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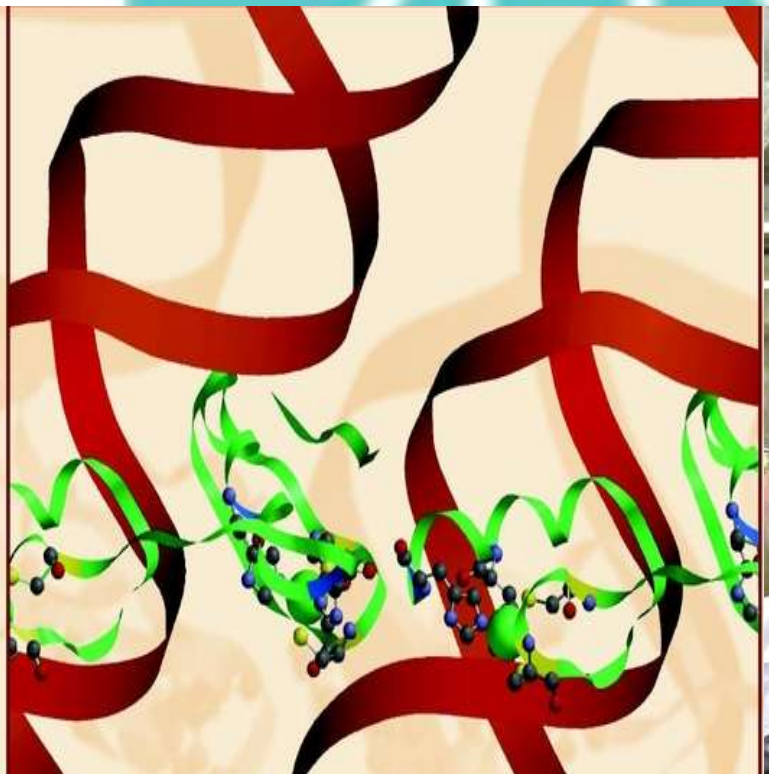
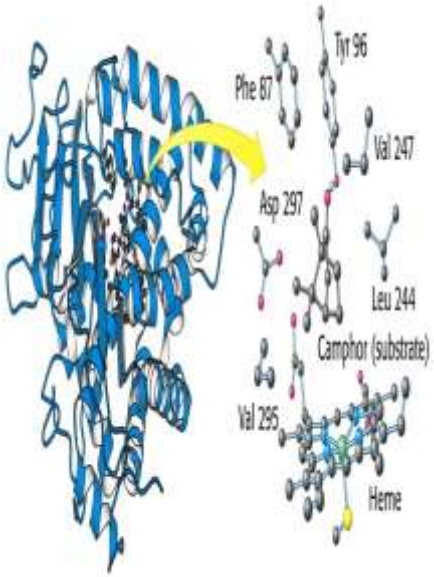


Enzymes
Catalysis,
Mechanism and Inhibition

Ehssan Alobaidy
PhD Clinical Biochemistry



Enzymes as catalysts

- ***It is necessary for biological reactions to occur much quicker than the ambient.***

  - **"Catalyst" a substance that when added to a chemical reaction, speeds it up without altering the final products or without itself being consumed.**

  - **Enzymes are biological catalysts**

Enzymes accelerate Reactions

Carbonic anhydrase: **an enzyme in the blood hydrating CO_2**

Transfer of CO_2 from the **tissue -> blood -> release** into air
-> one of the fastest enzymes -> hydrates 10^6 molecules/sec

TABLE 8.1 Rate enhancement by selected enzymes

Enzyme	Nonenzymatic half-life		Uncatalyzed rate ($k_{un}s^{-1}$)	Catalyzed rate ($k_{cat}s^{-1}$)	Rate enhancement ($k_{cat}s^{-1}/k_{un}s^{-1}$)
OMP decarboxylase	78,000,000	years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000	years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000	years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3	years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7	weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9	days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4	hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5	seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wofenden. *Science* 267 (1995):90–93.

Table 8-1
Biochemistry, Sixth Edition

Niacin is a precursor to nicotinamide

Enzymes require Cofactors

Apo enzyme + cofactor -> Holo enzyme

TABLE 8.2 Enzyme cofactors

Cofactor	Enzyme
Coenzyme	
Thiamine pyrophosphate	Pyruvate dehydrogenase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn	Superoxide dismutase
K^{+}	Propionyl CoA carboxylase

Table 8-2
Biochemistry, Sixth Edition

Niacin is a precursor to nicotinamide

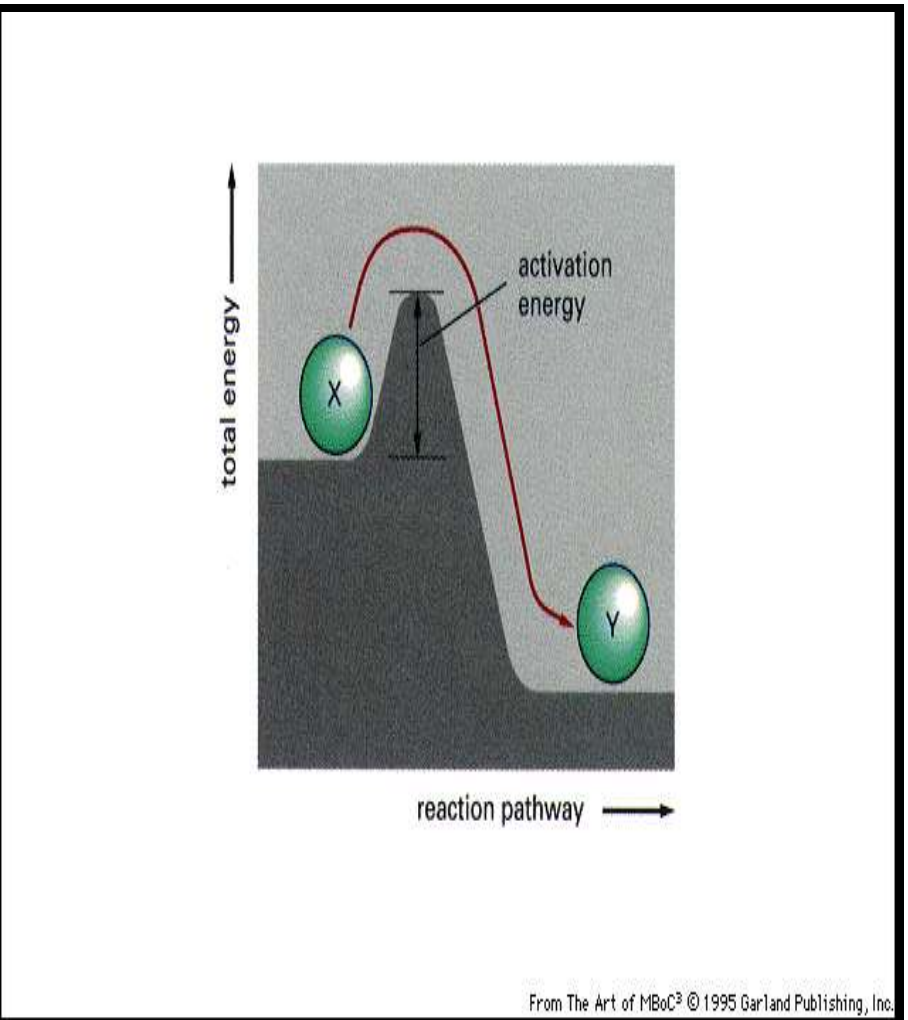
NAD+as Cofacter Hartnup disease pellagra-like dermatosis"

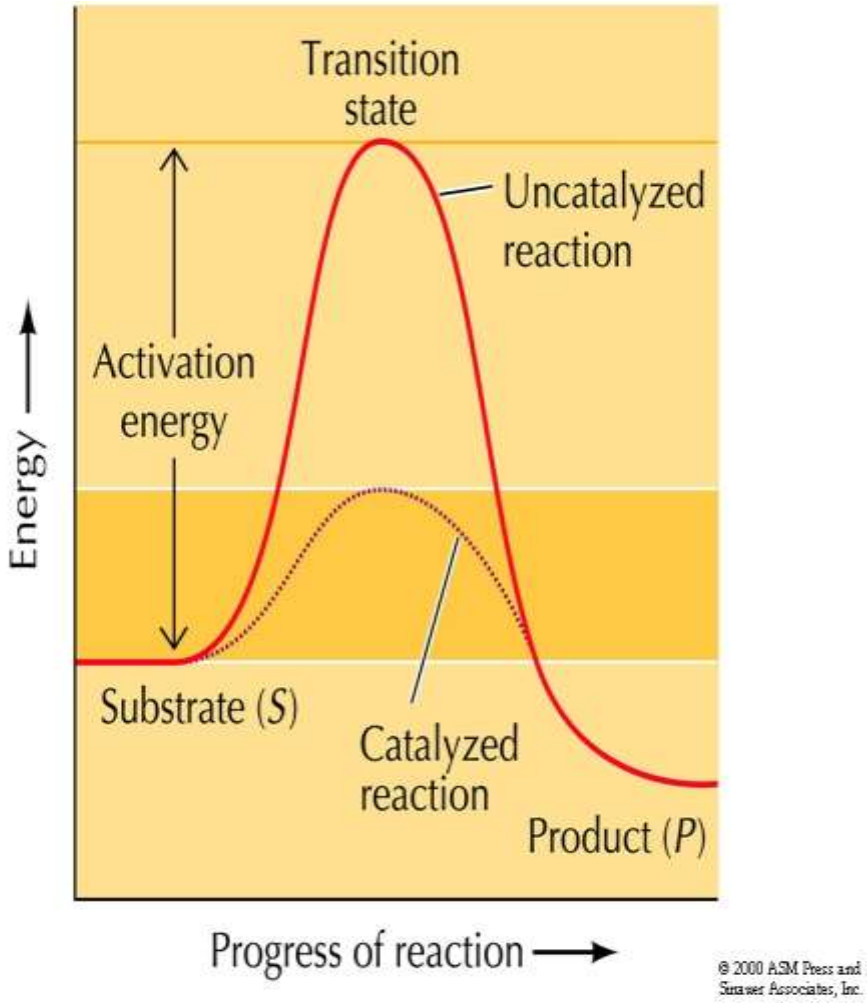
The enzyme classification(EC number)

TABLE 8.3 Six major classes of enzymes

Class	Type of reaction	Example	Chapter
1. Oxidoreductases	Oxidation-reduction	Lactate dehydrogenase	16
2. Transferases	Group transfer	Nucleoside monophosphate kinase (NMP kinase)	9
3. Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	Chymotrypsin	9
4. Lyases	Addition or removal of groups to form double bonds	Fumarase	18
5. Isomerases	Isomerization (intramolecular group transfer)	Triose phosphate isomerase	16
6. Ligases	Ligation of two substrates at the expense of ATP hydrolysis	Aminoacyl-tRNA synthetase	29

- **Enzymes DO NOT change the equilibrium constant of a reaction**
- **Enzymes DO NOT alter the amount of energy consumed or liberated in the reaction (standard free energy change, □G°)**
- **Enzymes DO increase the rate of reactions that are otherwise possible**
- **Enzymes DO decrease the activation energy of a reaction (□G°‡)**



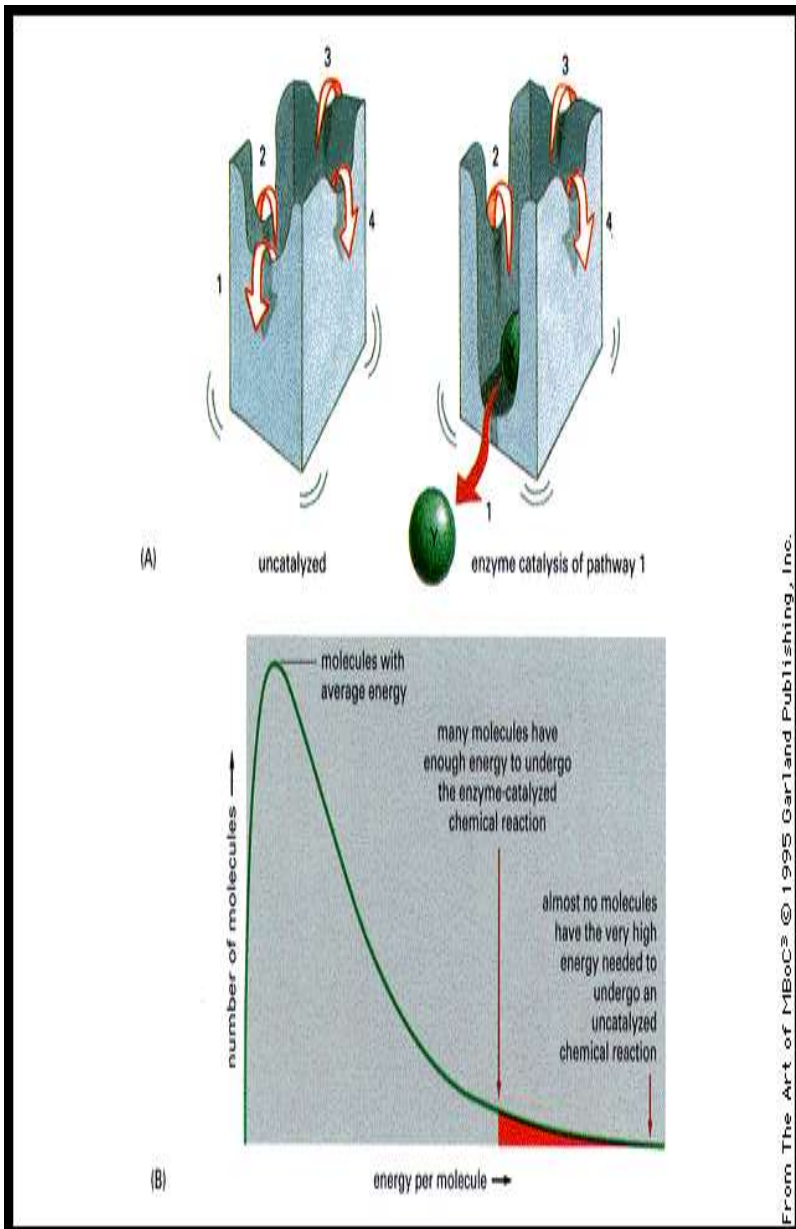


- **Enzymes DO increase the rate of reactions that are otherwise possible**
- **Enzymes DO decrease the activation energy of a reaction (□G°‡)**

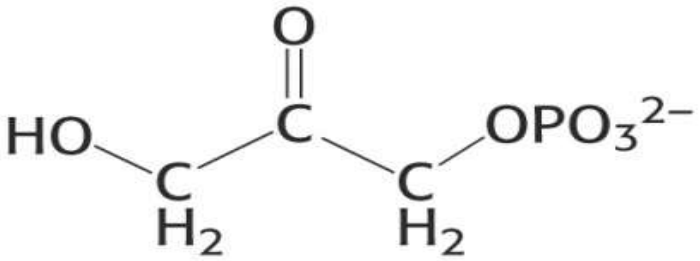
. In order for a reaction to take place between two molecules, the molecules must first find each other.

-This is why the rate of a reaction is dependent upon the concentrations of the reactants, since there is a higher probability that two molecules will collide at high concentrations.

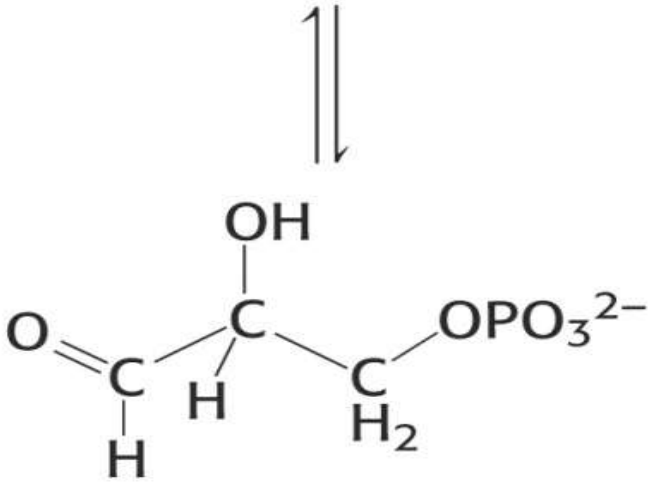
The enzyme organizes the reaction at the active site, thereby reducing the cost in terms of ENTROPY.



Example: conversion of DHAP into GAP

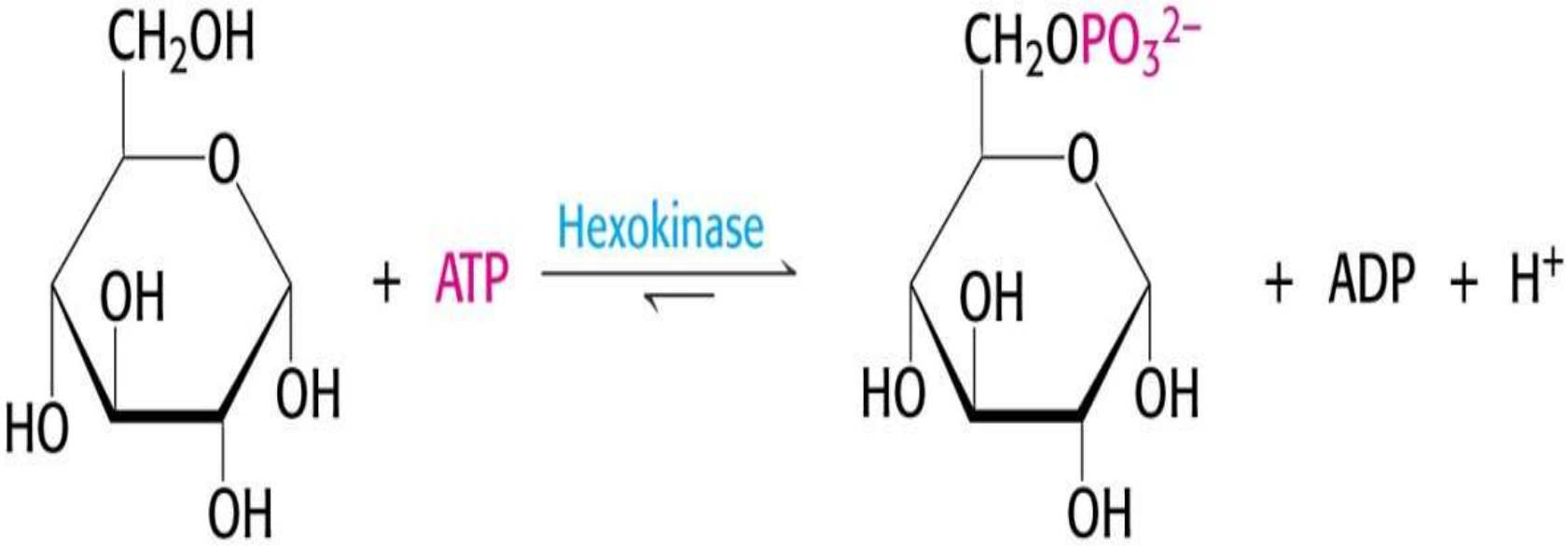


Dihydroxyacetone phosphate (DHAP)



Glyceraldehyde 3-phosphate (GAP)

Enzymes reactions



Glucose

Glucose 6-phosphate (G-6P)

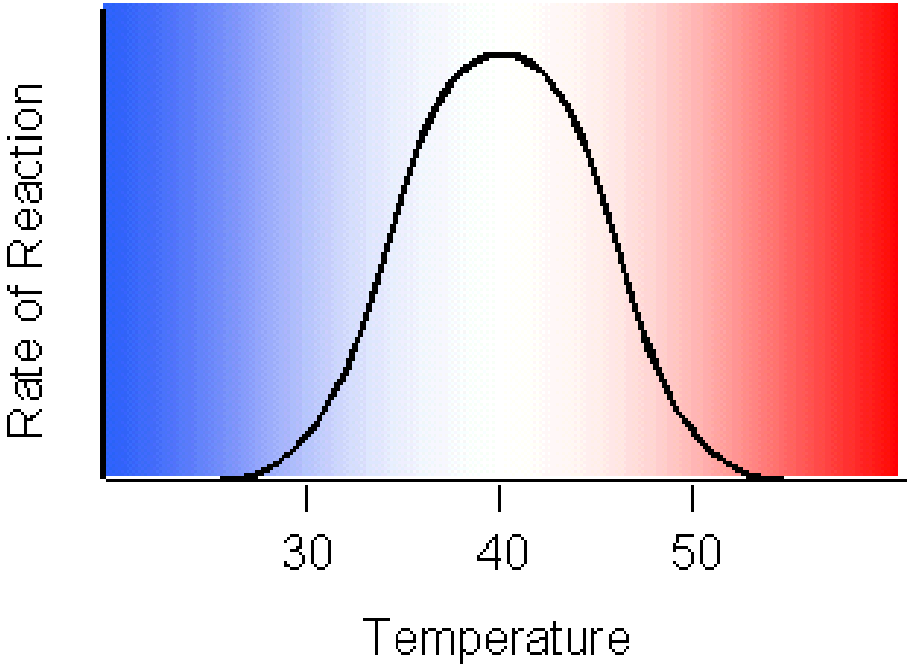
- **Enzymes provide many medical benefits**

  - ***key to understanding inborn errors of metabolism***
  - ***important in reactions***
  - ***targets of chemotherapy***
  - ***aid in diagnosis and monitoring therapy***
  - ***primary role of vitamins is as enzyme cofactors***
  - ***key to metabolic control and balance***

Enzyme properties

- **All enzymes are proteins**
  - *Molecular Weight range: 15 kd-1000 kd*
  - *enzymes show the same physical and chemical properties as all proteins*
    - *Denaturation by T and pH*
    - *Precipitation by heavy metals and salts*
    - *sensitivity to proteases..Inhibitors*
- **Enzymes are efficient biological catalysts which must operate at** 37^{o} C or below and at pH values **found in living cells ,Sastrate conc. and [S]conc.**

Factors Affecting Enzyme Activity

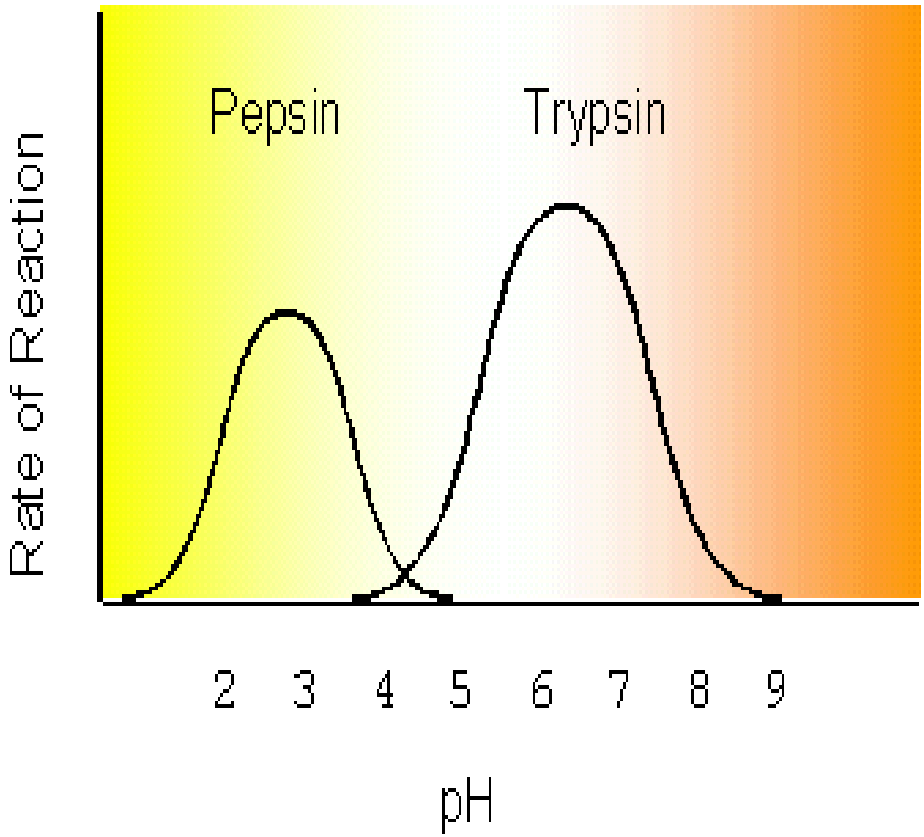


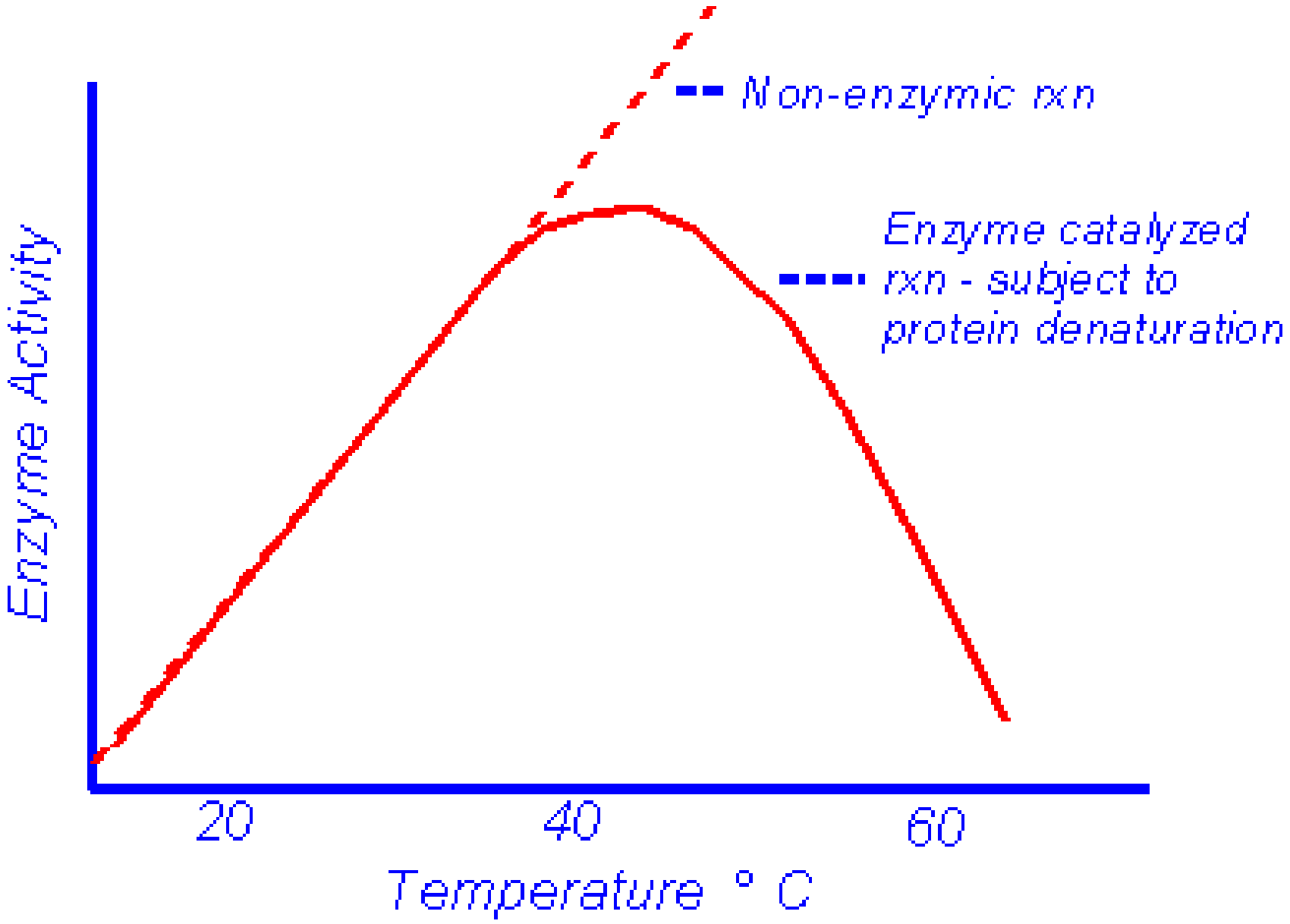
Temp

- are most active at an *optimum temperature* (usually 37 °C in humans)
- show little activity at low temperatures
- lose activity at high temperatures as denaturation occurs

pH

- are most active at *optimum pH*
- contain R groups of amino acids with proper charges at optimum pH
- lose activity in low or high pH as tertiary structure is disrupted




Non-enzymic rxn
Enzyme catalyzed rxn - subject to protein denaturation
Enzyme Activity
20
40
60
Temperature ° C

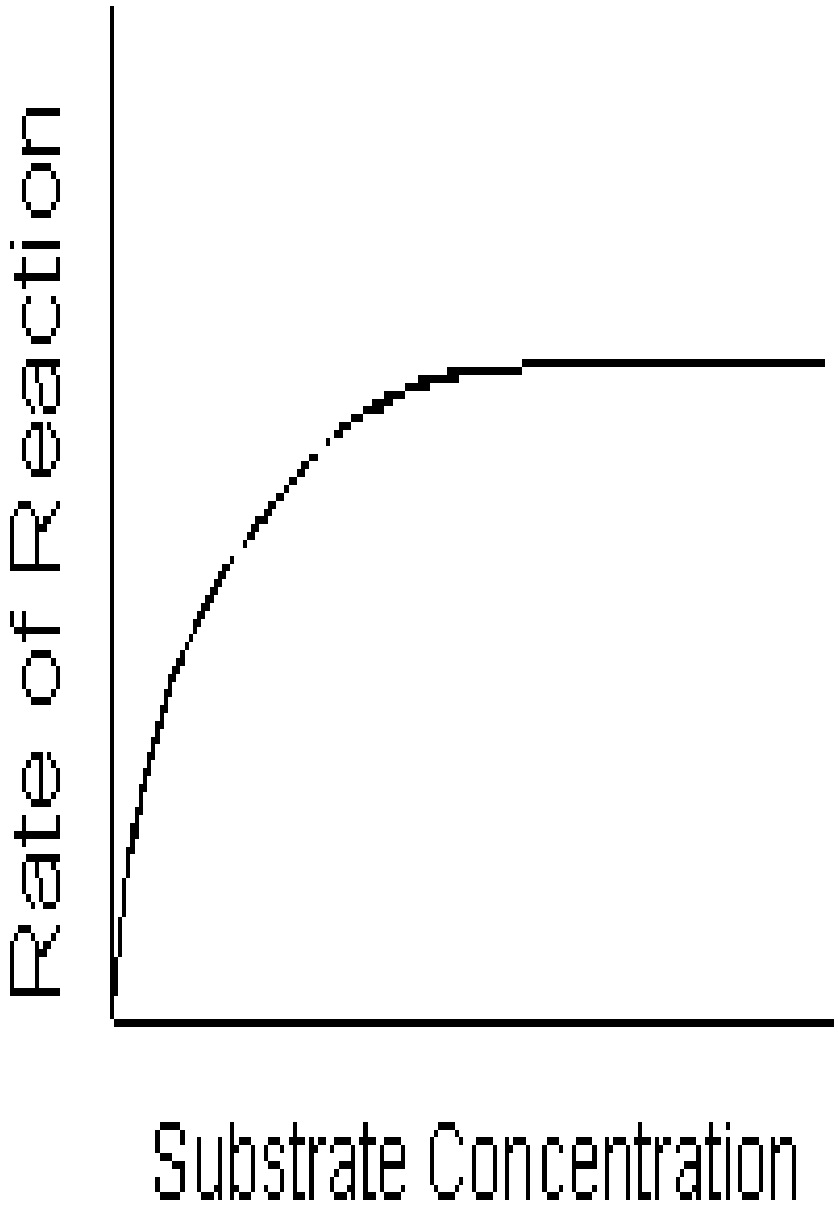
Optimum pH Values

. Enzymes in the body have an optimum pH of about 7.4

- certain have enzymes that operate at lower and higher optimum pH values

TABLE 20.5 Optimum pH for Selected Enzymes

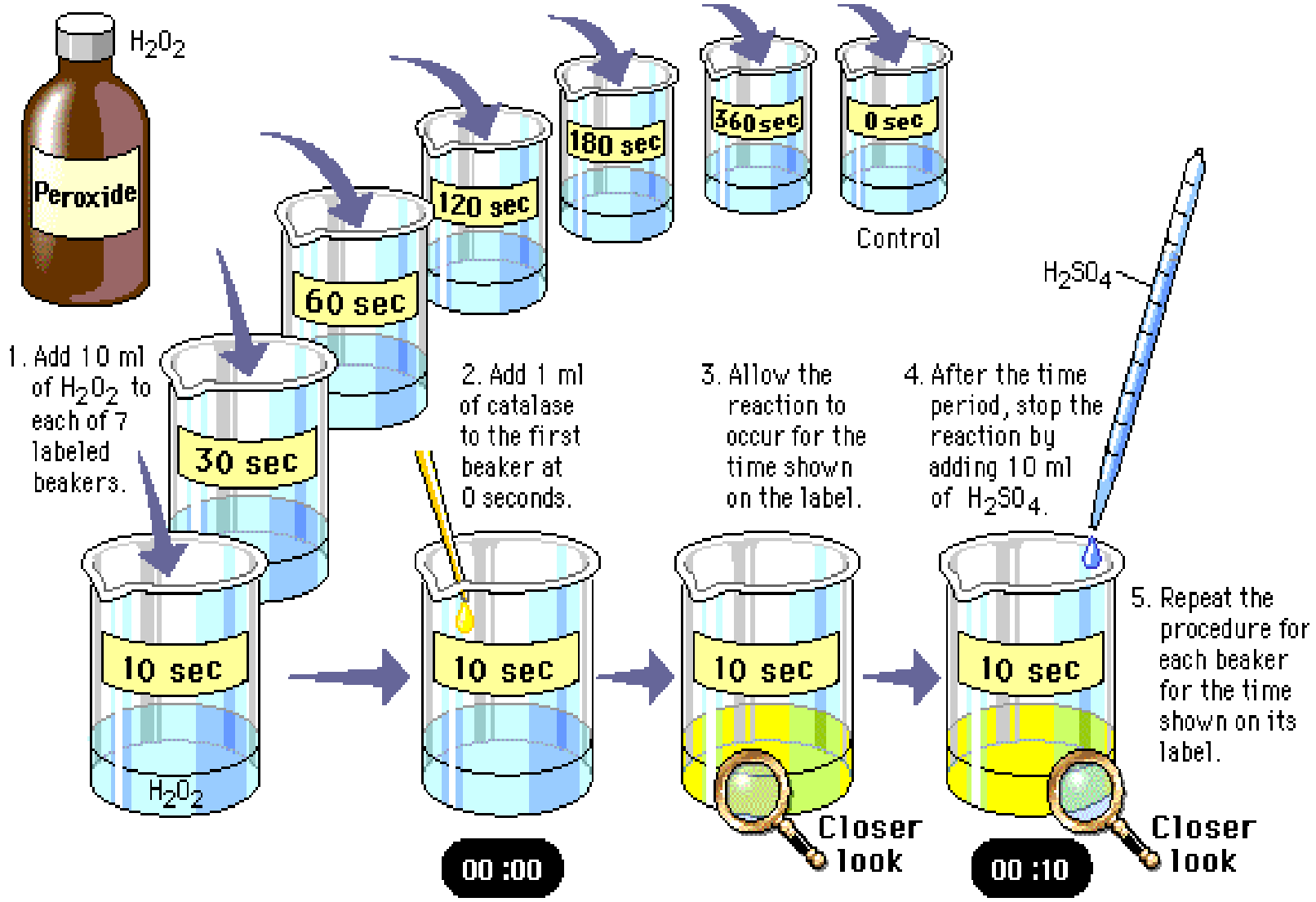
Enzyme	Location	Substrate	Optimum pH
Pepsin	Stomach	Peptide bonds	1.5–2.0
Sucrase	Small intestine	Sucrose	6.2
Amylase	Pancreas	Amylose	6.7–7.0
Urease	Liver	Urea	7.0
Trypsin	Small intestine	Peptide bonds	7.7–8.0
Lipase	Pancreas	Lipid (ester bonds)	8.0
Arginase	Liver	Arginine	9.7



[S]

…An increase in *substrate concentration*

- **increases the *rate of reaction* (at constant enzyme concentration)**
- **eventually saturates an enzyme with substrate to give maximum activity**


H_2O_2
Peroxide
360 sec
0 sec
180 sec
120 sec
60 sec
30 sec
10 sec
Control
1. Add 10 ml of H_2O_2 to each of 7 labeled beakers.
H_2O_2
2. Add 1 ml of catalase to the first beaker at 0 seconds.
00 :00
3. Allow the reaction to occur for the time shown on the label.
Closer look
4. After the time period, stop the reaction by adding 10 ml of H_2SO_4.
H_2SO_4
00 :10
Closer look
5. Repeat the procedure for each beaker for the time shown on its label.

- **The velocity (V) of an enzyme-catalyzed reaction is dependent upon the *substrate concentration [S]***

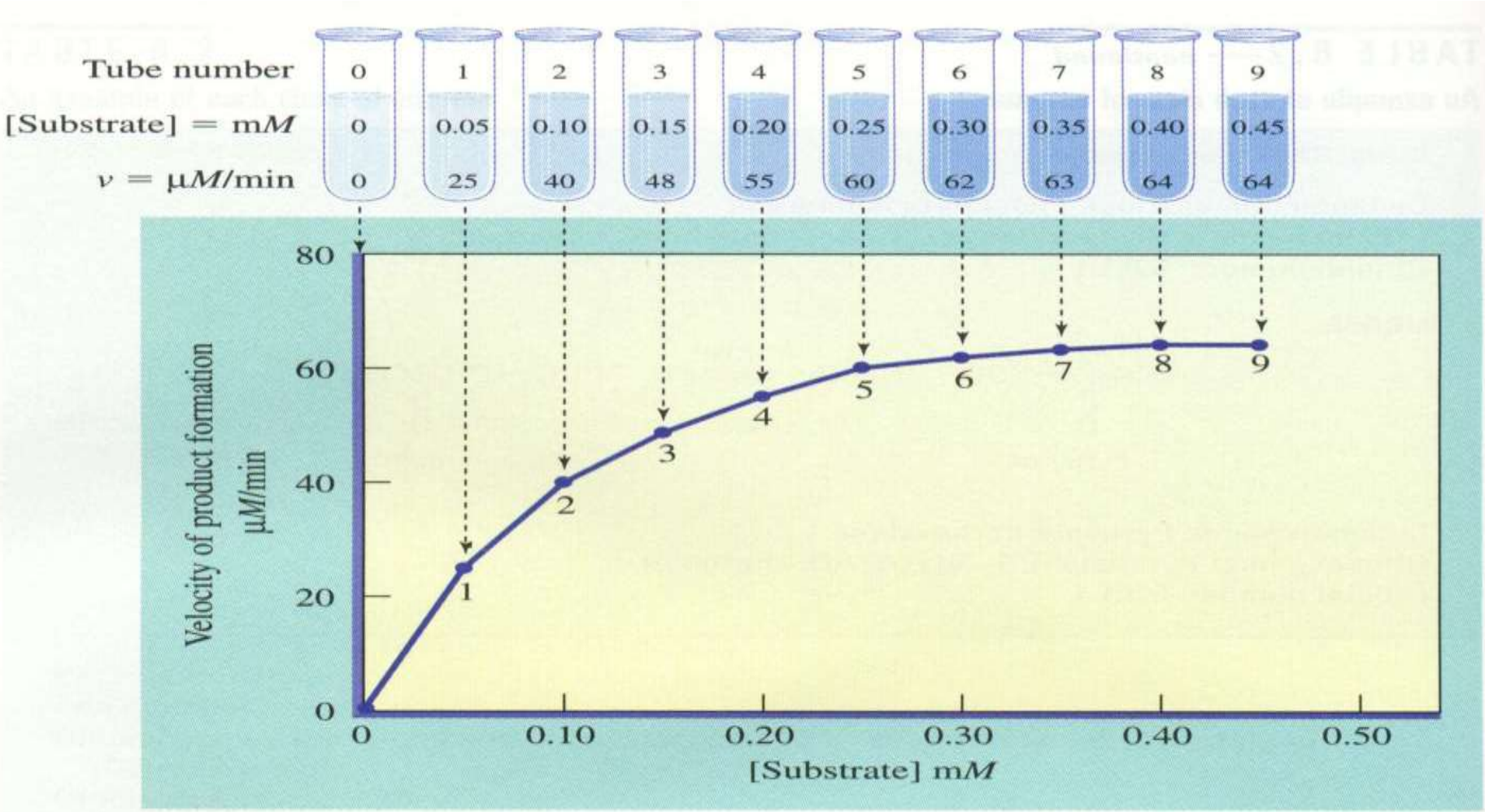


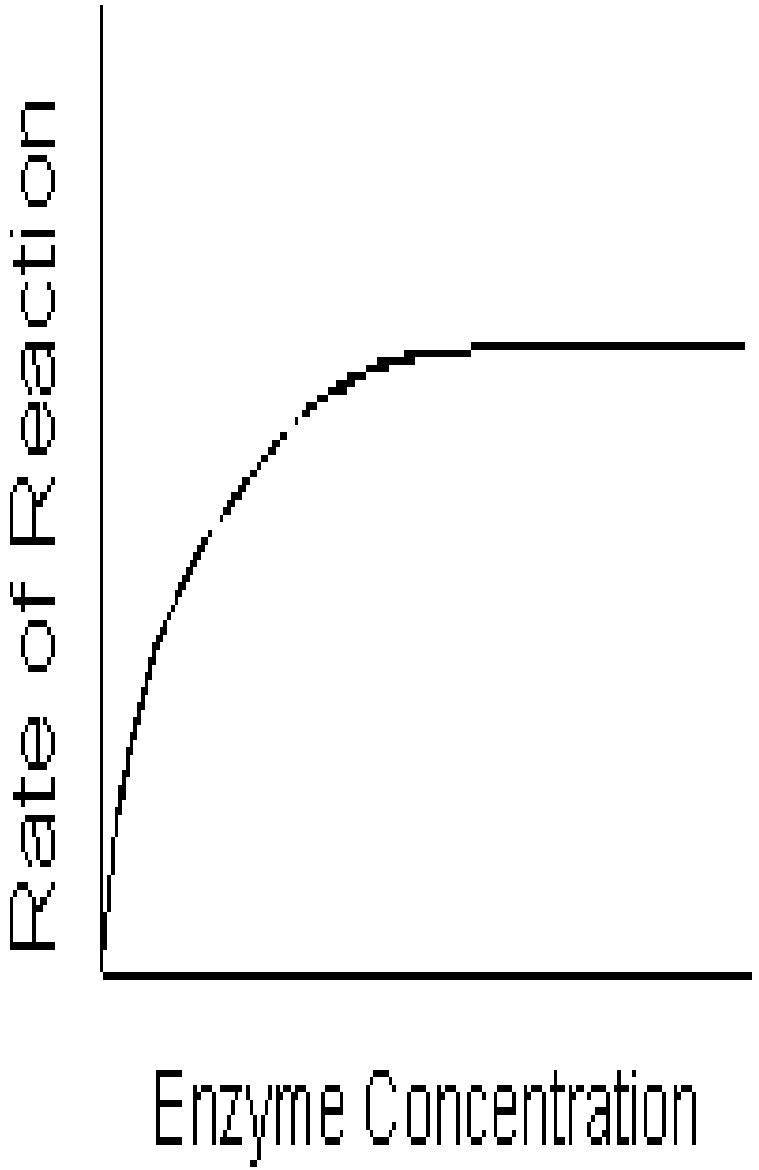
FIGURE 6.3

- ***A plot of V vs [S] is often hyperbolic (Michaelis-Menten plot)***

[E]

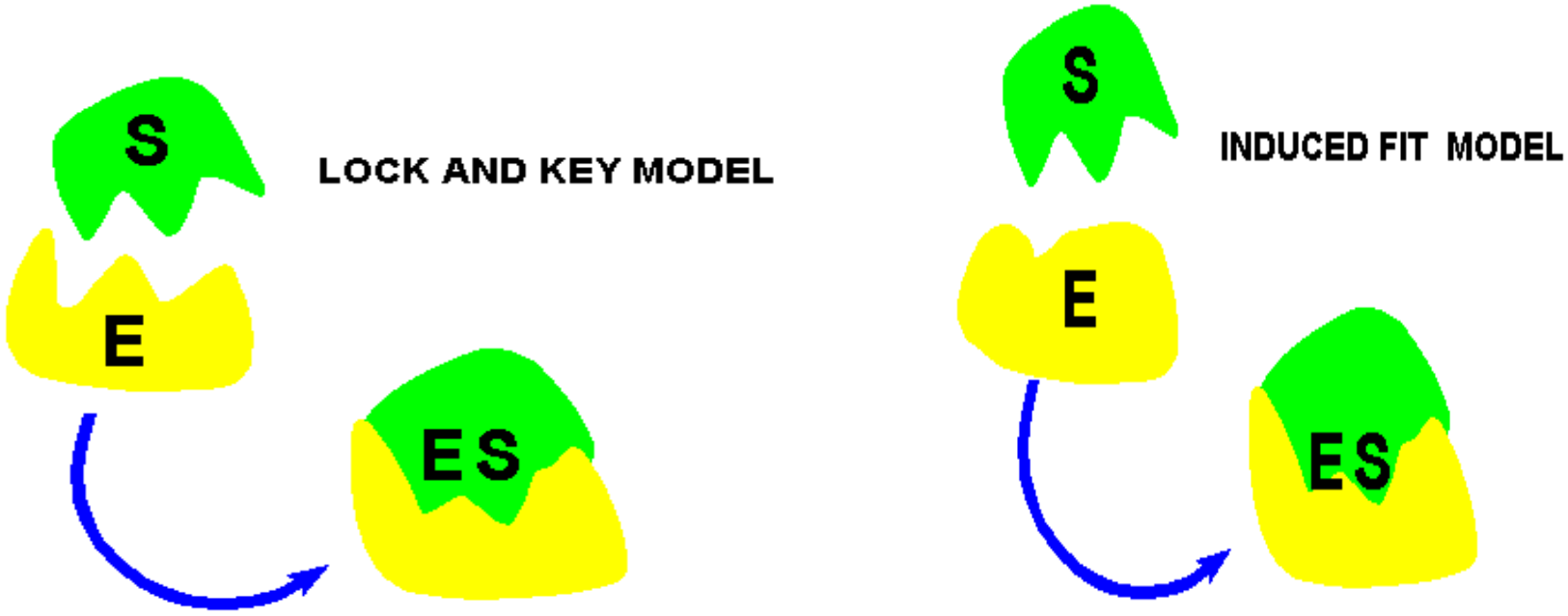
..An increase in enzyme concentration

- *increases the rate of reaction (at constant substrate concentration)*
- *binds more substrate with enzyme*



Enzyme Properties

- Enzymes are highly specific in their catalysis
  - they must bind (form a complex) with substrate into a region of the enzyme known as the "active site"
- How?



- ***Substrate***s bind to the enzyme's **active site**
  - pocket in the enzyme

- ***Substrates bind in active site by***
  - ***hydrogen bonding***
  - ***hydrophobic interactions***
  - ***ionic interactions***

Enzyme/Substrate Interactions

- **Lock and key model**
- substrate (key) fits into a perfectly shaped space in the enzyme (lock)

- **Induced fit model**
- substrate fits into a space in the enzyme, causing the enzyme to change conformation
- change in protein conformation leads to an exact fit of substrate with enzyme

FIGURE 6.10

The lock and key model to describe the formation of an ES complex. The substrate has a shape that is complementary or fits into a preformed site on the enzyme. Note that *a*, *b*, and *c* refer to specific types of interactions that form between substrate and enzyme.

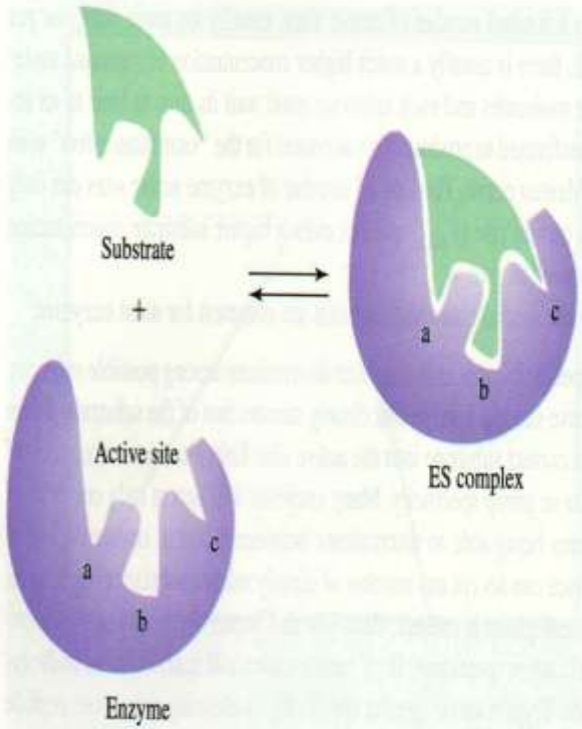
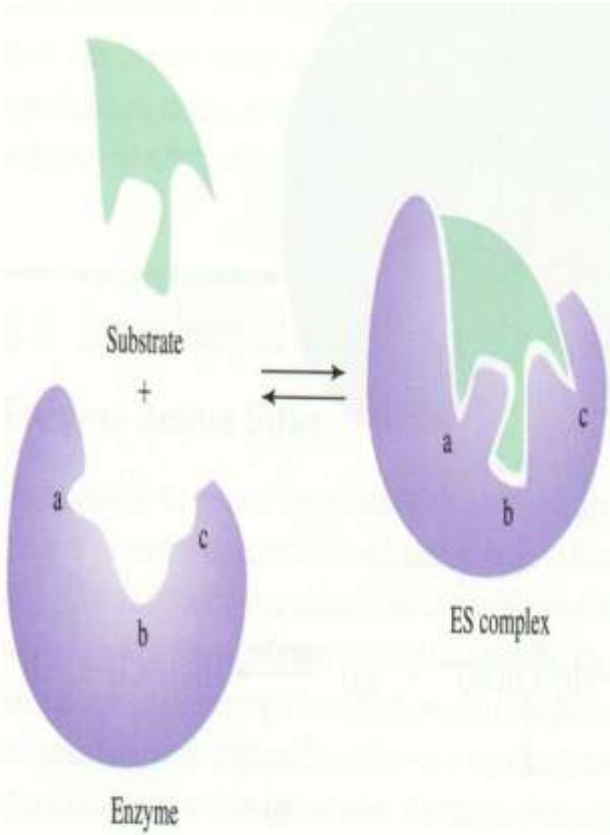


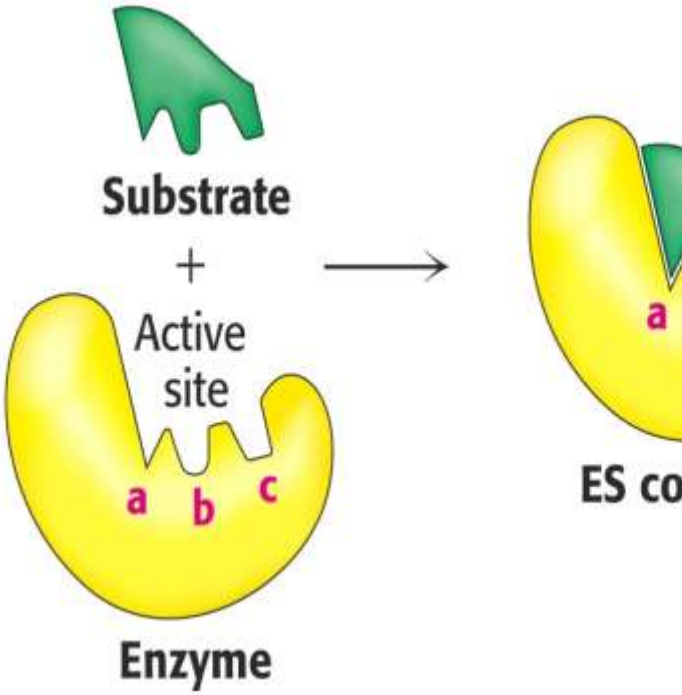
FIGURE 6.11

The induced-fit model to explain binding of a substrate to an enzyme active site. Initially the enzyme does not have a preformed site for substrate binding. Initial binding of the substrate induces specific conformational changes in the enzyme structure to make it more compatible to the substrate's size, shape, and polarity.



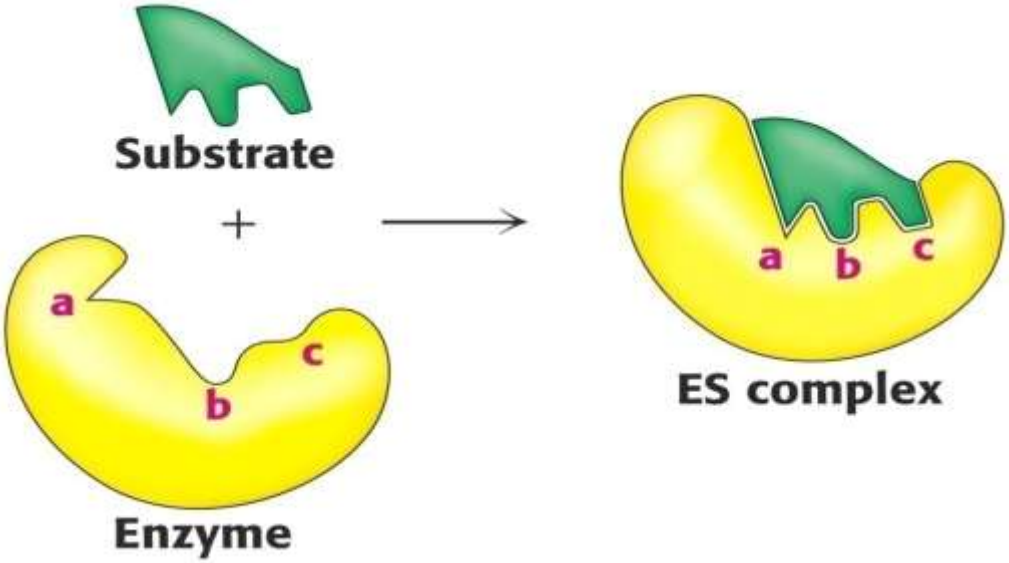
Binding specificity governed by 3D arrangement of atoms

Lock and Key model (E. Fisher, 1890)



Active site complementary to shape of substrate

Induced-Fit model (D.E. Koshland, 1958)



Active site forms a complementary shape of substrate after binding substrate

Enzyme Properties

- ****Enzymes also allow the regulation of reactions through activation or inhibition of the enzyme by effectors***

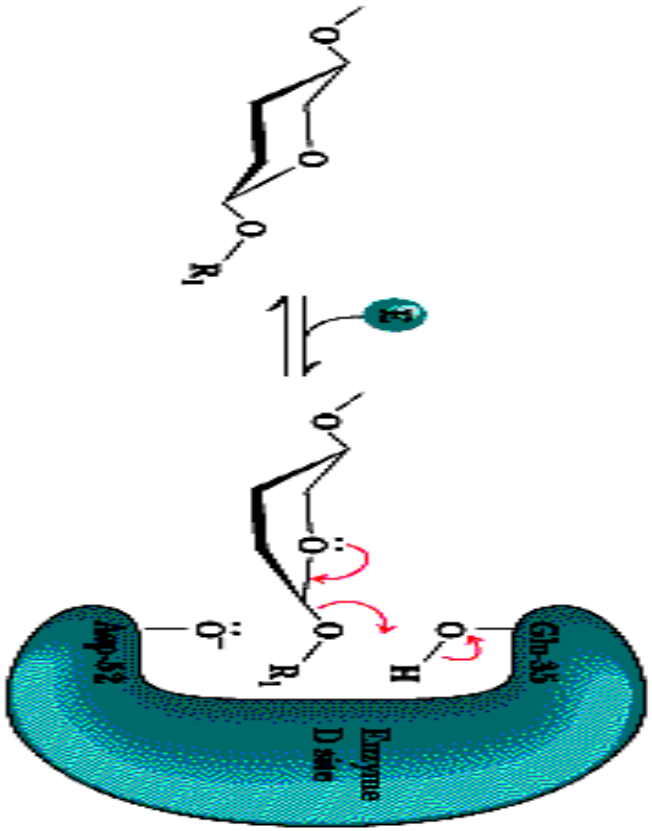
  ** All biological reactions are found to be enzyme catalyzed****

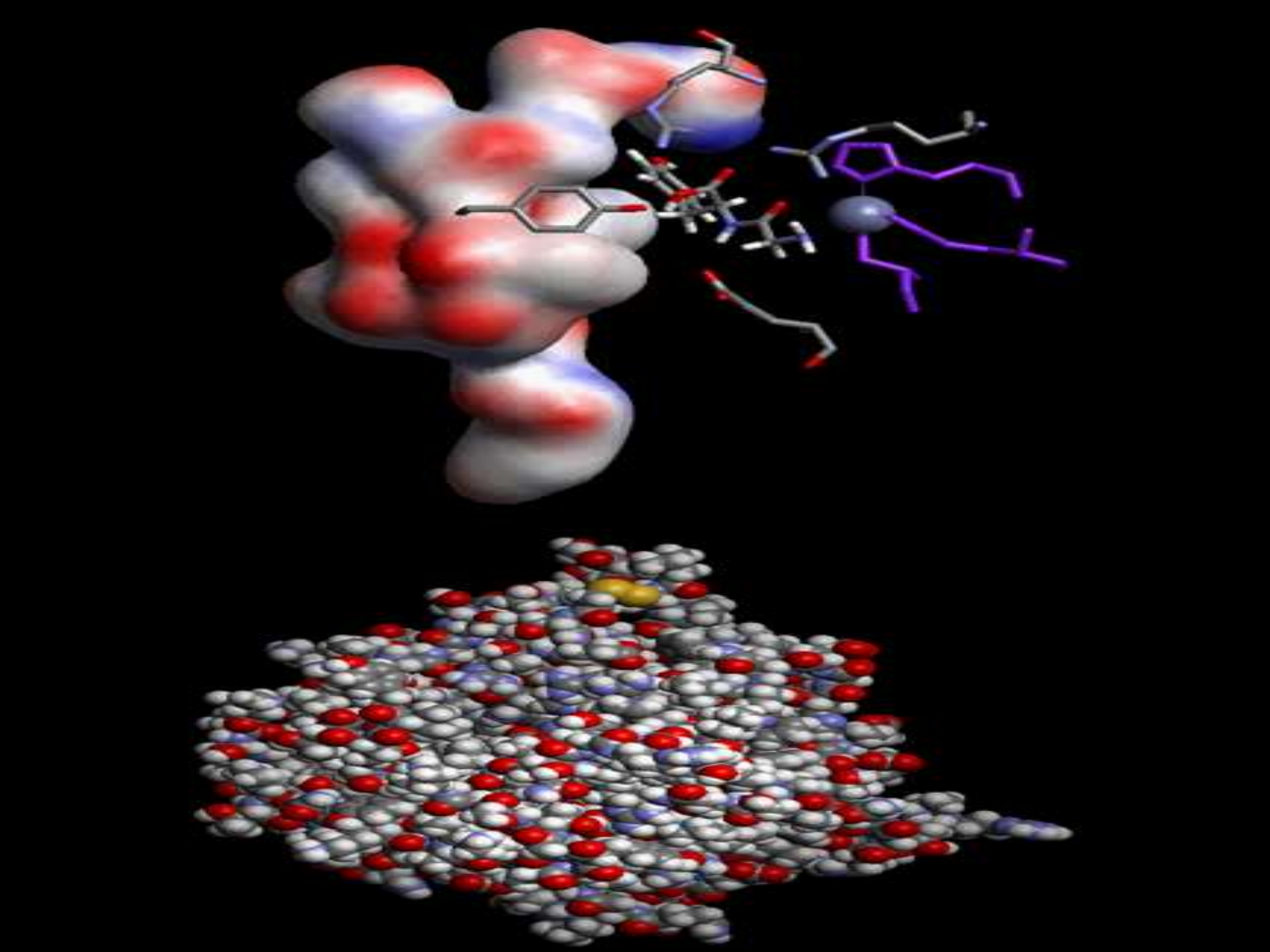
- **How do enzymes catalyze biochemical reactions?**
  - ***involves basic principles of organic chemistry***

- **What functional groups can be involved in catalysis?**

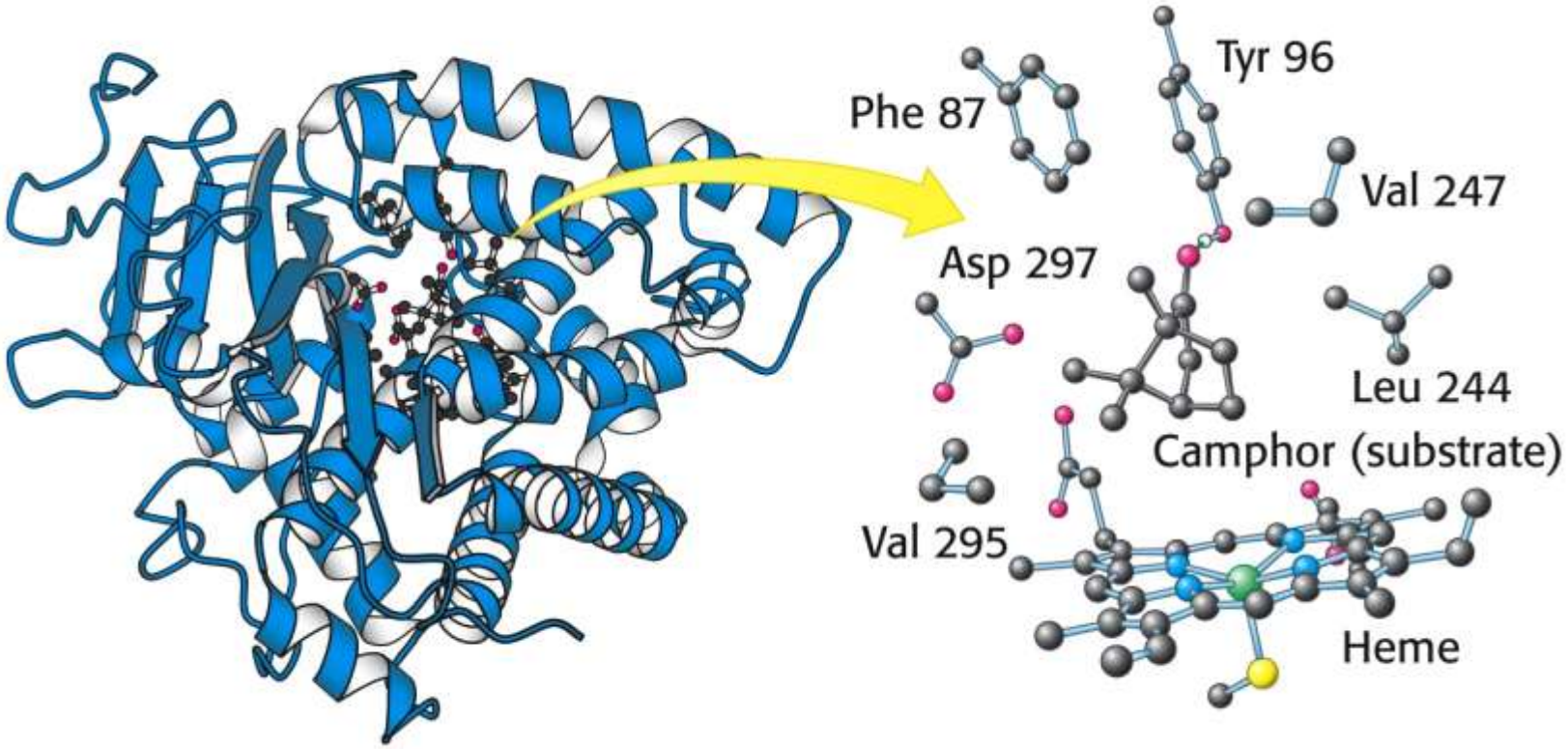
- **catalysis occurs when substrate is immobilized near these residues at the active site**

- **The binding of the substrate results in the distortion of the substrate in a way that makes the chemical reaction easier.**





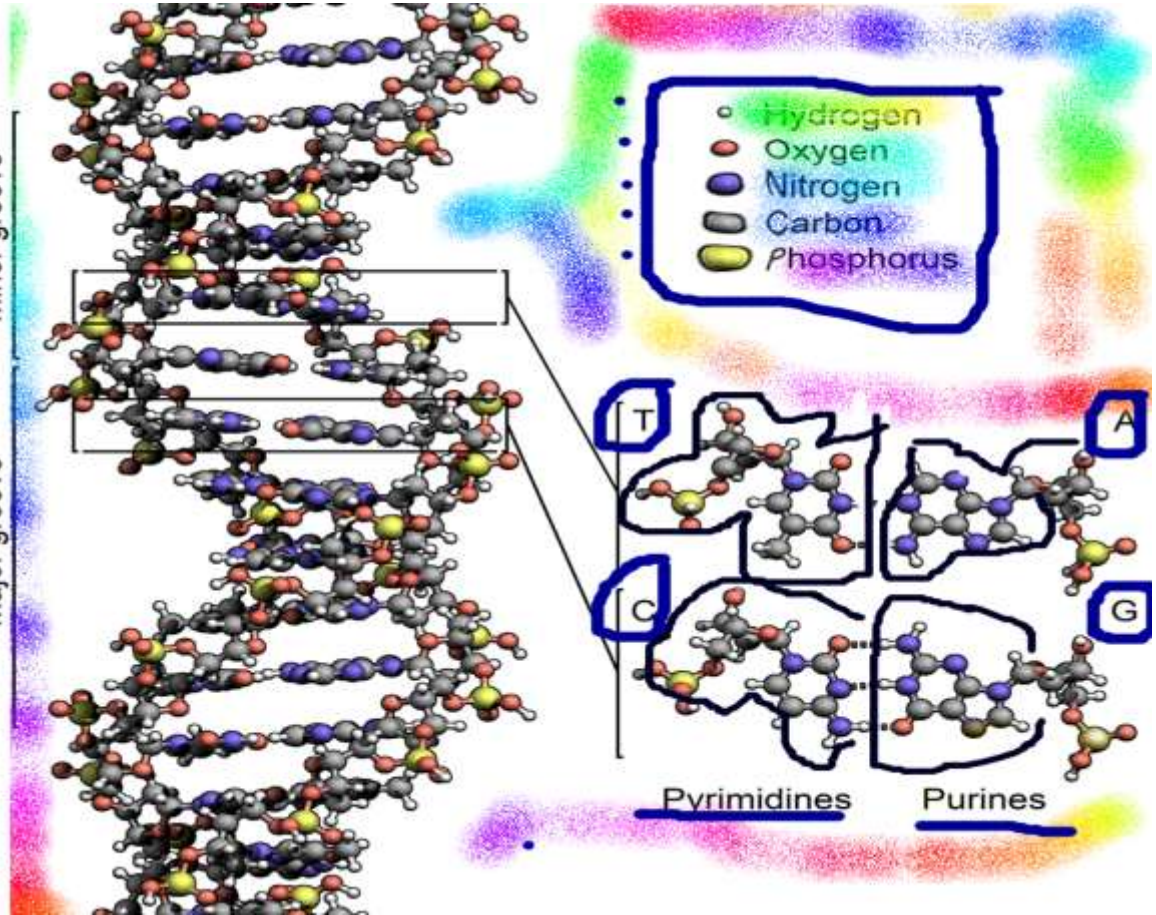
X-ray crystallography to "see" ES complexes

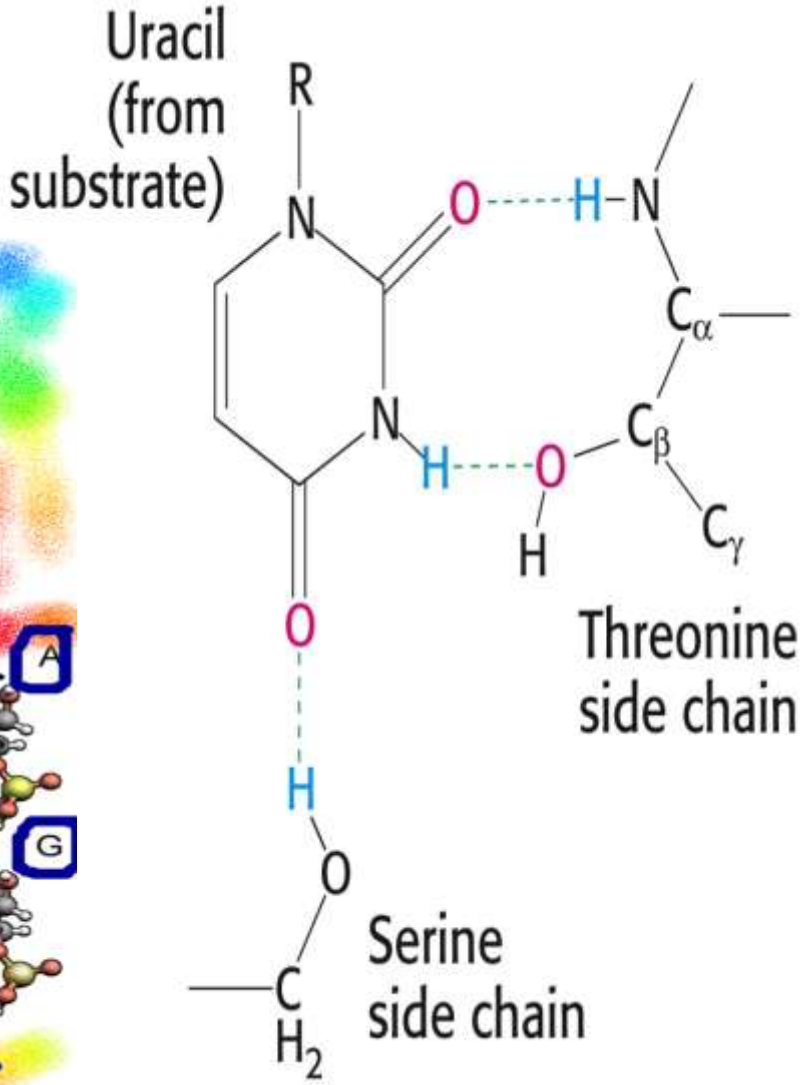


Cytochrome P450

Hydrogen bonding between substrate and active site

Ribonuclease enzym :
Forms H-bonds with uridine component of substrate





Michaelis and Menten

In 1913, Michaelis and Menten proposed the following mechanism for a saturating reaction rate

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

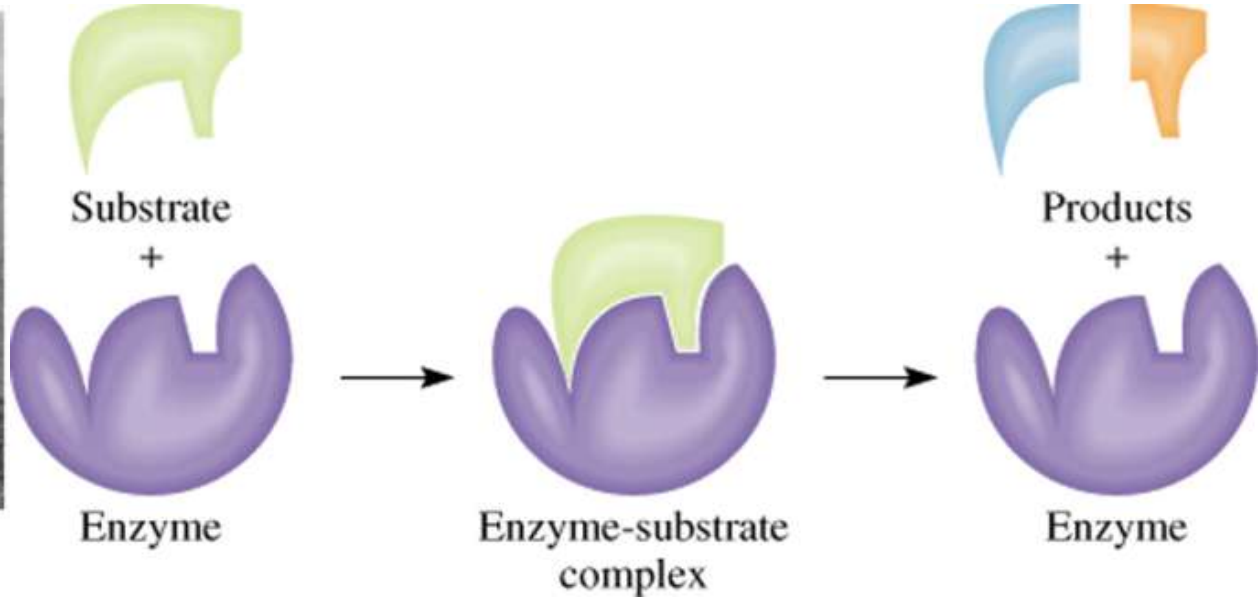
Complex.

no back reaction
-> no k_{-2}

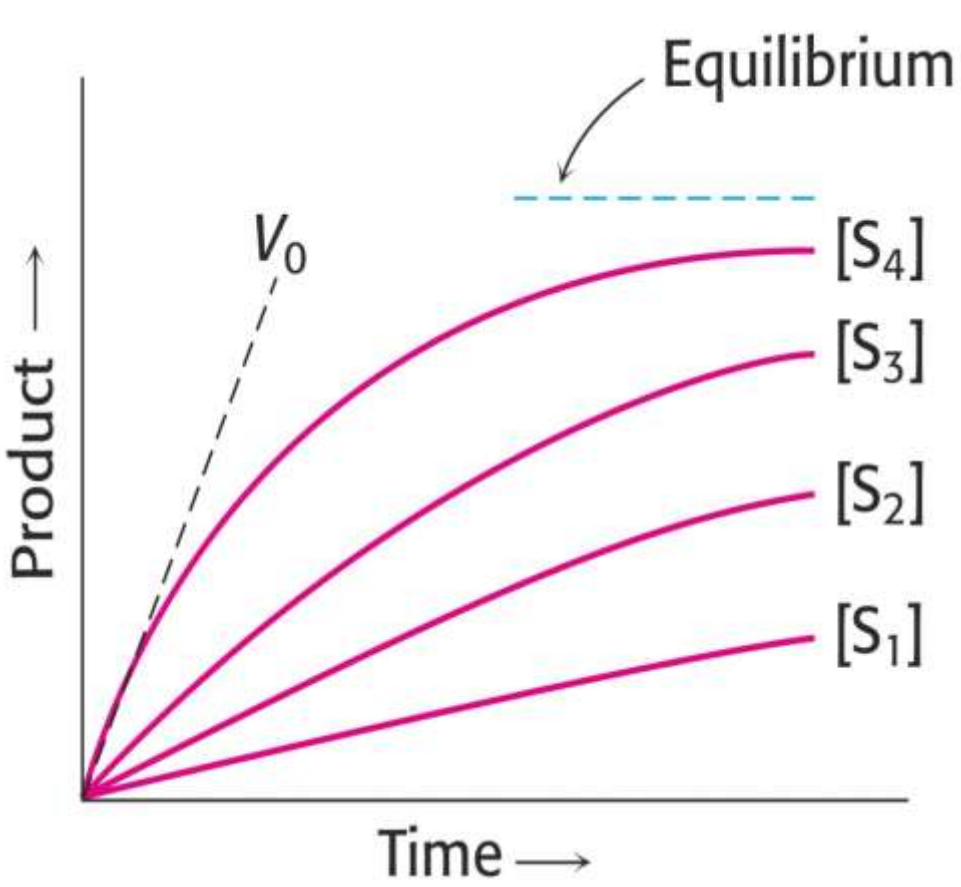
product

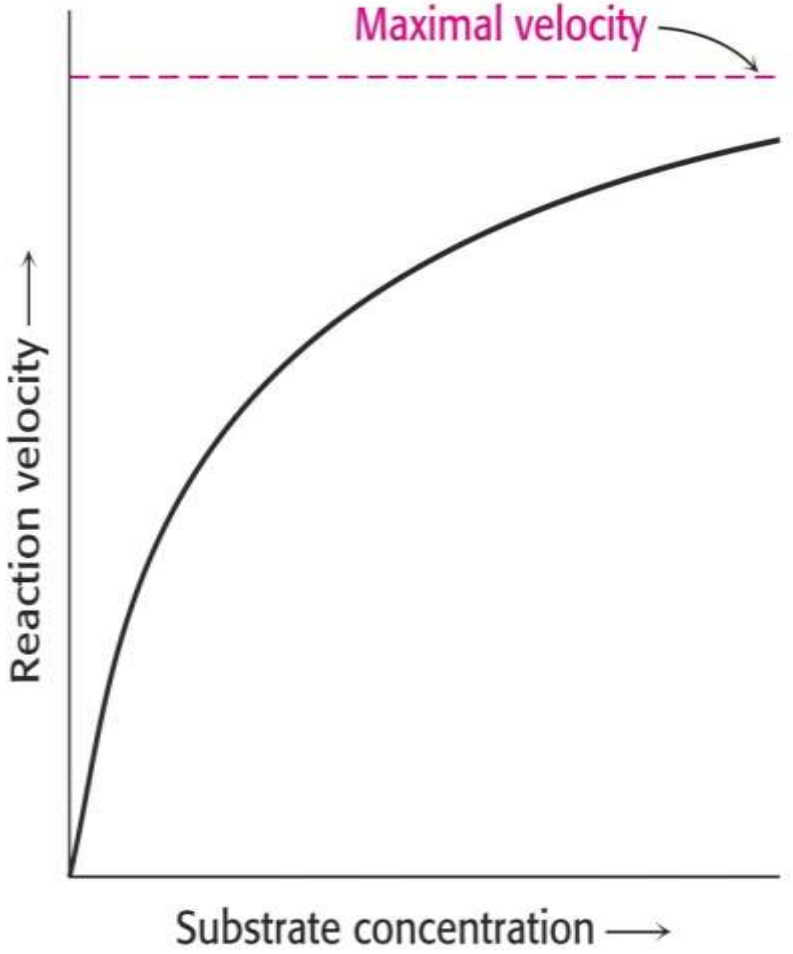
Leonor Michaelis
1875–1949

Maud Menten
1879–1960



Enzyme-catalyzed reaction progression curves





V_0 -> initial velocity -> product formed /sec at the beginning of the reaction (t=0)

-> V_0 changes with [S] -> V_0 rises when [S] rises -> until saturation (Max. Velocity)

- **The velocity (V) of an enzyme-catalyzed reaction is dependent upon the substrate concentration [S]**

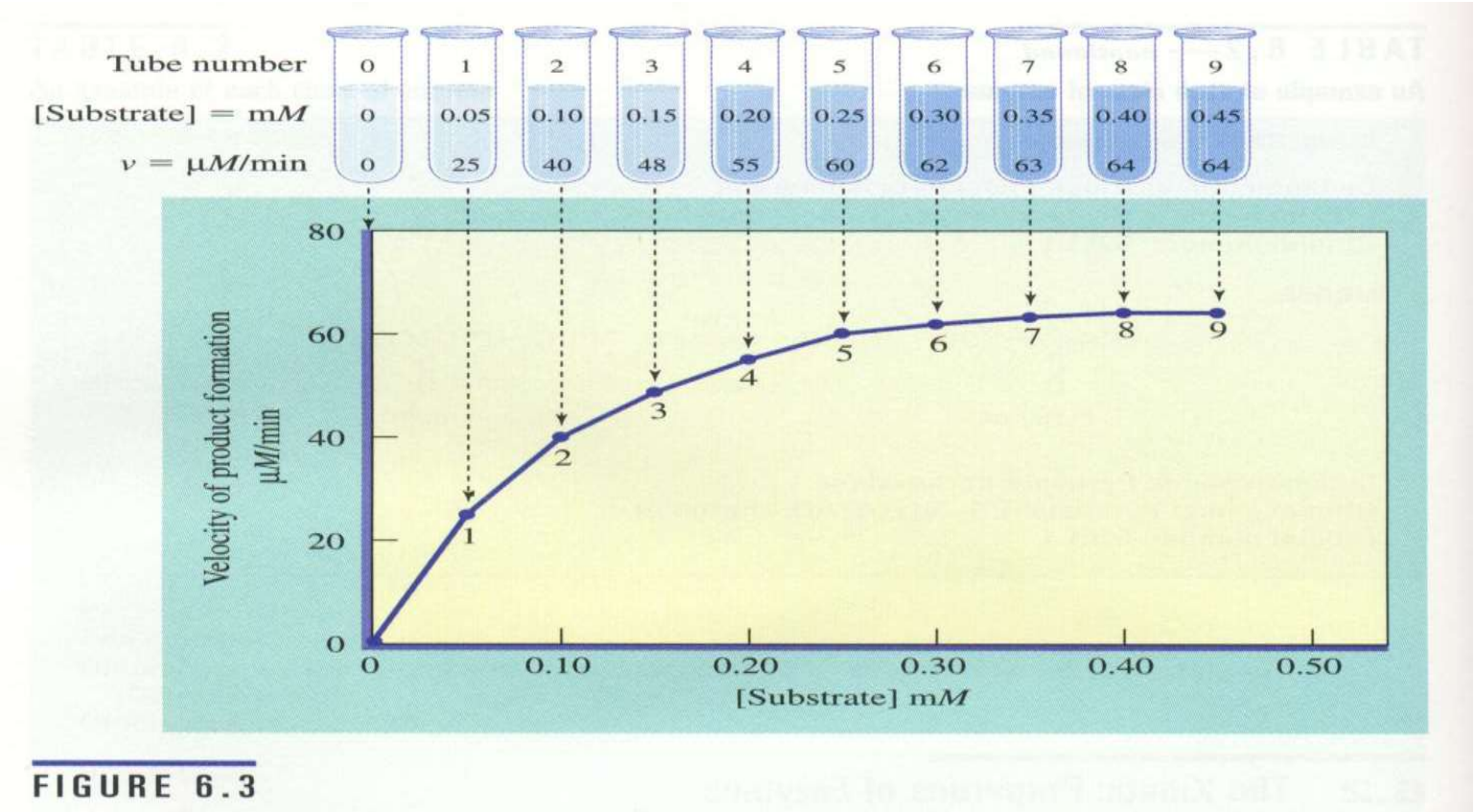
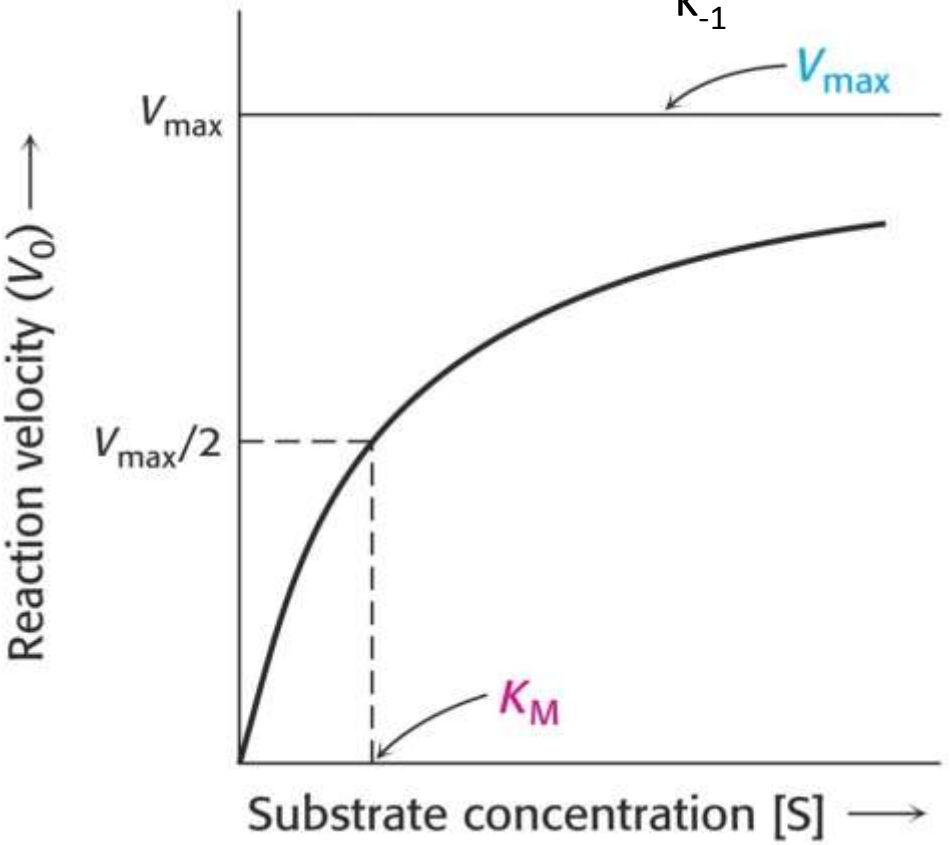


FIGURE 6.3

- A plot of V vs [S] is often hyperbolic (Michaelis-Menten plot)

Velocity vs Substrate Concentration: The Michaelis-Menten model

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$



- V_{max}: maximal velocity when all sites of E occupied
-
- K_m: Michaelis constant, when [S] gives $V_{max}/2$

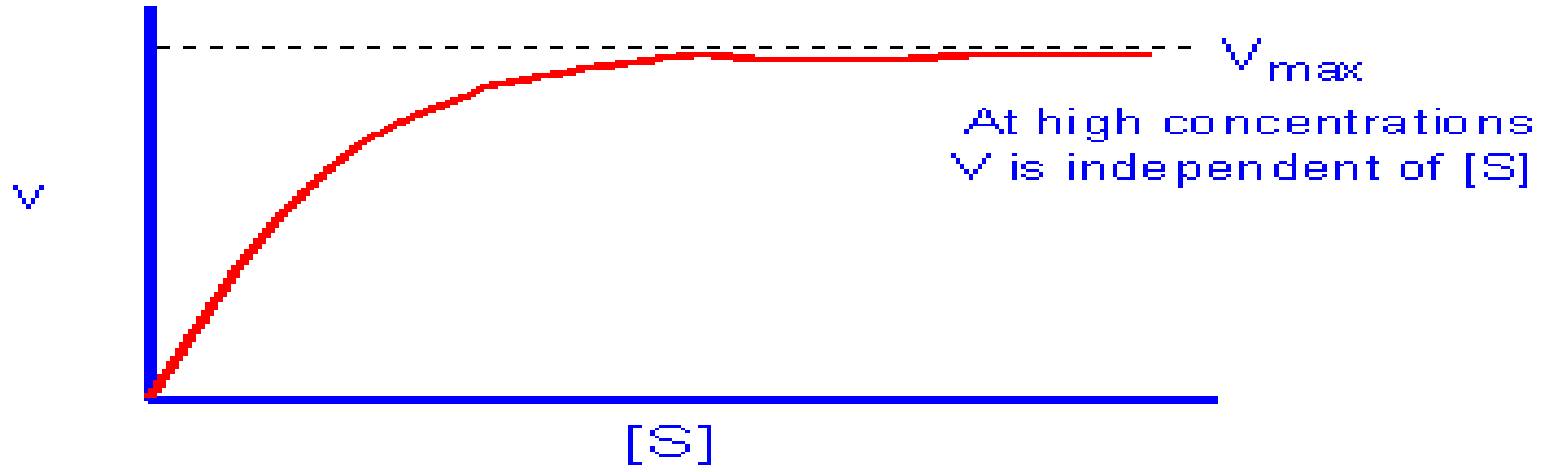
Enzyme Kinetics

- In 1913, Lenor Michaelis and Maude Menten made the assumption that the reversible step in the mechanism does achieve equilibrium

The Michaelis – Menten Model

Experimental Observations

1. Saturation



Enzyme Kinetics

Michealis-Menten equation

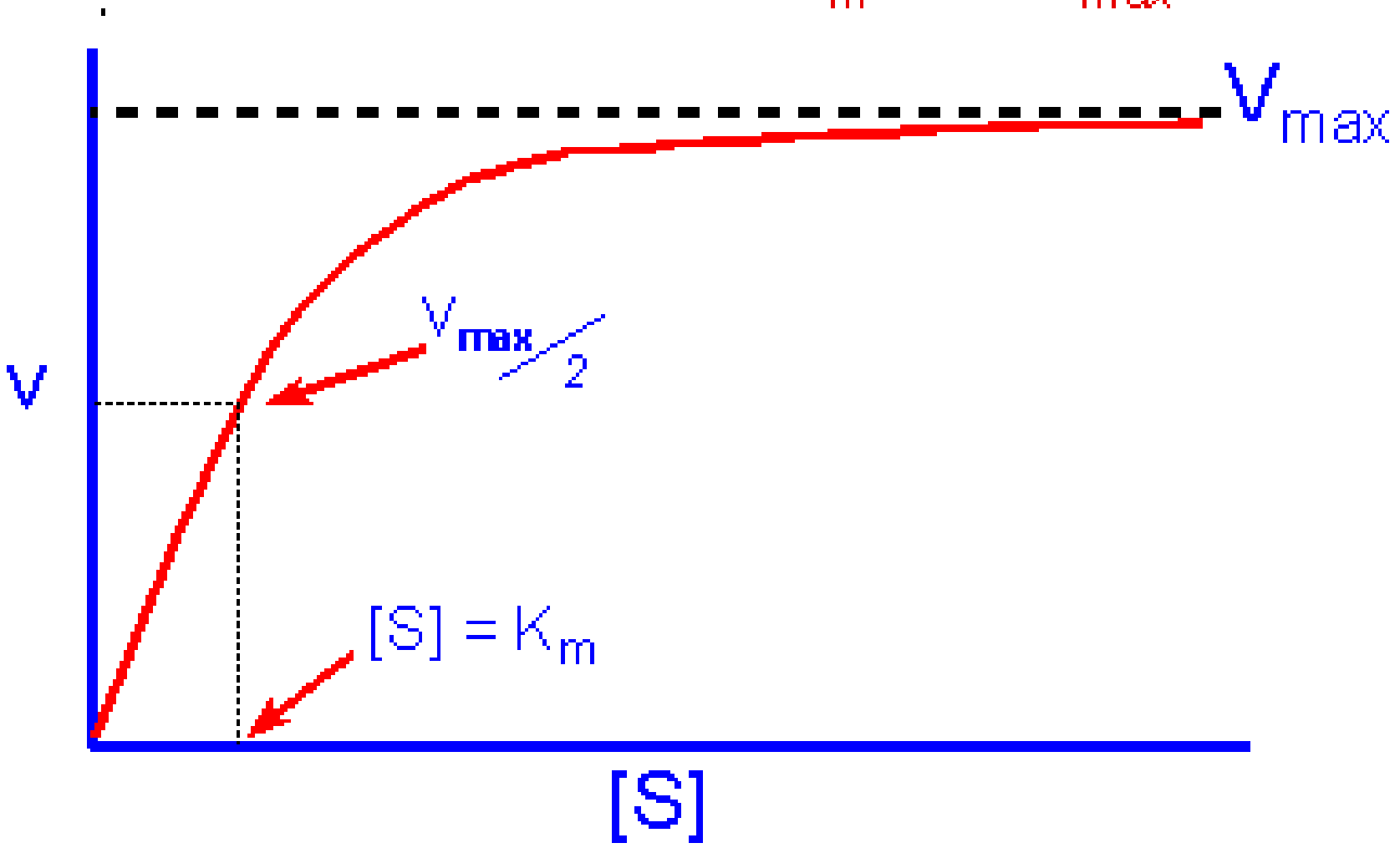
$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

V_{max}= the rate of reaction in which all of the active sites of the enzyme are consumed by substrate

K_m= a ratio of all rate constants involved. K_m also represents the substrate concentration at which the reaction rate is 1/2 of V_{max}

[S] = **the concentration of substrate binding to enzyme**

Measurement of K_m and V_{max}



Extrapolation to Vmax is quite difficult in such a plot and an alternative form of plot was sought which would be more useful

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

- **V =** is the reaction rate (velocity) at a substrate concentration [S]

- $V_{max=}$ is the maximum rate that can be observed in the reaction
  - substrate is present in excess
  - enzyme can be saturated (zero order reaction)

- $K_{M=}$ is the Michaelis constant
  - a constant that is related to the affinity of the enzyme for the substrate
  - units are in terms of concentration

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

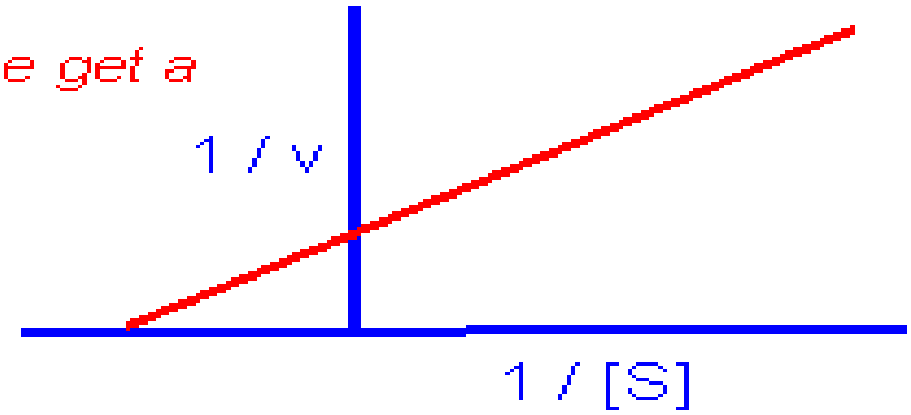
Begin with the Michaelis - Menten equation and take the reciprocal of both sides.

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

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

This equation has the form of a linear or straight line: $y = mx + b$

Thus if we plot 1/v vs 1/ [S] we get a straight line plot according to Lineweaver and Burke.



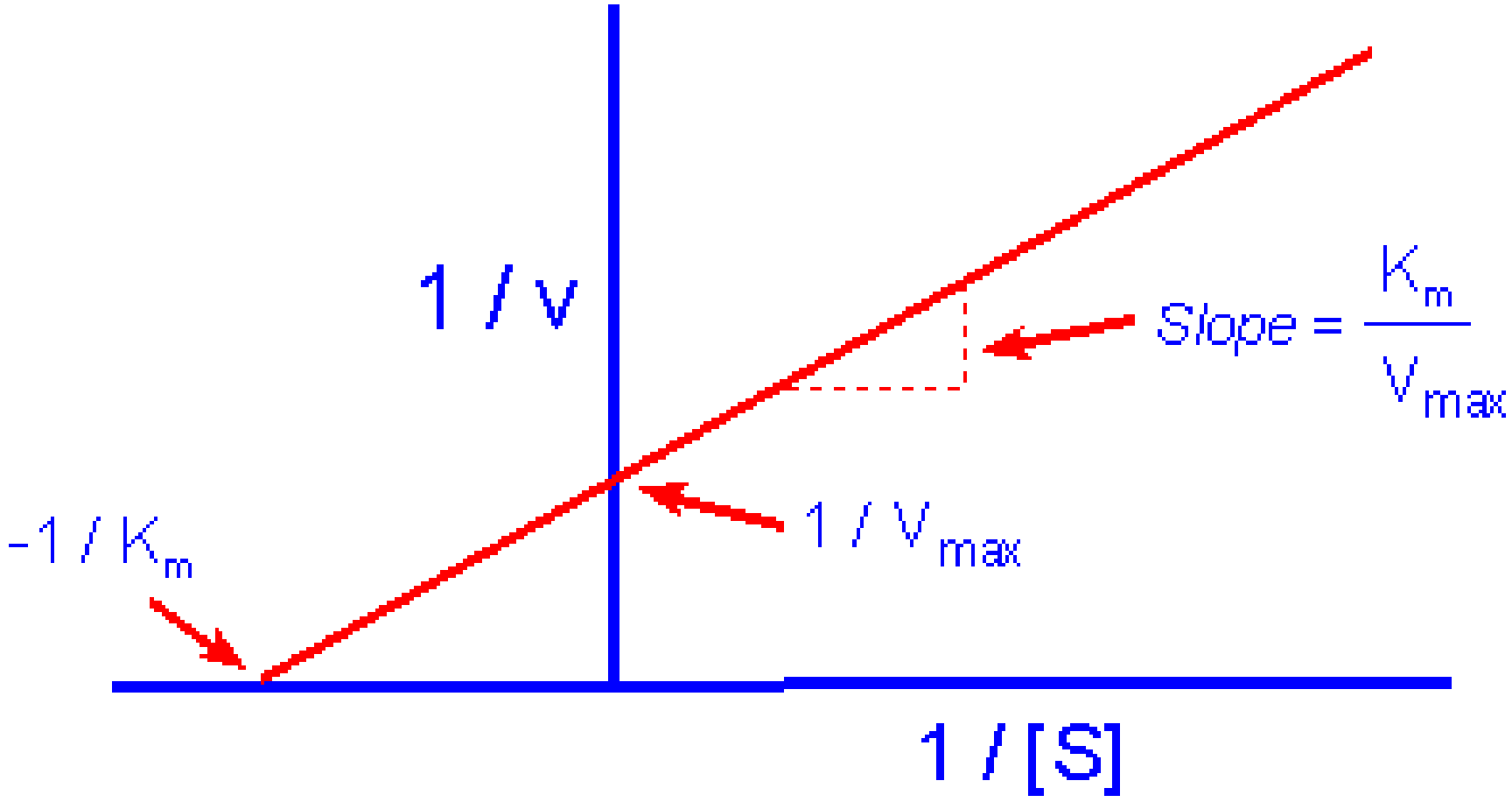
Lineweaver and Burke plot

Y= mx + b

I/V = Km/Vmax * I/[S] + 1/V

Slope=m

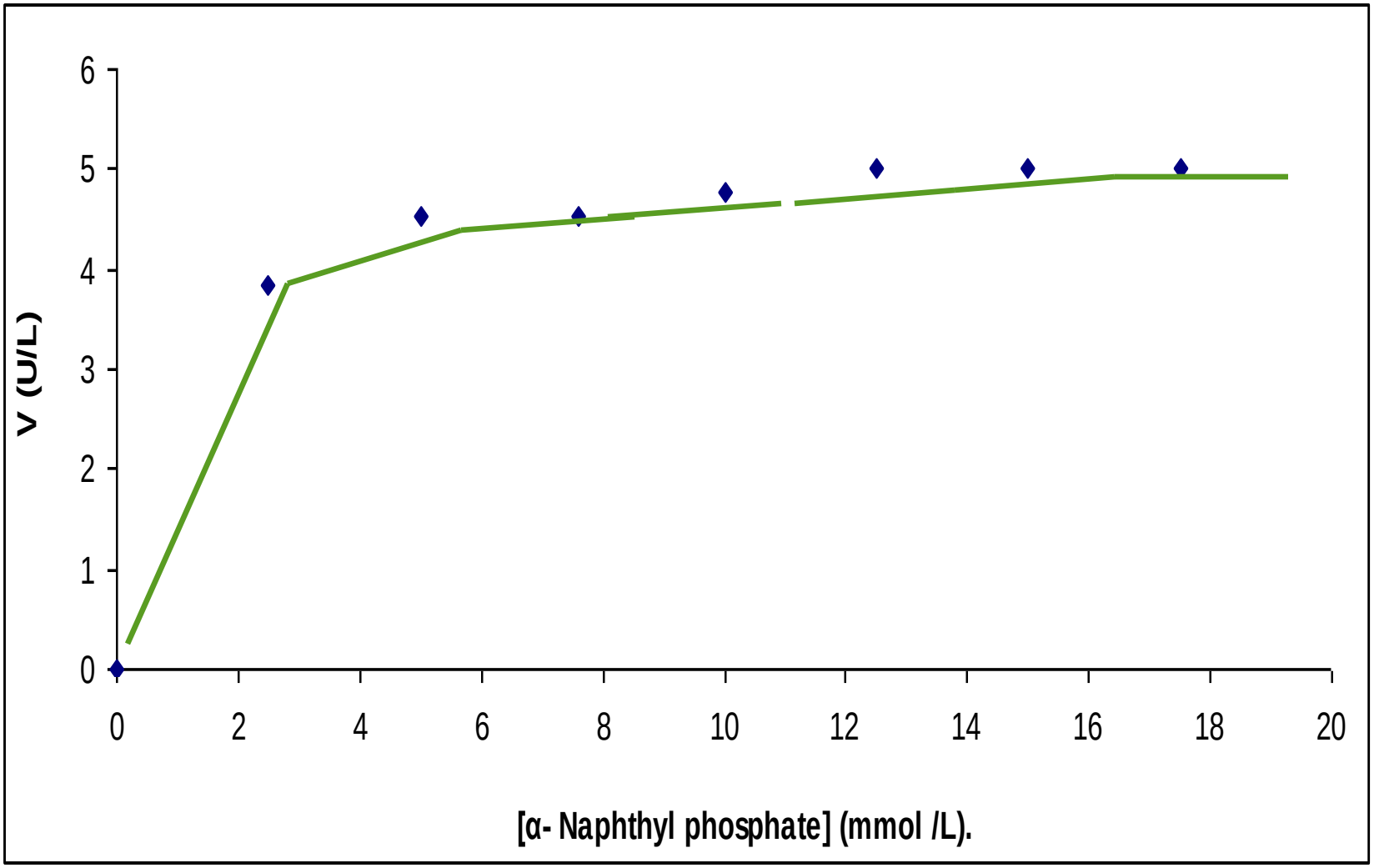
A Double Reciprocal or Lineweaver-Burke Plot



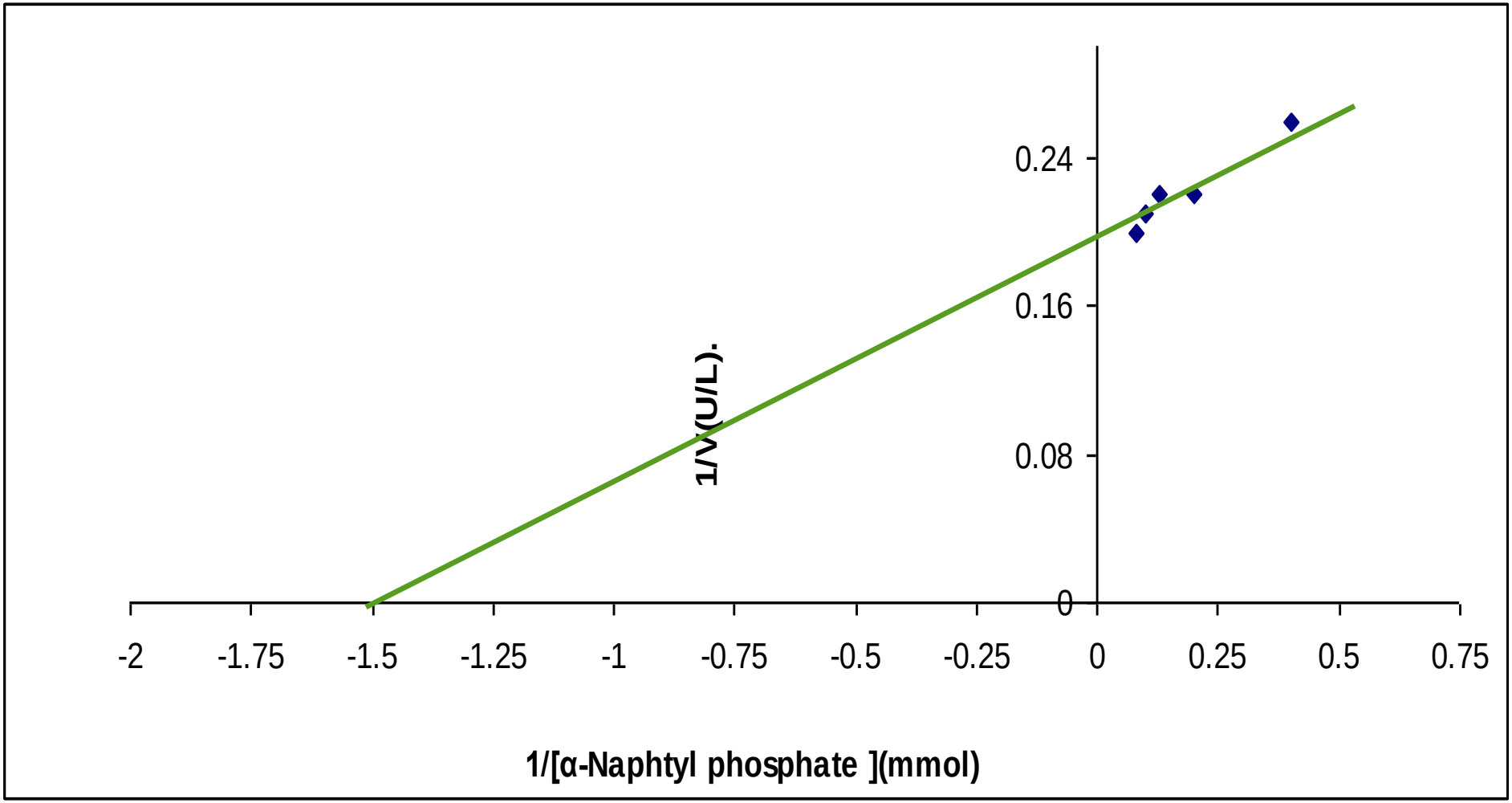
Remember that because this is a reciprocal plot that the origin represents infinite substrate concentration and therefore the maximum velocity.

Example__Effect of Selective Inhibitors on Activity of Prostatic Acid Phosphatase [PAP] in Sera of Patients

The purpose of this study is to determine PAP in patients with BPH activity ,and study the inhibitory effect of several compounds [Molybdate sodium, L-tartrate ,and Cupper sulfate]on the activity of enzyme .



Kinetic properties of (α-naphthyl phosphste) acid phosphatase in serum: *Michaelis –Menten plots* **.**



Kinetic properties of(α-naphtyl phosphste) acid phosphatase in serum (Line weaver Burk).

Michaelis-Menten Kinetics

When the enzyme is saturated with substrate, the reaction is progressing at its maximal velocity, V_{max}.

At saturation $[E]_T = [ES]$, and the equation for reaction velocity simplies to $V_{max} = k_2 [E]_T$

the Michaelis-Menten Equation of enzyme kinetics:

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

Michaelis-Menten Kinetics

What is K_M ?

- The concentration of substrate which gives $\frac{1}{2}$ of V_{max}.
- This means that low values of K_M imply the enzyme achieves maximal catalytic efficiency at low [S].

**K_M gives an idea of the range of [S] at which a reaction will occur.

-> indicates binding strength (substrate affinity)

The larger the K_M, the WEAKER the binding affinity of enzyme for substrate.

Michaelis-Menten Kinetics

TABLE 8.4 K_M values of some enzymes

Enzyme	Substrate	K_M (μM)
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

Table 8-4
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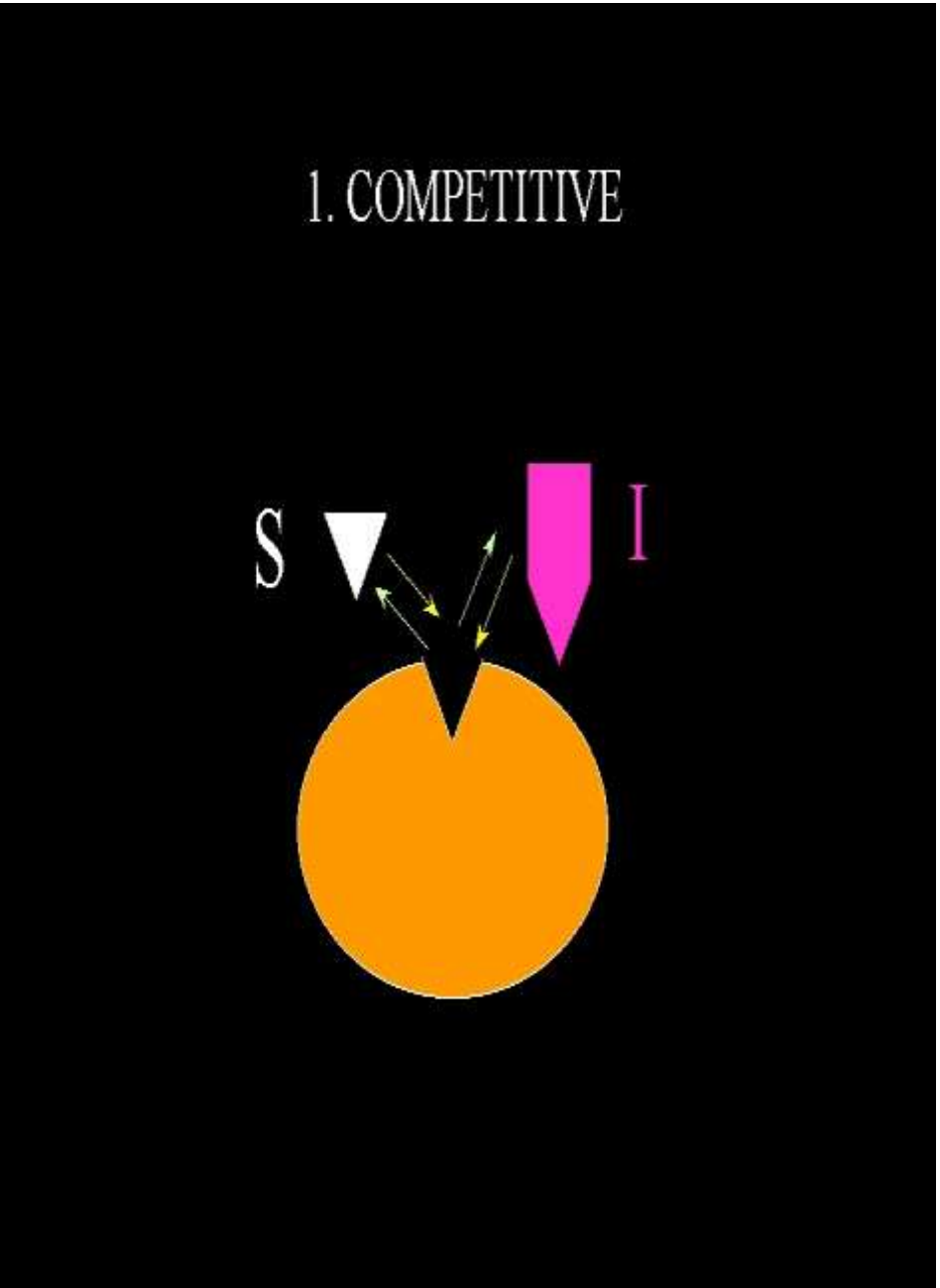
Enzyme Inhibition

- Inhibitors can halt the activity of an enzyme
  - ***results in a decreasing concentration of product formation***
  - **Drug therapy is based on the inhibition of specific enzymes**
- ***There are three major classes of inhibitors***
  - ***Competitive***
  - **Noncompetitive**
  - **Uncompetitive**

Competitive Inhibition



- *A molecule that fits*

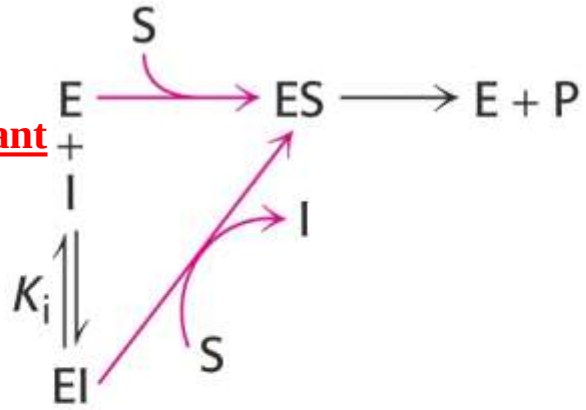
into the enzyme's active site but does not react with It

Enzyme will remain inactive until the inhibitor falls off

- *More substrate is needed*

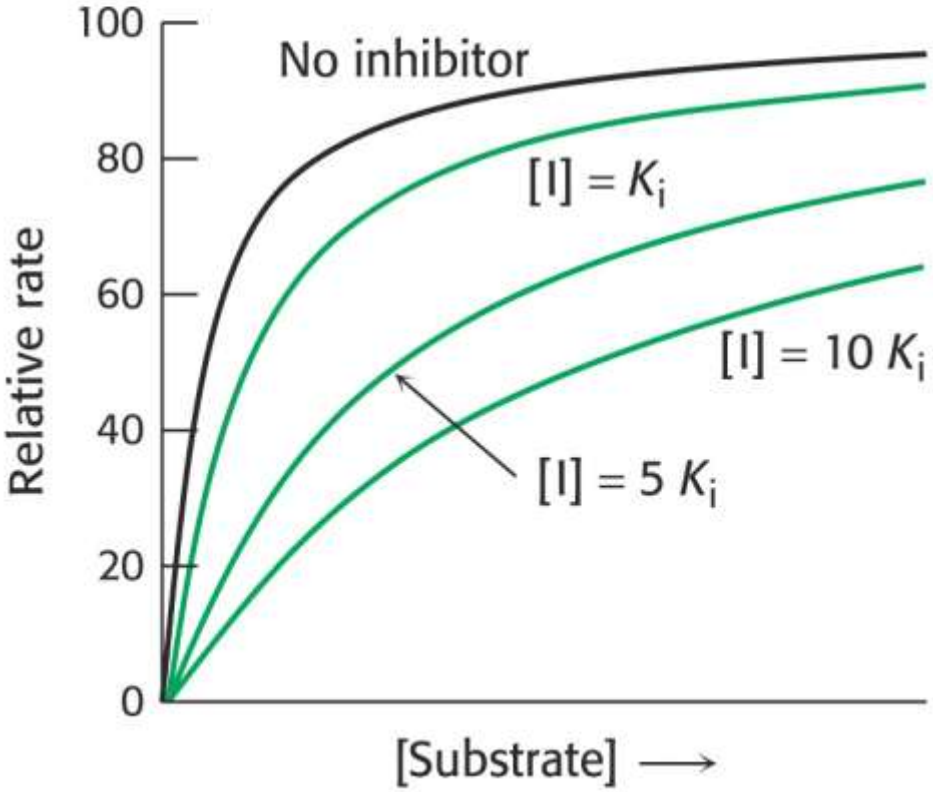
to get to the maximum rate, since substrate "competes" with inhibitor

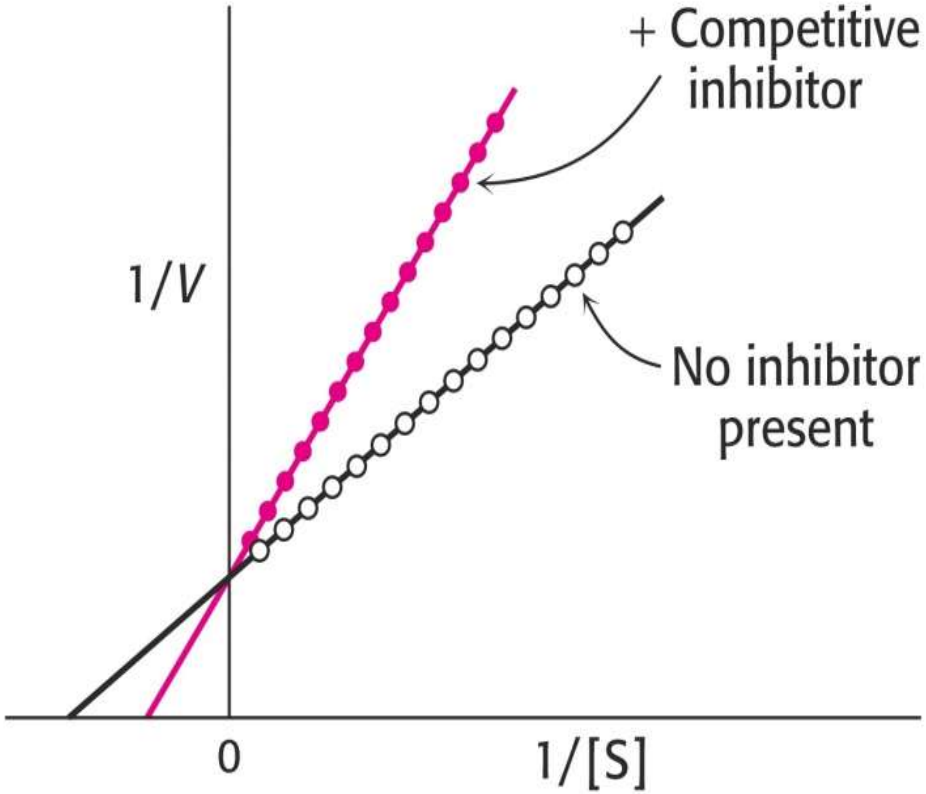
Competitive inhibition affects K_m

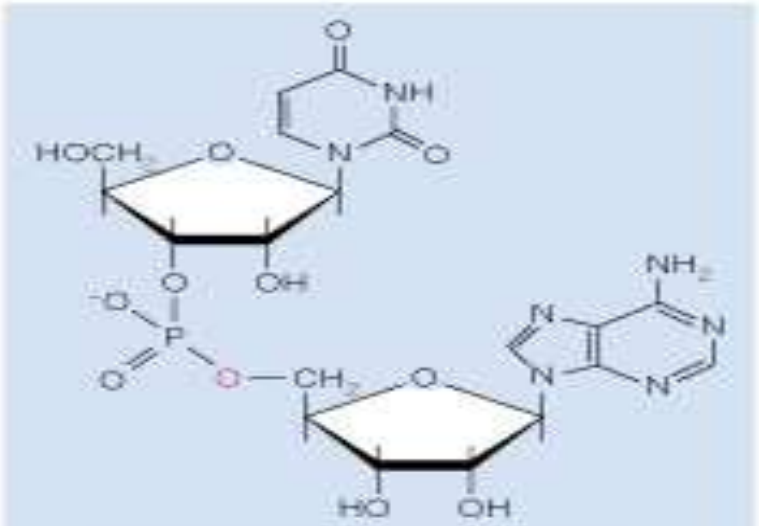


Cannot reactant

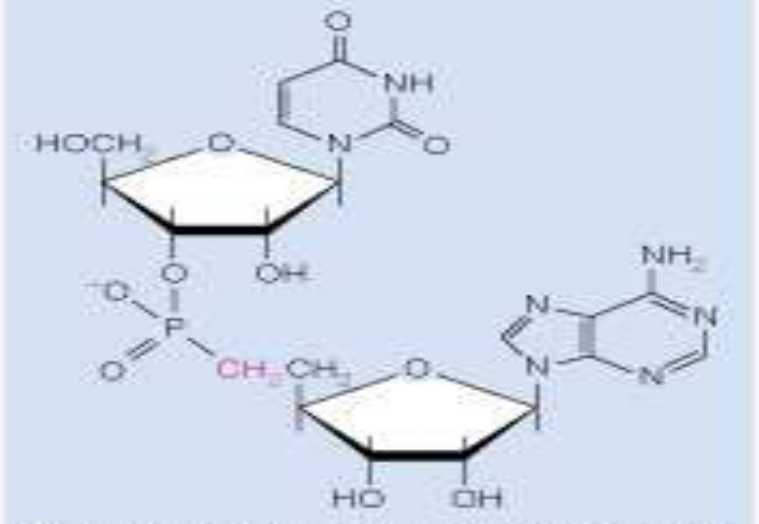
- Inhibition overcome by increase in substrate concentration
- K_m altered: apparent K_m value increased Vmax Constant





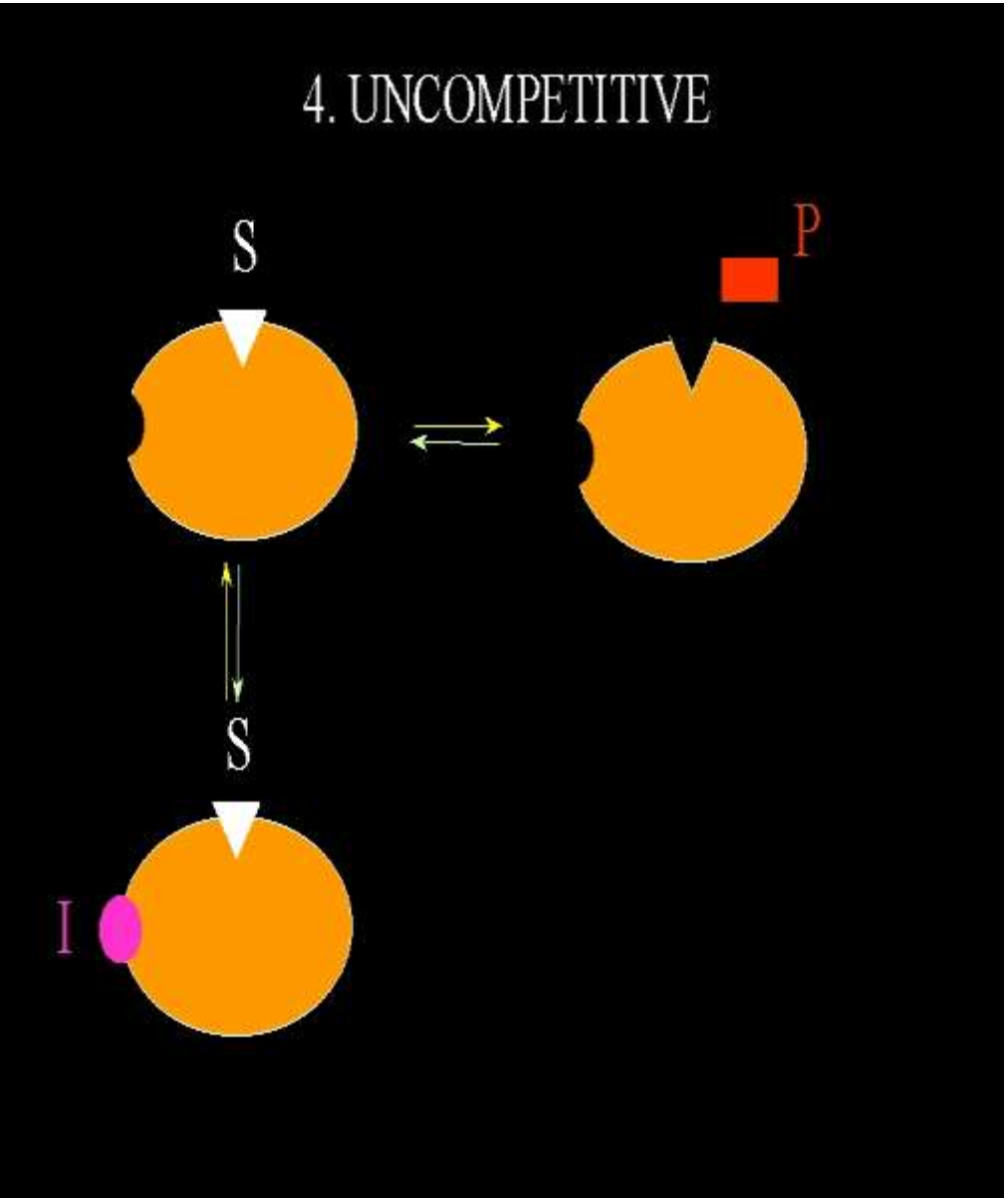


UpA: ribonuclease substrate



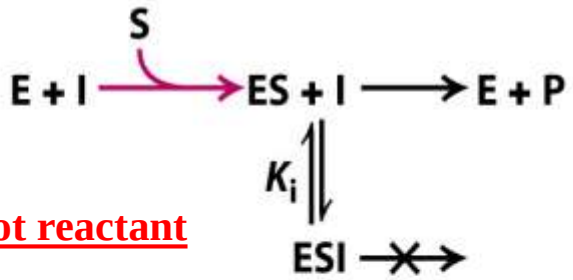
UpcA: competitive inhibitor of ribonuclease

Uncompetitive Inhibition



- ***Inhibitor binds to the enzyme only after enzyme-substrate complex forms***
- ***As a result, catalytic activity is blocked***

Un-Competitive inhibition affects V_{max} and K_m



Cannot reactant

- Inhibition cannot be overcome by increase in substrate concentration

-> V_{max} altered: apparent V_{max} value decreased

-> K_m altered: apparent K_m value decreased

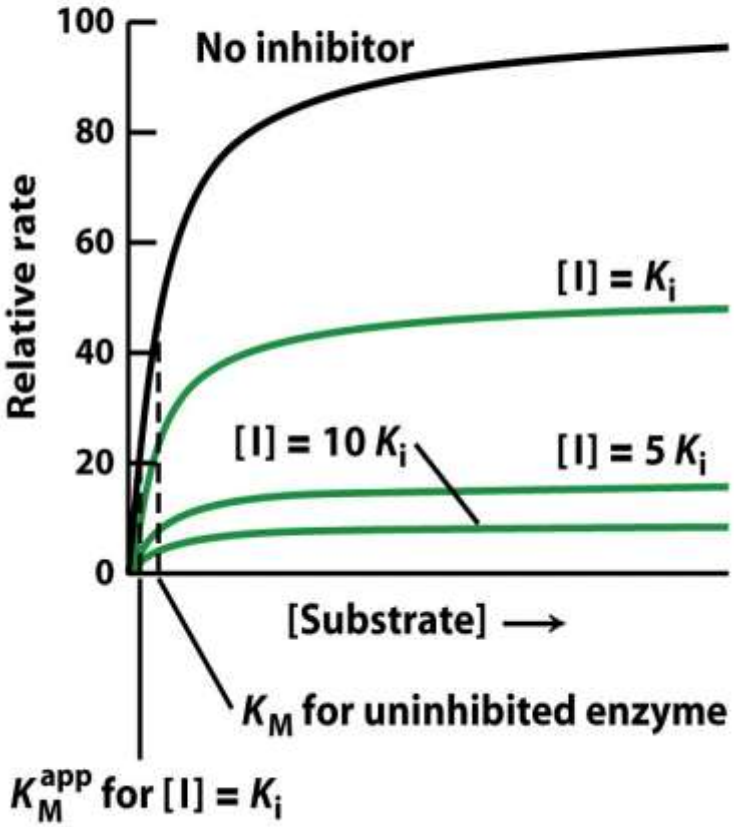


Figure 8-18
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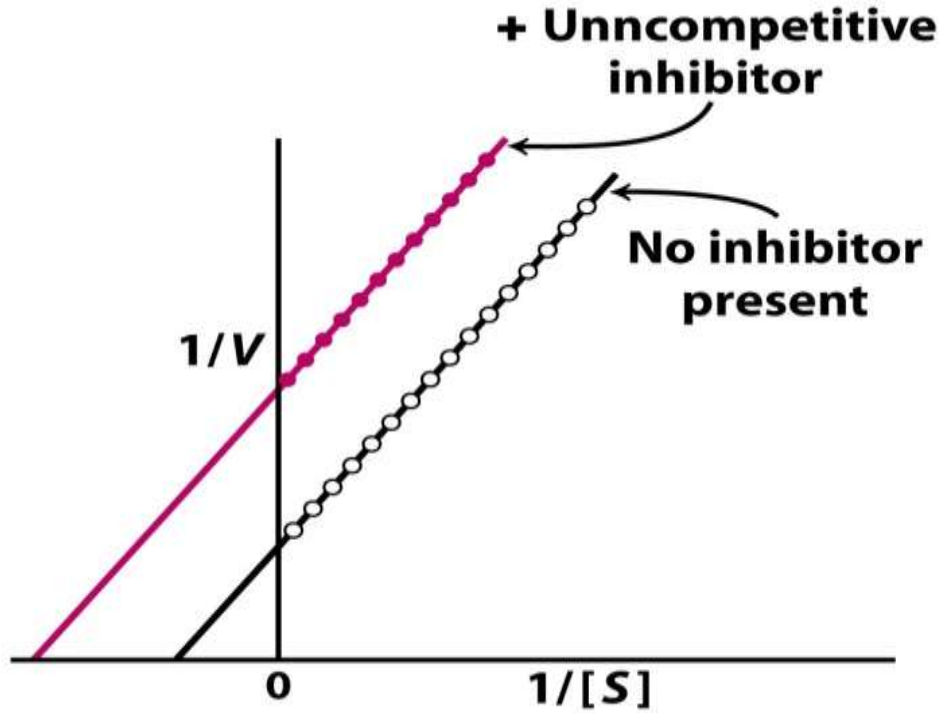

Figure 8-21
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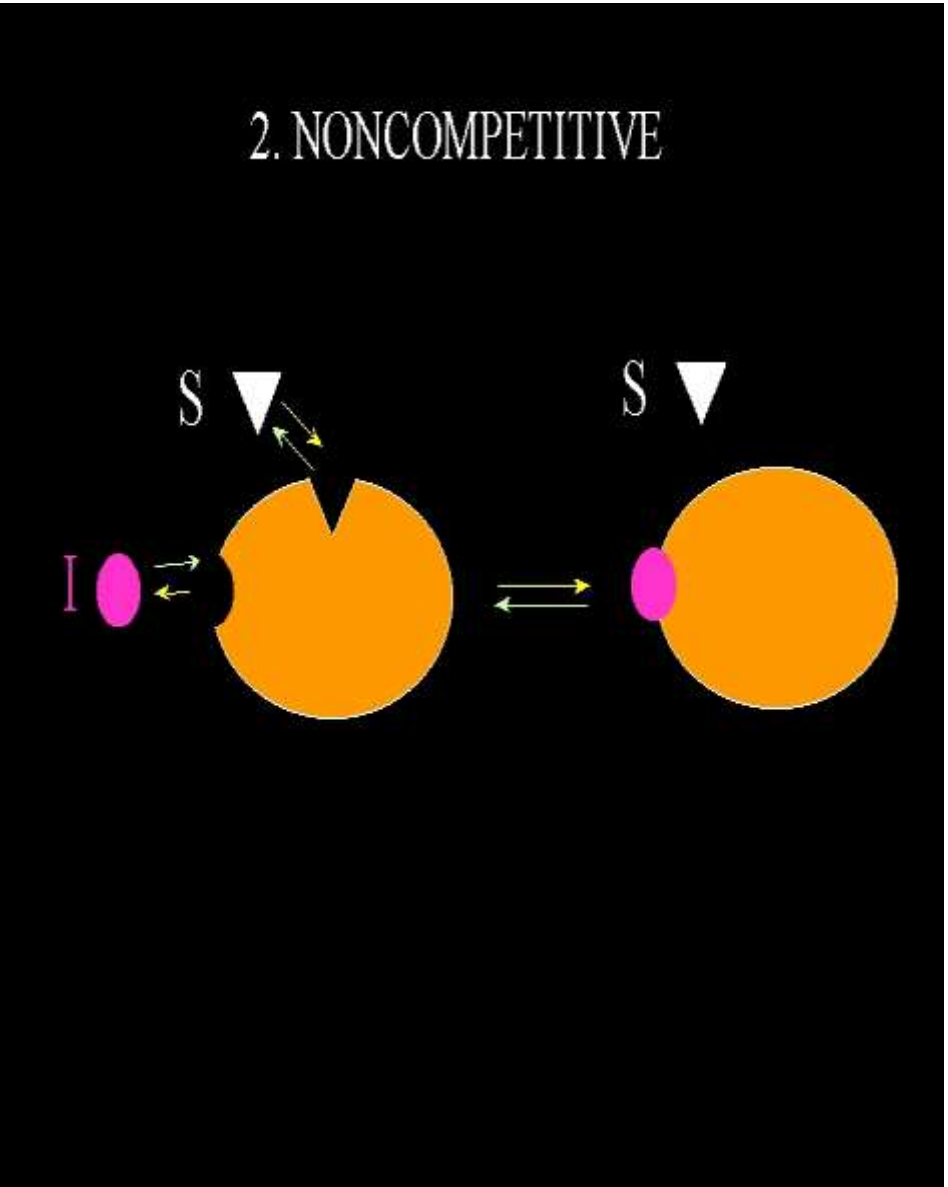
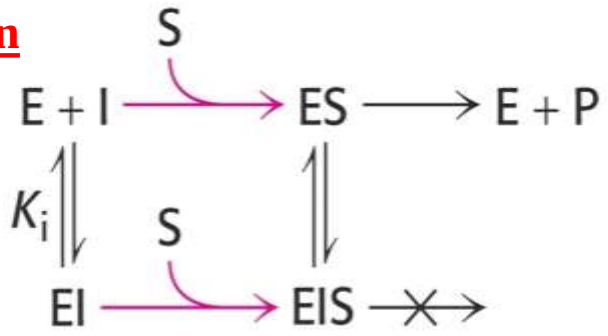
*Noncompetitive Inhibition * MIXED*



- **Inhibitor fits into a site on the enzyme different from the active site**
- ***As a result, the folding of the enzyme changes a bit, distorting the active site in a way that makes it less effective as a catalyst***
- **A decrease in the maximum rate would be observed since each catalyst has become less efficient**

Non-Competitive inhibition affects V_{max}

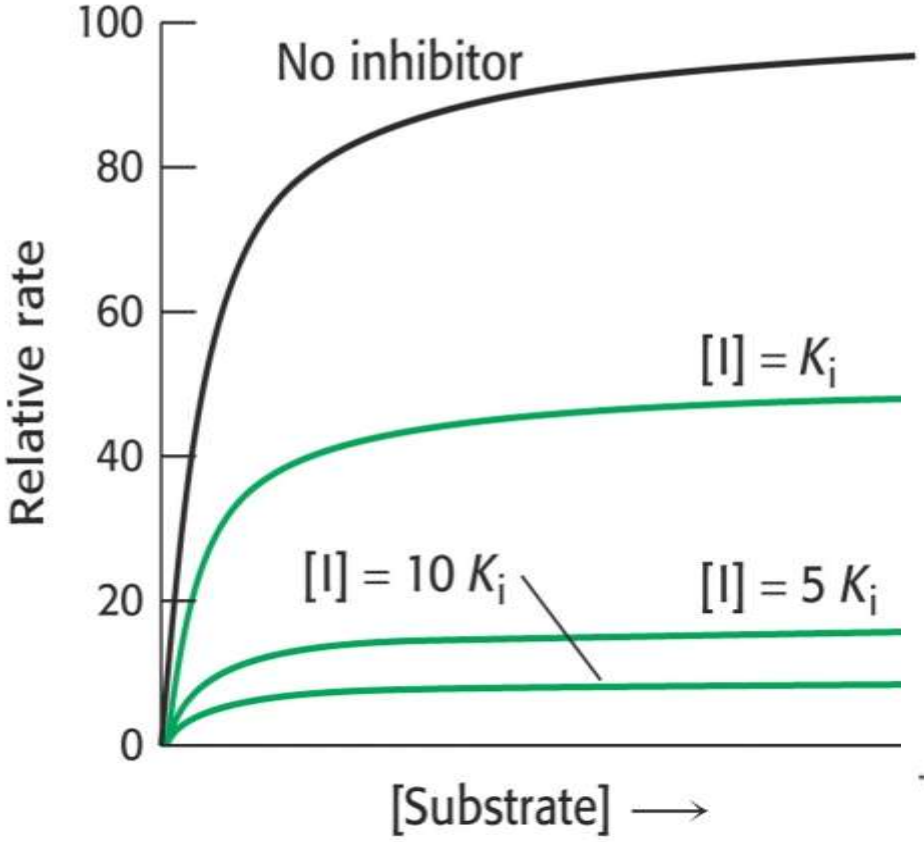
Neither can react

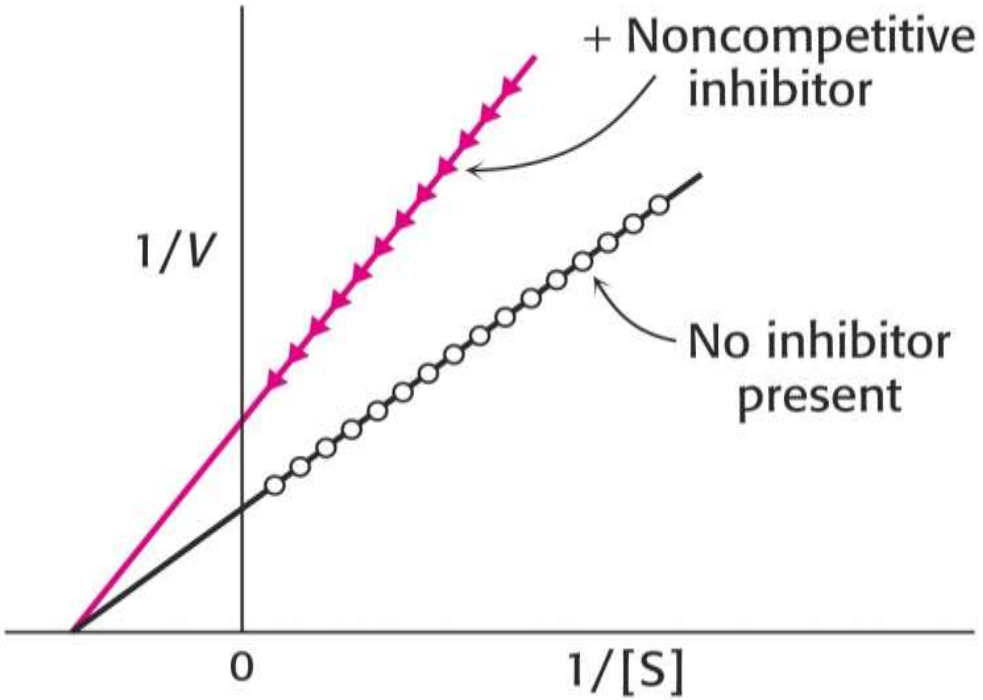


Max

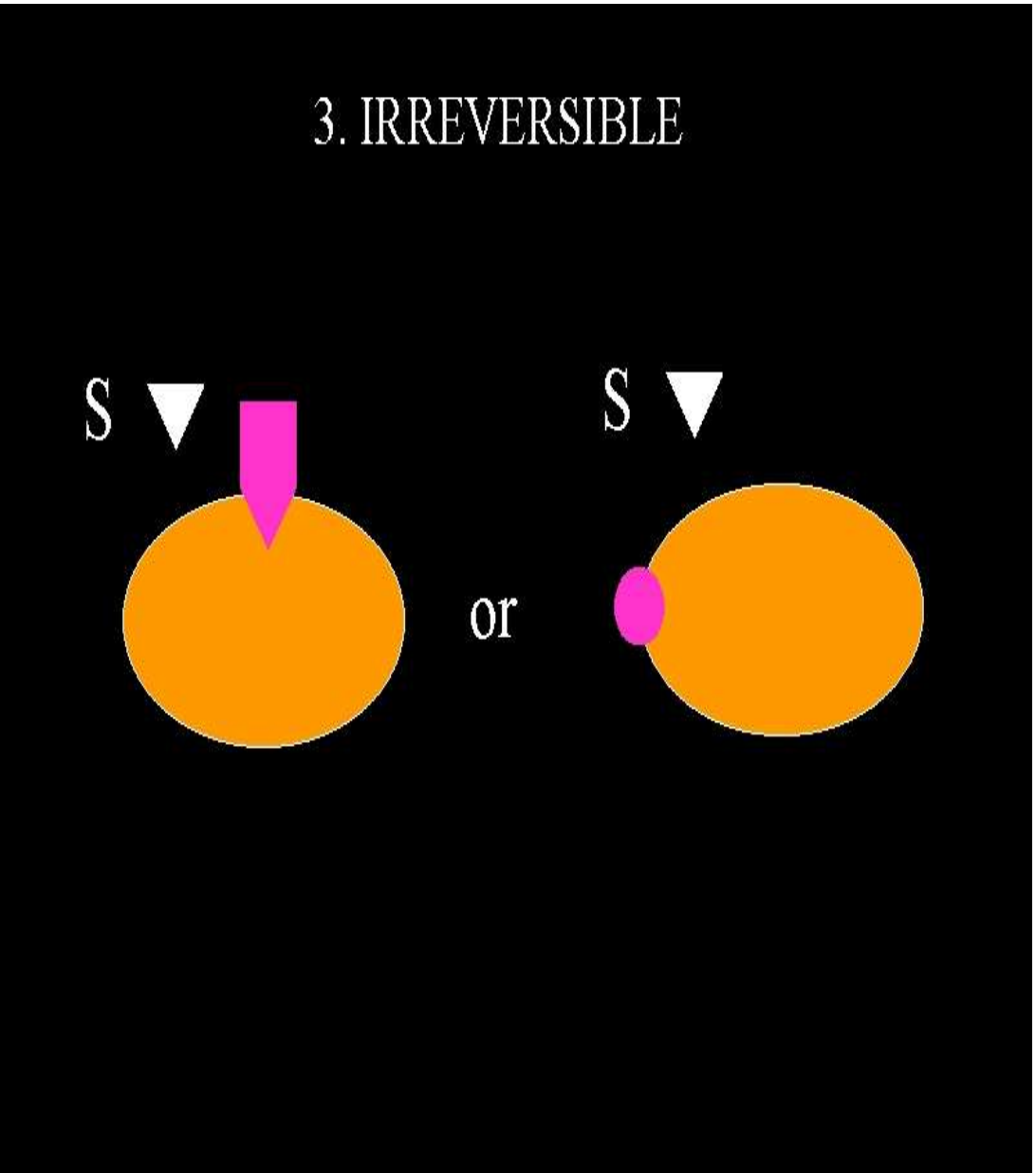
-> Inhibition cannot be overcome by increase in substrate concentration

-> V_{max} altered: apparent V_{max} value decreased



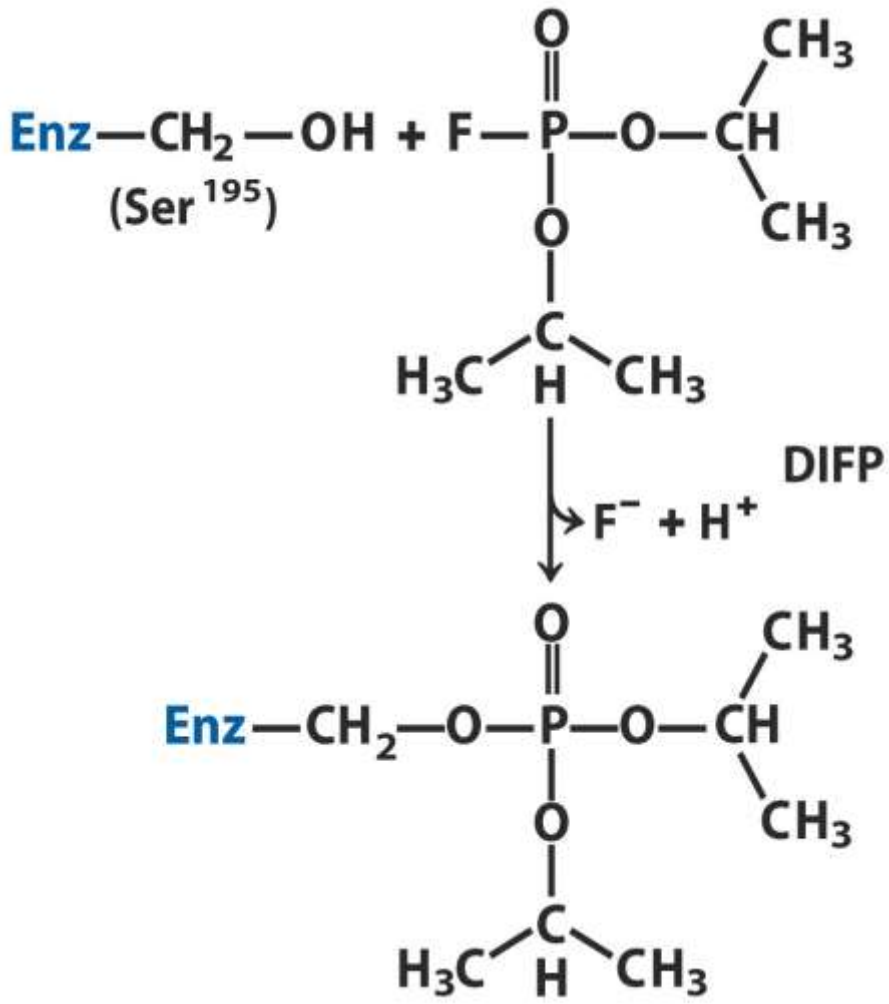


Irreversible Inhibition

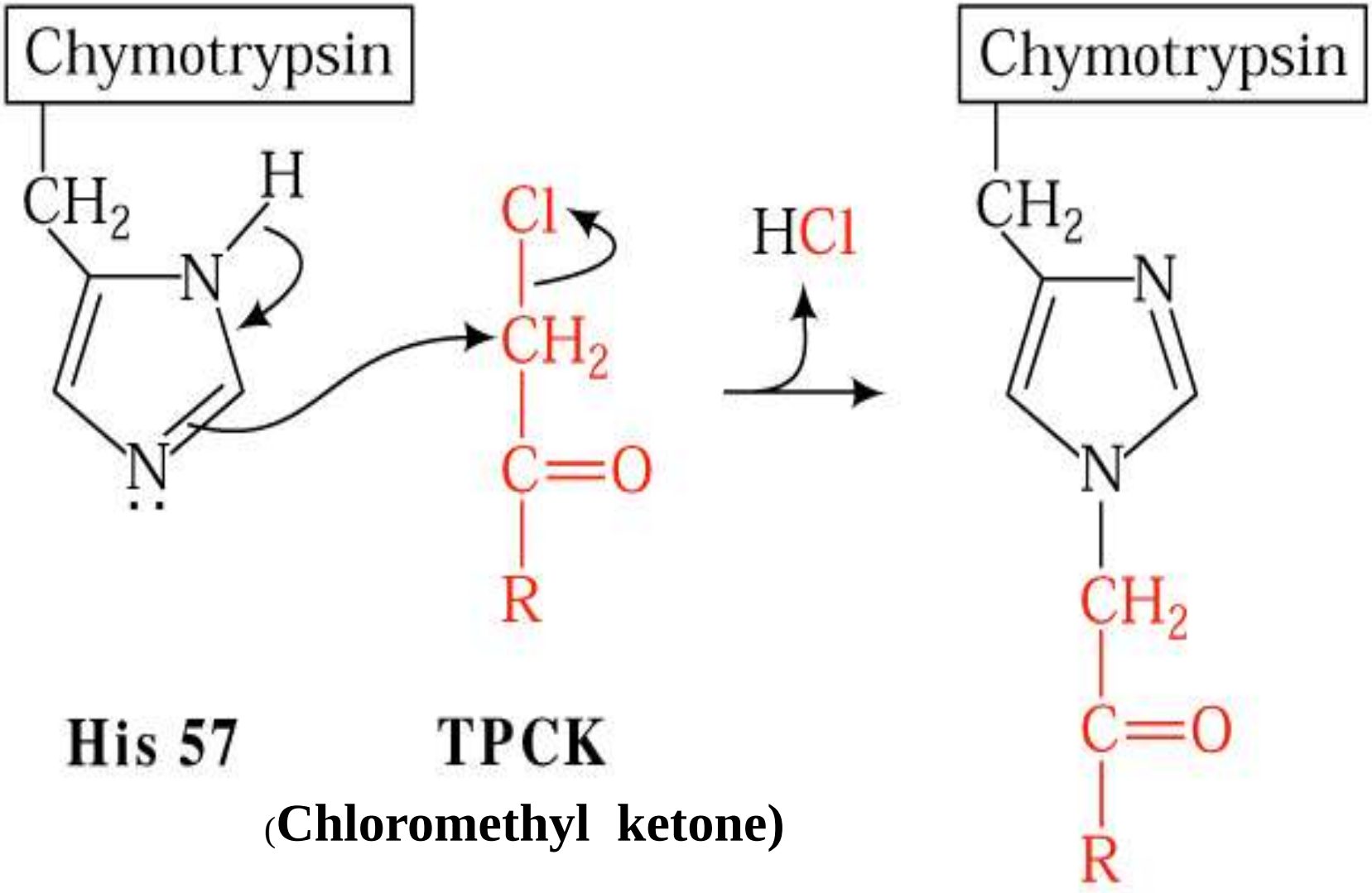


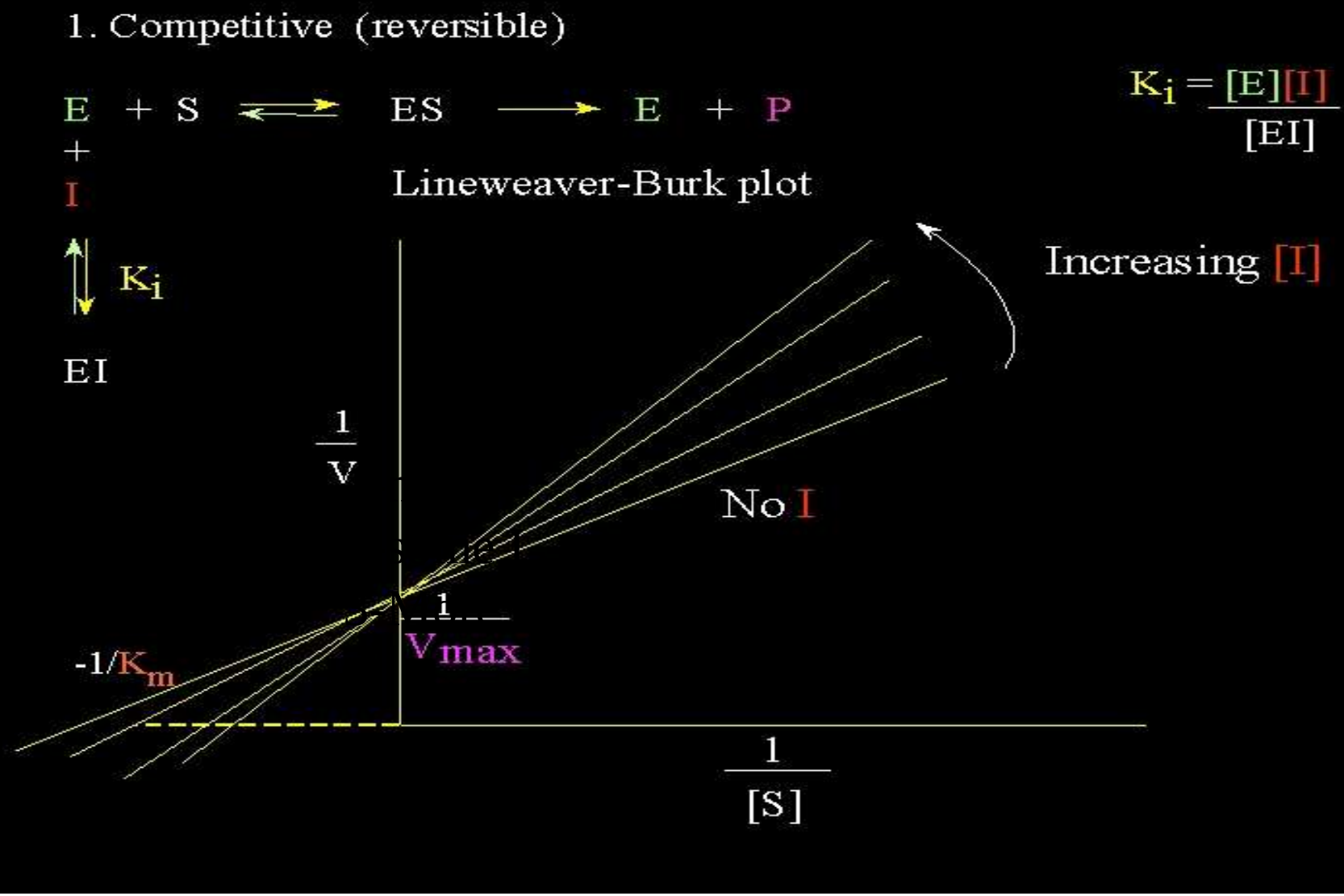
- ***Inhibitor may bind to the active site or alternative site***
- ***Next, inhibitor forms a covalent bond to the enzyme***
- ***Since inhibitor, will not fall off, the enzyme molecule is dead***

Irreversible Inhibition by Adduct Formation



(di-isopropyl-fluorophosphate)



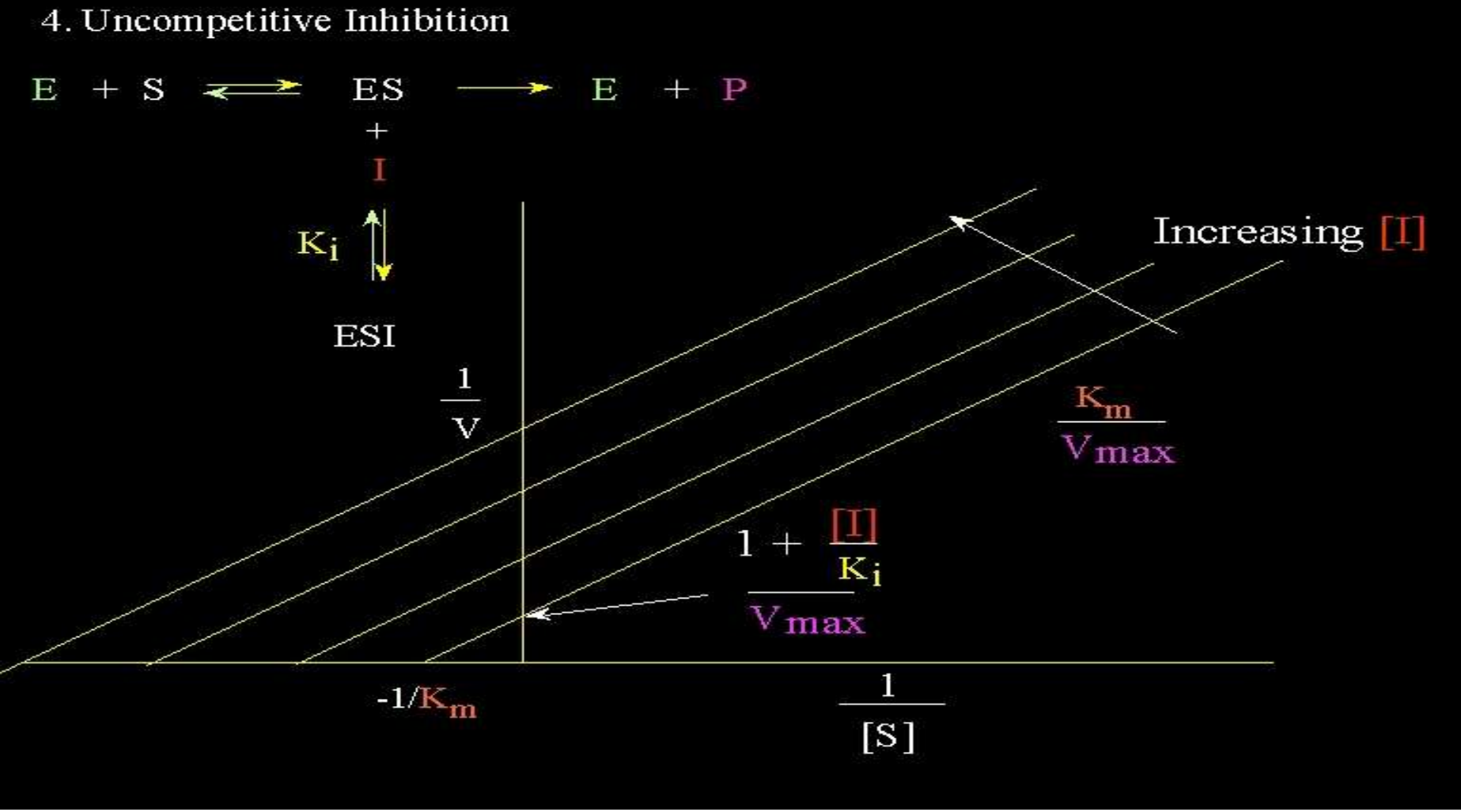


1-no I *Slope=Km/Vmax*

2-some I *Km increase↑and Vmax constant*

3-More I

Different slopes, same y-intercept ***(Km for substrate increases)***

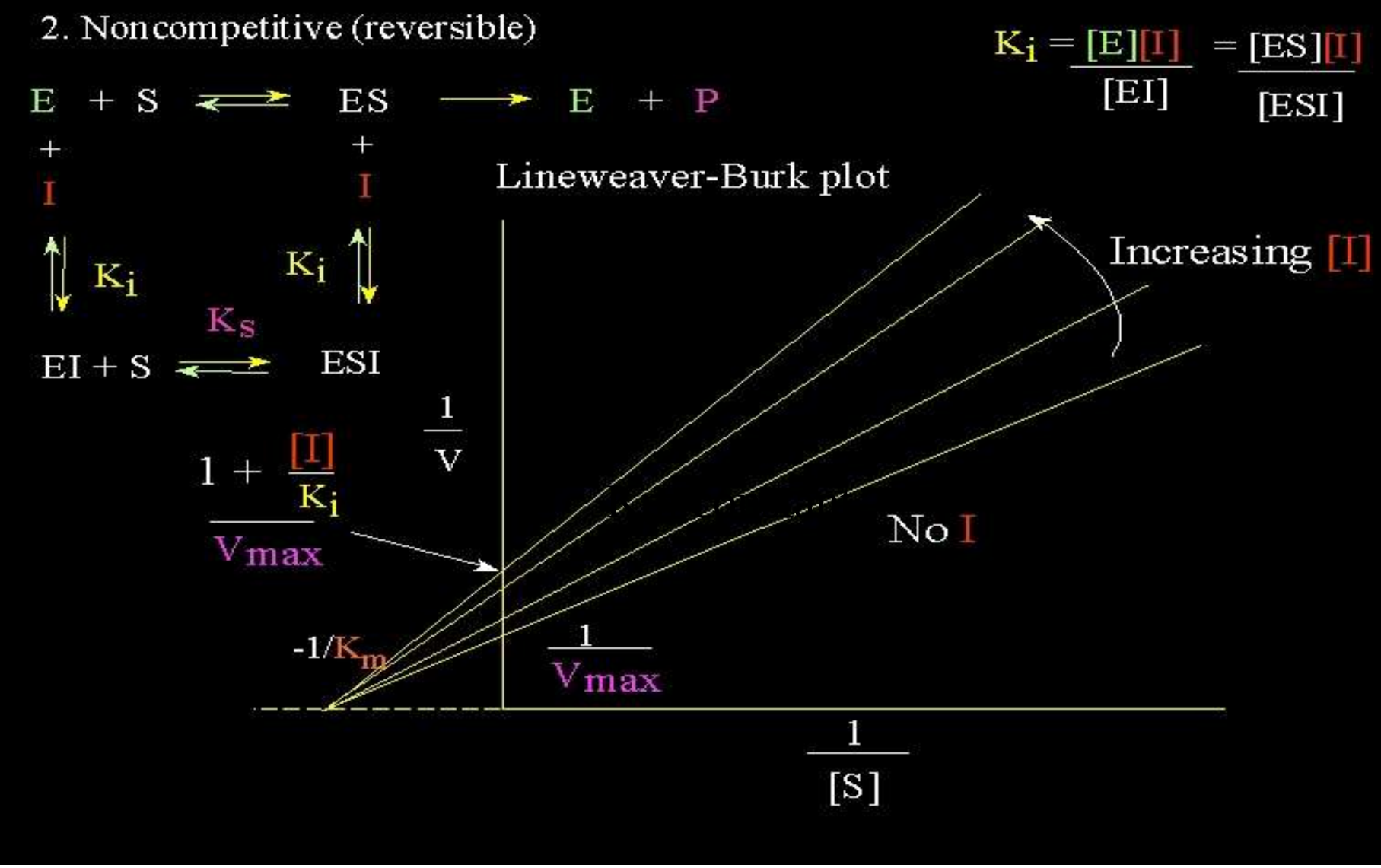


1-no I *Slope=Km/Vmax*

2-some I *Km decreas and Vmax decreas*

3-More I

Same slope, different x-intercept and y-intercept *(Equal change in both Km and Vmax)*



Different slopes, different y-intercept, same x-intercept (*Vmax decreases)* ↓ *Km constant*

Enzyme Inhibition-Irreversible

- Group-specific reagents
  -> e.g. S_N –Reaction
  Esterfication

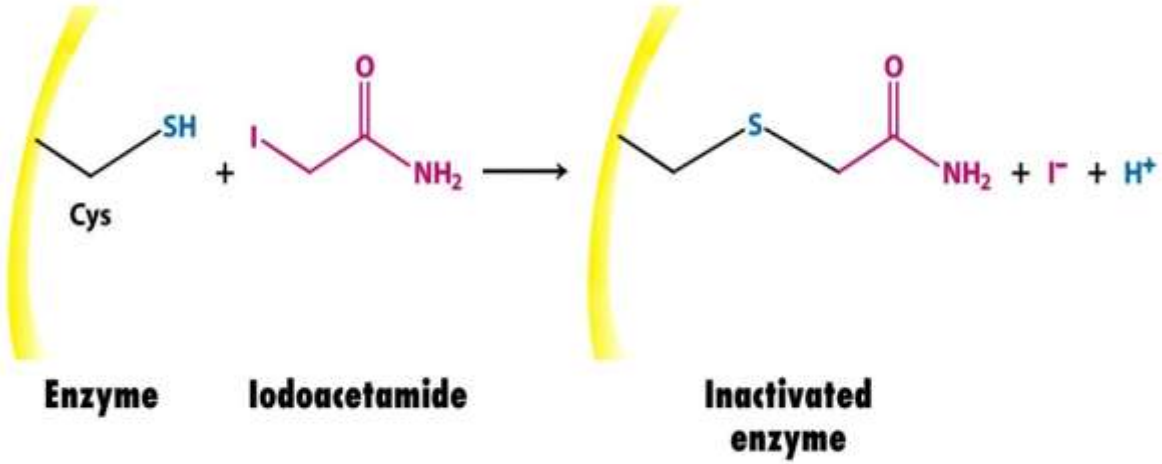


Figure 8-24
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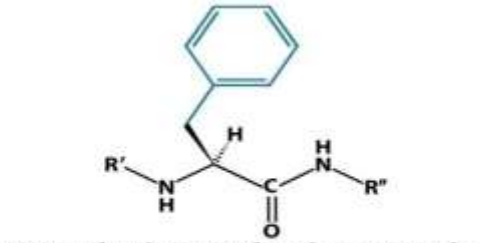
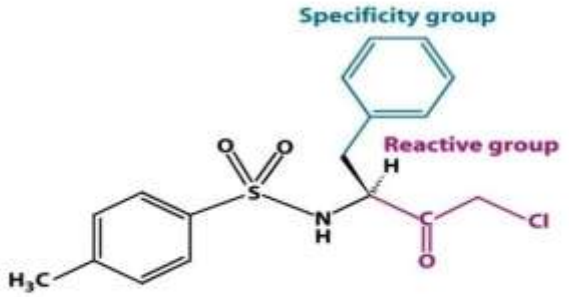
- *Substrate analog*



Natural substrate for chymotrypsin



Tosyl-L-phenylalanine chloromethyl ketone (TPCK

Figure 8-25a
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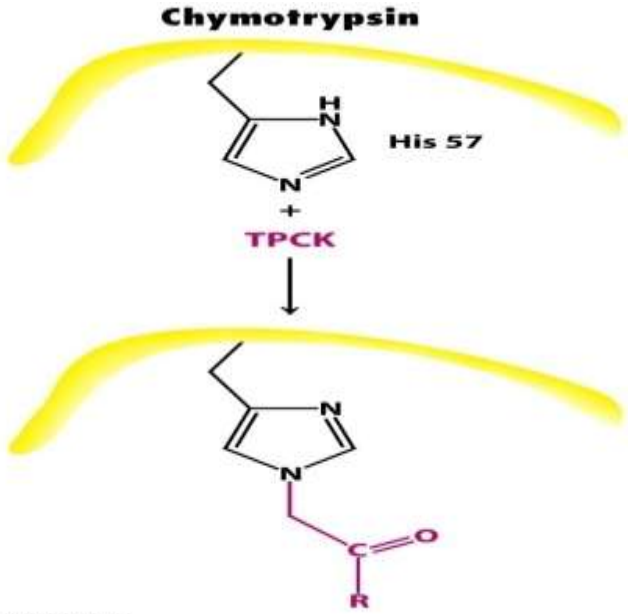

Figure 8-25b
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Summary

- Enzymes are biological catalysts, Enzyme as proteins
- Enzymes DO decrease the activation energy of a reaction ($\Delta G^{\circ\ddagger}$)
- Factors Affecting Enzyme Activity T, PH, [S] , [E] and Inhibiters
- General reaction $E+S \longrightarrow ES \longrightarrow P + E$
- Michaelis-Menten Equation $V_0 = Vmax * [S] / Km * [S]$
- 1-Lineweaver –Burke PLOT

. $I/V_0 = Km/Vmax.\ I/S + I/Vmax$ and Its proofed last equation

$Km \rightleftharpoons [S]$ When $V_0 = \frac{1}{2}$ Vmax

- enzyme has a small value of K_M, it achieves its maximum catalytic efficiency at low substrate
- Inhibiters –Competitive Km ↑ Vmax–Un competitive Km ↓ Vmax ↓ -Non-competitive Vmax ↓
- ↑[S] only in Competitive Inhibition .
- Tow types From Inhibiters Reversible and Irreversible .

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{[S]} \qquad (18)$$

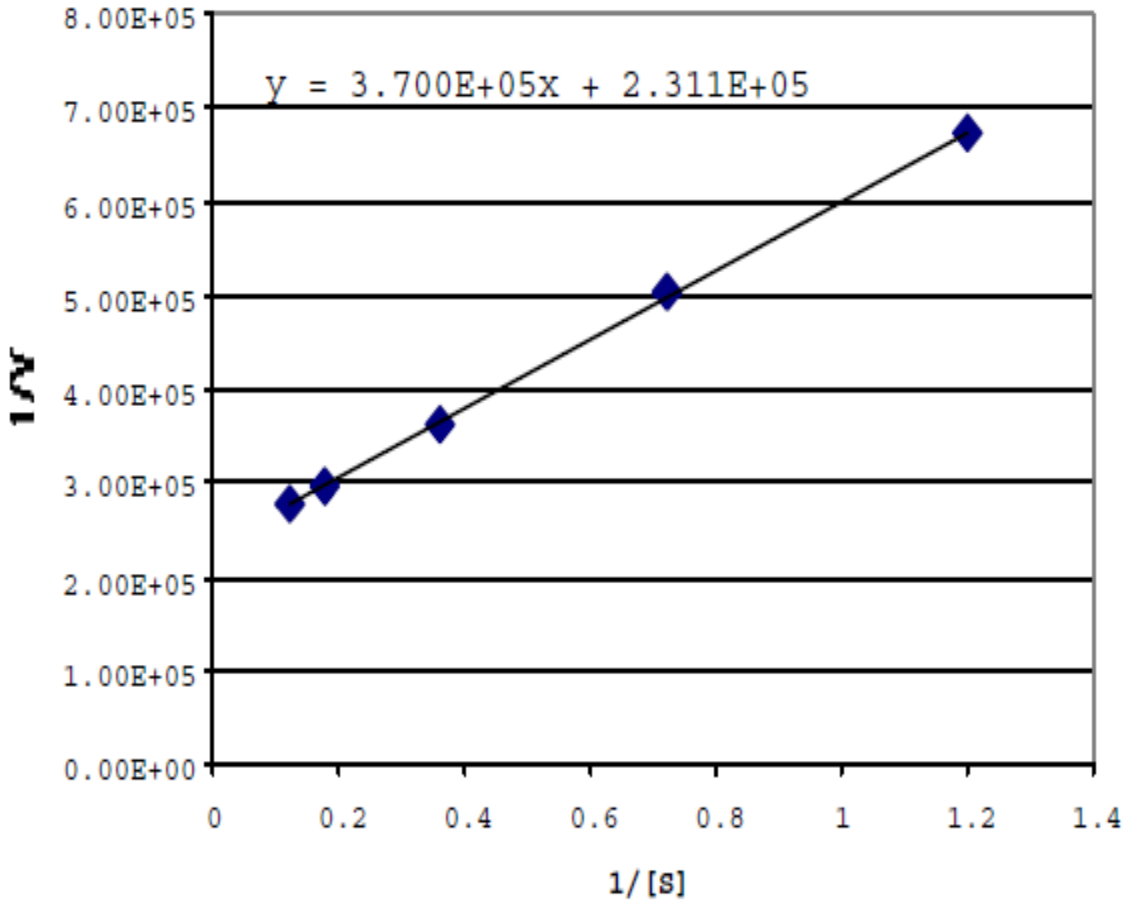
Determining K_M and V_{max} experimentally

To characterize an enzyme-catalyzed reaction K_M and V_{max} need to be determined. The way this is done experimentally is to measure the rate of catalysis (reaction velocity) for different substrate concentrations. In other words, determine V at different values of [S]. Then plotting 1/V vs. 1/[S] we should obtain a straight line described by equation (18). From the y-intercept and the slope, the values of K_M and V_{max} can be determined. For example, use EXCEL to plot the data shown below. Fit the data to a straight line, and from the equation of the straight line determine the values of K_M and V_{max}.

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{[S]} \qquad (18)$$

[S] (mM)	V (mM/sec)	1/[S] (mM-1)	1/V (sec/mM)
8.33	3.62E-06	0.12	2.76E+05
5.55	3.39E-06	0.18	2.95E+05
2.77	2.75E-06	0.36	3.64E+05
1.38	1.99E-06	0.72	5.02E+05
0.83	1.49E-06	1.2	6.73E+05

You should obtain a plot as shown in figure 6 below. From the fit to the data show that $K_M = 1.6$ for this data and V_{max} is 4.32×10^{-6} mM/sec



Significance of K_M

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{[S]} \qquad (18)$$

From equation 18, when $[S] = K_M$, then $V = V_{max}/2$. Hence K_M is equal to the substrate concentration at which the reaction rate is half its maximum value. In other words, if an enzyme has a small value of K_M, it achieves its maximum catalytic efficiency at low substrate concentrations. Hence, the smaller the value of K_M, the more efficient is the catalyst. The value of K_M for an enzyme depends on the particular substrate . It also depends on the pH of the solution and the temperature at which the reaction is carried out. For most enzymes K_M lies between 10^{-1} and 10^{-7} M.

Thank You