Advanced Reactor Design

Week 10
Enzymatic Reactions

Saba A. Gheni, Ph.D.
Chemical Engineering Department
ghenis@tu.edu.iq

COLLEGE OF ENGINEERING - كلية الهندسة
Tikrit University - جامعة تكريت

Introduction

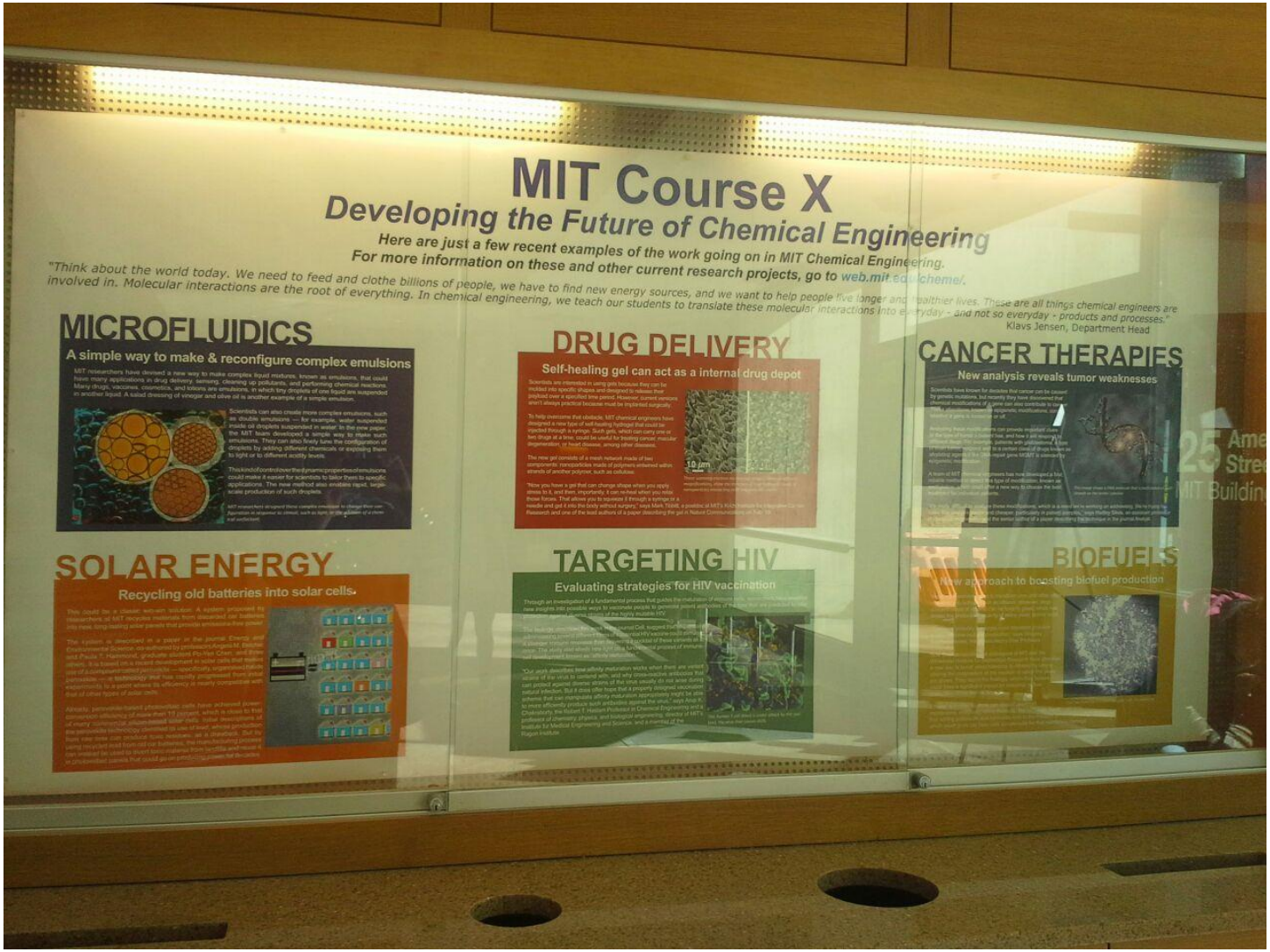
- • Definition of Enzymatic Reactions
- • Importance in Biological Systems
- • Role of Enzymes as Biocatalysts
- • Real-world Applications (e.g., medicine, industry, biotechnology)

Topics to be Covered

- 1. Basics of Enzymatic Reactions
- 2. Mechanism of Enzyme Action
- 3. Factors Affecting Enzyme Activity
- 4. Enzyme Kinetics (Michaelis-Menten Theory)
- 5. Types of Enzymes and Classification

Objectives

- • Understand the fundamentals of enzymatic reactions
- • Explore the mechanisms by which enzymes function
- • Identify key factors that influence enzyme activity
- • Analyze enzyme kinetics and classification


MIT Course X
Developing the Future of Chemical Engineering
Here are just a few recent examples of the work going on in MIT Chemical Engineering.
For more information on these and other current research projects, go to web.mit.edu/cheme/.
"Think about the world today. We need to feed and clothe billions of people, we have to find new energy sources, and we want to help people live longer and healthier lives. These are all things chemical engineers are involved in. Molecular interactions are the root of everything. In chemical engineering, we teach our students to translate these molecular interactions into everyday - and not so everyday - products and processes."
Klavs Jensen, Department Head
MICROFLUIDICS
A simple way to make & reconfigure complex emulsions
DRUG DELIVERY
Self-healing gel can act as a internal drug depot
CANCER THERAPIES
New analysis reveals tumor weaknesses
SOLAR ENERGY
Recycling old batteries into solar cells.
TARGETING HIV
Evaluating strategies for HIV vaccination
BIOFUELS
New approach to boosting biofuel production

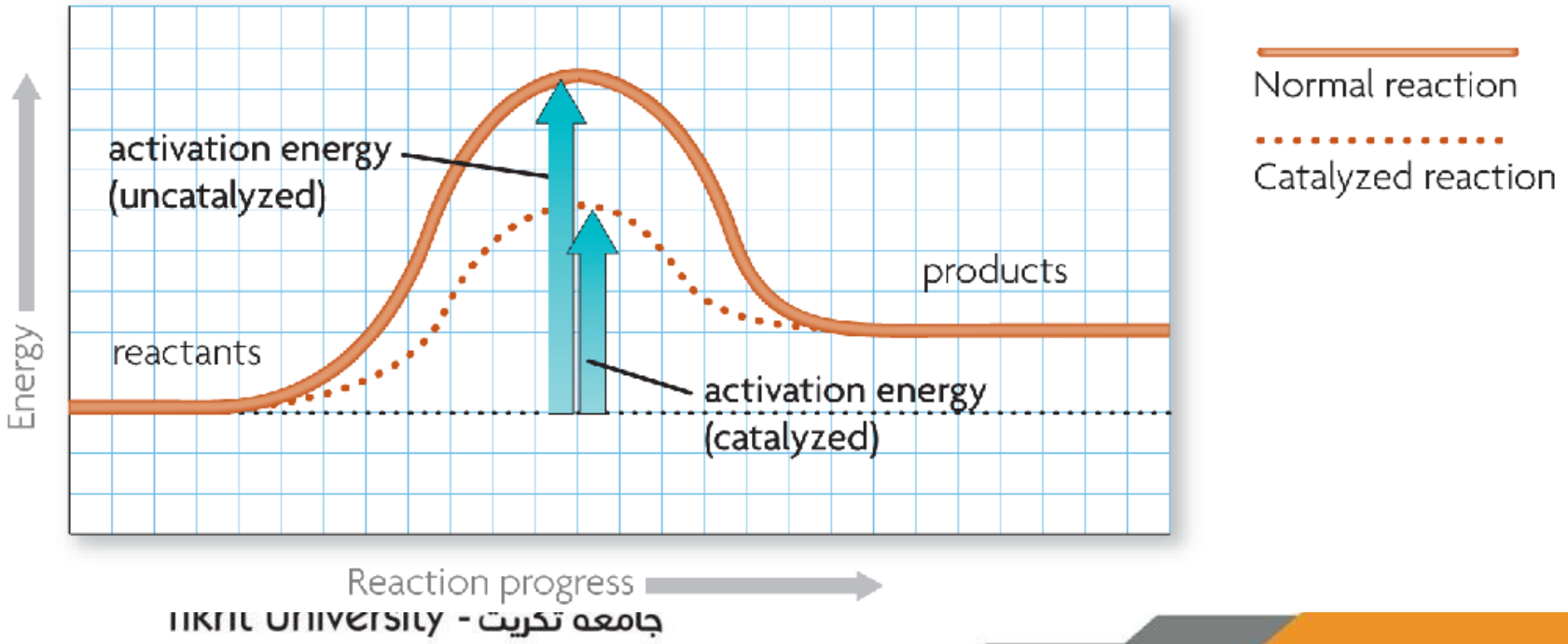
Robert S. Langer
Chemical Engineer, MIT

- **Robert** S. **Langer** is one of 12 Institute Professors at MIT; being an Institute Professor is the highest honor that can be awarded to a faculty member. Dr. **Langer** has written more than 1,480 articles.
- He also has over 1,360 issued and pending patents worldwide. Dr. **Langer**'s patents have been licensed or sublicensed to over 400 pharmaceutical, chemical, biotechnology and medical device companies.

A catalyst lowers activation energy

Catalysts are substances that speed up chemical reactions.

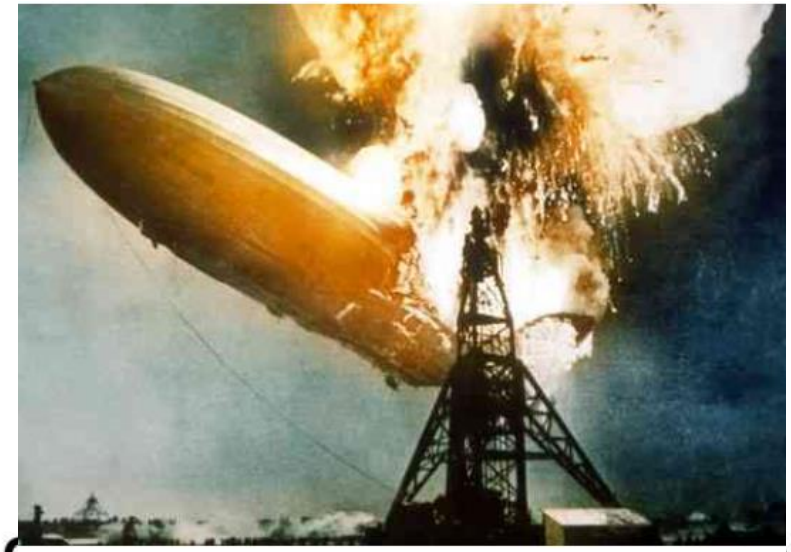
- decrease activation energy
- increase reaction rate



Enzymes allow chemical reactions to occur under tightly controlled conditions.

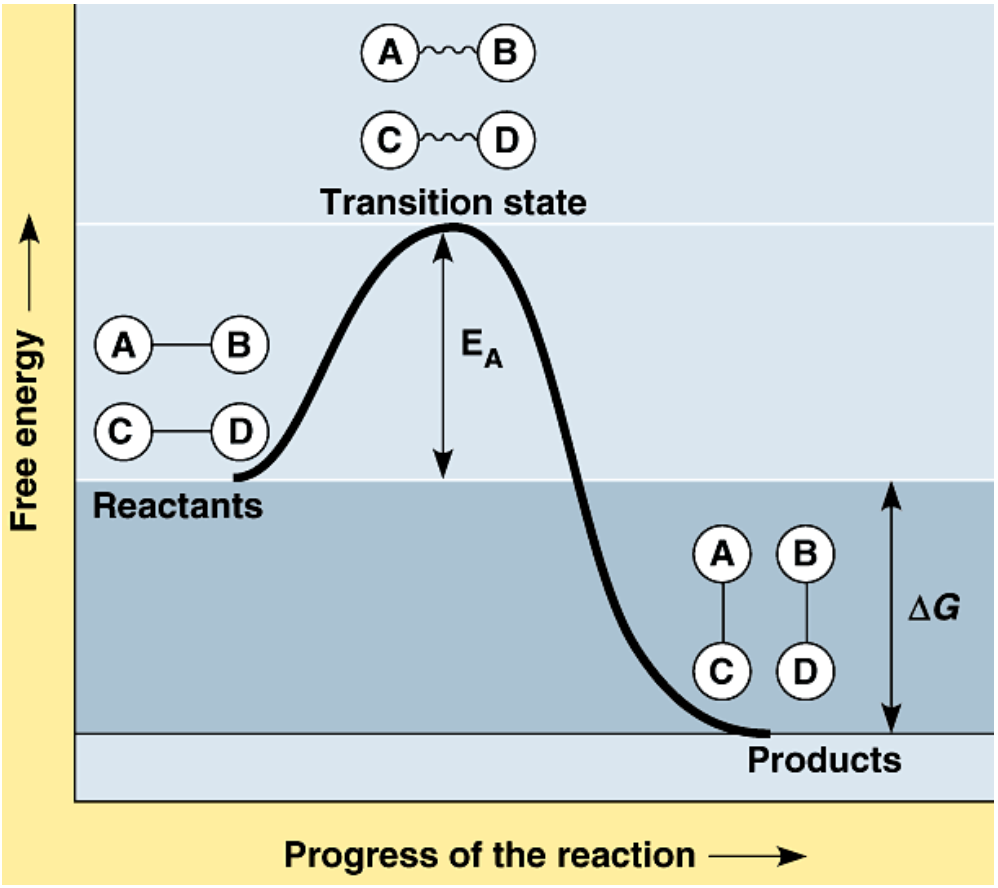
- Enzymes are catalysts in living things.
  - Enzymes are needed for almost all processes.
    - Most enzymes are proteins.

- If you mix two moles of hydrogen gas H_2 with one mole of oxygen gas-nothing happens.
- If you add a spark to the container, the following reaction occurs. KABOOM
  - $2H_2 + O_2 \rightarrow 2\ H_2O \quad \Delta G = -58$ kcal/mole

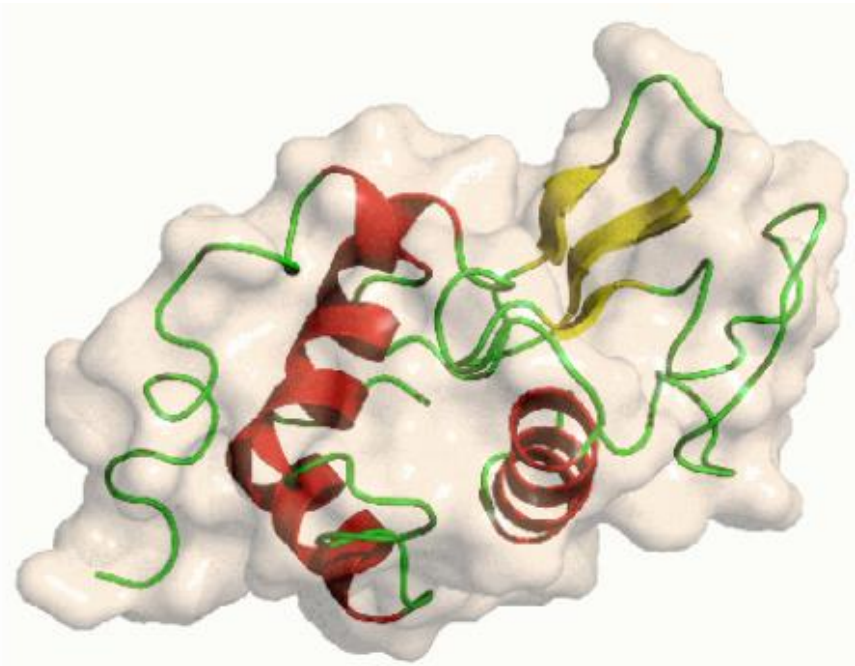


In order for water to be produced H_2 must become 2H and the O_2 must become 2O as this frees up the electrons tied up in covalent bonds, to form chemical bonds forming water, H_2O.

- The energy used to break the bonds in the reactants so they can be reformed in the products is called the energy of activation.



- Enzymes are biological catalysts that increase the reaction rate of biochemical reactions.



The enzyme shown is lysozyme

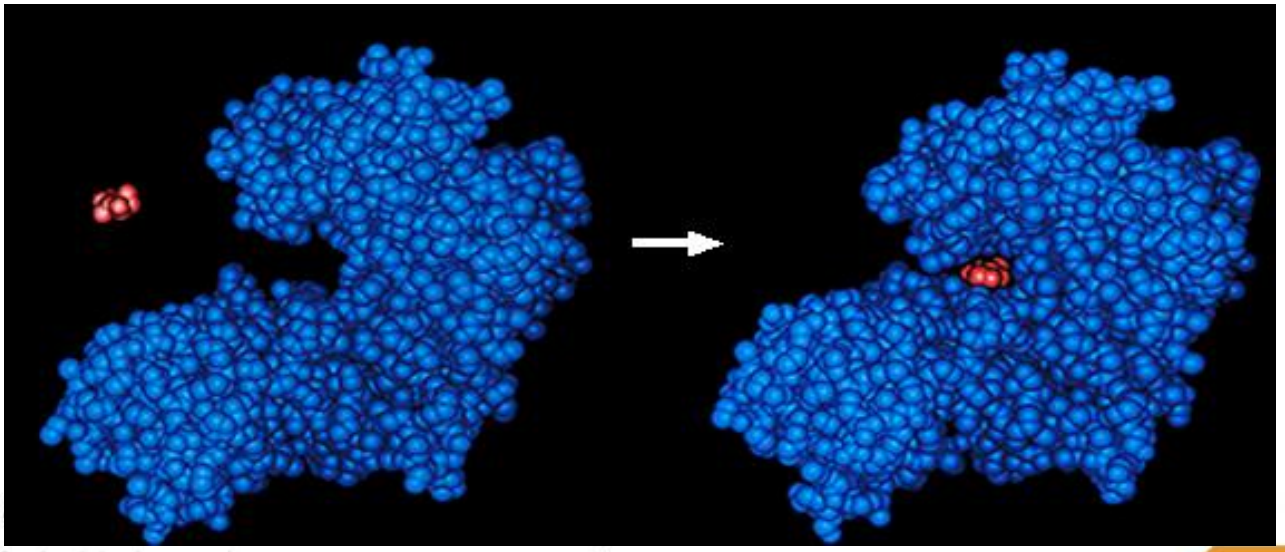
Characteristics of enzymes

A. Made of proteins (or RNA).

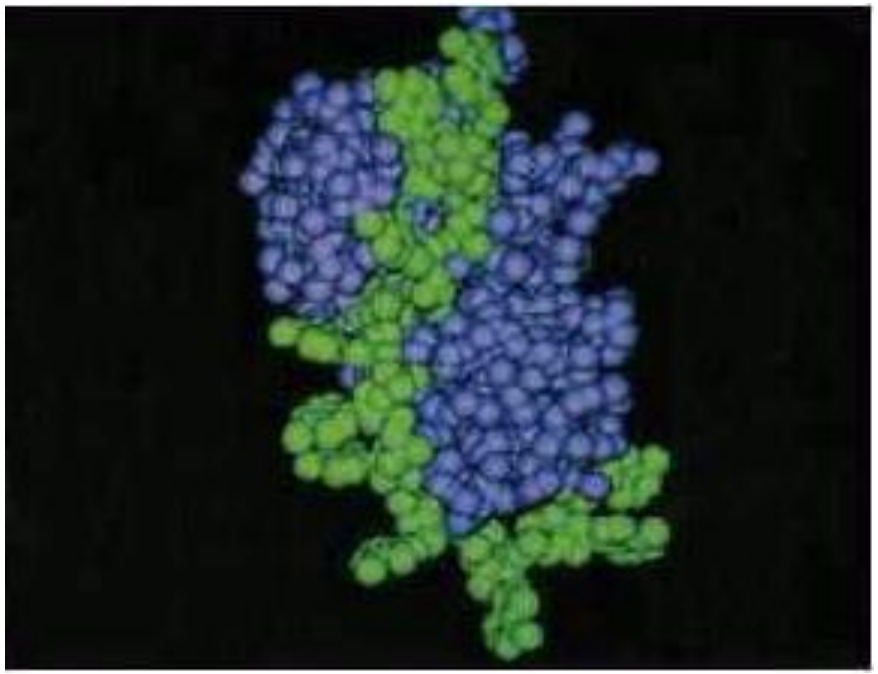
B. They are very specific and only work with a certain set of reactants or substrates that fit on their active site.

C. Enzymes can be used over and over again.

D. When an enzyme binds with the substrate, the substrate interacts with the enzyme causing it to change shape. This change in shape facilitates the chemical reaction to occur. This is called the induced fit.

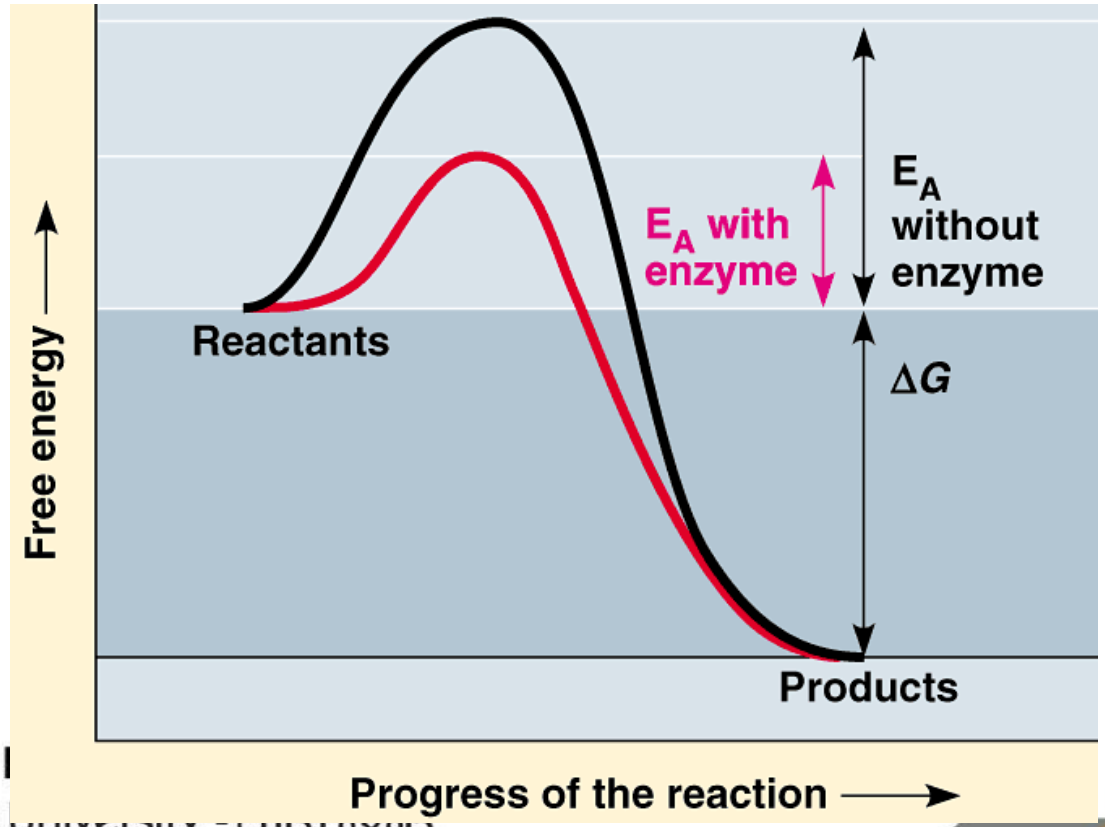


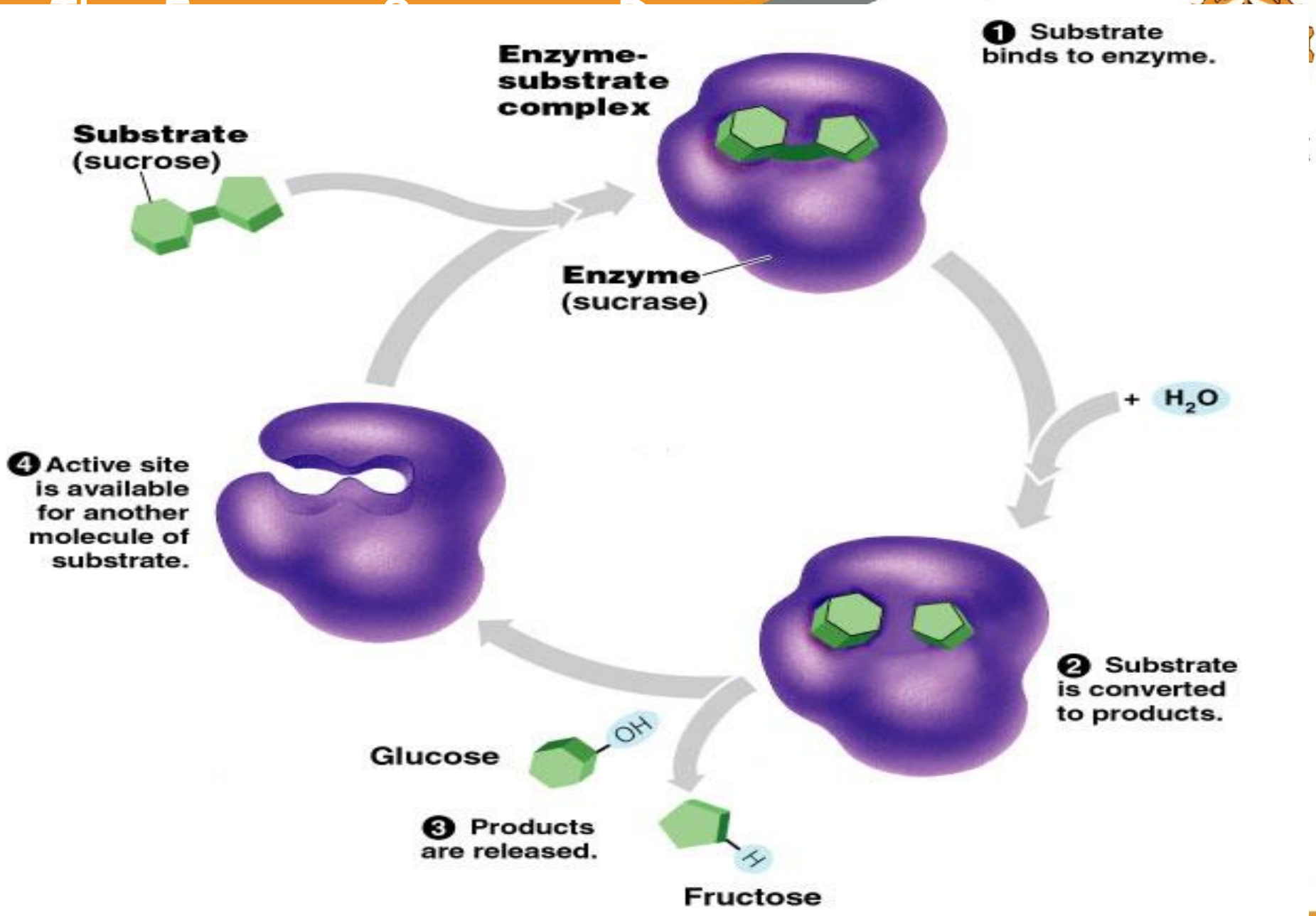
- Ribonuclease decomposes RNA, and the nucleotides can be recycled.
- The purple part is the enzyme; the green part is the substrate (RNA).



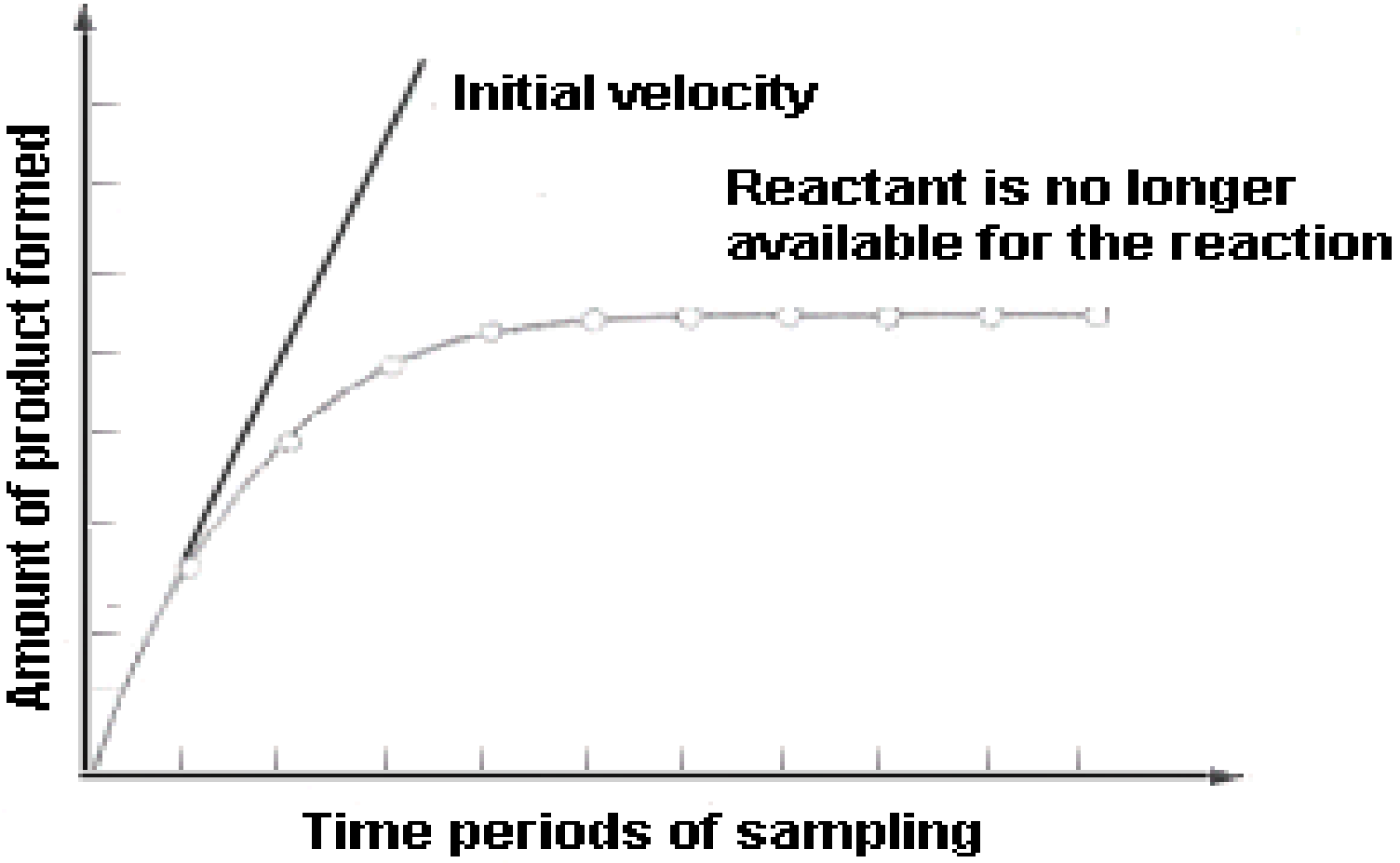
Enzymes Work by Lowering the Ener

E. Enzymes increase the reaction rate by lowering the energy of activation. They do NOT change Gibbs free energy or ΔG.

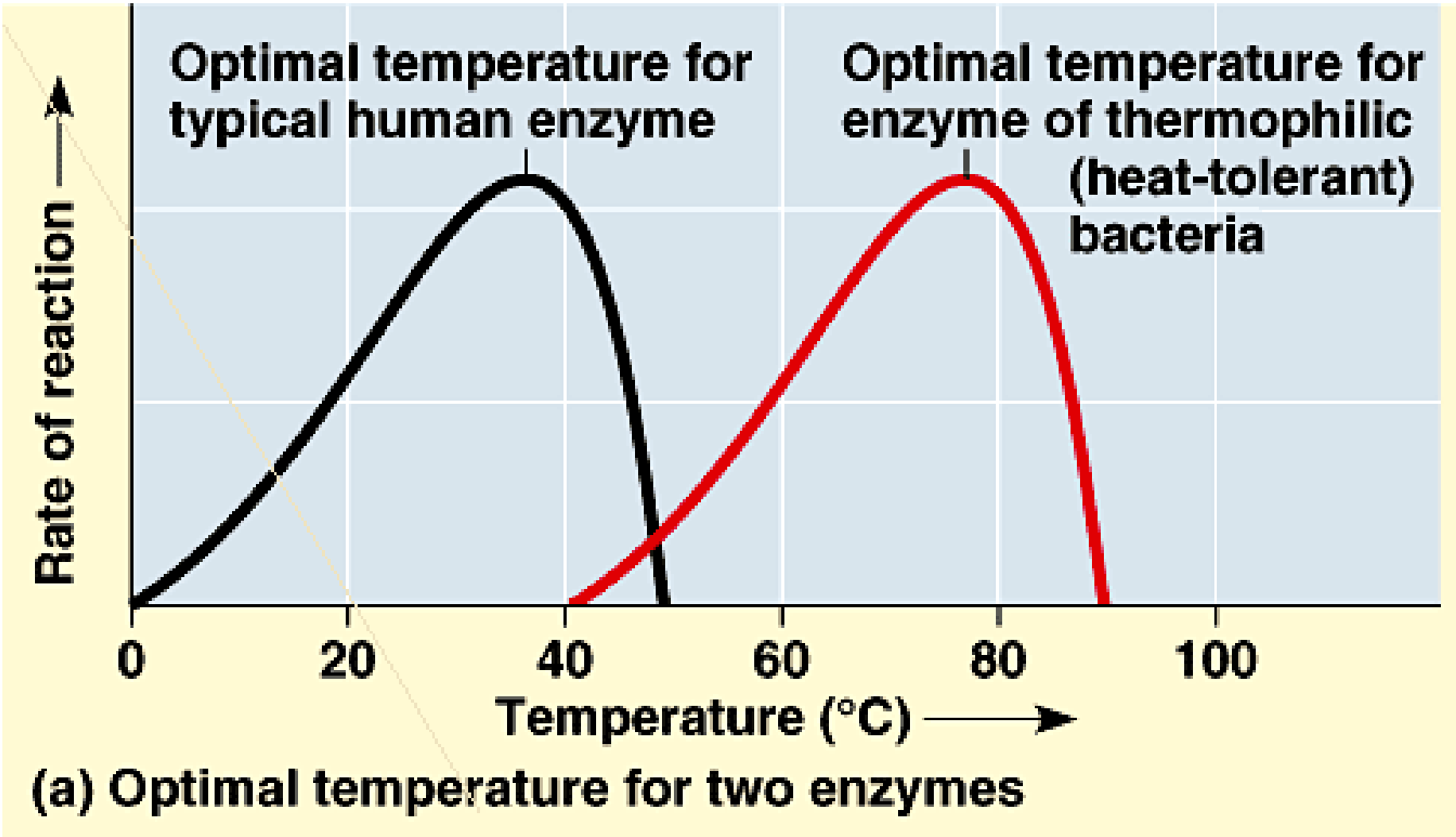



1 Substrate binds to enzyme.
Enzyme-substrate complex
Substrate (sucrose)
Enzyme (sucrase)
+ H_2O
2 Substrate is converted to products.
Glucose
OH
3 Products are released.
H
Fructose
4 Active site is available for another molecule of substrate.

- The reaction rate of an enzymatic reaction is always fastest at the beginning of the reaction when there is the greatest concentration of substrate. Why?

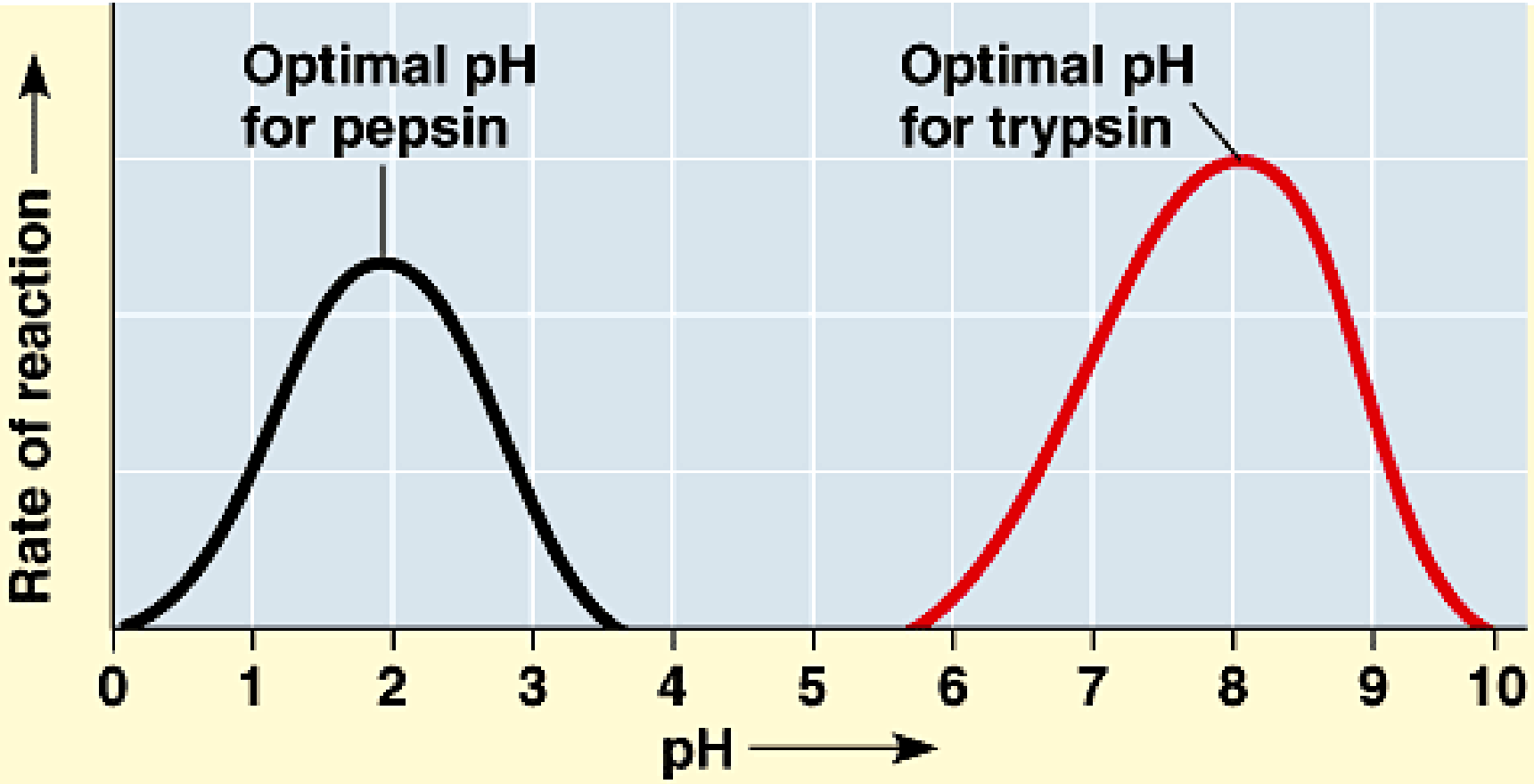


Effect of Increasing Temperature and

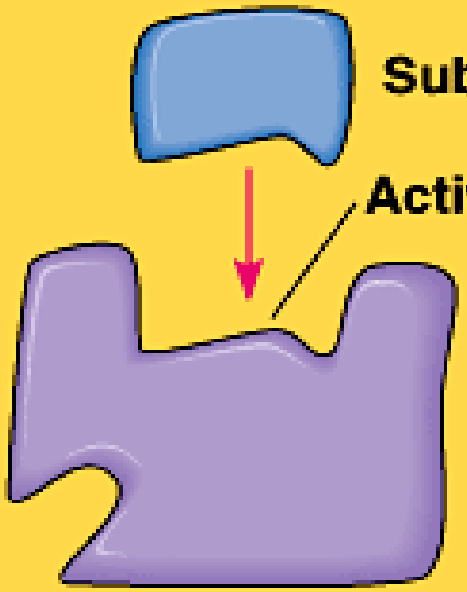


(a) Optimal temperature for two enzymes

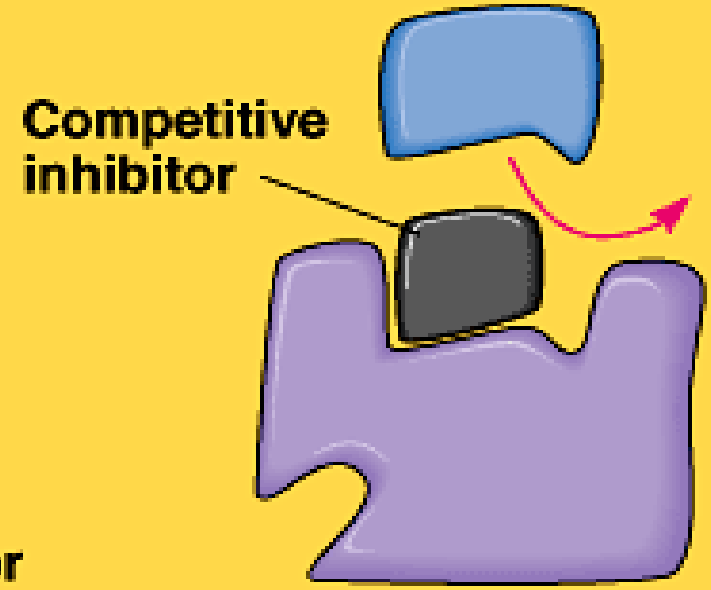
Effect of Varying pH and Enzymatic R



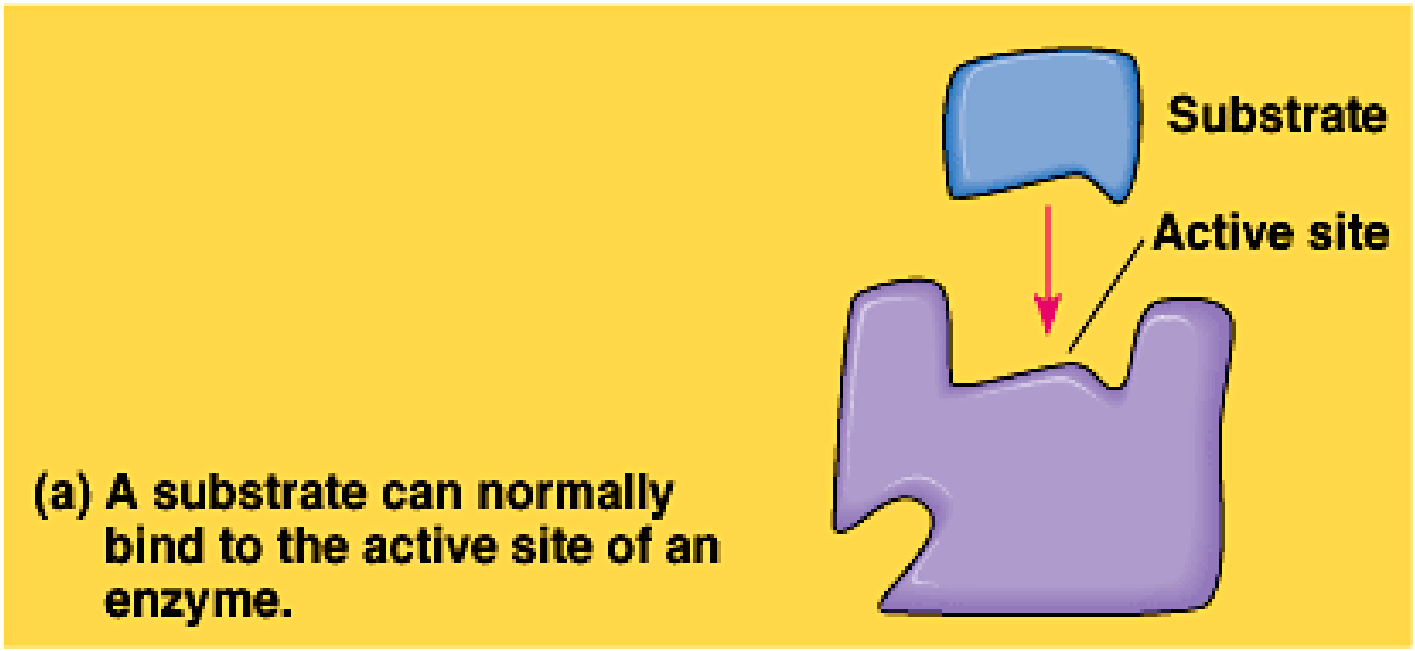
(b) Optimal pH for two enzymes



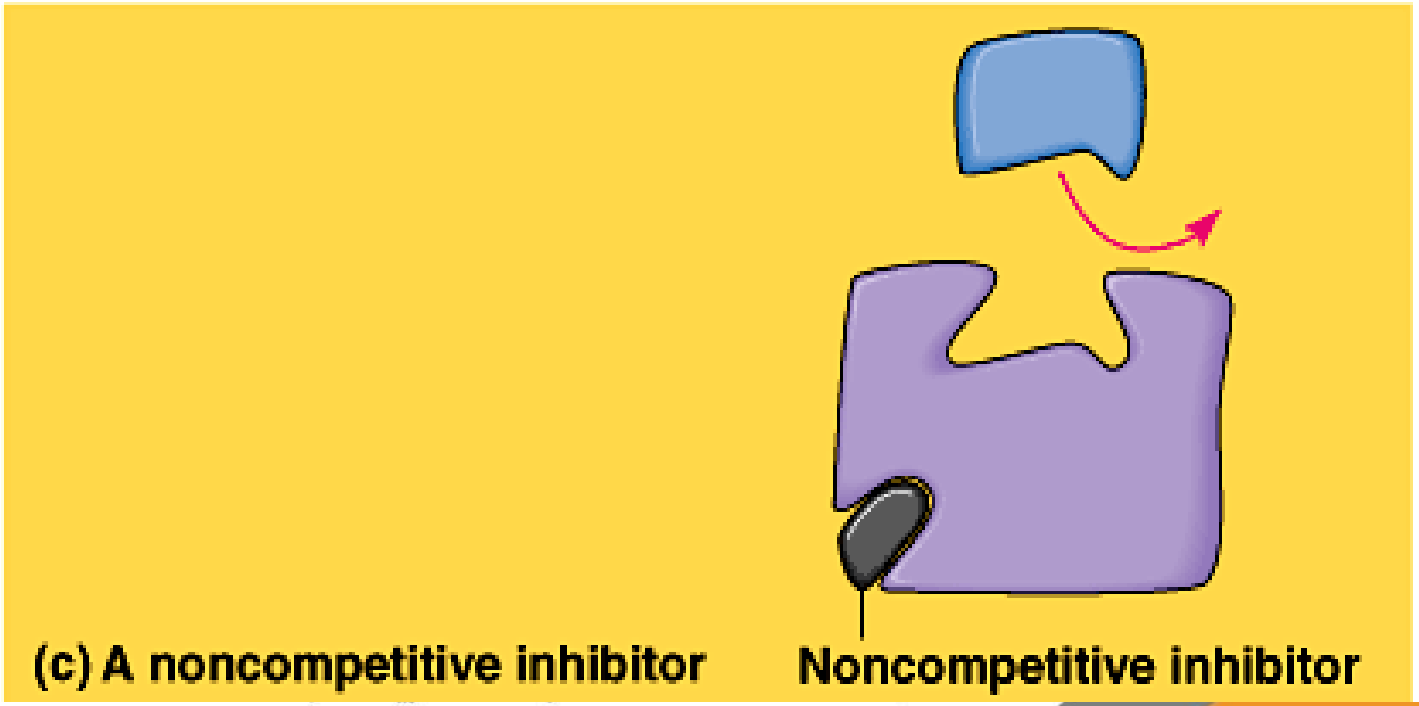
(a) A substrate can normally bind to the active site of an enzyme.



(b) A competitive inhibitor

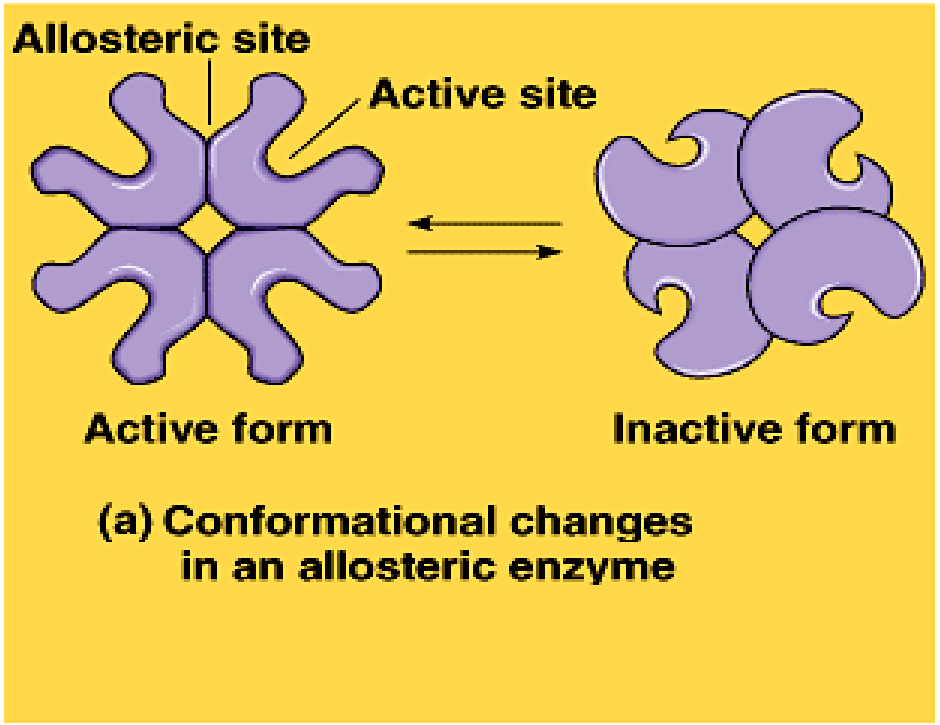


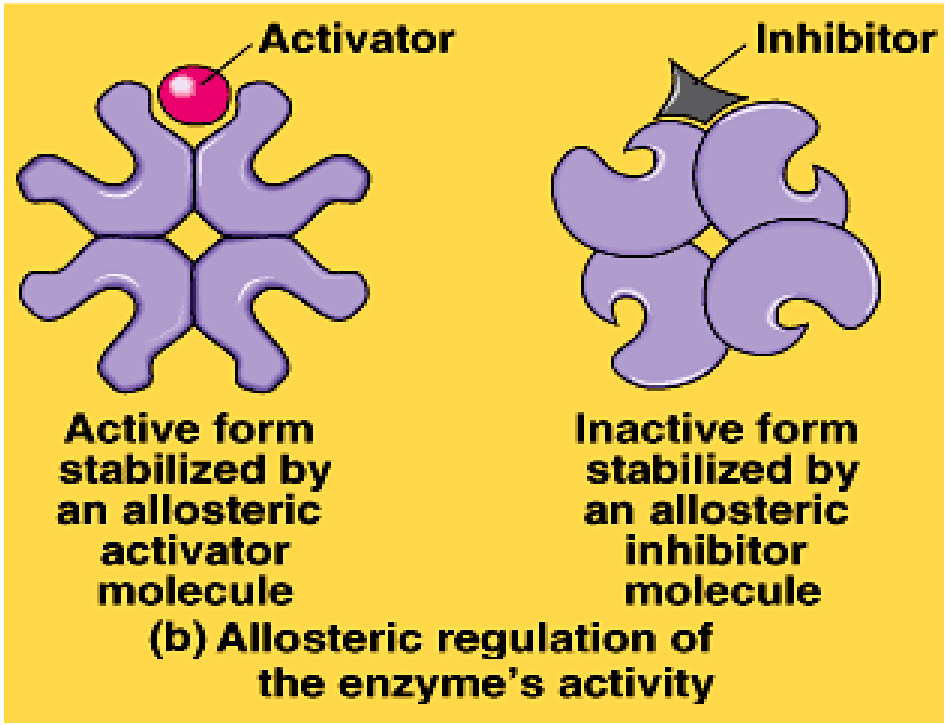
(a) A substrate can normally bind to the active site of an enzyme.

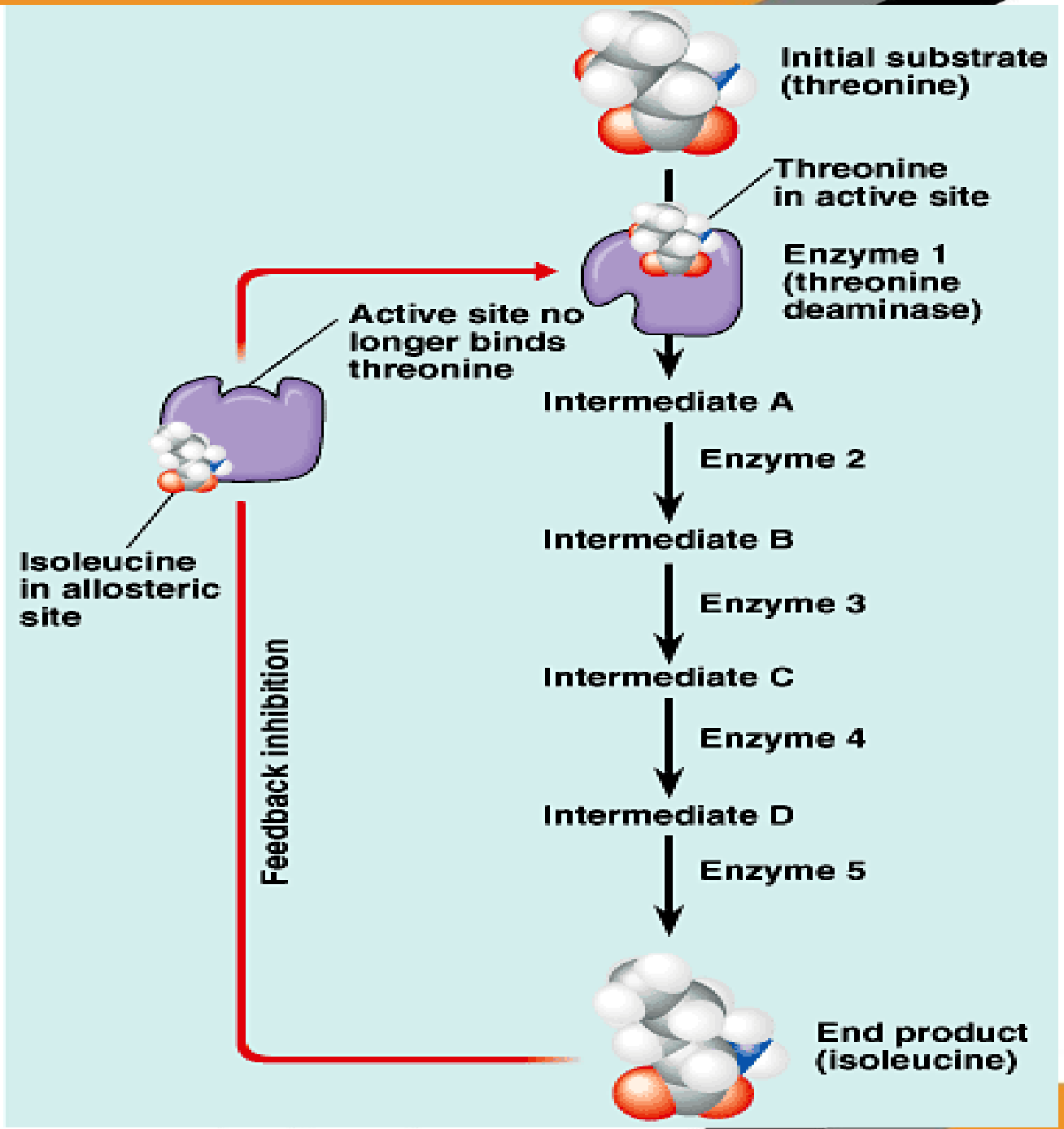


(c) A noncompetitive inhibitor

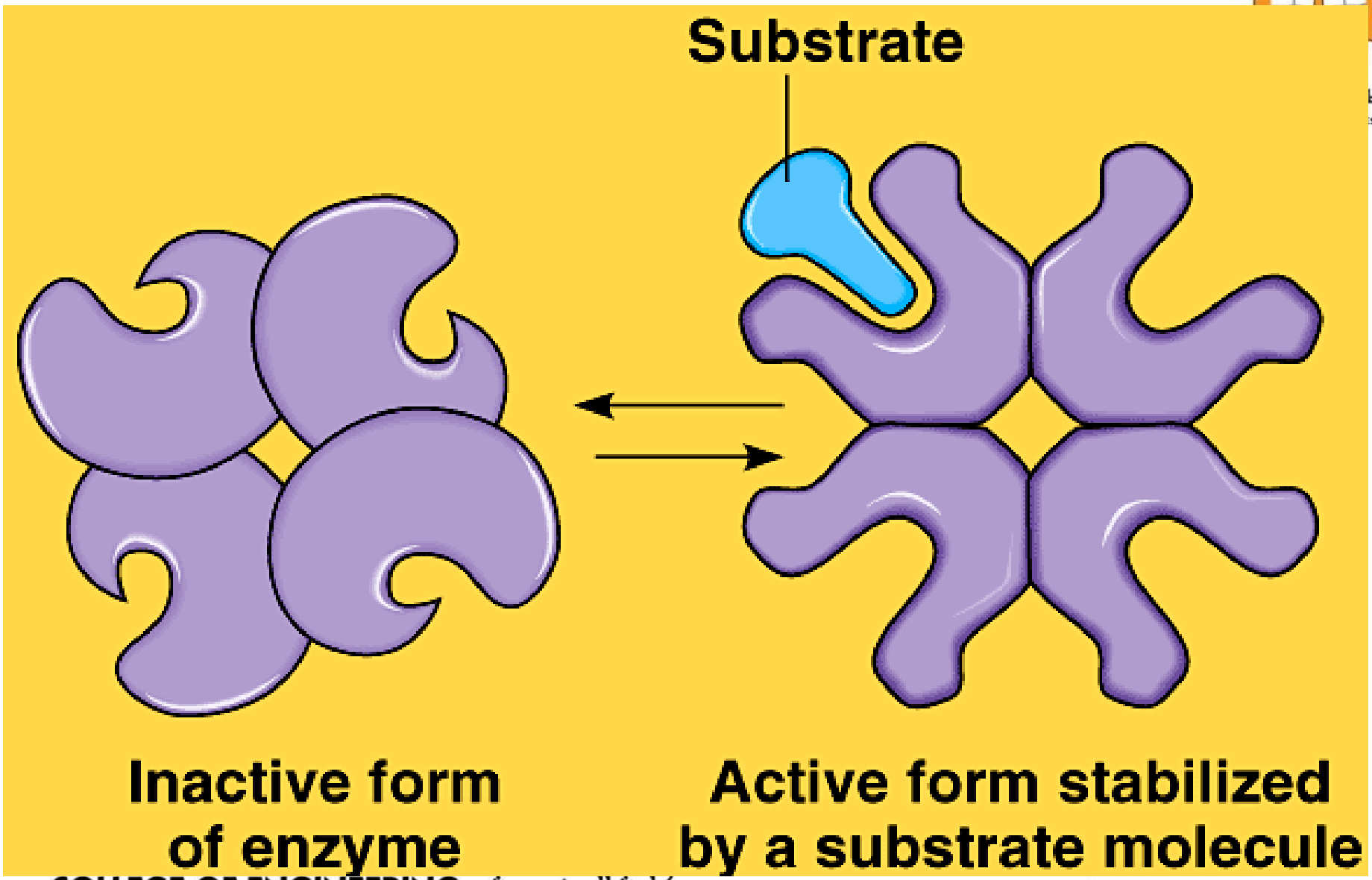
COLLE


Allosteric site
Active site
Active form
Inactive form
(a) Conformational changes in an allosteric enzyme


Activator
Inhibitor
Active form stabilized by an allosteric activator molecule
Inactive form stabilized by an allosteric inhibitor molecule
(b) Allosteric regulation of the enzyme's activity

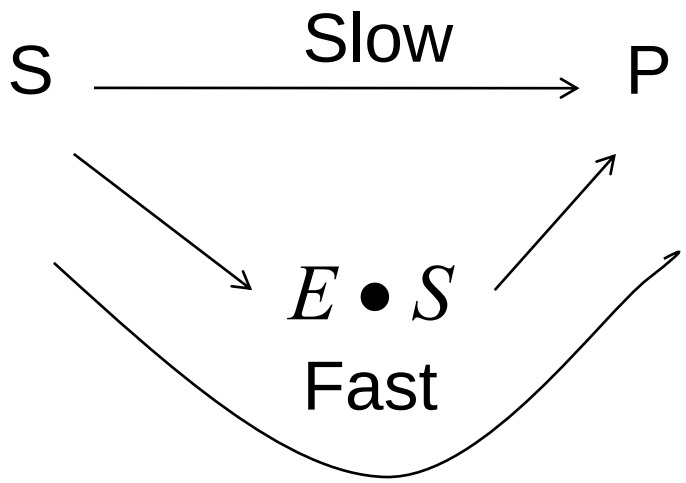

Initial substrate
(threonine)
Threonine
in active site
Enzyme 1
(threonine
deaminase)
Active site no
longer binds
threonine
Intermediate A
Enzyme 2
Intermediate B
Enzyme 3
Intermediate C
Enzyme 4
Intermediate D
Enzyme 5
Isoleucine
in allosteric
site
Feedback inhibition
End product
(isoleucine)

Cooperativity



Enzymes

Enzymes provide a pathway for the substrate to proceed at a faster rate. The substrate, S, reacts to form a product P.



A given enzyme can only catalyze only one reaction. Example, Urea is decomposed by the enzyme urease.

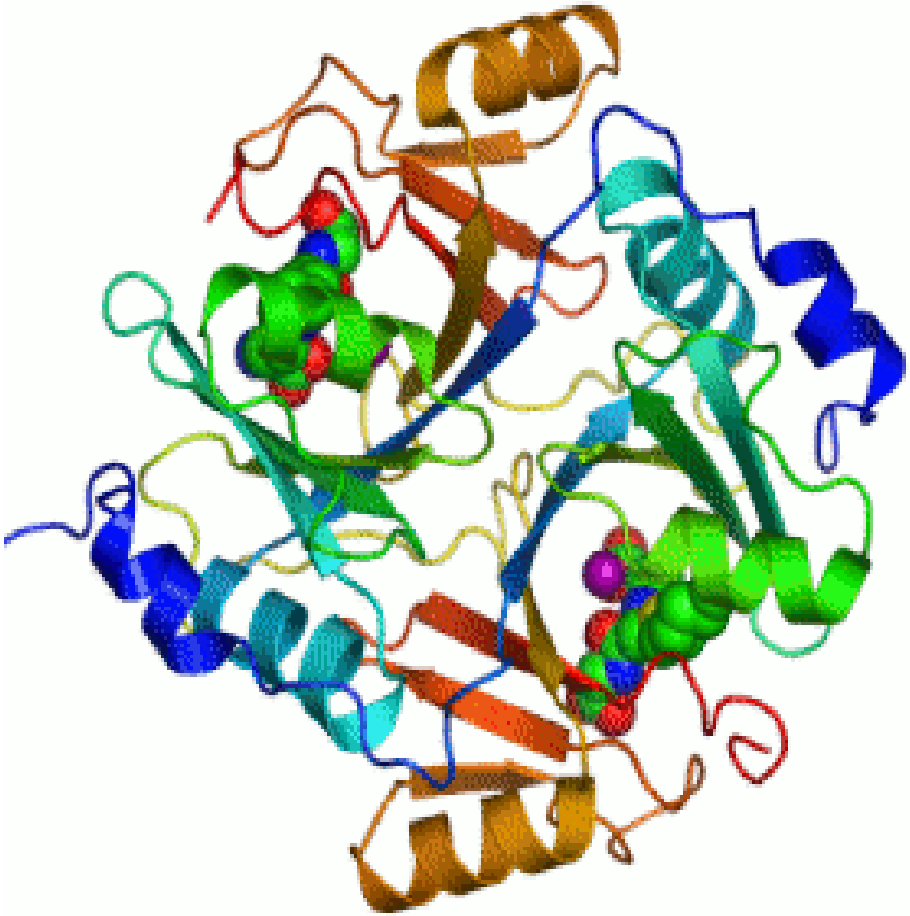
Enzymes…

- Have names that usually end in -_____.

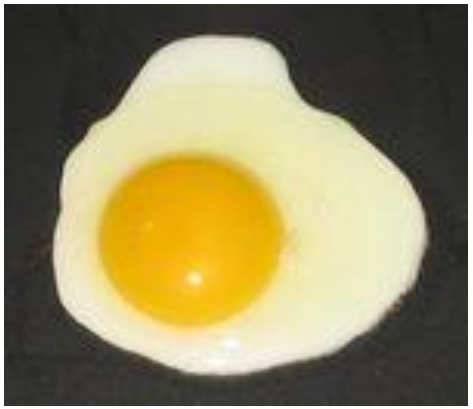
  -Sucrase >> Breaks down Sucrose into glucose molecules

  -Lactase >> Breaks down _____

  -Maltase >> breaks down _____



How do you stop an enzyme?



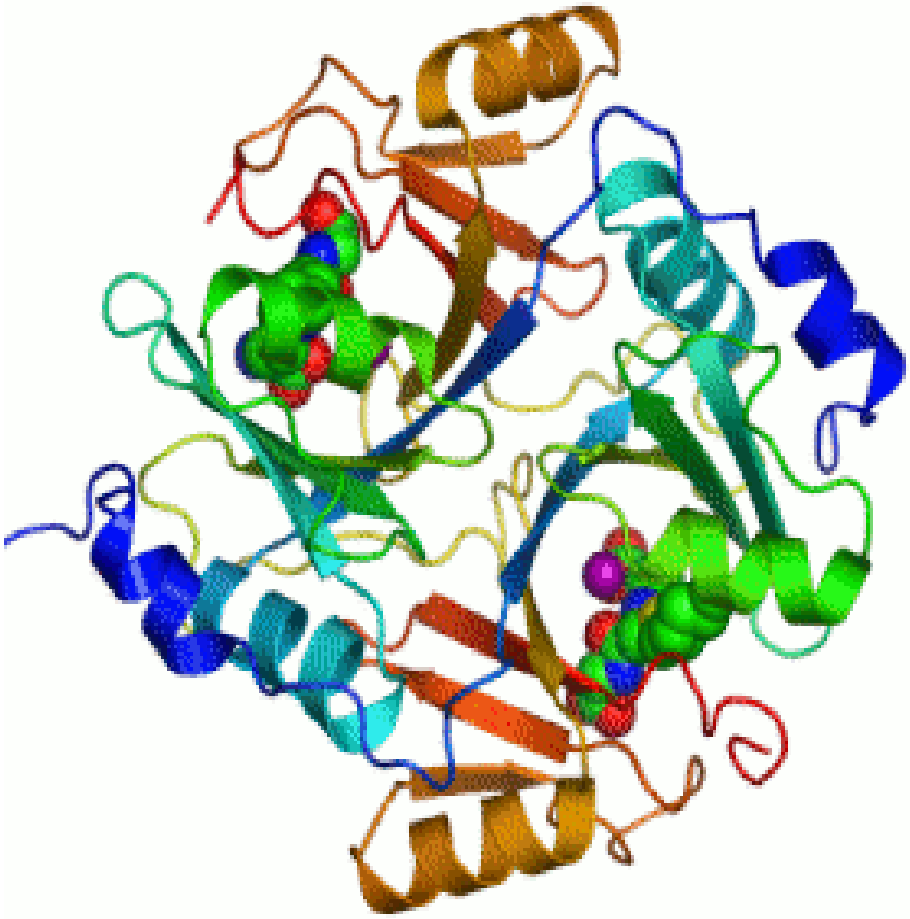
_____ _____!

- Alteration of a protein shape through some form of external stress
- Example, by applying heat or changing pH.
- Denatured protein can't carry out its cellular function .

Irreversible egg protein denaturation caused by high temperature (while cooking it).

Factors That Influence Enzyme Activity

- Temperature
- pH
- Cofactors & Coenzymes
- Inhibitors

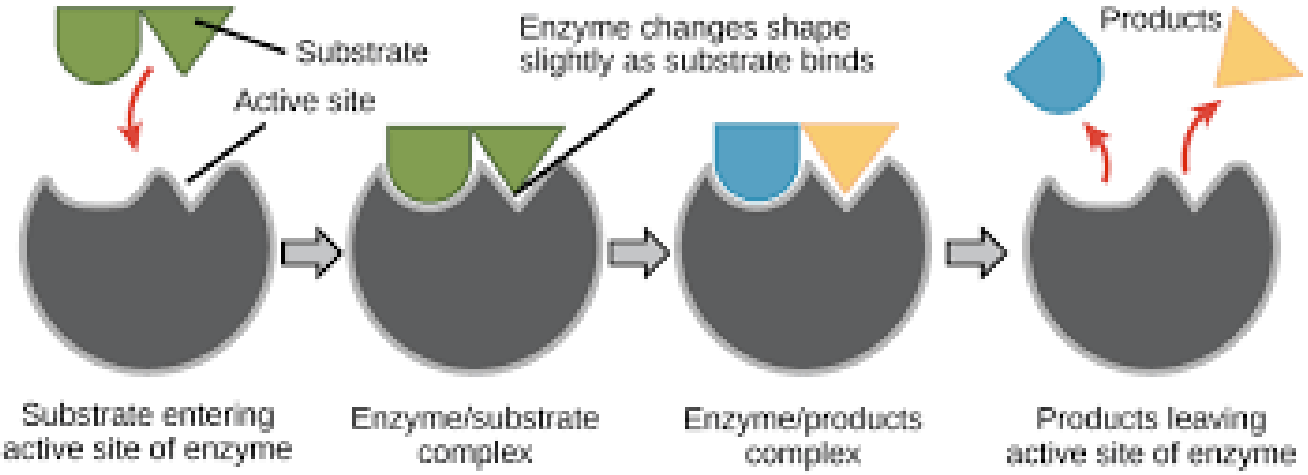


Six major classes of enzymes

- Oxidoreductases
- Transferases
- Hydrolases
- Lyases
- Isomerases
- Ligases

What Are Enzymes?

- Enzymes are Proteins
- Proteins are folded in specific shapes.
- Enzymes act as biological Catalysts. Catalysts speed up chemical reactions
- The enzyme is not permanently changed in the process. Animation



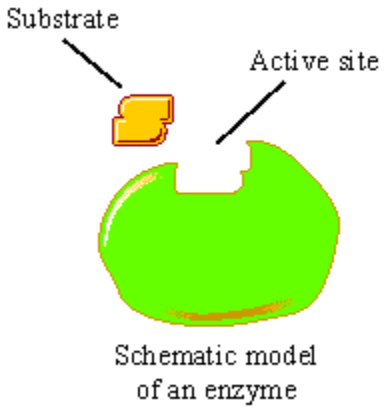
Enzymes

- Are specific for the substrate they will catalyze
- Are Reusable, which means they are not used up in the reaction.
- End in –*ase*

-Sucrase

-Lactase

-Maltase

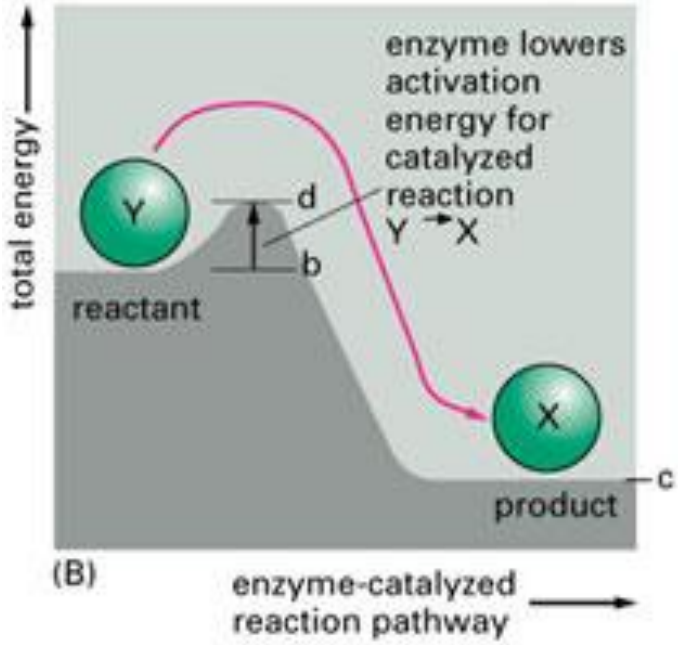


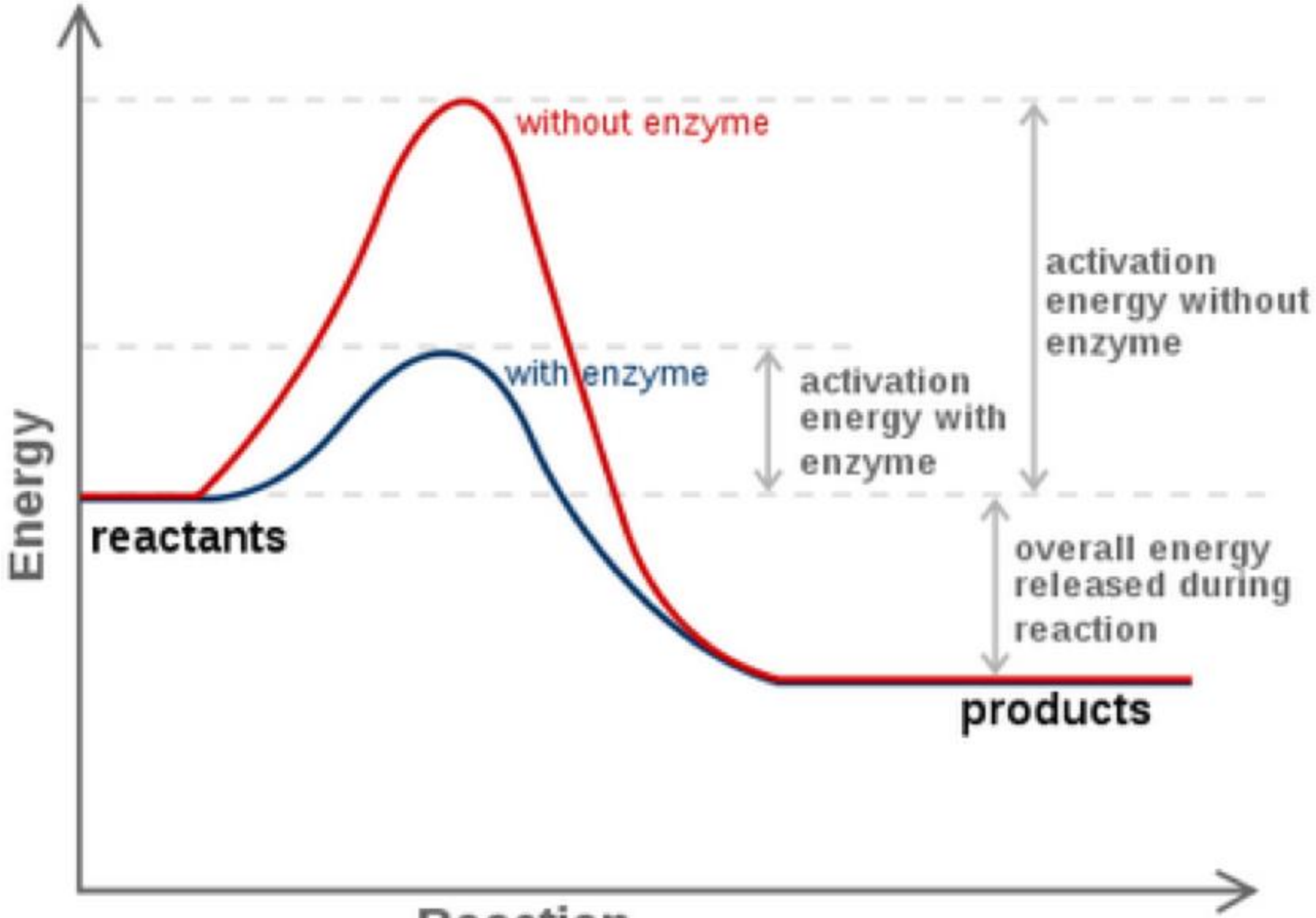
Schematic model of an enzyme

How do enzymes Work?

Enzymes work by weakening chemical bonds, which lowers the activation energy.

Molecules can be built up or broken down by the body.

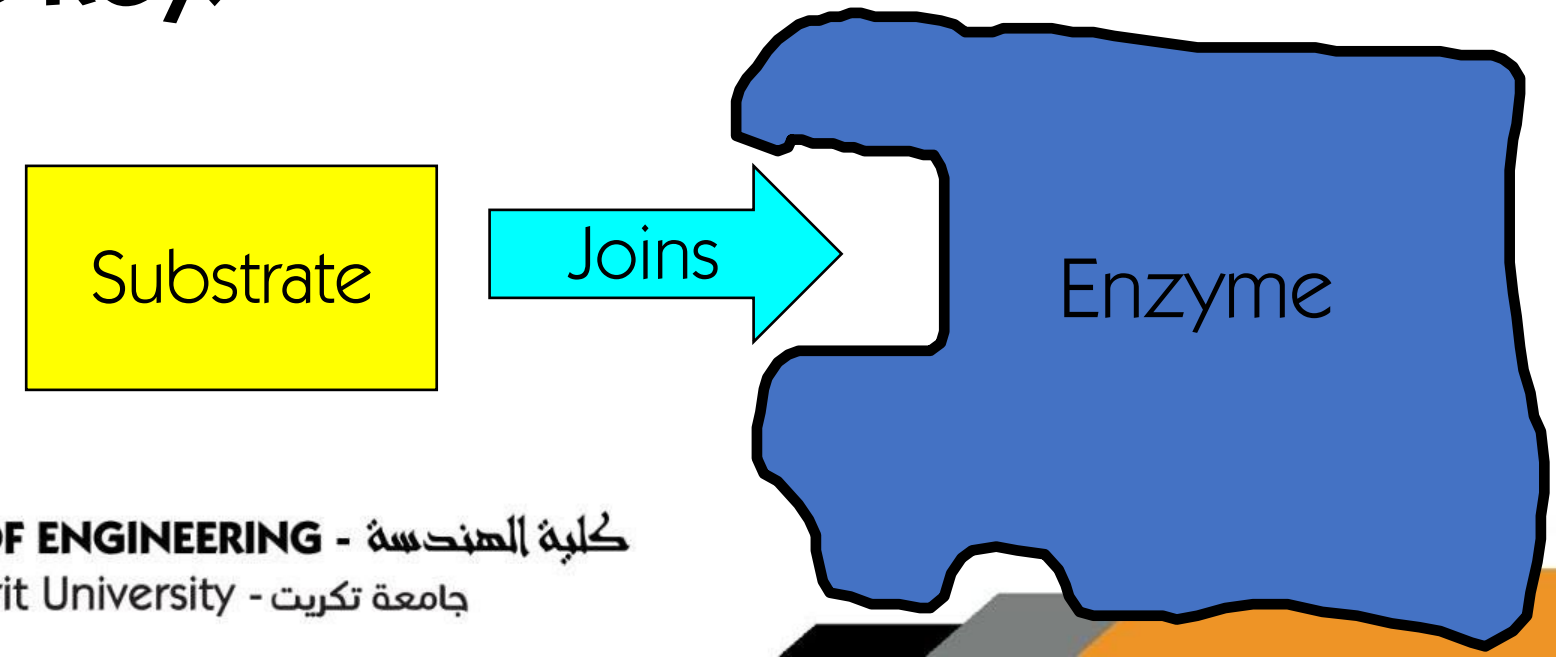



without enzyme
with enzyme
activation energy without enzyme
activation energy with enzyme
overall energy released during reaction
reactants
products
Energy
Reaction

Enzyme-Substrate Complex

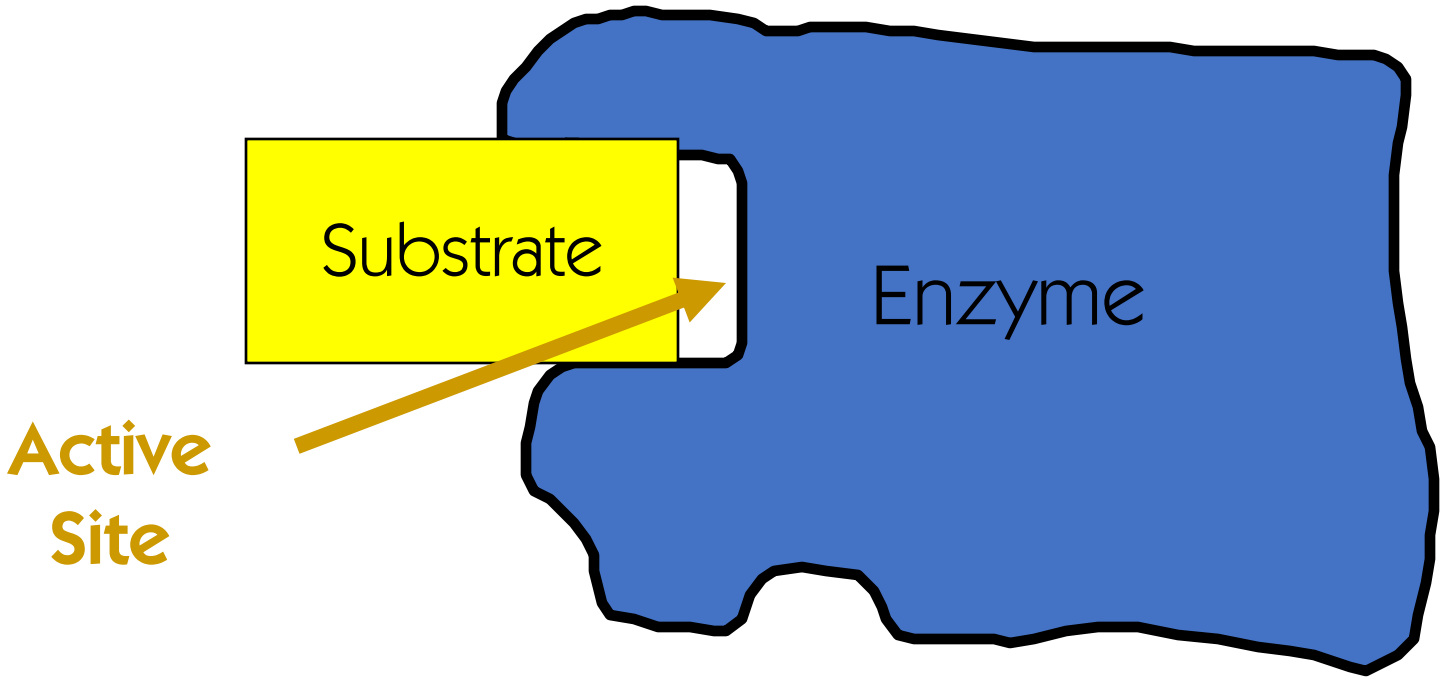
The **substance** (reactant) an **enzyme** acts on is the **substrate**

The lock and key analogy is that the enzyme is the lock and the substrate is the key.



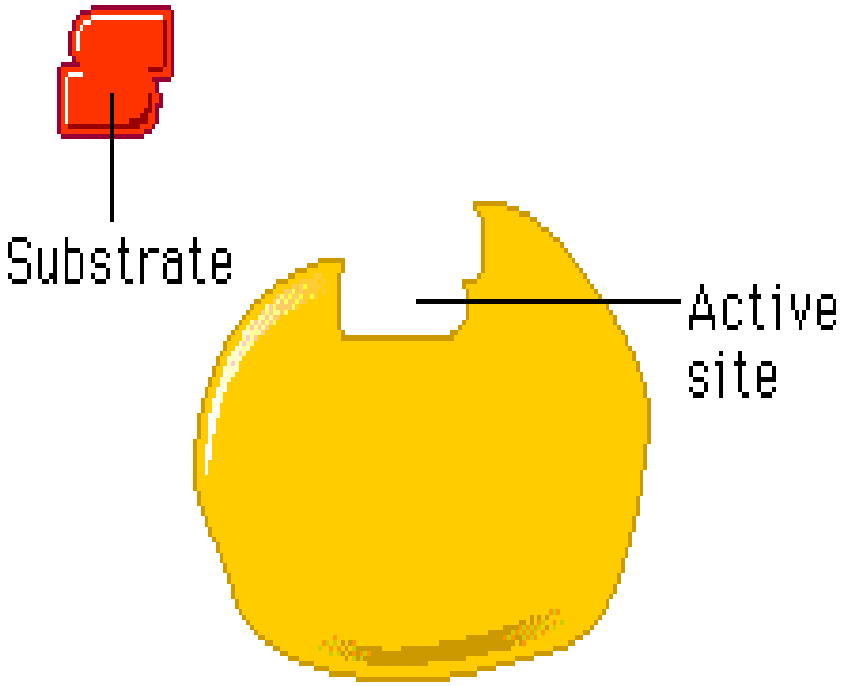
Active Site

- Where the substrate temporarily fits into the active site during the metabolic reaction..



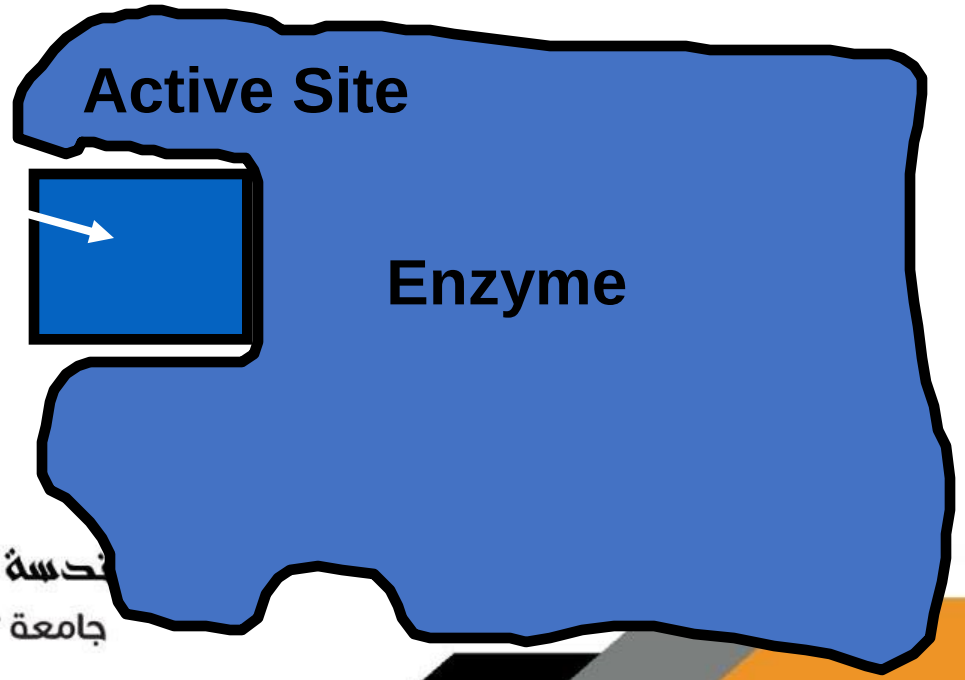
Induced Fit

- A change in the shape of an enzyme's active site
- Induced by the substrate
- The lock and key analogy is that the enzyme is the lock and the substrate is the key.



Induced Fit

- A change in the configuration of an enzyme's active site (H+ and ionic bonds are involved).
- Induced by the substrate.



What Affects Enzyme Activity?

- Three factors:
  1. Environmental Conditions
  2. Cofactors and Coenzymes
  3. Enzyme Inhibitors

1. Environmental Conditions

1. Extreme Temperature are the most dangerous

- high temps may denature (unfold) the enzyme. When an enzyme becomes denatured, it is essentially deactivated.

2. pH (most like 6 - 8 pH near neutral)

3. Ionic concentration (salt ions)

What is pH?

Water molecules naturally dissociate into a hydrogen ion(H+) and a hydroxide ion (OH-)

Ions are charged particles.

$$H_2O \rightleftarrows H^+ + OH^-$$

Hydrogen Ion

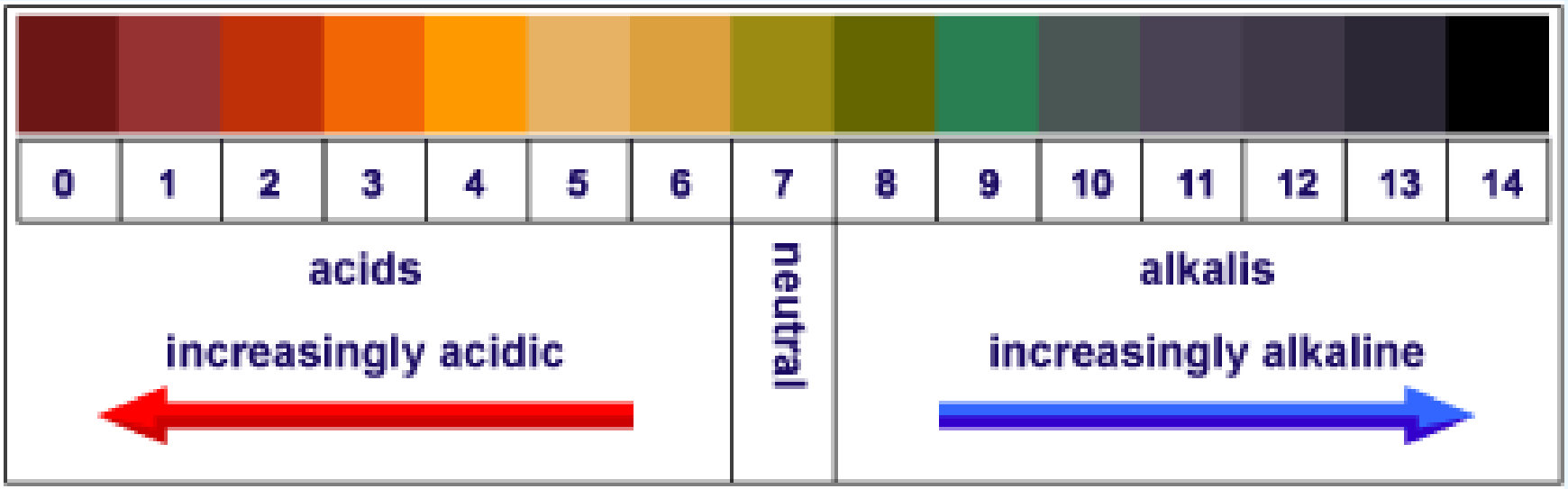
Hydroxide Ion

Acid

Base

The pH scale

- Indicates the concentration of H+ ions.
- The scale ranges from 0-14.
- 7 is neutral pH
- 0-6 is an acid
- 8-14 is a base



What do buffers have to do with pH?

Buffers are weak acids or bases that react with strong acids or bases to prevent sharp, sudden changes in pH.

These buffering systems are integral to maintaining homeostasis in organisms.

Homeostasis means to maintain stable internal conditions.

2. Cofactors and Coenzymes

- Inorganic substances (zinc, iron) and vitamins (respectively) are sometimes needed for proper enzymatic activity.

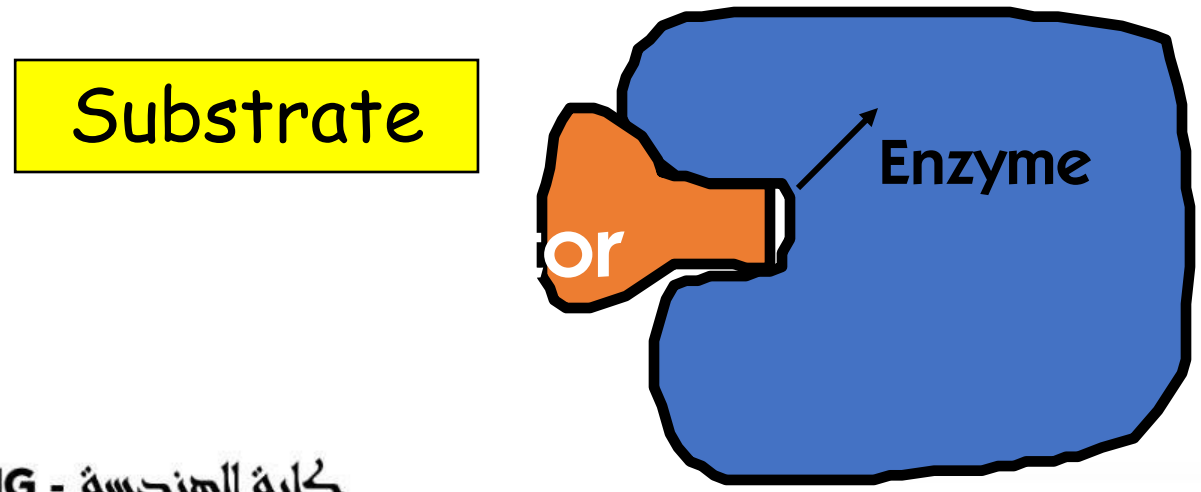
- Example:

  Iron must be present in the quaternary structure - hemoglobin in order for it to pick up oxygen.

Two examples of Enzyme Inhibitors

طريقك إلى النجاح
YOUR WAY TO SUCCESS

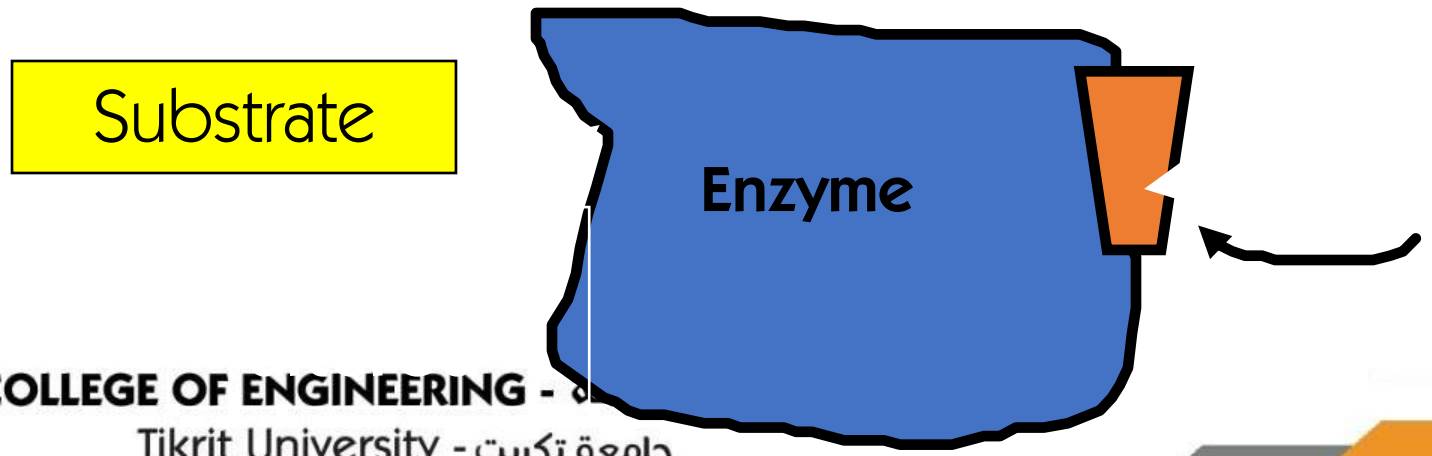
a. Competitive inhibitors: are chemicals that resemble an enzyme's normal substrate and compete with it for the active site.



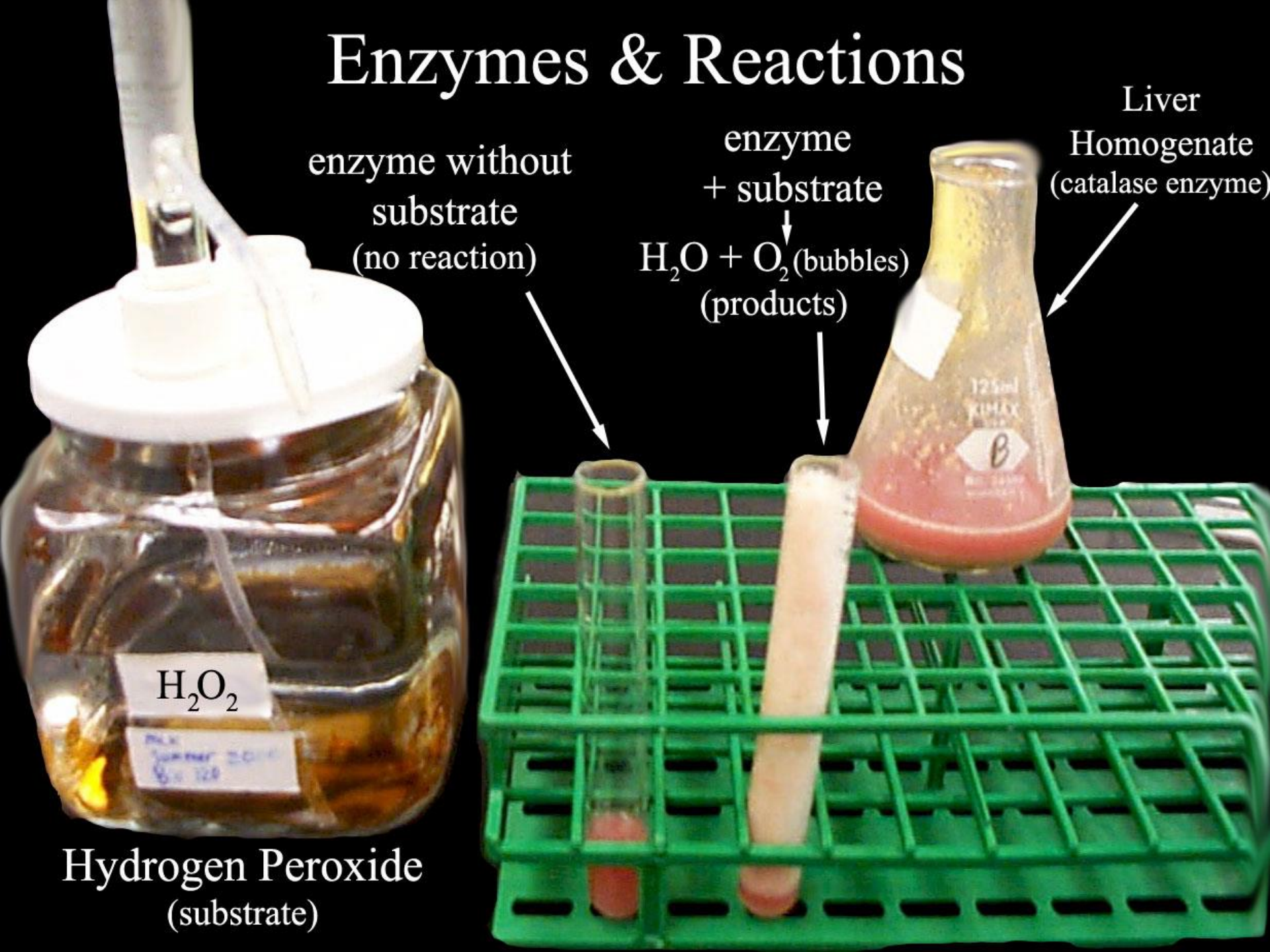
Inhibitors

b. Noncompetitive inhibitors:

Inhibitors that do not enter the active site, but bind to another part of the enzyme causing the enzyme to change its shape, which in turn alters the active site.



Enzymes & Reactions



Enzymes - Urease

A given enzyme can only catalyze only one reaction. Urea is decomposed by the enzyme urease, as shown below.

$$NH_2CONH_2 + UREASE \xrightarrow{H_2O} 2NH_3 + CO_2 + UREASE$$

$$S + E \xrightarrow{H_2O} P + E$$

The corresponding mechanism is:

$$E + S \xrightarrow{k_1} E \bullet S$$

$$E \bullet S \xrightarrow{k_2} E + S$$

$$E \bullet S + W \xrightarrow{k_3} P + E$$

Enzymes - **Michaelis-Menten Kinetics**

$$r_P = k_3(E \bullet S)(W)$$

$$r_{E \bullet S} = 0 = k_1(E)(S) - k_2(E \bullet S) - k_3 W(E \bullet S)$$

$$(E \bullet S) = \frac{k_1(E)(S)}{k_2 + k_3 W}$$

$$E_t = (E) + (E \bullet S)$$

$$(E) = \frac{E_t}{1 + \left(\frac{k_1 S}{k_2 + k_3 W}\right)}$$

Enzymes - **Michaelis-Menten Kinetics**

$$r_P = k_3(E \bullet S)(W) = \frac{\overbrace{k_3 W}^{k_{cat}} E_t S}{\underbrace{\frac{k_2 + k_3 W}{k_1}}_{K_M} + S} = \frac{\overbrace{k_{cat} E_t}^{V_{\max}} S}{K_M + S}$$

$$r_P = k_3(E \bullet S)(W) = \frac{V_{\max} S}{K_m + S}$$

Enzymes - **Michaelis-Menten Kinetics**

$$V_{max} = k_{cat} E_t$$

Turnover Number: k_{cat}

Number of substrate molecules (moles) converted to product in a given time (s) on a single enzyme molecule *(molecules/molecule/time)*

For the reaction: $H_2O_2 + E \xrightarrow{k_{cat}} H_2O + O + E$

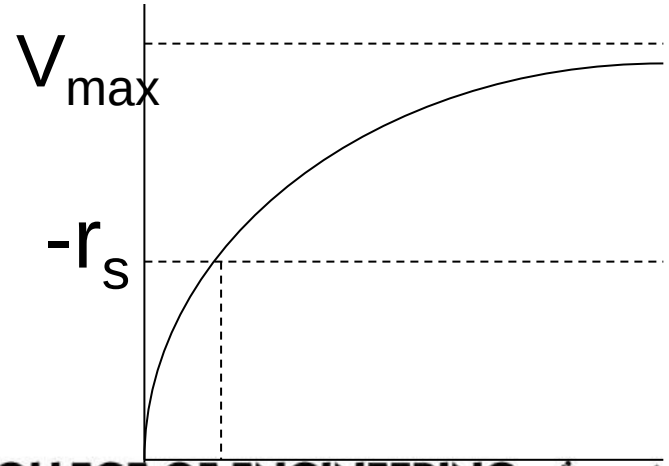
40,000,000 molecules of H_2O_2 converted to product per second on a single enzyme molecule.

Enzymes - **Michaelis-Menten Kinetics**

Michaelis-Menten Equation

$$r_P = -r_S = \frac{V_{max} S}{K_M + S}$$

(Michaelis-Menten plot)



Solving: $\frac{V_{max}}{2} = \frac{V_{max} S_{1/2}}{K_M + S_{1/2}}$

$K_M = S_{1/2}$

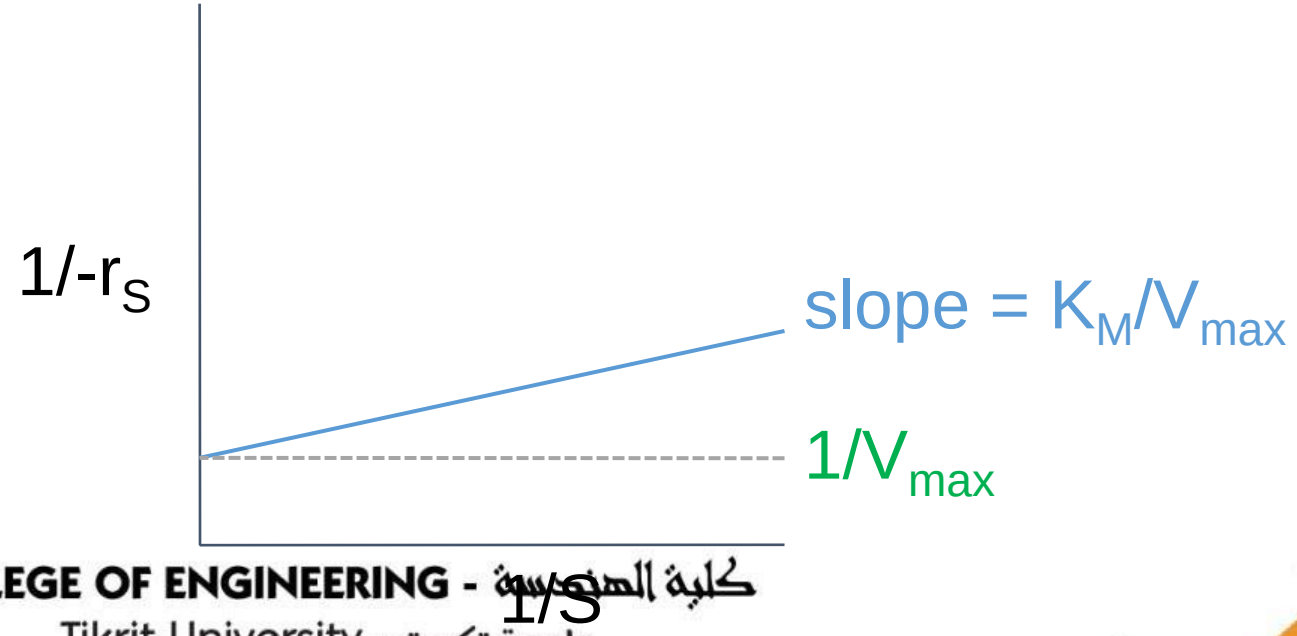
therefore K_M is the concentration at which the rate is half the maximum rate.

Enzymes - **Michaelis-Menten Kinetics**

Inverting yields:

$$\frac{1}{-r_S} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\left(\frac{1}{S}\right)$$

Lineweaver-Burk Plot



Types of Enzyme Inhibition

Competitive

$$E + I \Leftrightarrow I \bullet E \text{ (inactive)}$$

Uncompetitive

$$E \bullet S + I \Leftrightarrow I \bullet E \bullet S \text{ (inactive)}$$

Non-competitive

$$E \bullet S + I \Leftrightarrow I \bullet E \bullet S \text{ (inactive)}$$

$$I \bullet E + S \Leftrightarrow I \bullet E \bullet S \text{ (inactive)}$$

Competitive Inhibition

Competitive inhibition pathway

$$E + S \rightleftharpoons E \cdot S \longrightarrow E + P$$
$$+$$
$$I$$
$$\Big\| K_I$$
$$E \cdot I$$

Reaction Steps

(1) $E + S \xrightarrow{k_1} E \bullet S$

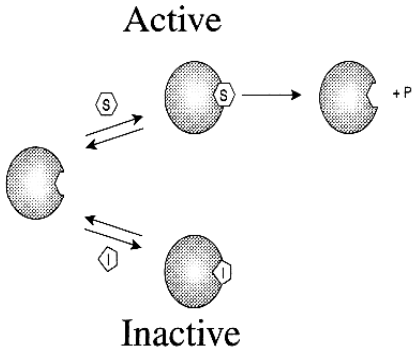
(2) $E \bullet S \xrightarrow{k_2} E + S$

(3) $E \bullet S \xrightarrow{k_3} P + E$

(4) $I + E \xrightarrow{k_4} E \bullet I$ (inactive)

(5) $E \bullet I \xrightarrow{k_5} E + I$

Competitive Inhibition Pathway



(a) Competitive inhibition. Courtesy of D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 3rd ed. (New York: Worth Publishers, 2000), p. 266.

Competitive Inhibition

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} E \bullet S \xrightarrow{k_3} E + P$$

$$E + I \underset{k_5}{\overset{k_4}{\rightleftarrows}} E \bullet I \text{ (inactive)}$$

1) Mechanisms:

$$E + S \rightarrow E \cdot S \qquad E \cdot S \rightarrow E + S$$

$$E \cdot S \rightarrow P + E \qquad E + I \rightarrow E \cdot I$$

$$E \cdot I \rightarrow E + I$$

$$r_P = k_3 C_{E \cdot S}$$

Competitive Inhibition

2) Rate Laws:

$$r_{E\cdot S} = 0 = k_1 C_S C_E - k_2 C_{E\cdot S} - k_3 C_{E\cdot S}$$

$$C_{E\cdot S} = \frac{k_1 C_S C_E}{k_2 + k_3} = \frac{C_S C_E}{K_m}$$

$$r_P = \frac{k_3 C_S C_E}{K_m}$$

$$r_{I\cdot E} = 0 = k_4 C_I C_E - k_5 C_{I\cdot E}$$

$$C_{I\cdot E} = \frac{C_I C_E}{K_I} \qquad K_I = \frac{k_5}{k_4}$$

Competitive Inhibition

$$C_{Etot} = C_E + C_{E\cdot S} + C_{I\cdot E}$$

$$C_E = \frac{C_{Etot}}{1 + \frac{C_S}{K_m} + \frac{C_I}{K_I}}$$

$$r_P = \frac{k_3 C_{Etot} C_S}{K_m + C_S + \frac{C_I K_m}{K_I}}$$

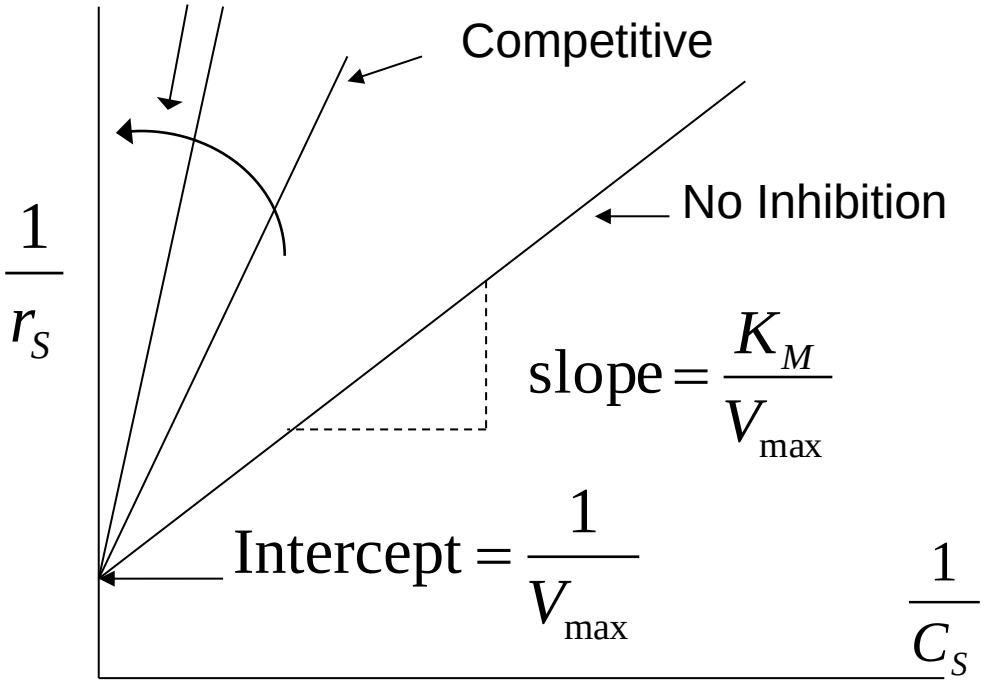
$$-r_S = \frac{V_{max} C_S}{C_S + K_m\left(1 + \frac{C_I}{K_I}\right)}$$

$$\frac{1}{-r_S} = \frac{1}{V_{max}} + \frac{k_m}{V_{max}}\left(1 + \frac{C_I}{K_I}\right)\frac{1}{C_S}$$

Competitive Inhibition

From before (no competition): $\frac{1}{-r_S} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \frac{1}{C_S}$

Increasing C_I



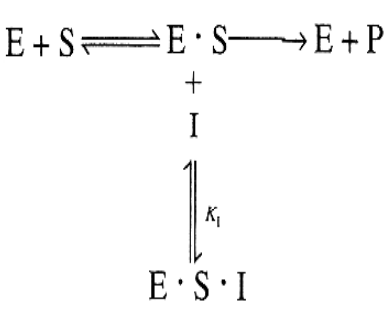
Competitive

$$\frac{1}{-r_S} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \left(1 + \frac{C_I}{K_I}\right) \frac{1}{C_S}$$

Intercept does not change, slope increases as inhibitor concentration increases

Uncompetitive Inhibition

Uncompetitive inhibition pathway



Reaction Steps

$$(1)\quad E + S \xrightarrow{k_1} E \bullet S$$

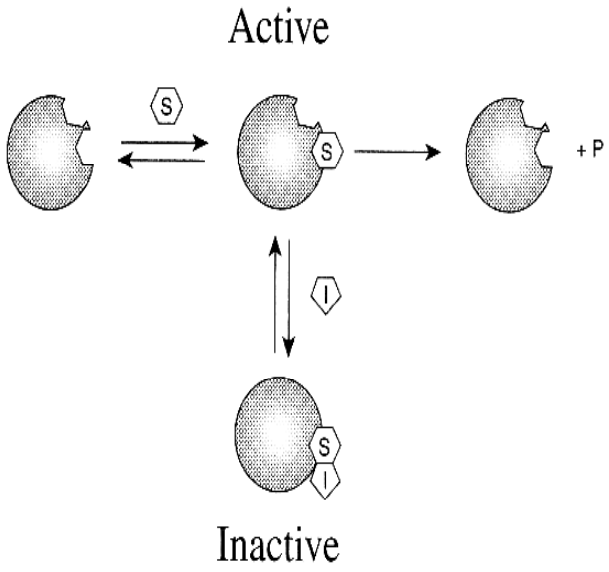
$$(2)\quad E \bullet S \xrightarrow{k_2} E + S$$

$$(3)\quad E \bullet S \xrightarrow{k_3} P + E$$

$$(4)\quad I + E \bullet S \xrightarrow{k_4} I \bullet E \bullet S \text{ (inactive)}$$

$$(5)\quad I \bullet E \bullet S \xrightarrow{k_5} I + E \bullet S$$

Uncompetitive Pathway



Uncompetitive Inhibition

Inhibition only has affinity for enzyme-substrate complex

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} E \bullet S \xrightarrow{k_3} P$$

$$I + E \bullet S \underset{k_5}{\overset{k_4}{\rightleftarrows}} I \bullet E \bullet S \text{ (inactive)}$$

Developing the **rate law:**

$$r_P = -r_S = k_{cat}\left(E \bullet S\right)$$

$$r_{E\bullet S} = 0 = k_1\left(E\right)\left(S\right) - k_2\left(E \bullet S\right) - k_{cat}\left(E \bullet S\right) - k_4\left(I\right)\left(E \bullet S\right) + k_5\left(I \bullet E \bullet S\right) \quad (1)$$

$$r_{I\bullet E\bullet S} = 0 = k_4\left(I\right)\left(E \bullet S\right) - k_5\left(I \bullet E \bullet S\right) \quad (2)$$

Uncompetitive Inhibition

Adding (1) and (2)

$$k_1(E)(S) - k_2(E \bullet S) - k_{cat}(E \bullet S) = 0$$

$$(E \bullet S) = \frac{k_1(E)(S)}{k_2 + k_{cat}} = \frac{(E)(S)}{K_M}$$

From (2)

$$(I \bullet E \bullet S) = \frac{k_4}{k_5}(I)(E \bullet S) = \frac{(I)(E \bullet S)}{K_I} = \frac{(I)(E)(S)}{K_I K_M}$$

$$K_I = \frac{k_5}{k_4}$$

$$r_p = k_{cat}(E \bullet S) = \frac{k_{cat}(E)(S)}{K_M}$$

Uncompetitive Inhibition

Total enzyme

$$E_t = (E) + (E \bullet S) + (I \bullet E \bullet S)$$

$$= (E)\left(1 + \frac{(S)}{K_M} + \frac{(I)(S)}{K_I K_M}\right)$$

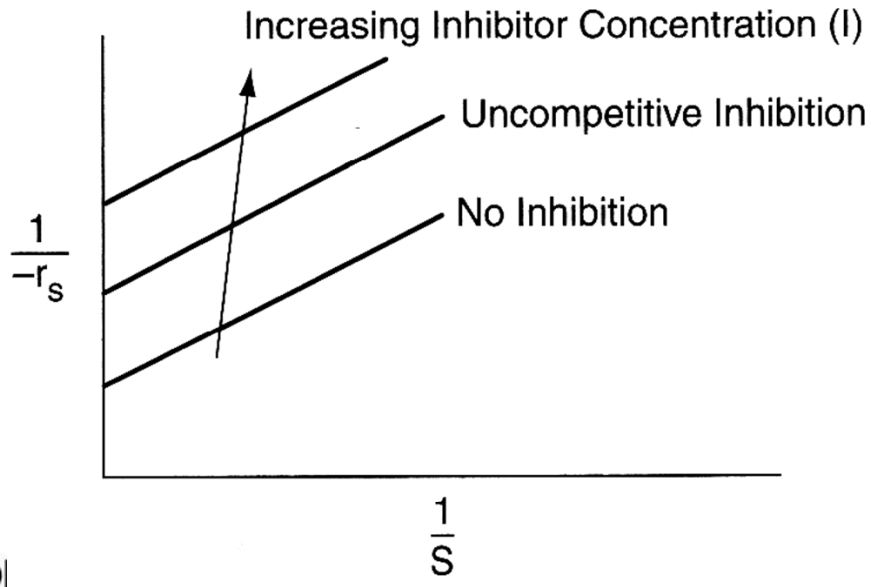
$$r_p = \frac{k_{cat} E_t (S)}{K_M \left(1 + \frac{(S)}{K_M} + \frac{(I)(S)}{K_I K_M}\right)}$$

$$-r_S = r_P = \frac{V_{\max}(S)}{K_M + (S)\left(1 + \frac{(I)}{K_I}\right)}$$

Uncompetitive Inhibition

$$\frac{1}{-r_S} = \frac{1}{V_{\max}(S)}\left(K_M + (S)\left(1 + \frac{(I)}{K_I}\right)\right)$$

$$\frac{1}{-r_S} = \frac{K_M}{V_{\max}}\left(\frac{1}{(S)}\right) + \frac{1}{V_{\max}}\left(1 + \frac{(I)}{K_I}\right)$$



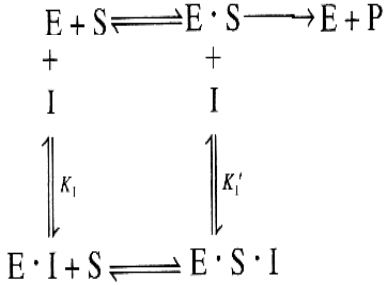
Slope remains the same but intercept changes as inhibitor concentration is increased

Lineweaver-Burk Plot for uncompetitive inhibition

Non-competitive Inhibitio

Reaction Steps

Mixed inhibition



(1) $E + S \rightleftharpoons E \bullet S$

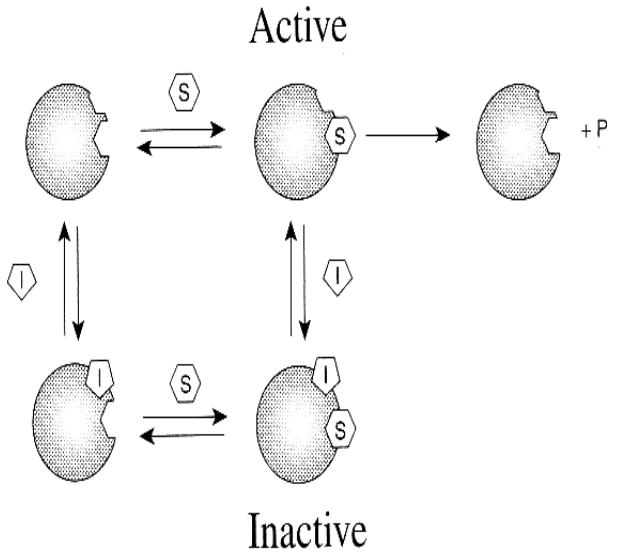
(2) $E + I \rightleftharpoons I \bullet E$ (inactive)

(3) $I + E \bullet S \rightleftharpoons I \bullet E \bullet S$ (inactive)

(4) $S + I \bullet E \rightleftharpoons I \bullet E \bullet S$ (inactive)

(5) $E \bullet S \longrightarrow P + E$

Noncompetitive Pathway



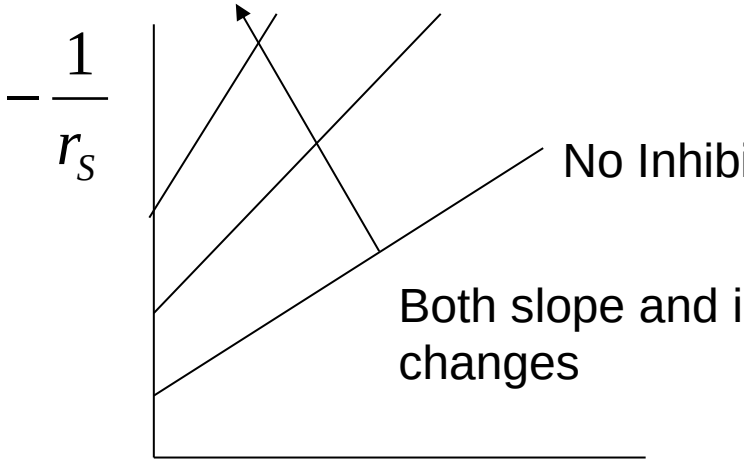
Non-competitive Inhibitio

$$E + S \rightleftharpoons E{\cdot}S \longrightarrow P + E$$

$$+I \uparrow\downarrow -I \qquad -I \uparrow\downarrow +I$$

$$(\text{inactive})\, I.E + S \rightleftharpoons I.E.S\ (\text{inactive})$$

Increasing I

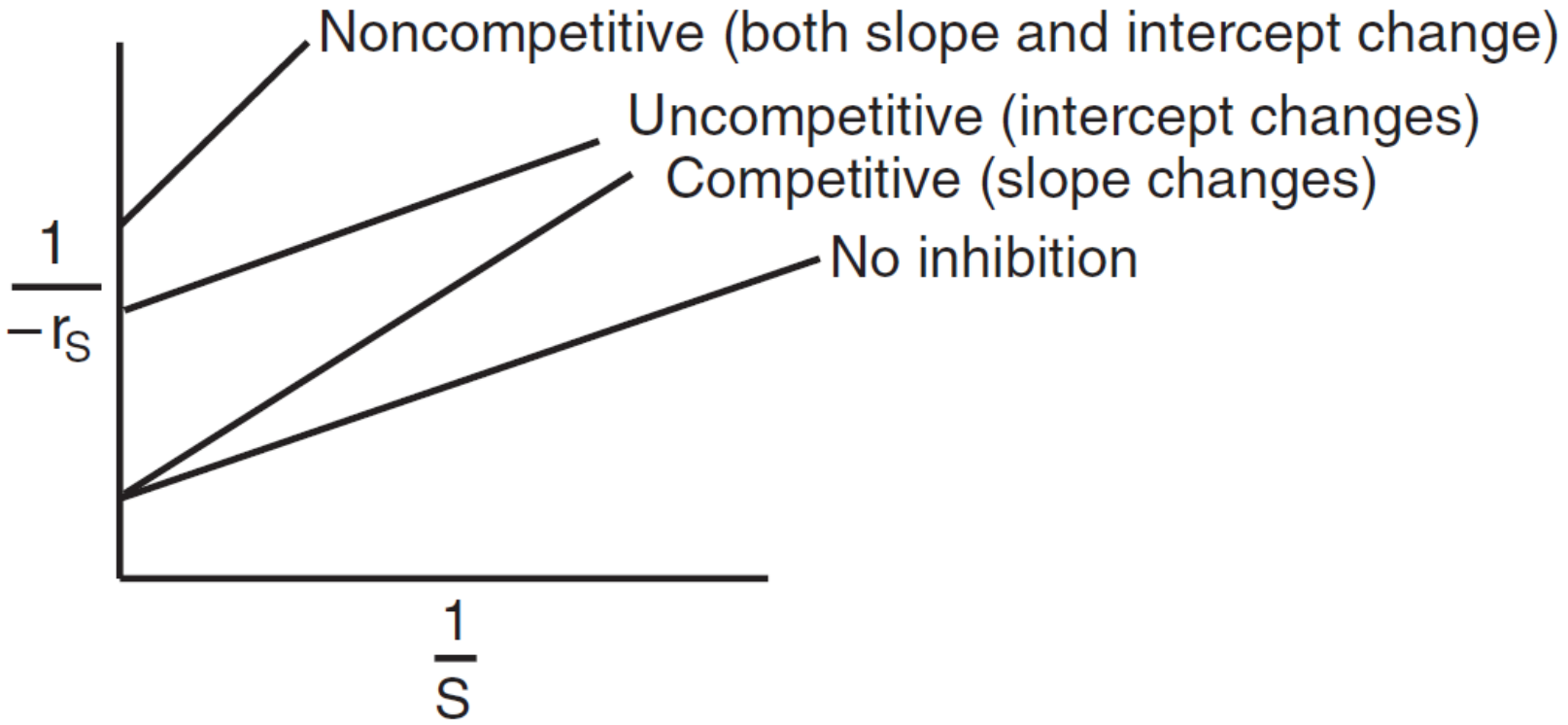


$$-r_S = \frac{V_{\max} C_S}{\left(k_M + C_S\right)\left(1 + \frac{C_I}{k_I}\right)}$$

$$\frac{1}{-r_S} = \frac{1}{V_{\max}}\left(1 + \frac{C_I}{k_I}\right) + \frac{k_M}{V_{\max}}\left(\frac{1}{C_S}\right)\left(1 + \frac{C_I}{k_I}\right)$$

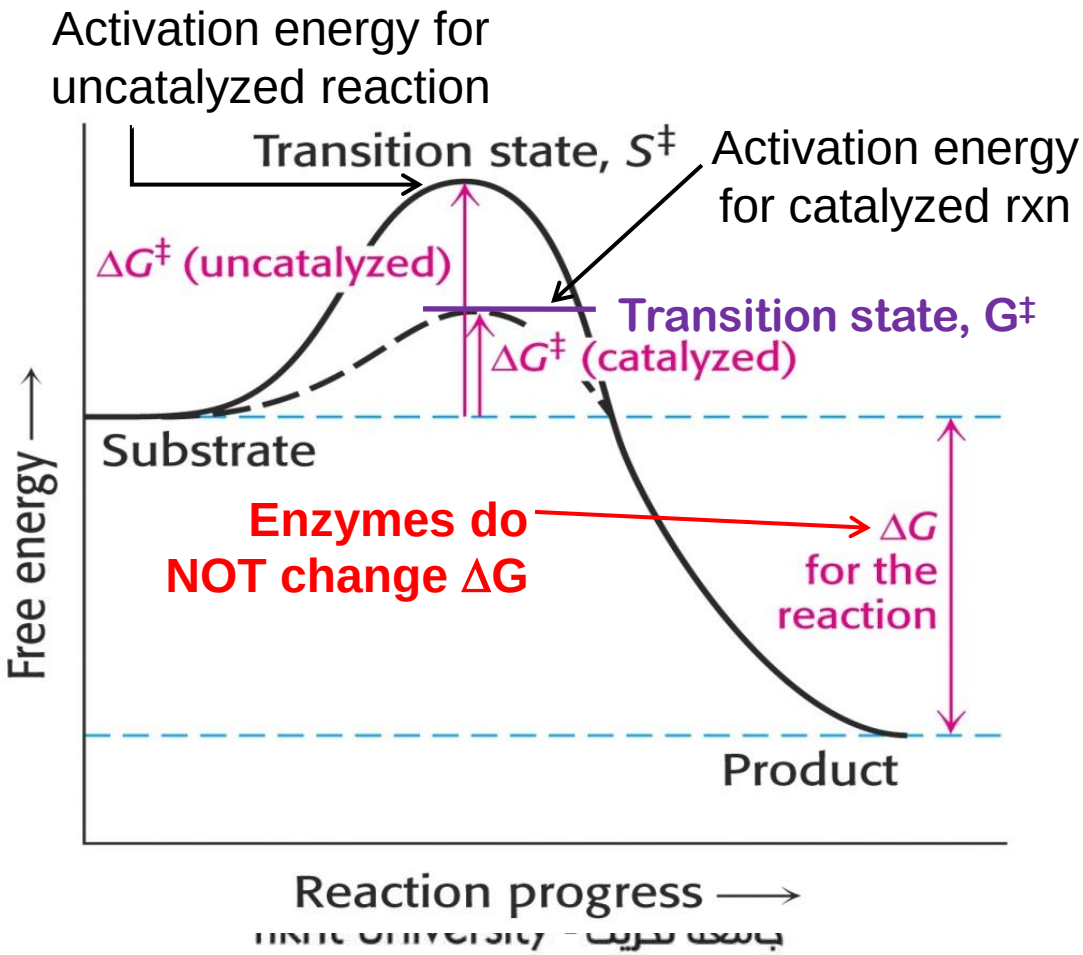
Summary: Types of Enzyme Inhibition

Lineweaver–Burk plots for three types of enzyme inhibition.



Enzymes Increase Reaction Rate

- **Effects the reaction rate (kinetics), NOT equilibrium (thermo)**
- Lower activation energy $\Delta G^{\ddagger}$ increases reaction rate, reach equilibrium faster
- ΔG is unchanged, so ratio of products to reactants at equilibrium is the same



$$\text{Kinetic}: A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$$

$k_{1,cat} > k_{1,uncat}$

$\Delta G^{\ddagger}$ determines rxn rate

$\Delta G^{\ddagger} = -RT \ln(k)$

Enzymes change $\Delta G^{\ddagger}$

Thermodynamic:

$$K = \frac{[C]}{[A][B]} = \frac{k_1}{k_{-1}}$$

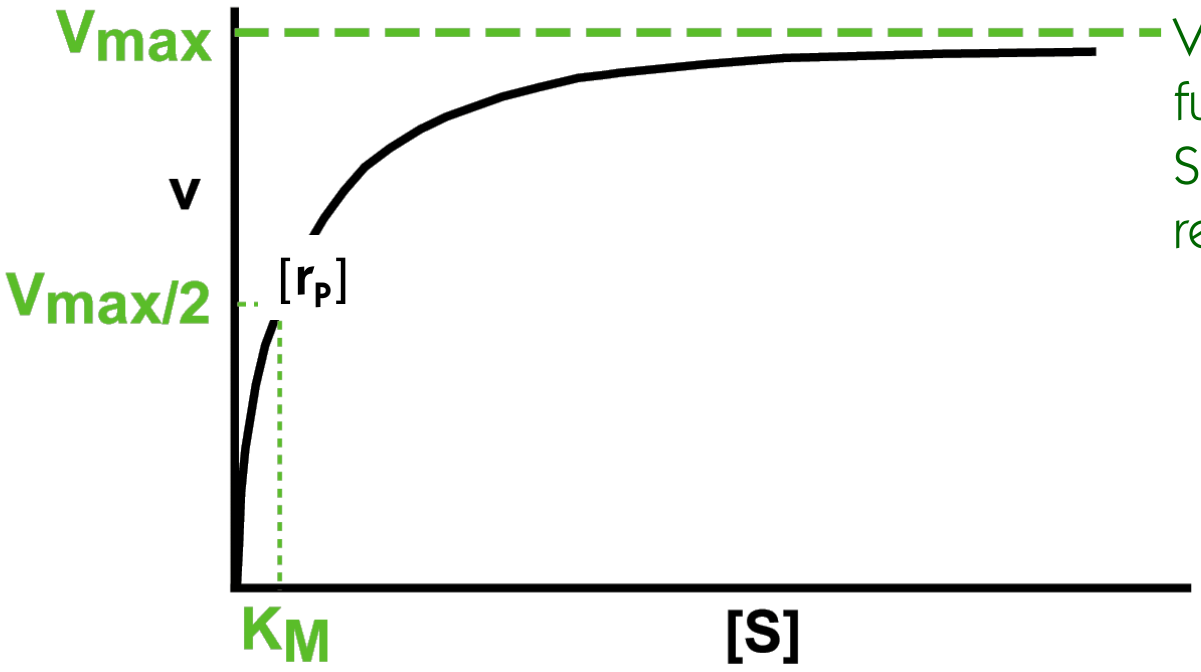
$K_{cat} = K_{uncat}$

ΔG determines equilibrium

$\Delta G = -RT \ln(K)$

Enzymes do NOT change ΔG

Michaelis-Menten (M-M) Equation



V_{max}: maximum reaction rate, further increases in substrate, S, no longer increase the reaction velocity, v

v = reaction velocity = $r_P = -r_S$

K_m = substrate concentration where reaction velocity v = $V_{max}/2$

[S] = substrate concentration [P]: product concentration

Empirically found the Michaelis-Menten equation:

$$v = \frac{V_{max} C_S}{K_m + C_S}$$

Where V_{max} depends on the amount of enzyme

Rate Equation for Enzyme Reaction

$$v = r_P = \frac{V_{max} S}{K_m + S}$$ Goal: derive this experimentally determined reaction rate

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

E: enzyme S: substrate

ES: enzyme-substrate complex

$$\text{rate of product formation}: \quad v = r_P = \frac{dC_P}{dt} = k_2 C_{ES}$$

We cannot measure C_{ES}, so we need to get C_{ES} in terms of species we can measure. Start by writing the rate equation for C_{ES} :

$$\frac{dC_{ES}}{dt} = k_1 C_S C_E - (k_{-1} + k_2) C_{ES}$$

The free enzyme concentration C_E is also difficult to measure. Use the mass balance to get C_E in terms of C_{ES} and C_{E0}.

$$C_E = C_{E0} - C_{ES} \qquad \text{where } C_{E0} = C_{E,t=0}$$

Substitute into rate eq for C_E:

$$\frac{dC_{ES}}{dt} = k_1 C_S (C_{E0} - C_{ES}) - (k_{-1} + k_2) C_{ES}$$

C_{ES} in Measurable Quantities

$$\rightarrow \frac{dC_{ES}}{dt} = k_1 C_S (C_{E0} - C_{ES}) - (k_{-1} + k_2) C_{ES}$$

Pseudo-steady state assumption: ES is a reactive intermediate, so $\frac{d[ES]}{dt} = 0$

$$\frac{dC_{ES}}{dt} = 0 = k_1 C_S (C_{E0} - C_{ES}) - (k_{-1} + k_2) C_{ES}$$ Now solve for C_{ES}

Multiply out and rearrange $\rightarrow k_{-1} C_{ES} + k_2 C_{ES} = k_1 C_S C_{E0} - k_1 C_S C_{ES}$

Bring C_{ES} to left side of equation $\rightarrow k_{-1} C_{ES} + k_2 C_{ES} + k_1 C_S C_{ES} = k_1 C_S C_{E0}$

Factor out C_{ES} $\rightarrow C_{ES} (k_{-1} + k_2 + k_1 C_S) = k_1 C_S C_{E0}$

Divide by quantity in bracket $\rightarrow C_{ES} = \frac{k_1 C_S C_{E0}}{k_{-1} + k_2 + k_1 C_S}$

Divide top & bottom by k_1 $\rightarrow C_{ES} = \frac{C_S C_{E0}}{\frac{k_{-1} + k_2}{k_1} + C_S}$

Plug this expression for C_{ES} into dC_p/dt

Derivation of the M-M Equation

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

E: enzyme S: substrate

ES: enzyme-substrate complex

$$\text{rate of product formation: } v = r_P = \frac{dC_P}{dt} = k_2 C_{ES}$$

$$\rightarrow C_{ES} = \frac{C_S C_{E0}}{\frac{k_{-1} + k_2}{k_1} + C_S}$$

Plug this expression for C_{ES} into dC_p/dt

$$r_P = \frac{dC_P}{dt} = \frac{k_2 C_{E0} C_S}{\frac{k_{-1} + k_2}{k_1} + C_S}$$

Compare to experimentally observed rate eq:

$$v = r_P = \frac{V_{max} C_S}{K_m + C_S}$$

$$\boxed{V_{max} = k_2 C_{E0}}$$

V_{max} occurs when enzyme is fully saturated with S (in ES form)

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

When $C_S >> K_m$, then:

$$r_P = -r_s \approx V_{max}$$

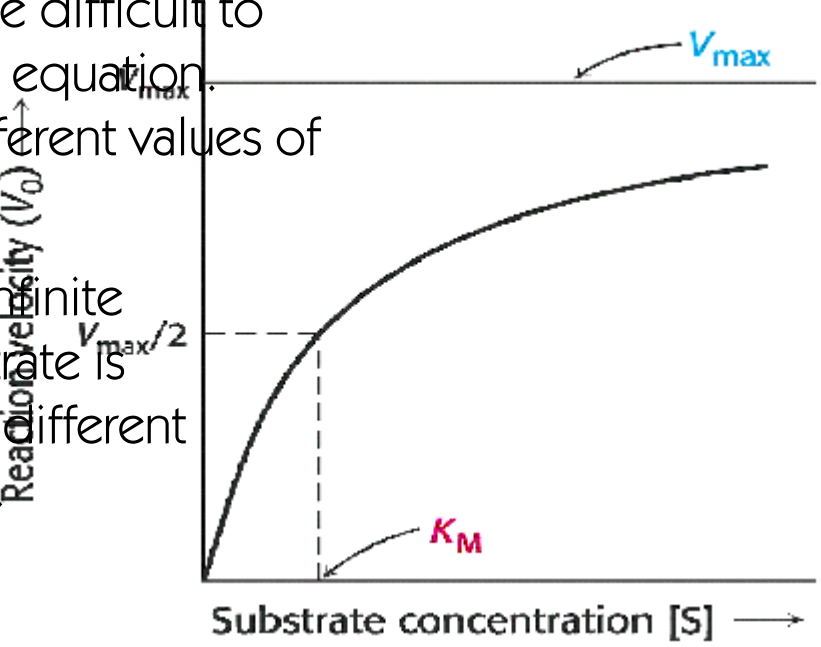
When $C_S << K_m$, then:

$$r_P = -r_S = \frac{V_{max} C_S}{K_m}$$

Complications with Measuring Rates with the M-M Equation

In practice, V_{max} can be difficult to estimate using the MM equation. Everyone reported different values of V_{max}.

Since a solution with infinite concentration of substrate is impossible to make, a different equation was needed.



Lineweaver-Burk Equation

Lineweaver & Burk inverted the MM equation

$$r_P = \frac{V_{max} C_S}{K_m + C_S}$$

$$\rightarrow \frac{1}{r_P} = \frac{K_m + C_S}{V_{max} C_S}$$

$$\rightarrow \frac{1}{r_p} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{C_S}\right) + \frac{1}{V_{max}}$$

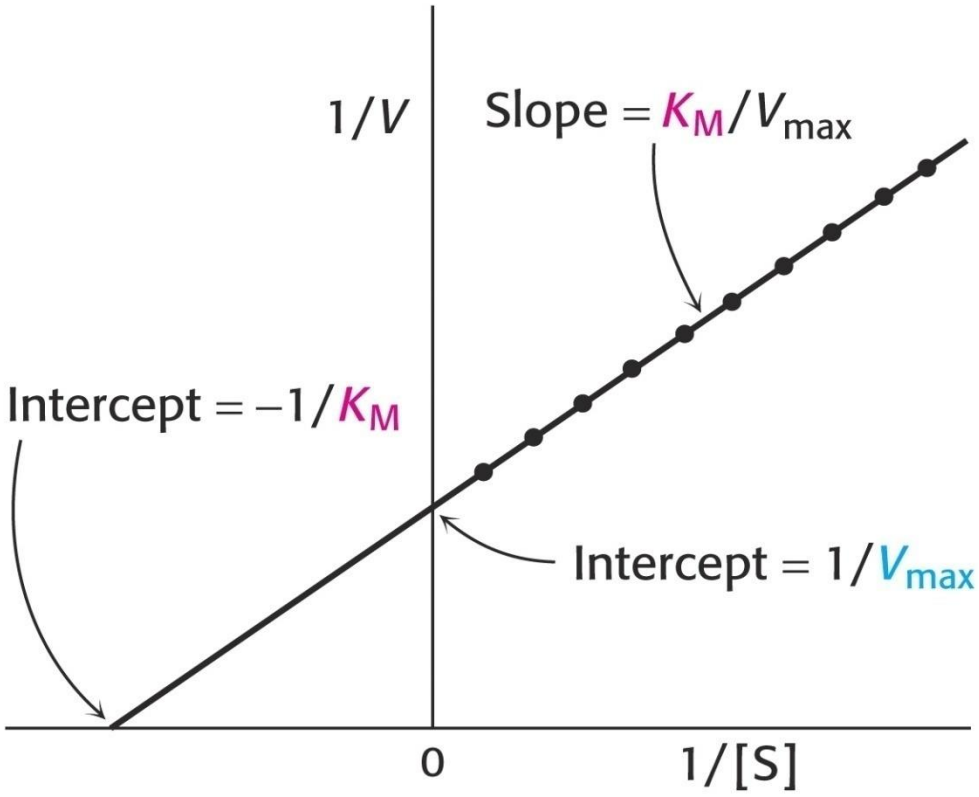
$$y = \quad (m)\ (x) + b$$

By plotting 1/ v vs $1/C_S$, a linear plot is obtained:

Slope = K_m/V_{max}

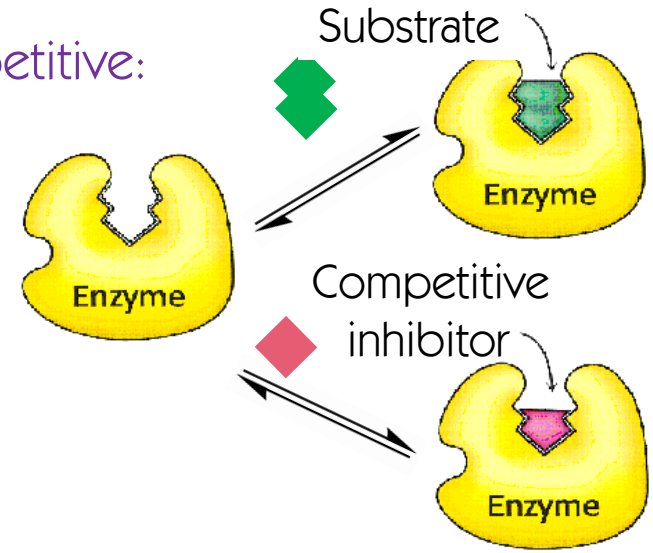
y-intercept = $1/V_{max}$

x-intercept= $-1/K_m$



Types of Reversible Inhibition

1. Competitive:



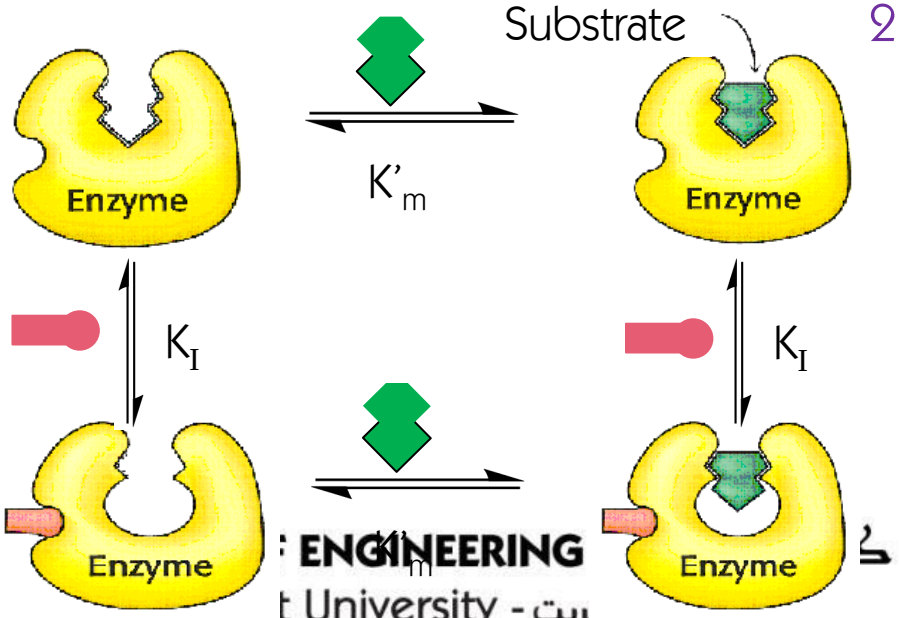
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + I \rightleftharpoons EI \quad (K_I)$$

I is the inhibitor

- Binds to active site & blocks substrate binding
- Reduces the C_{Enzyme} available for binding

2. Noncompetitive



$$E + S \underset{}{\overset{K'_m}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + I \rightleftharpoons EI \quad (K_I) \qquad ES + I \rightleftharpoons ESI \quad (K_I)$$

$$EI + S \rightleftharpoons ESI \quad (K'_m)$$

- Inhibitor binds to some other site
- Does not affect substrate binding

ENGINEERING
t University - ريت

1. Competitive inhibition

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

$$E + I \overset{K_I}{\rightleftharpoons} EI$$

Substrate and inhibitor compete for same site

Competitive inhibition

$$r_p = \frac{V_{max} C_S}{K_m\left(1 + \frac{C_I}{K_I}\right) + C_S}$$

vs **No inhibition**

$$r_p = \frac{V_{max} C_S}{K_m + C_S}$$

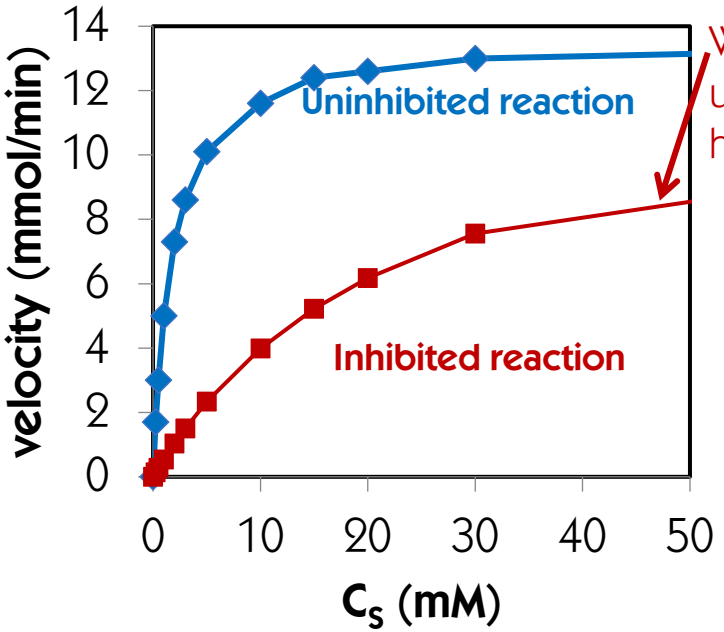
$K_{m,\,app} > K_m$

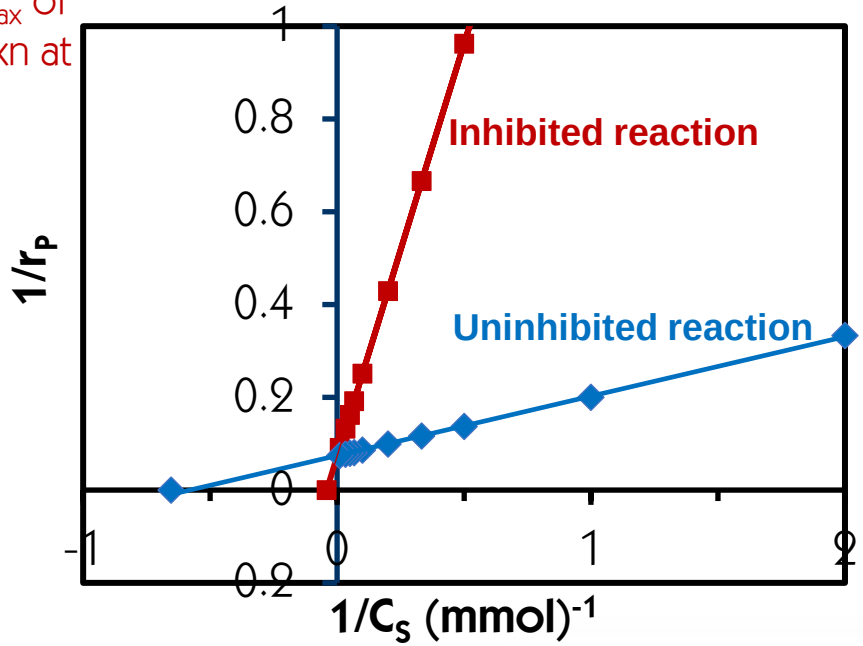
$V_{max,\,app} = V_{max}$

K_m observed w/ competitive inhibitor →

$$K_{m,app} = K_m\left(1 + \frac{[I]}{K_I}\right)$$

$$\frac{1}{r_P} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{C_S}\right) + \frac{1}{V_{max}}$$

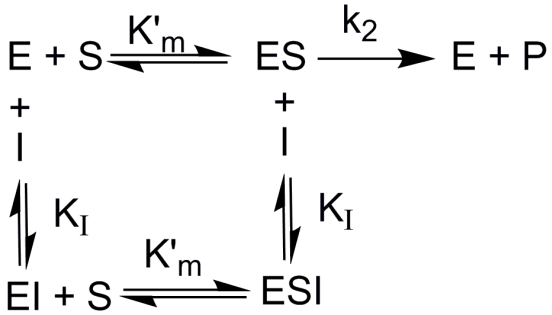




Slope = K_m/V_{max} y-int = $1/V_{max}$ x-int= $-1/K_m$

Can be overcome by high substrate concentration

2. Noncompetitive Inhibition

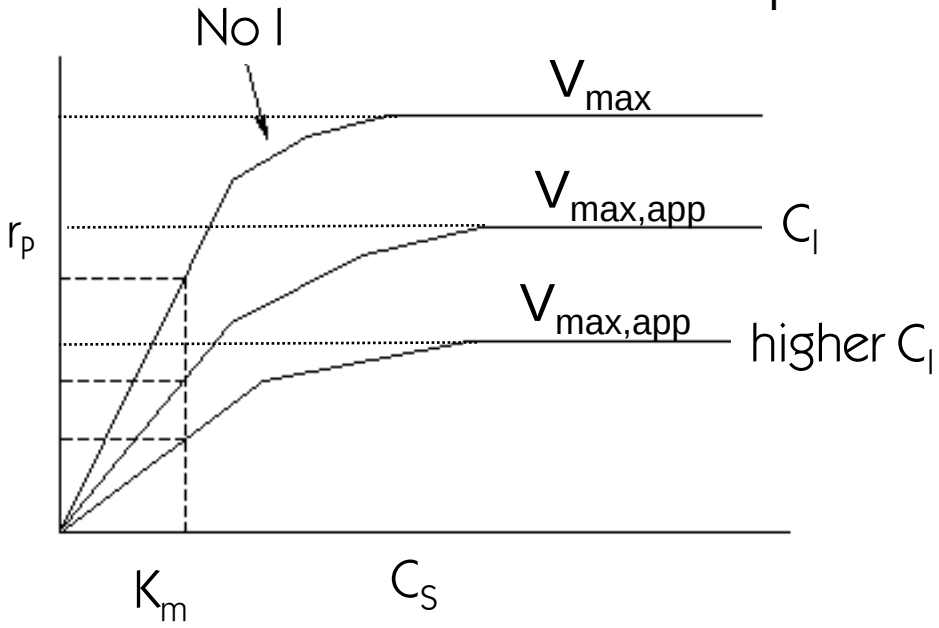


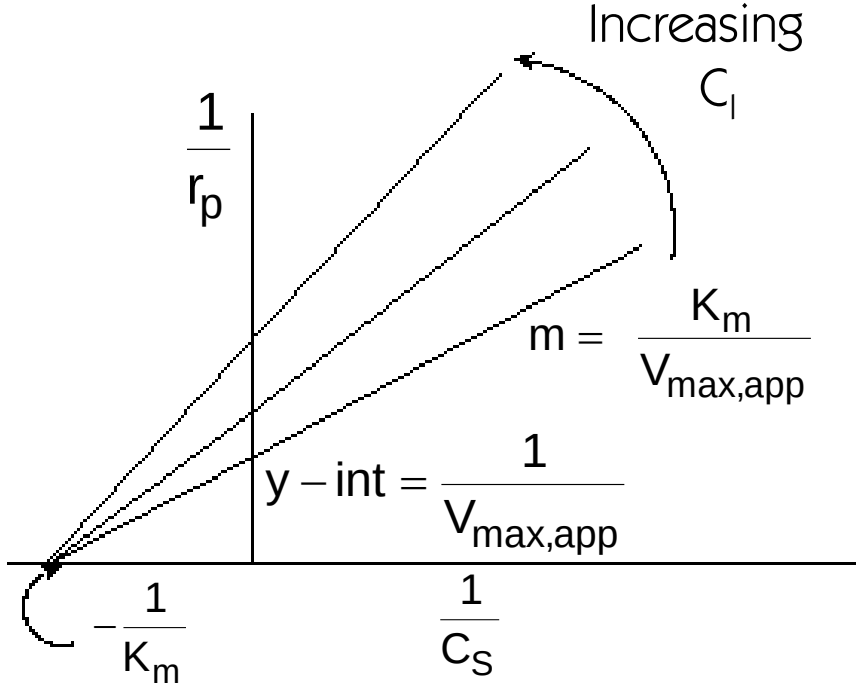
Competitive inhibition **No inhibition**

$$v = r_P = \frac{(V_{max}/(1+C_I/K_I))C_S}{C_S + K_m} \quad \text{vs} \quad r_p = \frac{V_{max}C_S}{K_m + C_S}$$

V_{max} observed w/ noncompetitive inhibitor → $V_{max,app} = \dfrac{V_{max}}{1+\dfrac{C_I}{K_I}}$

substrate and inhibitor bind different sites



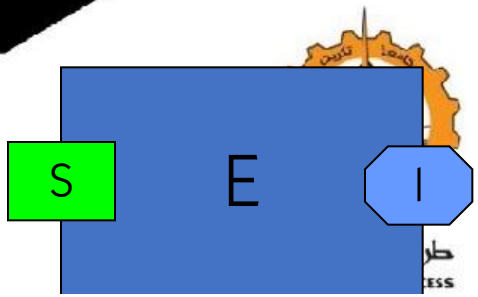


$V_{max,app} < V_{max}$

$K_{m,app} = K_m$

$$\frac{1}{r_P} = \frac{K_m}{V_{m,app}}\left(\frac{1}{C_S}\right) + \frac{1}{V_{m,app}}$$

3. Uncompetitive Inhibition



$$v = r_p = \frac{(V_{max}/(1+C_I/K_I))C_S}{C_S + [K_m/(1+C_I/K_I)]} \text{ vs } r_p = \frac{V_{max}C_S}{K_m + C_S}$$

substrate & inhibitor bind different sites but I only binds after S is bound

$$V_{max,app} = \frac{V_{max}}{\left(1+\frac{C_I}{K_I}\right)}$$

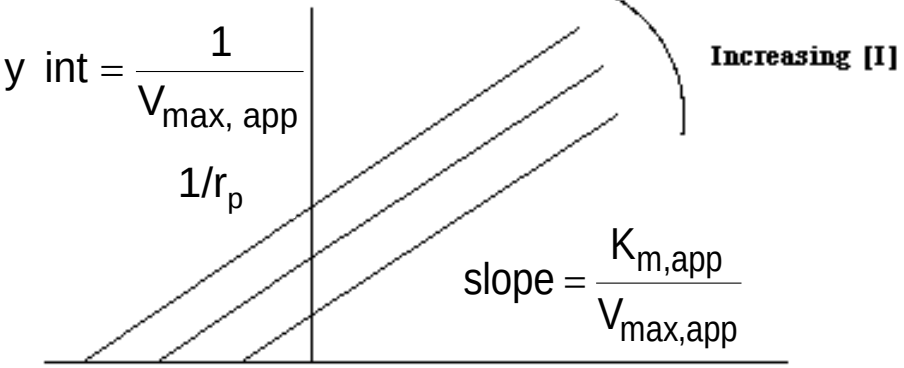
$$K_{m,app} = \frac{K_m}{\left(1+\frac{C_I}{K_I}\right)}$$

$$E + S \underset{}{\overset{K'_m}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$+ \; I \;\; \overset{K_I}{\rightleftharpoons} \;\; ESI$$

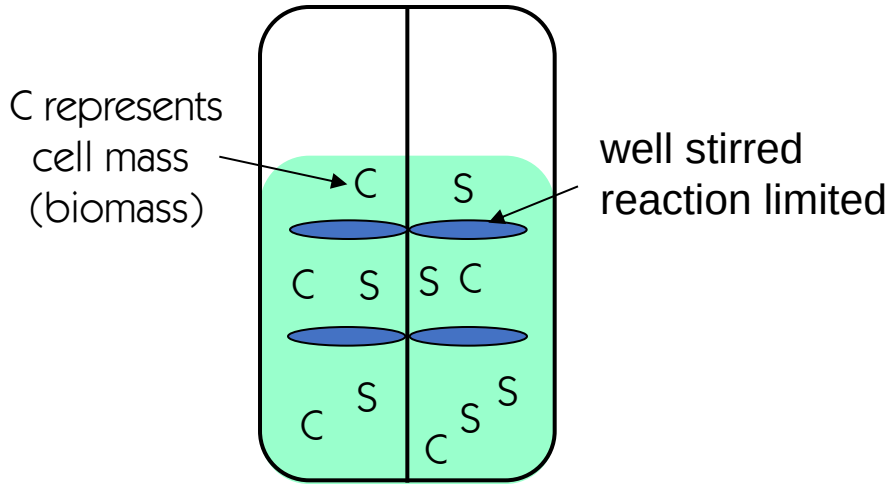
$V_{max,\,app} < V_{max}$

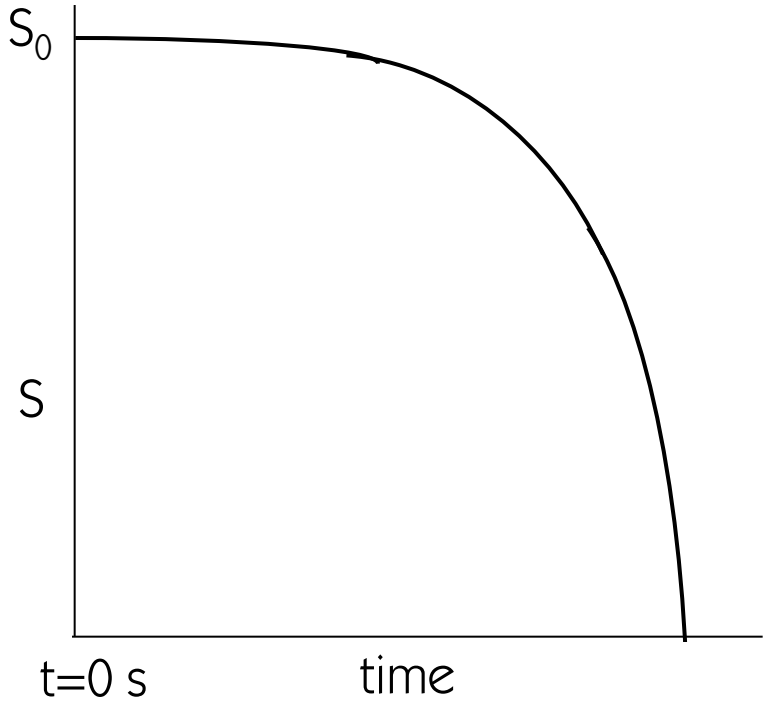
$K_{m,\,app} < K_m$



$$\frac{1}{r_P} = \frac{K_{m,app}}{V_{max,app}}\left(\frac{1}{C_S}\right) + \frac{1}{V_{max,app}}$$

Batch Bioreactor or Fermenter





Batch Reactor

In a reactor with substrate at concentration C_{S0} and C_C =0

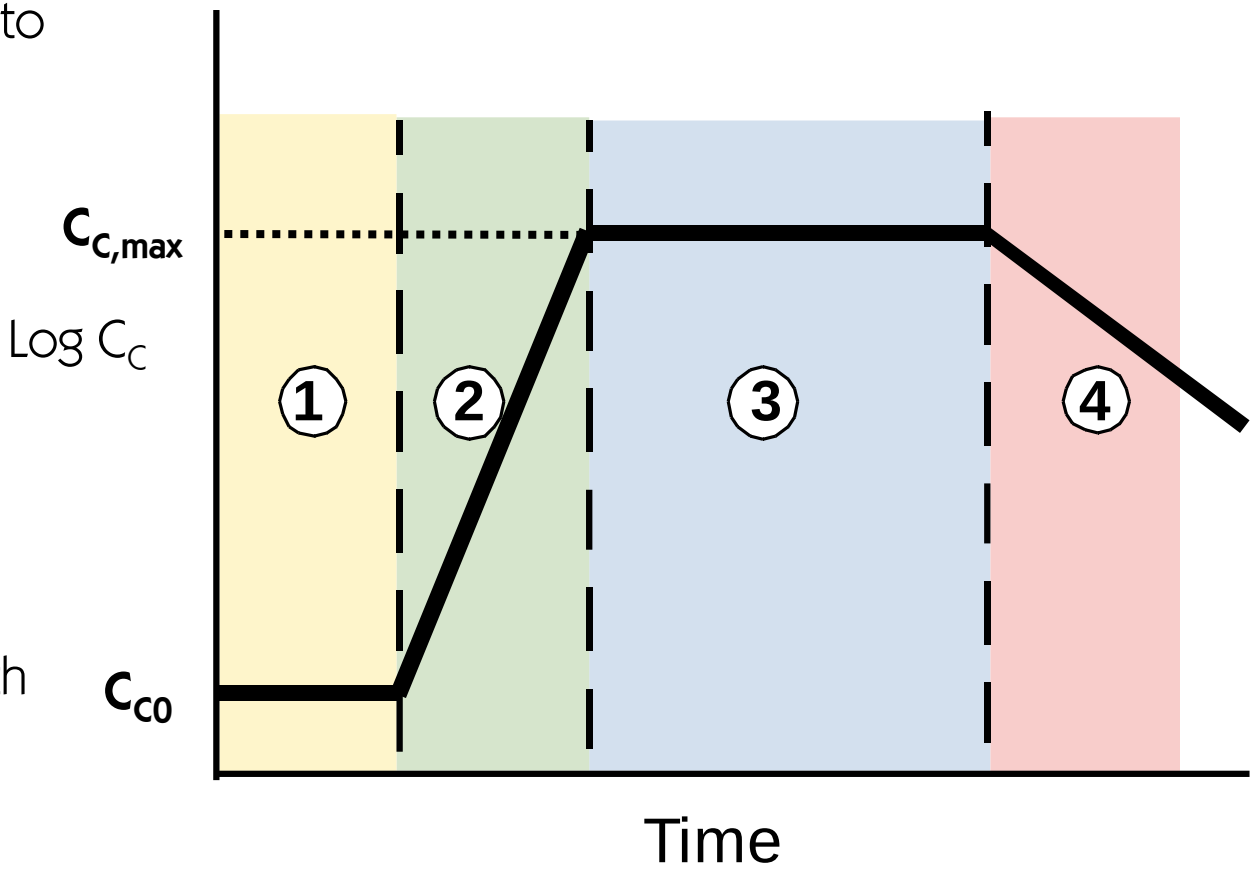
add cells at concentration C_{C0} at t = 0

add no more S or cells after t = 0

monitor C_S and C_C with time

Kinetics of Microbial Growth (Batch or Semi-Batch)

- **Region 1: Lag phase**
  - microbes are adjusting to the new substrate
- **Region 2: Exponential growth phase**
  - microbes have acclimated to the conditions
- **Region 3: Stationary phase**
  - limiting substrate or oxygen limits the growth rate
- **Region 4: Death phase**
  - substrate supply is exhausted



Quantifying Growth Kinetics

- Relationship of the specific growth rate to substrate concentration exhibits the form of saturation kinetics
- Assume a single chemical species, S, is growth-rate limiting
- Apply Michaelis-Menten kinetics to cellular system→ called the **Monod equation**

$$\text{Monod equation: } r_g = C_C \frac{\mu_{max} C_S}{K_s + C_S}$$

- μ_{max} is the maximum specific growth rate when $S >> K_s$
- C_S is the substrate concentration
- C_C is the cell concentration
- K_s is the saturation constant or half-velocity constant. Equals the rate-limiting substrate concentration, S, when the specific growth rate is ½ the maximum
- Semi-empirical, experimental data fits to equation, assumes that a single enzymatic reaction, and therefore substrate conversion by that enzyme, limits the growth-rate

Monod Model (1942) – Nobel Prize

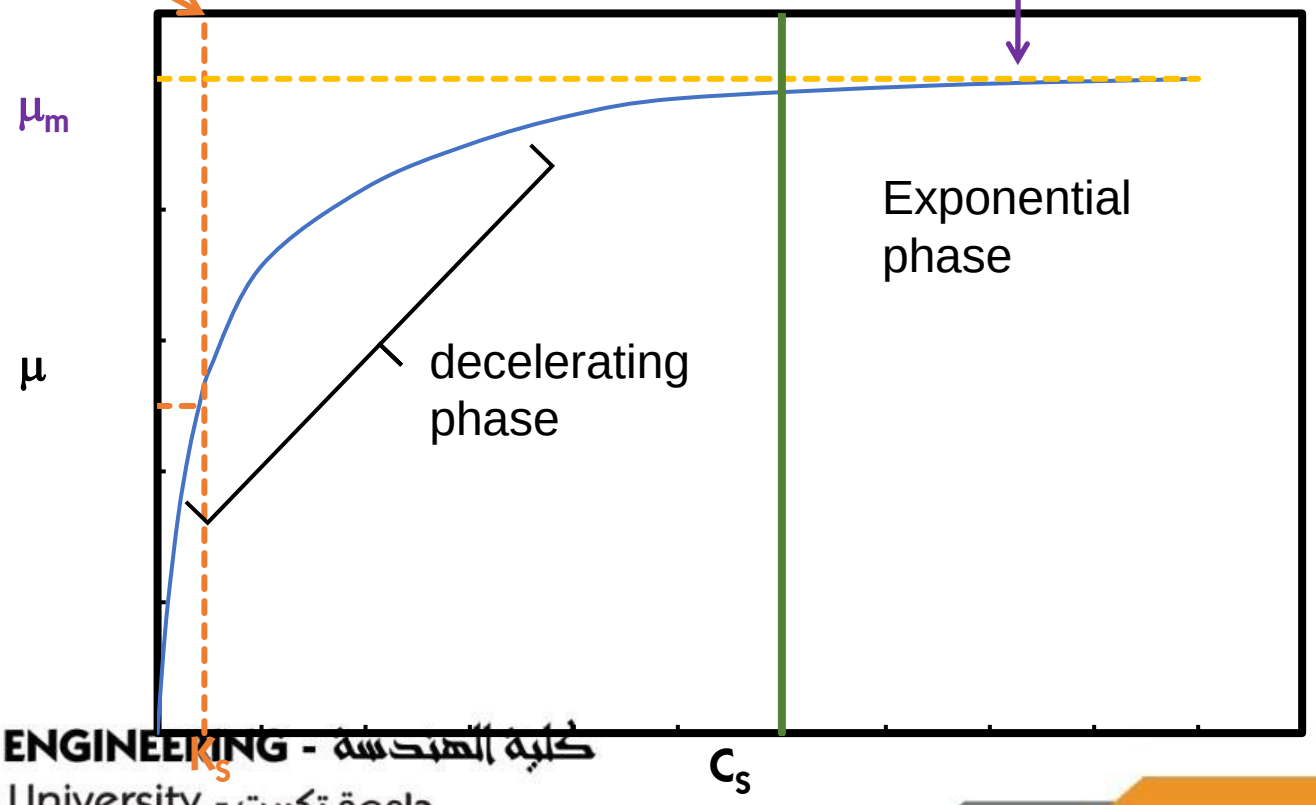
First-order kinetics:

$$C_S \ll K_S \rightarrow r_g = C_C \frac{\mu_m C_S}{K_s}$$

$$r_g = C_C \frac{\mu_m C_S}{K_s + C_S}$$

Zero-order kinetics:

$$C_S >> K_S \rightarrow r_g = \mu_m$$



Mass Balance on Cell Growth with Products

Overall balance for cells growing on carbohydrate with products:

$$CH_mO_n + a\,O_2 + b\,NH_3 \rightarrow c\,CH_\alpha O_\beta N_\delta + d\,CH_xO_yN_z + e\,H_2O + f\,CO_2$$

Carbohydrate (can be any organic material)

Nitrogen source

Cell material (biomass)

Product

Individual elemental balances:

1) Carbon: $1 = c + d + f$
2) Hydrogen: $m + 3b = c\alpha + dx + 2e$
3) Oxygen: $n + 2a = c\beta + dy + e + 2f$
4) Nitrogen: $b = c\delta + dz$

Yield Coefficients

Cell yield for substrate: $$Y_{C/S} = -\frac{\Delta C}{\Delta S}$$ ← ΔC: cell mass formed; ← ΔS: substrate consumed

Cell yield for O_2: $$Y_{C/O_2} = -\frac{\Delta C}{\Delta O_2}$$ ← ΔC: cell mass formed; ← ΔO_2: oxygen consumed

Product yield for substrate: $$Y_{P/S} = -\frac{\Delta P}{\Delta S}$$ ← ΔP: product mass formed; ← ΔS: substrate consumed

Summary

- • Enzymes are crucial biological catalysts
- • They operate through specific mechanisms influenced by various factors
- • Understanding enzyme kinetics helps in multiple scientific and industrial applications