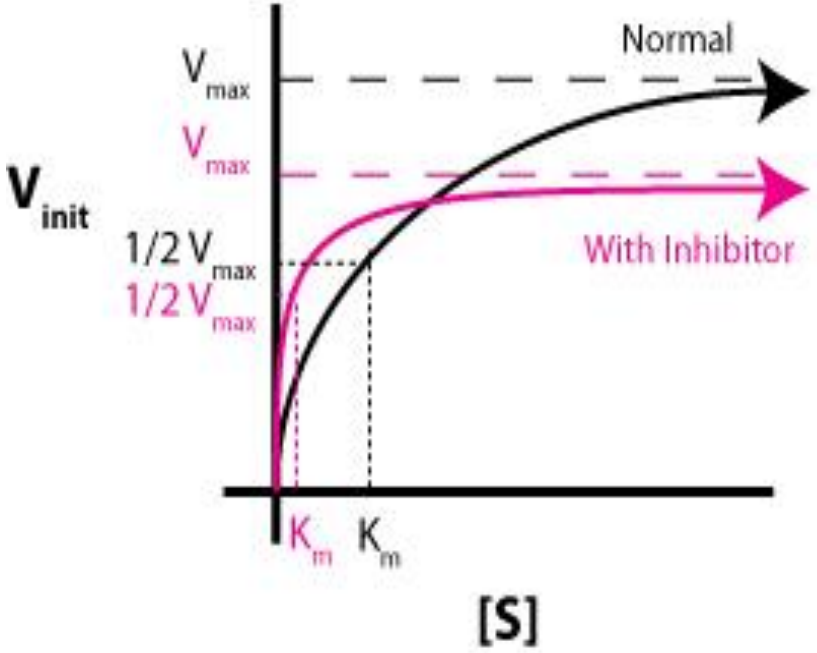
MM

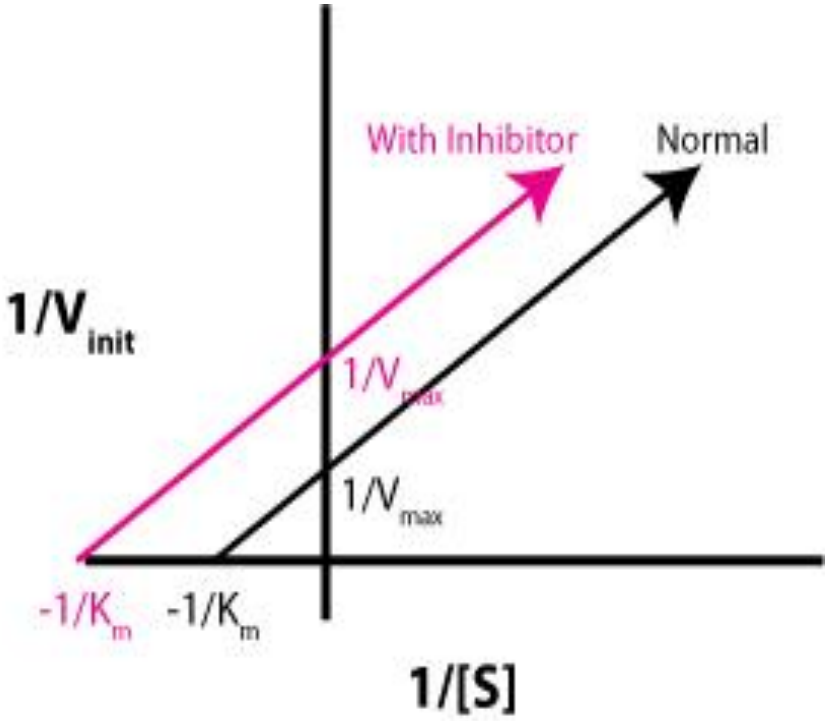
LB

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

Where Ki = $\frac{[E][I]}{[EI]}$

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

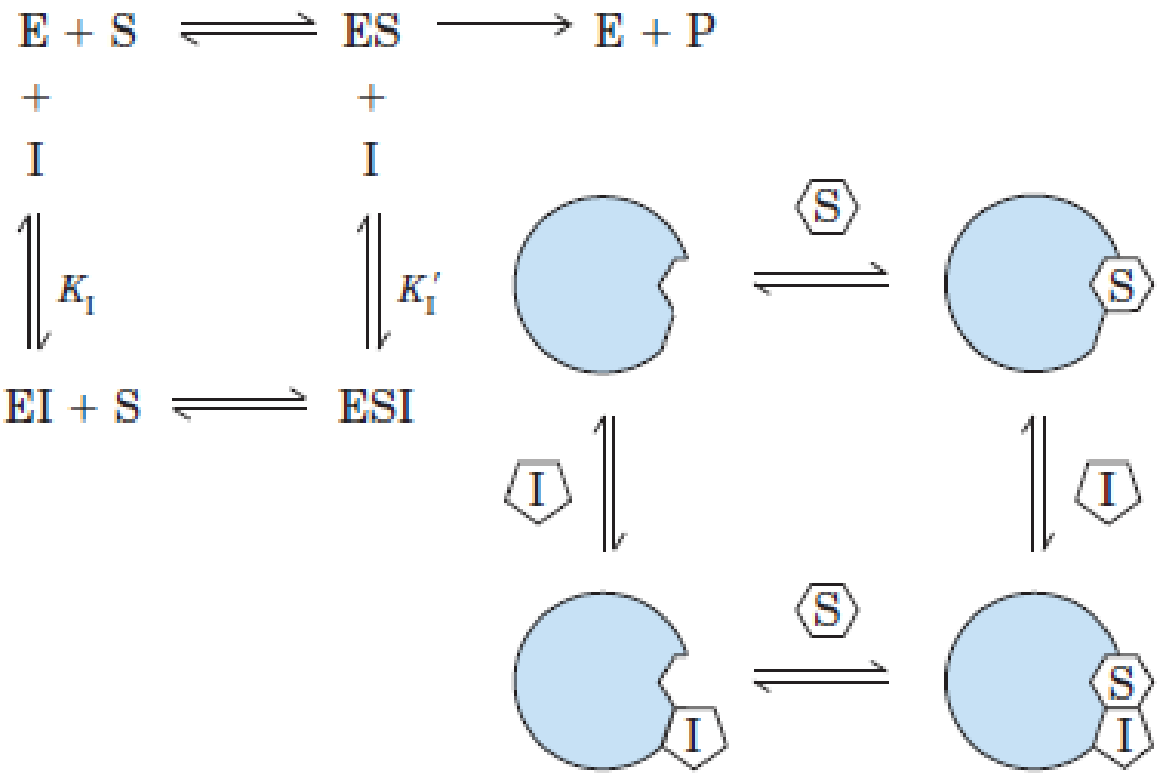




Non-competitive inhibition

- May either be reversible or irreversible.
- Inhibitors have no structural similarity with substrate.
- Binds other than substrate binding site. Its binding probably changes the 3D conformation of the enzyme resulting in failure of catalysis or release of products.
- It lower the initial velocity
- As it does not compete with the substrate, does not affect the substrate affinity, and hence Km unaltered.
- As it binds at site other than substrate binding site, it may bind with free enzyme or ES complex, and therefore termed as mixed inhibition.
- By increasing the substrate concentration, the inhibitor cannot dislodge from EI or ESI complex. So, the original Vmax cannot be regained. Thus Vmax is reduced to $V_{max}/(1+[I]/Ki)$.

(c) Mixed inhibition



- Inhibition of thiol enzyme by iodoacetate/idoacetamide, some enzyme by metal ions.

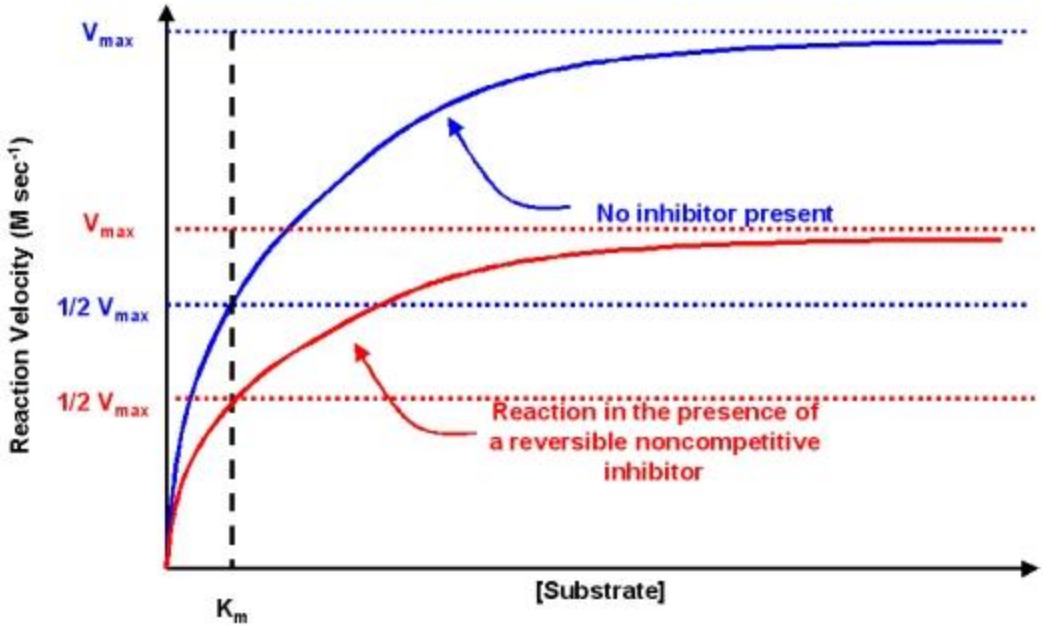
MM

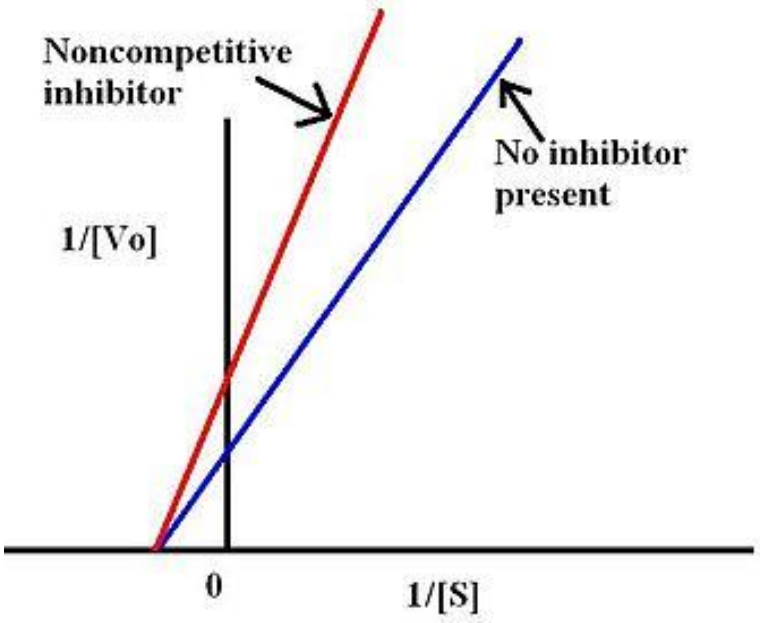
LB

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

Where Ki = $\frac{[E][I]}{[EI]}$

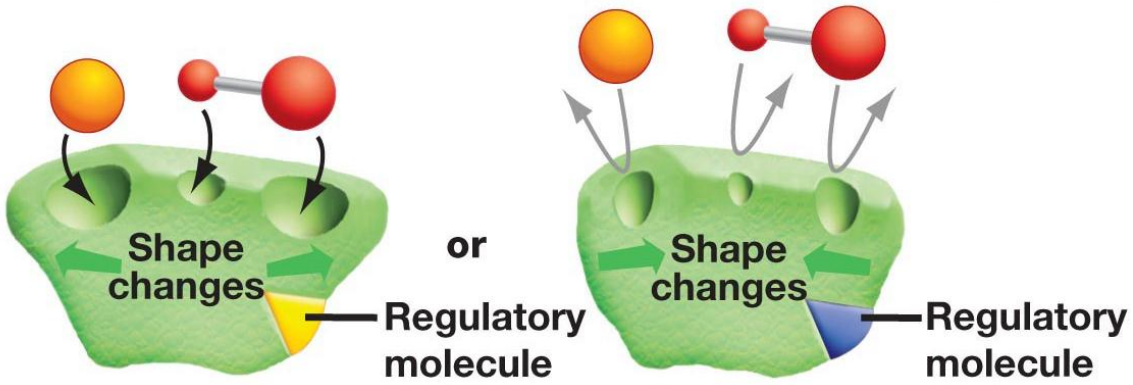
$$\frac{1}{V_o} = \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right) \times \frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$





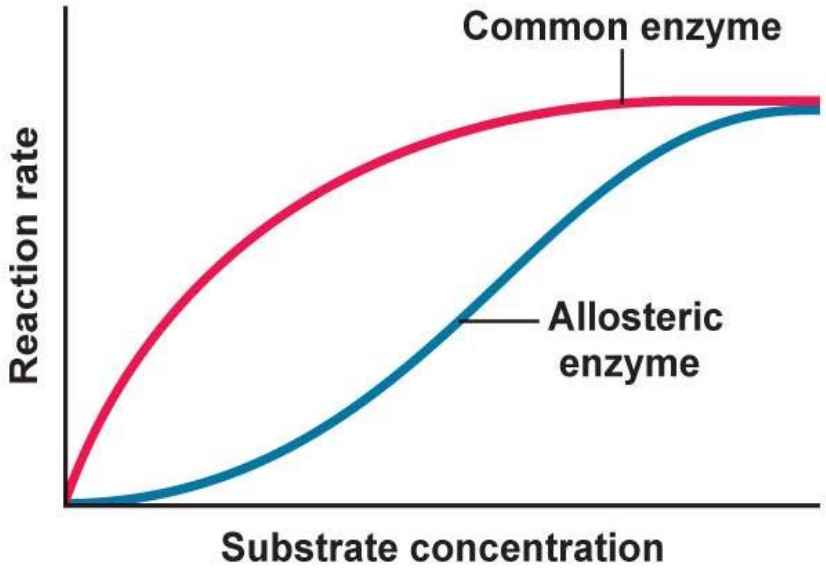
Allosteric modulation

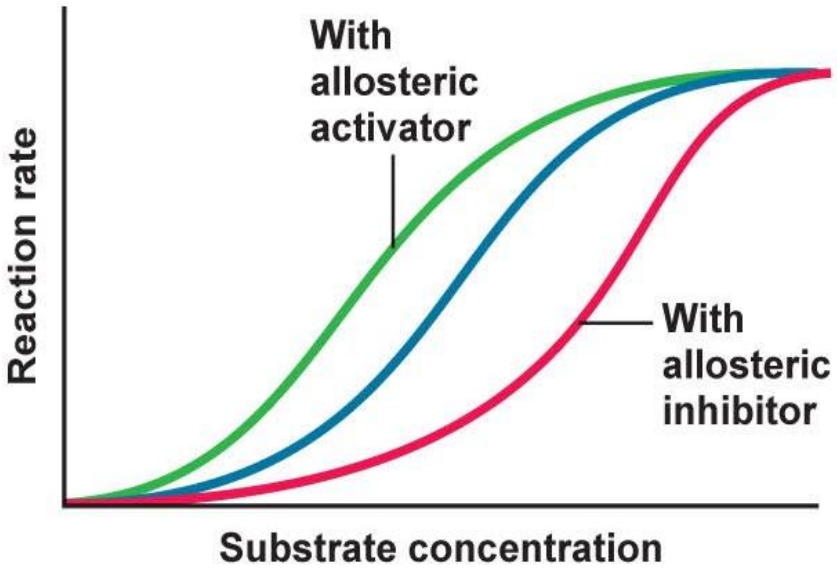
- Some enzyme are either activated or inhibited by the binding of specific low-MW ligands to specific sites of the enzyme, termed allosteric site.
- This sites are different from its active site.
- Such regulatory substances are known as allosteric modulators or effectors.
- They may be positive or negative modulator.
- The modulators play important roles in the in vivo regulation of enzyme activity.
- Sometimes, allosteric modulators protect the active site from denaturation.
- The allosteric property of an enzyme may be destroyed by mutation, radiation, mercurials, protease, high ionic strength, pH and temperature, but the enzymatic activity may still remain unaffected.
- The active site and allosteric sites may occur on different peptide subunit of oligomeric allosteric enzymes.



Allosteric activation
The active site becomes available to the substrates when a regulatory molecule binds to a different site on the enzyme.

Allosteric deactivation
The active site becomes unavailable to the substrates when a regulatory molecule binds to a different site on the enzyme.





Enzymes	Allosteric activators	Allosteric inhibitors
Acetyl-CoA carboxylase	Citrate	Palmitoyl-CoA
Aspartate transcarbamoylase		CTP
Fructose 1,6-bis phosphatase		Fructose 2,6-bisphosphate
Glycogen synthase	Glucose 6-P	
Phosphofructokinase 1	Fructose 2,6-bisphosphate	ATP
Pyruvate carboxylase	Acetyl-CoA	

- Allosteric enzymes are enzymes that change their conformation upon binding with an effector, which results in an apparent change in binding affinity at a different ligand binding site.
- Allosteric specificity: separate site of binding activator or inhibitor, may have both activator and inhibitor or only one.
- Positive and negative cooperativity of allosteric modulators with substrate.
- Allosteric activators, on binding to allosteric site, promote either the binding of substrate at active site or catalytic activity of the enzyme. Allosteric inhibitors imparts opposite effect. The cooperativity between the substrate and allosteric modulators is called heterotropic allosteric effects.

- An allosteric enzyme possesses more than one substrate binding site on its peptide subunit, and can bind to as many substrate molecules.
- Positive cooperativity between substrate of an allosteric enzyme. Bindings of one substrate molecule at one of the substrate binding site of an enzyme produces such conformational change in the enzyme that promote the binding of more substrates to the other substrate binding site of the enzyme. This phenomenon called homotropic effect.
- Follow sigmoidal kinetics instead hyperbola.
- Often an intermediate or product of a metabolic pathway allosterically inhibits an enzyme catalyzing an earlier step of that pathway, termed allosteric feedback inhibition.
- Classification:
  - K-series: Changes the Km not Vo or Vmax
  - V-series: Changes the Vmax/Vo but not Km

$$Vo = \frac{Vmax\ [S]^n}{K' + [S]^n}$$

K’ constant different from Km of MM equation

n is Hill co-efficient. The value of n is determined by the number of substrate-binding site on an enzyme molecule and the number, strength and types of interaction between those substrate binding site.

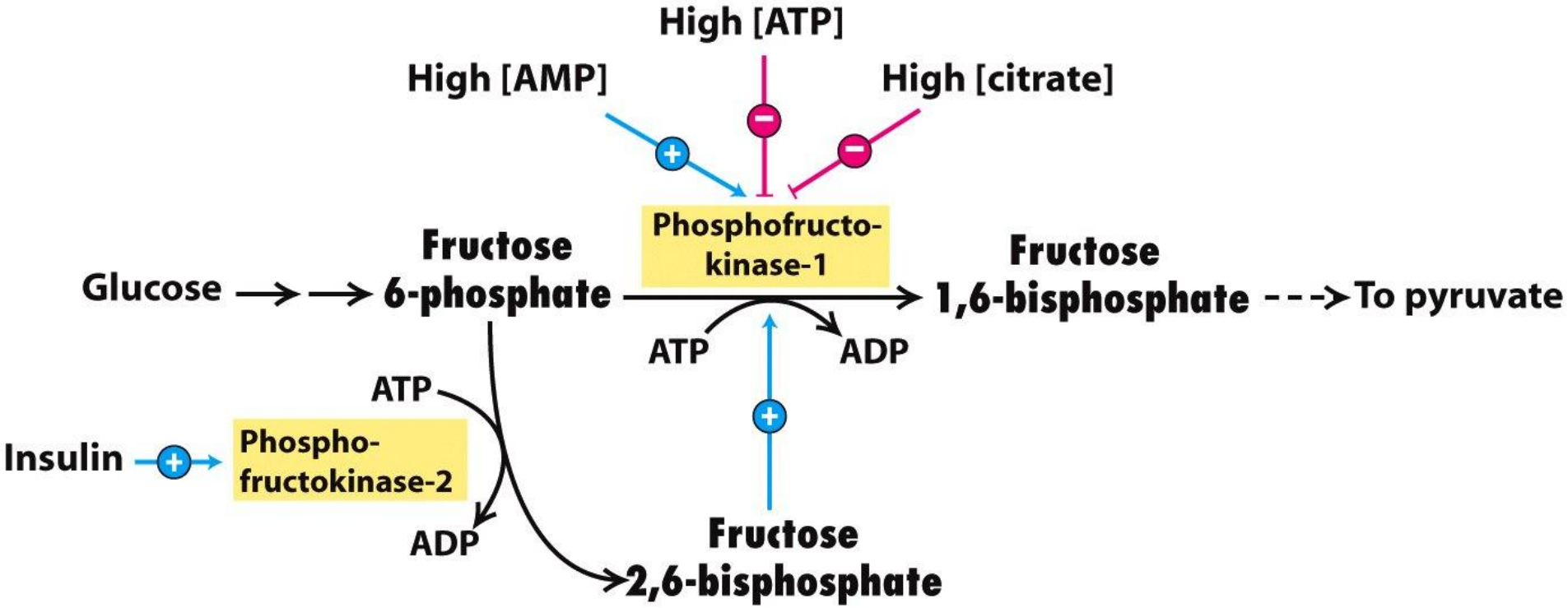
n>1 ***: Positively cooperative binding***: Once one ligand molecule is bound to the enzyme, its affinity for other ligand molecules increases.

n<1 ***: Negatively cooperative binding***: Once one ligand molecule is bound to the enzyme, its affinity for other ligand molecules decreases.

n=1 : ***Non-cooperative binding***

Rate Limiting Step

- In chemical kinetics, the rate (or velocity) of a reaction mechanism with several steps is often determined by the slowest step, known as the rate-determining step (RDS) or rate-limiting step.
- Have least affinity for substrate, and therefore the step is slow.
- In a reaction coordinate, the transition state with the highest energy is the rate-determining step of a given reaction.
- Prone to inhibit (or activate) by ligands.
- Ideally takes part in the early step of metabolic processes.
- Example: Phosphofructokinase1 of EMP pathway, citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase complex for TCA cycle


High [ATP]
High [AMP]
High [citrate]
Phosphofructo-
kinase-1
Fructose
6-phosphate
Fructose
1,6-bisphosphate
Glucose
To pyruvate
ATP
ADP
ATP
Insulin
Phospho-
fructokinase-2
ADP
Fructose
2,6-bisphosphate

Isozymes

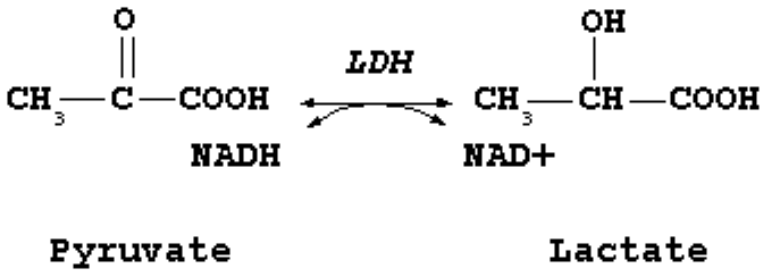
- **Isozymes** were first described by R. L. Hunter and Clement Markert (1957) who defined them as different variants of the same enzyme having identical functions and present in the same individual.
- Isozymes (also known as isoenzymes or more generally as Multiple forms of enzymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction.
- These enzymes usually display different kinetic parameters (e.g. different Km values), or different regulatory properties.
- They usually differ on the basis of their 3D structure, electrophoretic mobility, immunogenic properties, pH and temp. optimum, relative susceptibility to inhibitors, degree of denaturation.
- The existence of isozymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage.

- Synthesized from different genes. Oligomeric in nature.

- An example of an isozyme is glucokinase, a variant of hexokinase which is not inhibited by glucose 6-phosphate.

- The enzyme Lactate Dehydrogenase is made of two(H-form and M-Form) different sub units, combines in different Permutations and Combinations in depending on the tissue in which it is present

Type	Composition	Location
LDH1	HHHH	Heart and Erythrocyte
LDH2	HHHM	Heart and Erythrocyte
LDH3	HHMM	Brain and Kidney
LDH4	HMMM	Skeletal Muscle and Liver
LDH5	MMMM	Skeletal Muscle and Liver



- H4 isozyme is formed under condition of plentiful oxygen and show optimum action under aerobic metabolism, predominantly in adult stage
- M4 isozyme formed under low oxygen tension and show optimal activity in anaerobic metabolism, predominantly in embryonic stage

- They are separated on the basis of their properties

- In oxygen depleted muscle, NAD+ is regenerated in the lactate dehydrogenase reaction. Active muscle tissue becomes anaerobic and produces pyruvate from glucose via glycolysis.

- It needs LDH to regenerate NAD+ from NADH, so glycolysis can continue. The lactate produced is released into blood.

- The muscle LDH isozyme (M4) works best in the NAD+ regenerating direction.

- Heart tissue is aerobic and uses lactate as a fuel, converting it to pyruvate via LDH and using the pyruvate to fuel the citric acid cycle to obtain energy. The heart LDH isozyme is inhibited by excess pyruvate, so the fuel won't be wasted.

Hexokinase:

- Type I to Type IV: according to electrophoretic mobility
- Type IV have high Km for glucose, known as glucokinase.
- Type I: Brain, heart and kidney; Type II: Skeletal muscle and adipose tissue; Type III: Liver and spleen; Type IV: Liver

Co-enzyme and Co-factor

- A cofactor is a non-protein chemical compound that is required for the protein's biological activity.
- Cofactors can be subdivided into either one or more inorganic ions, or a complex organic or metalloorganic molecule called a coenzyme; most of which are derived from vitamins.
- A cofactor that is tightly or even covalently bound is termed a prosthetic group.
- An inactive enzyme without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is called a holoenzyme [Apoenzyme + cofactor = Holoenzyme].

Name of the conjugate	Nature of the conjugate	Attachment to apoenzyme
Coenzyme	Non-protein organic molecule	Loosely bound
Prosthetic group	Non-protein organic molecule	Tightly bound
Metal-activated enzyme	Inorganic metal ions	Loosely bound
Metalloenzyme	Inorganic metal ions	Tightly bound

Classification

- Cofactors can be divided into two broad groups: organic cofactors, such as flavin or heme, and inorganic cofactors, such as the metal ions Mg^{2+}, Cu^{+}, Mn^{2+}, or iron-sulfur clusters.
- Organic cofactors are sometimes further divided into coenzymes and prosthetic groups. The term coenzyme refers specifically to enzymes and, as such, to the functional properties of a protein. On the other hand, "prosthetic group" emphasizes the nature of the binding of a cofactor to a protein (tight or covalent) and, thus, refers to a structural property.
- Inorganic: metal ions, iron-sulfer clusters
- Organic: vitamin-derived and non-vitamin derived

Ion	Examples of enzymes containing this ion
Cupric	Cytochrome oxidase
Ferrous or Ferric	Catalase Cytochrome (via Heme) Nitrogenase Hydrogenase
Magnesium	Glucose 6-phosphatase Hexokinase DNA polymerase
Manganese	Arginase
Molybdenum	Nitrate reductase Nitrogenase
Nickel	Urease
Zinc	Alcohol dehydrogenase Carbonic anhydrase DNA polymerase

Cofactor	Vitamin	Additional component	Chemical group(s) transferred
Thiamine pyrophosphate [25]	Thiamine (B_1)	None	2-carbon groups, α cleavage
NAD^+ and $NADP^+$ [26]	Niacin (B_3)	ADP	Electrons
Pyridoxal phosphate [27]	Pyridoxine (B_6)	None	Amino and carboxyl groups
Methylcobalamin [28]	Vitamin B_{12}	Methyl group	acyl groups
Cobalamine [4]	Cobalamine (B_{12})	None	hydrogen, alkyl groups
Biotin [29]	Biotin (H)	None	CO_2
Coenzyme A [30]	Pantothenic acid (B_5)	ADP	Acetyl group and other acyl groups
Tetrahydrofolic acid [31]	Folic acid (B_9)	Glutamate residues	Methyl, formyl, methylene and formimino groups
Menaquinone [32]	Vitamin K	None	Carbonyl group and electrons
Ascorbic acid [33]	Vitamin C	None	Electrons

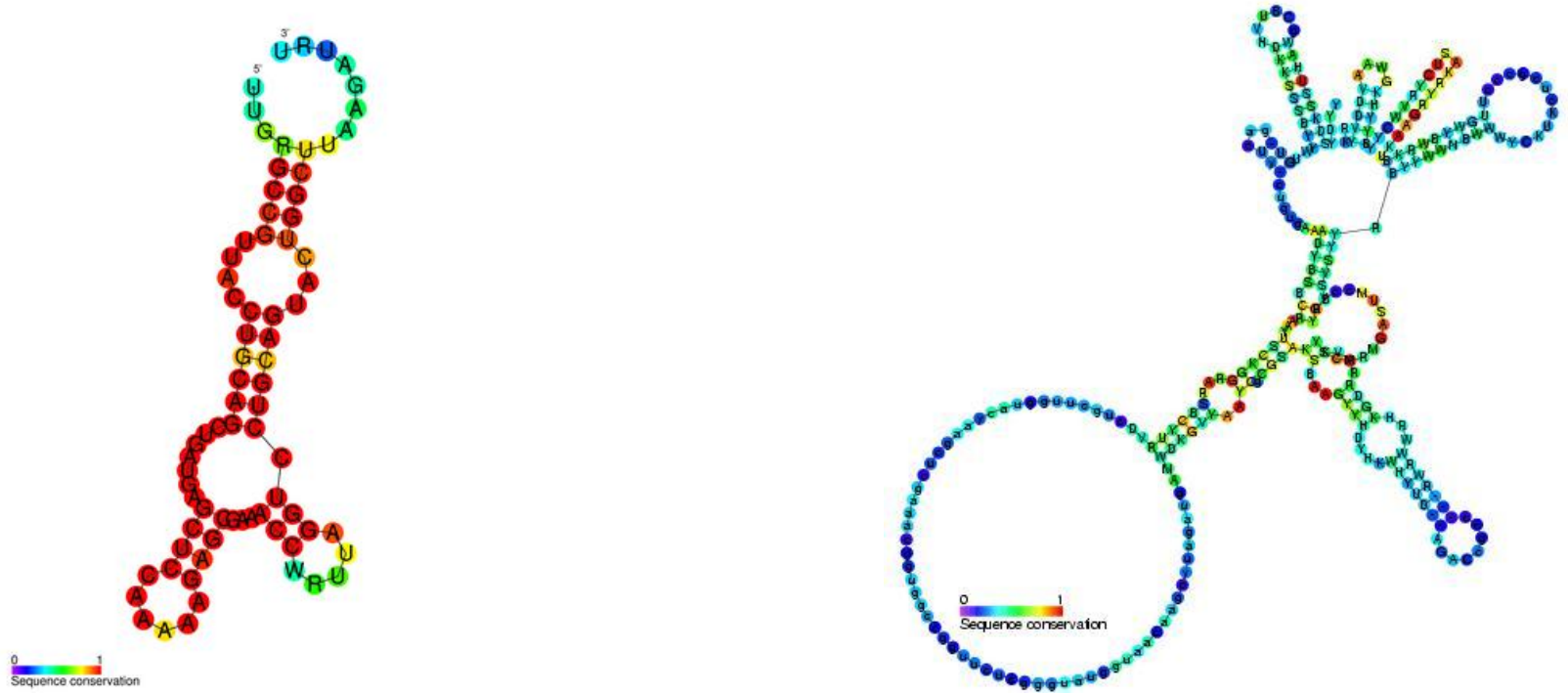
Non-vitamins [edit]

Cofactor	Chemical group(s) transferred
Adenosine triphosphate [36]	Phosphate group
S-Adenosyl methionine [37]	Methyl group
Coenzyme B [38]	Electrons
Coenzyme M [39][40]	Methyl group
Coenzyme Q [41]	Electrons
Cytidine triphosphate [42]	Diacylglycerols and lipid head groups
Glutathione [43][44]	Electrons
Heme [45]	Electrons
Lipoamide [4]	Electrons, acyl groups
Methanofuran [46]	Formyl group
Molybdopterin [47][48]	Oxygen atoms
Nucleotide sugars [49]	Monosaccharides
3'-Phosphoadenosine-5'-phosphosulfate [50]	Sulfate group
Pyrroloquinoline quinone [51]	Electrons
Tetrahydrobiopterin [52]	Oxygen atom and electrons
Tetrahydromethanopterin [53]	Methyl group

Non-protein enzyme: Ribozyme

- A ribozyme (ribonucleic acid enzyme) is an RNA molecule that is capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes.
- The first ribozymes were discovered in the 1980s by Thomas R. Cech, who was studying RNA splicing in the ciliated protozoan *Tetrahymena thermophila* and Sidney Altman, who was working on the bacterial RNase P complex.
- These ribozymes were found in the intron of an RNA transcript, which removed itself from the transcript, as well as in the RNA component of the RNase P complex.
- In 1989, Thomas R. Cech and Sidney Altman won the Nobel Prize in chemistry for their "discovery of catalytic properties of RNA."
- The discovery of ribozymes demonstrated that RNA can be both genetic material (like DNA) and a biological catalyst (like protein enzymes), and contributed to the RNA world hypothesis.

- The study of posttranscriptional processing of RNA molecules led to one of the most exciting discoveries in modern biochemistry—the existence of RNA enzymes
- The best-characterized ribozymes are the self-splicing group I introns, RNase P, and the hammerhead ribozyme, hairpin ribozyme
- Many ribozymes have either a hairpin – or hammerhead – shaped active center and a unique secondary structure that allows them to cleave other RNA molecules at specific sequences
- Ribozymes vary greatly in size. A self-splicing group I intron may have more than 400 nucleotides. The hammerhead ribozyme consists of two RNA strands with only 41 nucleotides in all.



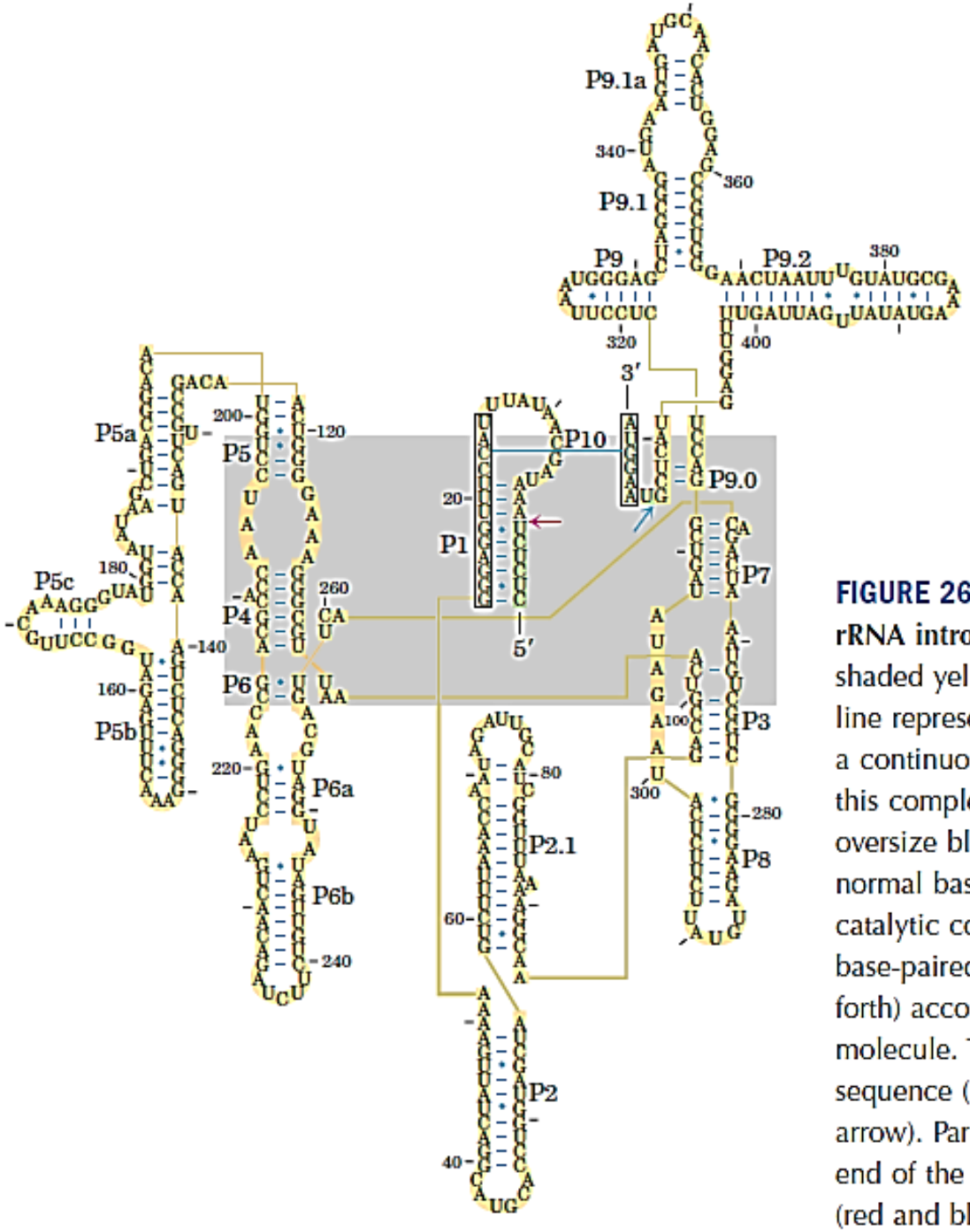


FIGURE 26–26 Secondary structure of the self-splicing rRNA intron from *Tetrahymena*. Intron sequences are shaded yellow, exon sequences green. Each thick yellow line represents a bond between neighboring nucleotides in a continuous sequence (a device necessitated by showing this complex molecule in two dimensions; similarly an oversize blue line between a C and G residue indicates normal base pairing); all nucleotides are shown. The catalytic core of the self-splicing activity is shaded. Some base-paired regions are labeled (P1, P3, P2.1, P5a, and so forth) according to an established convention for this RNA molecule. The P1 region, which contains the internal guide sequence (boxed), is the location of the 5′ splice site (red arrow). Part of the internal guide sequence pairs with the end of the 3′ exon, bringing the 5′ and 3′ splice sites (red and blue arrows) into close proximity. The three-

Enzymatic Properties of Group I Introns

- Binding of the guanosine cofactor group I rRNA intron is saturable.
- Splicing of group I introns is processed by two sequential ester-transfer reactions.
- The exogenous guanosine (exoG) first docks onto the active G-binding site located in P7, and its 3'-OH is aligned to attack the phosphodiester bond at the 5' splice site located in P1, resulting in a free 3'-OH group at the upstream exon and the exoG being attached to the 5' end of the intron.
- Then the terminal G of the intron swaps the exoG and occupies the G-binding site to organize the second ester-transfer reaction: the 3'-OH group of the upstream exon in P1 is aligned to attack the 3' splice site in P10, leading to the ligation of the adjacent upstream and downstream exons and release of the catalytic intron.