كلية الصيدلة - جامعة الزيتونة الأردنية
Faculty of Pharmacy
Al-Zaytoonah University of Jordan

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Biochemistry for Nursing

0201163

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Subjects for part 2

- **Buffers**
- **Enzymes**

Buffers

pH is a measure of the concentration of H^+ [H_3O^+] ions in a solution.

Only the concentration of H^+ and OH^- molecules determine the pH.

$[H^+] = [OH^-]$, the solution is neutral.

$[H^+] > [OH^-]$, the solution is acidic

$[OH^-] > [H^+]$, the solution is basic.

Acid: H^+ donor

Base: H^+ acceptor

(H^+: Proton)

$$pH = -\log [H^+]$$

pH is a measure of acidity:

$\uparrow[H^+] \rightarrow \uparrow$ acidity $\rightarrow \downarrow$pH

pH is inversely proportional with log $[H^+]$, which means that small changes in pH means BIG changes in acidity.

Each 1 unit change of pH is equivalent to 10 folds change in [H+] (e.g., if pH decreased from 3 to 2, this means that [H+] has increased by 10 times).

What is buffer?

A buffer is a solution that can resist change in pH.

Buffers can resist change in pH when adding small amounts of acids or bases.

The buffer is composed of either:

A weak acid + its conjugate base ($HA + H_2O \leftrightarrow H^+ + A^-$)

OR

A weak base + its conjugate acid ($A + H_2O \leftrightarrow AH^+ + OH^-$)

Weak acid: Partial dissociation.

e.g.:

Acetic acid (CH_3COOH), Phosphoric acid (H_3PO4)

Strong acid: Full dissociation.

e.g.:

Hydrochloric acid (HCl), Sulfuric acid (H_2SO4)

To make a buffer, the conjugate acid or base are added as a salt.

e.g.:
CH_3COONa "sodium acetate"

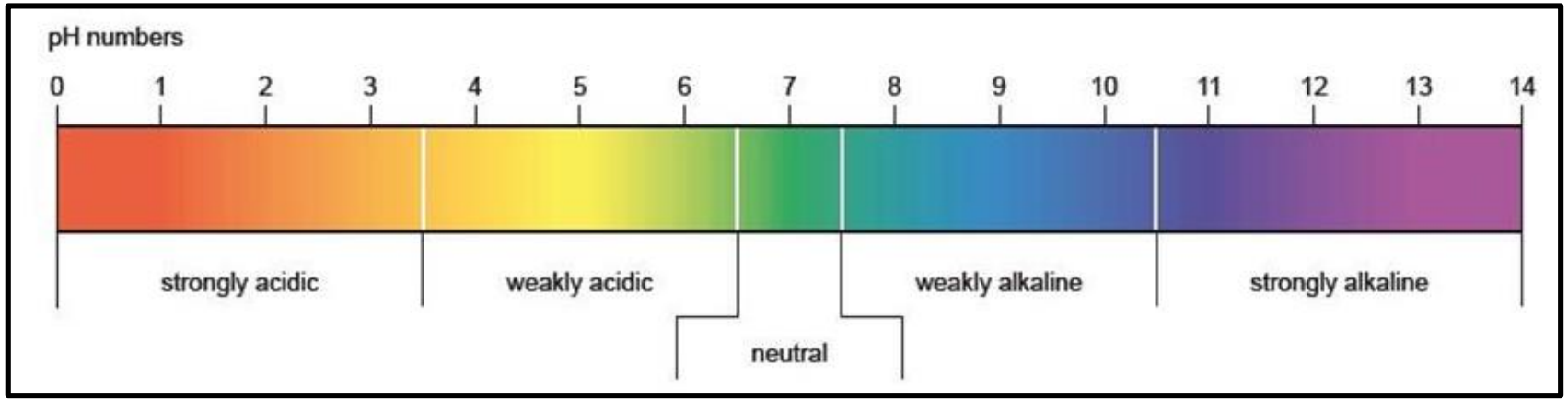
Examples:

1. Acetic acid and acetate ion:

$CH_3COOH + H_2O \leftrightarrow CH_3COO^- + H^+$

2. Ammonia and ammonium:

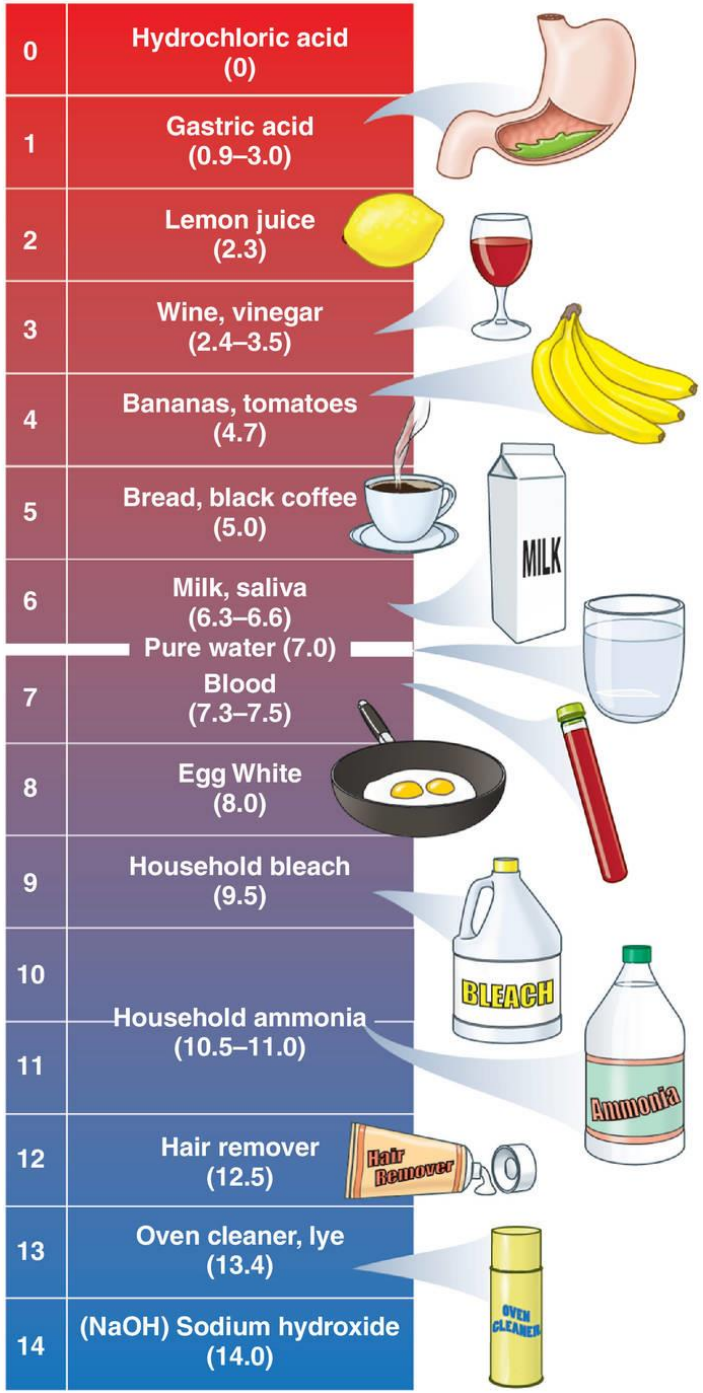
$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$



A color chart for universal indicator that shows the pH scale

pH scale and pH values of common materials

Material	pH
10% HCl	1.0
Gastric juice	1.0–5.0
0.1% HCl	3.0
☆ Pure water (neutral) at 25°C	7.0
☆ Blood plasma	7.35–7.45
Pancreatic juice	8.4–8.9
0.1% NaOH	11.0
10% NaOH	13.0



- **The Dissociation constant of weak acids (Ka):**

$HA + H_2O \leftrightarrow H^+ + A^-$

$$Ka = \frac{[Products]}{[Reactants]} = \frac{[H^+][A^-]}{[HA]}$$

pKa= - log Ka

pKa indicates the degree of dissociation.

↓Ka → ↓dissociation →↑pKa → The weaker the acid, and *vice versa*.

Thus, only weak acids have pKa, strong acids have no pKa.

Acids can be either:

- **Monoprotic:** with one proton to lose

e.g.: HCl, CH_3COOH

- **Polyprotic**: with more than one proton to lose

e.g.: H_2SO_4, H_3PO_4

Polyprotic acids have >1 pKa (one for each proton).

[…]: Molar concentration = #mols / L

• **Buffering capacity**:

Buffers can resist changes in pH only within a certain range = pKa ±1.

Maximum Buffering capacity: when **pH=pKa**.

(e.g.: pKa of acetic acid buffer = 4.8 → the buffering range= 3.8 – 5.8, the maximum buffering capacity is at pH = 4.8).

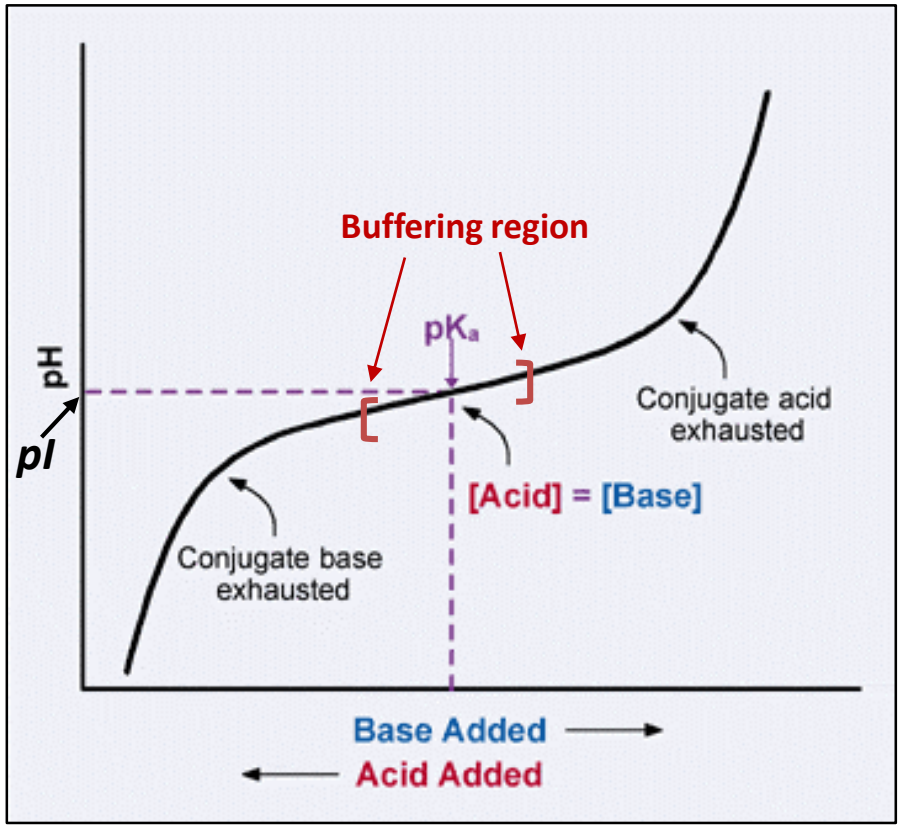
• **Handerson- Hasselbalch equation:**

$$pH = pKa + \log\frac{[A^-]}{[HA]}$$

conjugate base

weak acid

Used to calculate the pH of a buffer or the concentration of its components, and to calculate the **isoelectric point (pI)** of proteins.



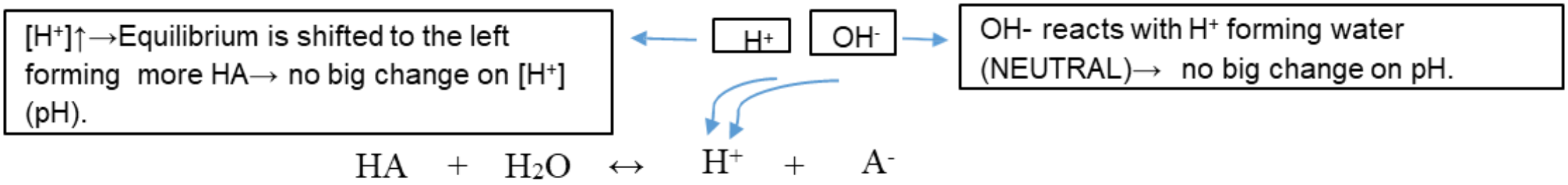
Buffers can resist changes in pH only within a certain range = pKa ±1.

How do buffers work?

Buffers resist big changes in pH upon adding limited amounts of acids or bases:

- When adding an acid (H^+): the concentration of H^+ increases, so the equilibrium is shifted to the left forming HA. HA does not affect pH since the H is not free.

- When adding a base (OH^-): OH^- reacts with H^+ forming water. Water is neutral → no big change on pH.



Commonly used buffers in biological systems:

Important buffer used for physiological application: Phosphate Buffered Saline (**PBS**), with pH: 7.4

Clinical Correlations

Normal blood pH is the arterial blood pH between: **7.35 – 7.45** (normal pH is almost 7, i.e., neutral).

Acidemia: arterial blood pH < 7.35

Alkalemia: arterial blood pH >7.45

Acidosis: abnormal condition lowering arterial pH
Alkalosis: abnormal condition raising arterial pH

Buffering systems in the body:

1) Bicarbonate buffering system in blood:

$$H_2O + CO_2 \leftrightarrow \underset{\text{Weak acid}}{H_2CO_3} \leftrightarrow H^+ + \underset{\text{Conjugate base}}{HCO_3^-}$$

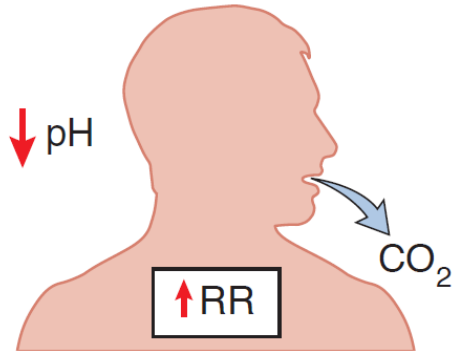
2) Respiratory system: through controlling the rate of ventilation.

Hyperventilation/Hypoventilation

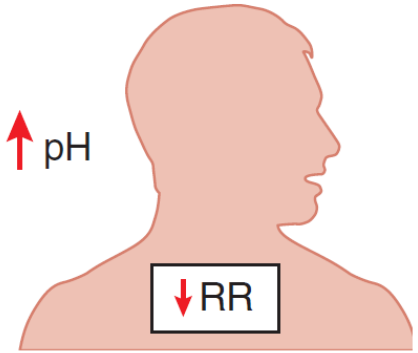
3) Renal system: through controlling the secretion of H^+, and the generation of ammonia (NH_3) and bicarbonate ($HCO3^-$).

Respiratory compensation

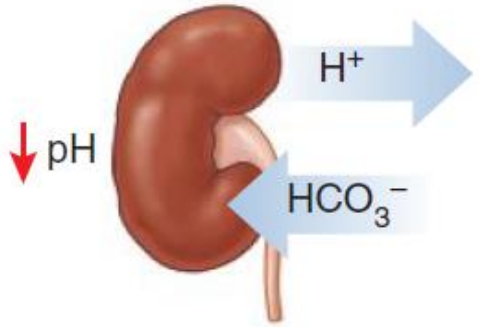
- If the pH is too low, as in metabolic acidosis, the respiratory center increases the rate of respirations. The increased respiratory rate "blows off" CO_2, which raises pH.



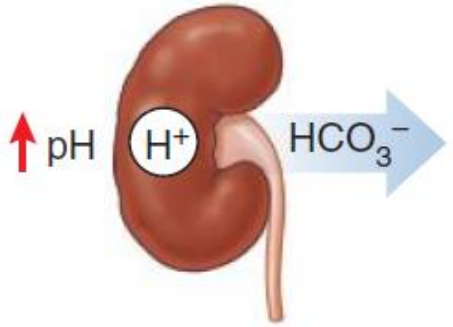
- In metabolic alkalosis, the pH is too high. Breathing slows, allowing CO_2 to accumulate, and pH drops.



Renal compensation



- In response to acidosis, the kidneys eliminate H^+ and reabsorb more bicarbonate.



- In response to alkalosis, the kidneys conserve H^+ and excrete more bicarbonate.

The cause of pH disturbance could be metabolic or respiratory, thus there are two types of acidosis and alkalosis:

Acidosis

A. Metabolic acidosis:

1. Lactic acidosis: due to Hypoxia or Hypovolemia
2. Diabetic ketoacidosis (DKA): increases ketoacids in the blood → ↓pH.
3. Renal failure: insufficiency of the kidneys to generate bicarbonate ($HCO3^-$) → ↓pH.

B. Respiratory acidosis:

Hypoventilation.

Hypoventilation → ↑PCO_2→ ↑$[H^+]$

Hypoventilation could be due to:

a) pulmonary disorder (such as COPD)
b) brain trauma or tumor (affecting respiratory centers in the brain)
c) toxins or drugs (inhibiting respiratory enzymes).

Alkalosis

A. Metabolic alkalosis:

due to hyponatremia (blood Na^+ low) and hypokalemia (blood K^+ low)

kidneys retain Na^+ and K^+ at the expense of H^+ leading to alkalosis.

B. Respiratory alkalosis: due to hyperventilation.

Hyperventilation → ↓ PCO_2→ ↓$[H^+]$

For Your Info.

Hypoxia: ↓PO_2 → poor tissue oxygenation → Anaerobic metabolism → production and accumulation of lactic acid → lactic acidosis.

Hypovolemia: ↓blood volume (due to dehydration, bleeding…) poor tissue oxygenation → Anaerobic metabolism → production and accumulation of lactic acid → lactic acidosis ↓pH.

Chronic obstructive pulmonary disease, or COPD, refers to a group of diseases that cause airflow blockage and breathing-related problems

Enzymes

Enzymes are protein molecules that act as catalysts
i.e., they increase the rate of the chemical reactions without being consumed in the overall reaction.

Chemical reactions can proceed 10^3-10^8 times faster than the uncatalyzed reaction.

The following equation shows the general enzymatic reaction:

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

- **Substrates** (S) or reactants
- Enzyme is used to form a reaction intermediate called **activated** or **transition state complex** (ES)
- When **transition state complex** breaks down or interact with another reactant releasing **free enzyme** (E) and **product** (P) arise
- Thus, enzymes are **not consumed** in the reaction.

Enzymes Classes and Nomenclature

Enzyme is assigned two names:

1. **The first** is its short, recommended name, convenient for everyday use;

example: pepsin, trypsin.

Also the word ending in **–ase:**

- Derived from its substrate : lactase- reacts lactose/ lipase - reacts lipid

OR

- The chemical reaction it catalyzes

Example: Oxidase – catalyzes oxidation

Hydrolase – catalyzes hydrolysis

2. **The second** is the more complete systematic name, which is used when an enzyme must be identified without ambiguity.

- The international union of Biochemistry and Molecular Biology (IUBMB) developed a nomenclature system for enzymes, the **Enzyme Class (EC) numbers**
- Each enzyme class is designated by a number from 1 to 6, preceded by "EC".

Enzymes Classes and Nomenclature

CLASS	DESIGNATION	FUNCTION
EC1	Oxidoreductases	catalyze oxidation/reduction reactions
EC2	Transferases	transfer a functional group (e.g. a methyl or phosphate group)
EC3	Hydrolases	catalyze the hydrolysis of various bonds
EC4	Lyases	cleave various bonds by means other than hydrolysis and oxidation
EC5	Isomerases	catalyze isomerization changes within a single molecule
EC6	Ligases	join two molecules covalent bonds.

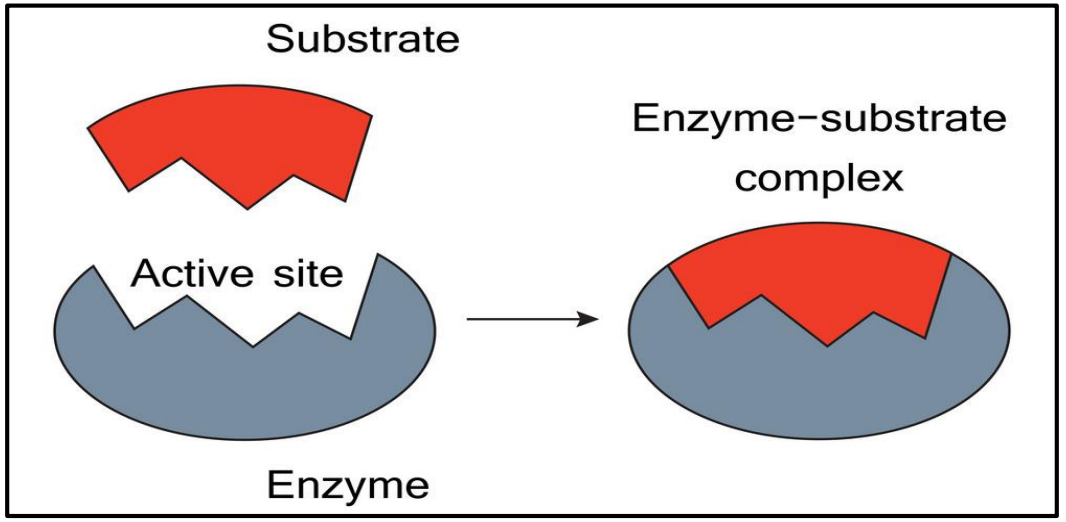
These sections are subdivided by other features such as the substrate, products, and chemical mechanism. An enzyme is fully specified by four numerical designations.

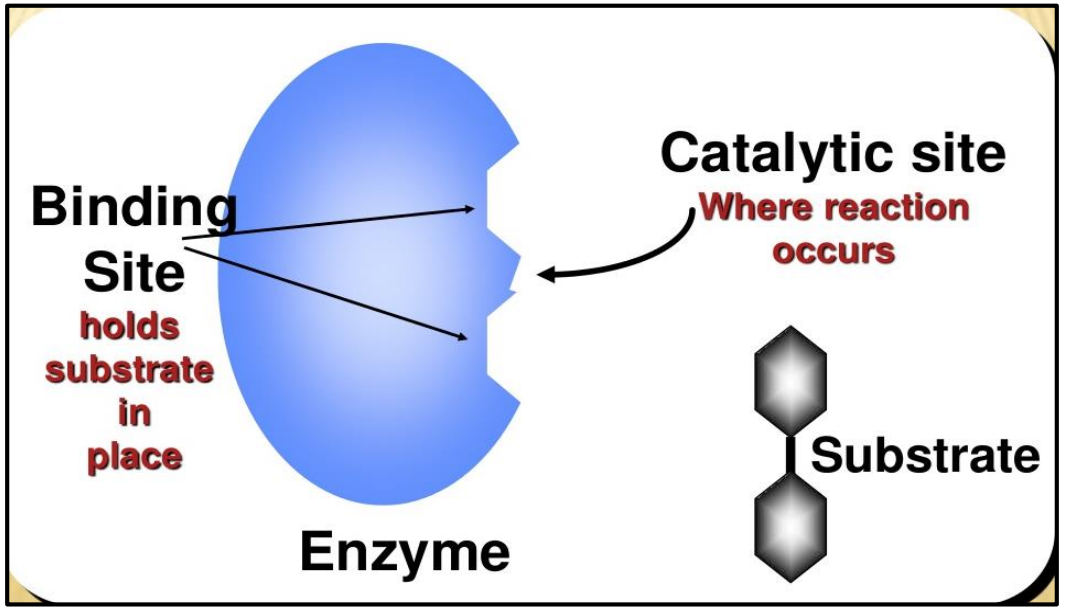
For example, hexokinase (**EC 2.7.1.1**) is a transferase (EC 2) that adds a phosphate group (EC 2.7) to a hexose sugar, a molecule containing an alcohol group (EC 2.7.1)

Enzymes Properties

1. Active site: a special cleft in the enzyme that is composed of a binding site, and a catalytic site

- **Binding site** is the part that has the right shape and functional groups to **bind with substrate**.
- **Catalytic site** provide an environment that favors the catalytic events (**where the reaction occurs**)





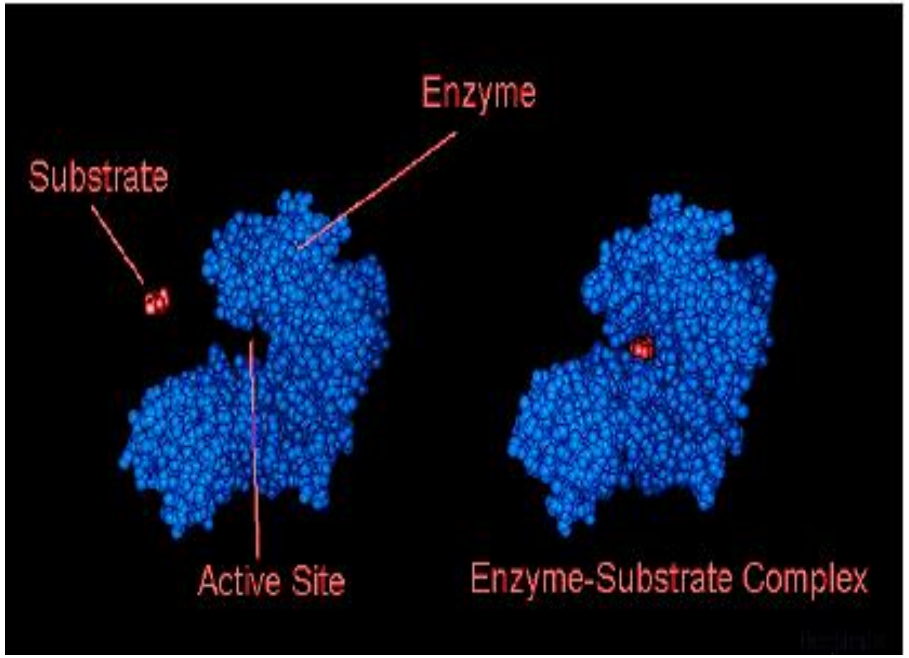
2. **Specificity**: Enzymes are highly specific and sensitive

Highly specific:

- Interacting with **one or a few** specific substrates with **high-affinity** binding
- Catalyzing one type of chemical reaction.

Highly sensitive:

- to any change in the substrate conformation.



3. Catalytic efficiency:

- The turnover number (Kcat) indicates the catalytic power of the enzyme
- Kcat: the number of substrates converted into product per enzyme per second.
- Kcat (for most enzymes) = 10^2 -10^4 (transforming 100-10000 substrate molecules into product per second)

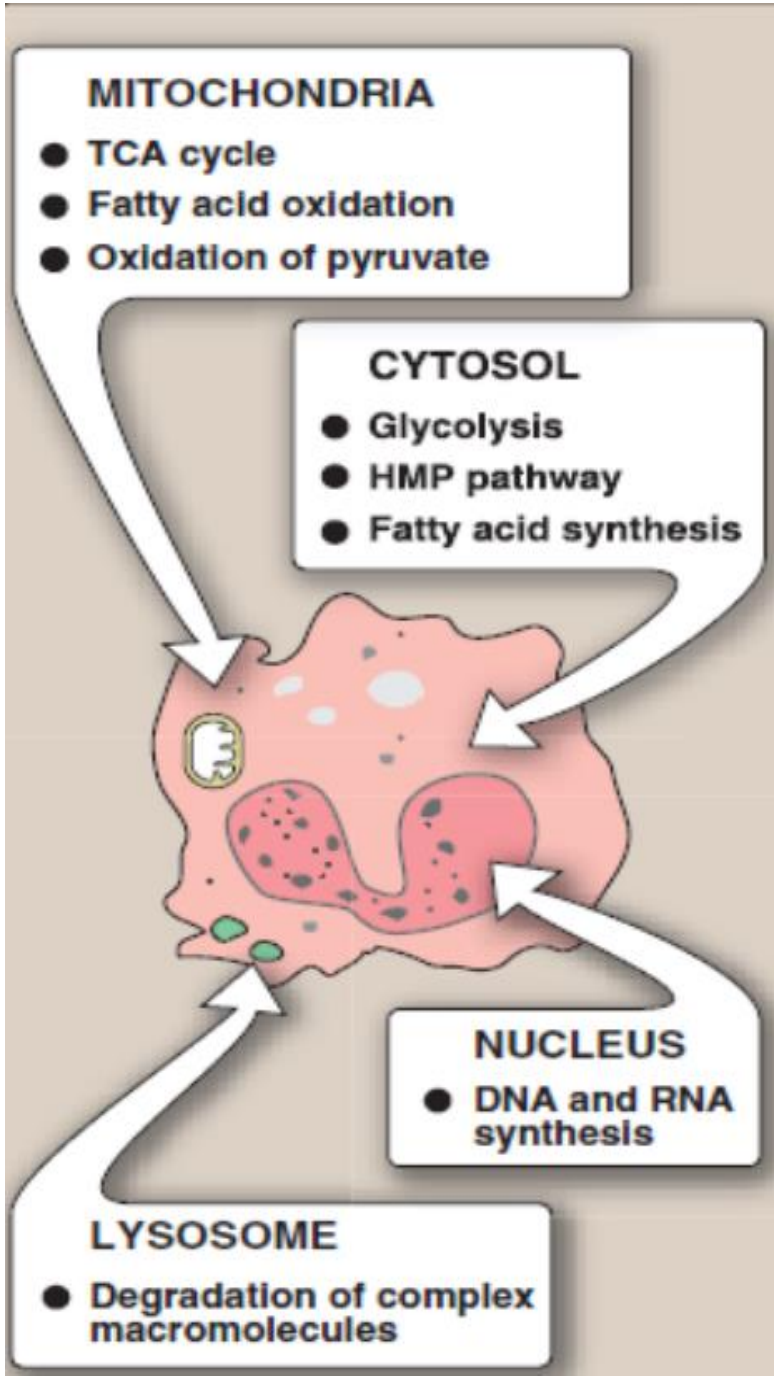
4. Regulation: enzymes can be regulated (activated or inhibited) according to physiological needs.

5. Enzymes are usually globular proteins.

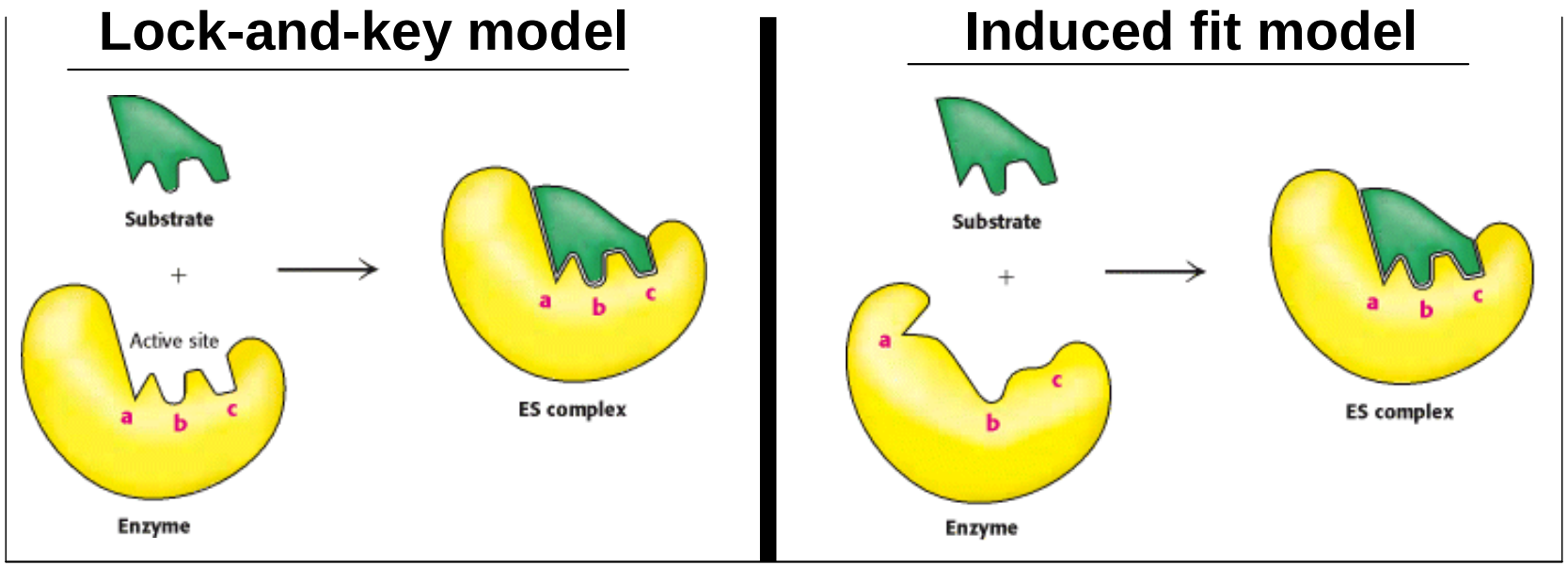
6. Localization

Enzymes are **tissue-specific**; i.e., different tissues have a different profiles of enzymes depending on their function.

- At the cell level, many enzymes are located in specific organelles in the cell. An enzyme location within the cell affects cell metabolism.
- For example, some enzymes are localized in the cytosol, others in the mitochondria, lysosomes, endoplasmic reticulum… etc.



- **Two models (hypotheses) of substrate binding to enzymes:**



Lock and key hypothesis

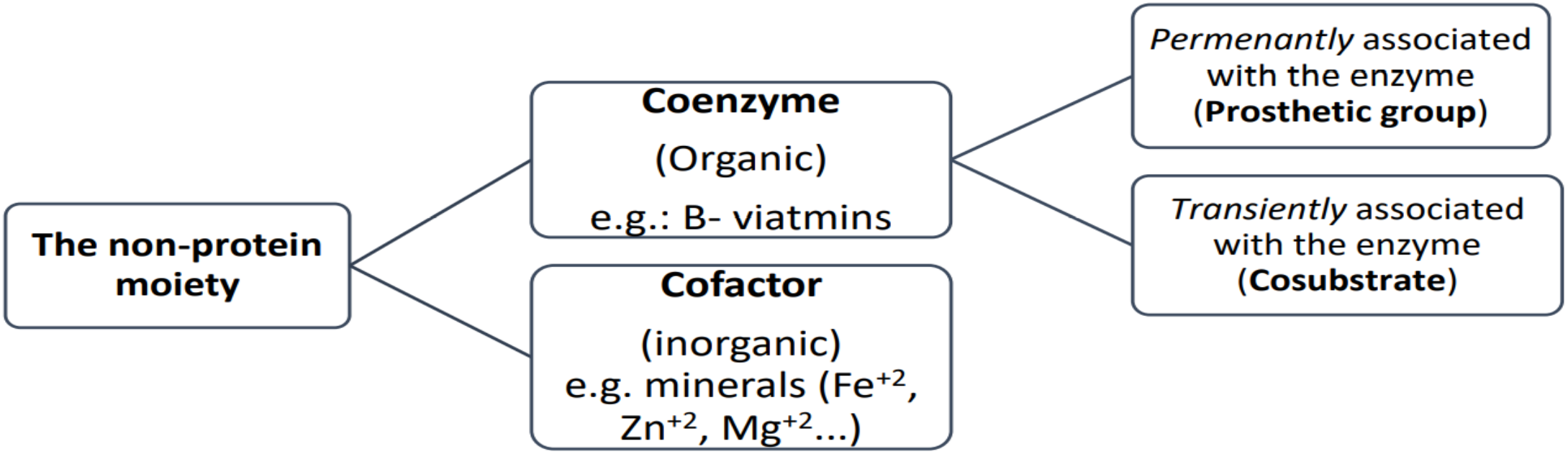
This is the simplest model to represent how an enzyme works. The substrate simply fits into the active site to form a reaction intermediate.

Induced fit hypothesis

In this model the enzyme molecule *changes* shape as the substrate molecules gets close. The change in shape is 'induced' by the approaching substrate molecule. This more sophisticated model relies on the fact that protein structure is *flexible*.

Protein and a non-protein moiety of enzyme

- Most enzymes (protein) associate with **non-protein moiety** that is needed for enzymatic activity (**in order to be functional)**



Isozyme

Enzymes with different structures but with the same function.

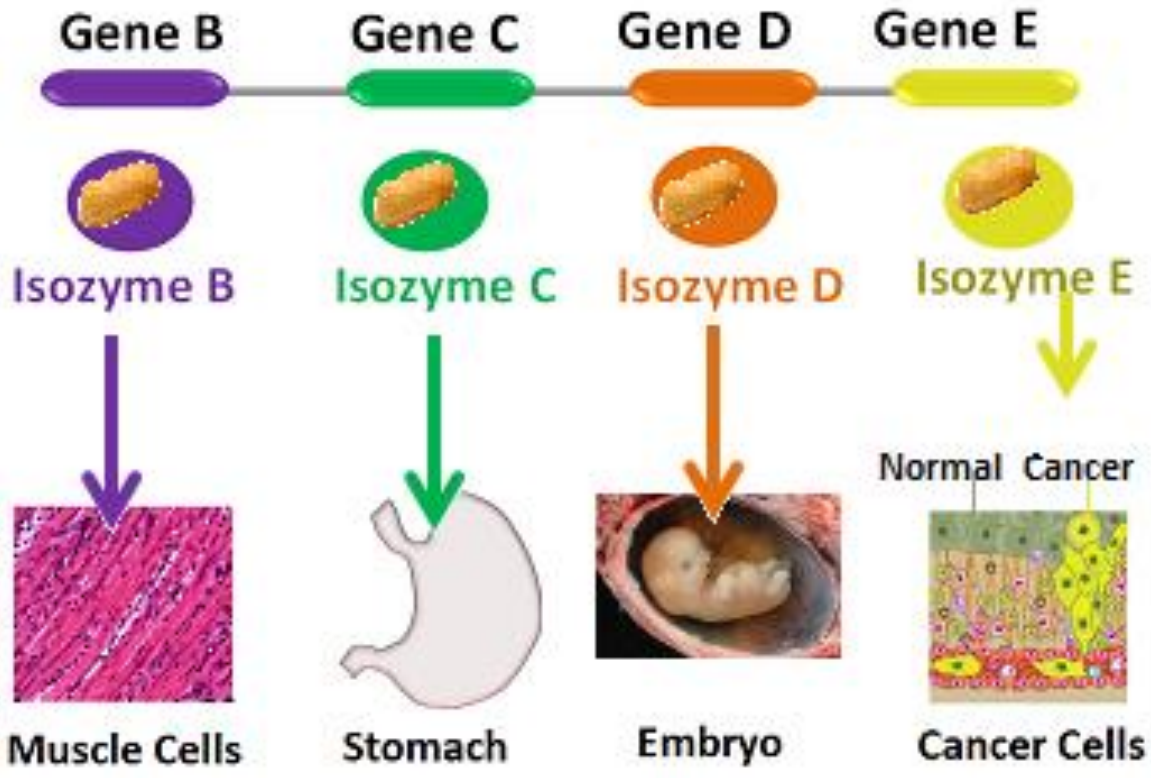
Catalyze the same chemical reaction.

Various isozymes are present in different tissues or at different developmental stages.

Examples:

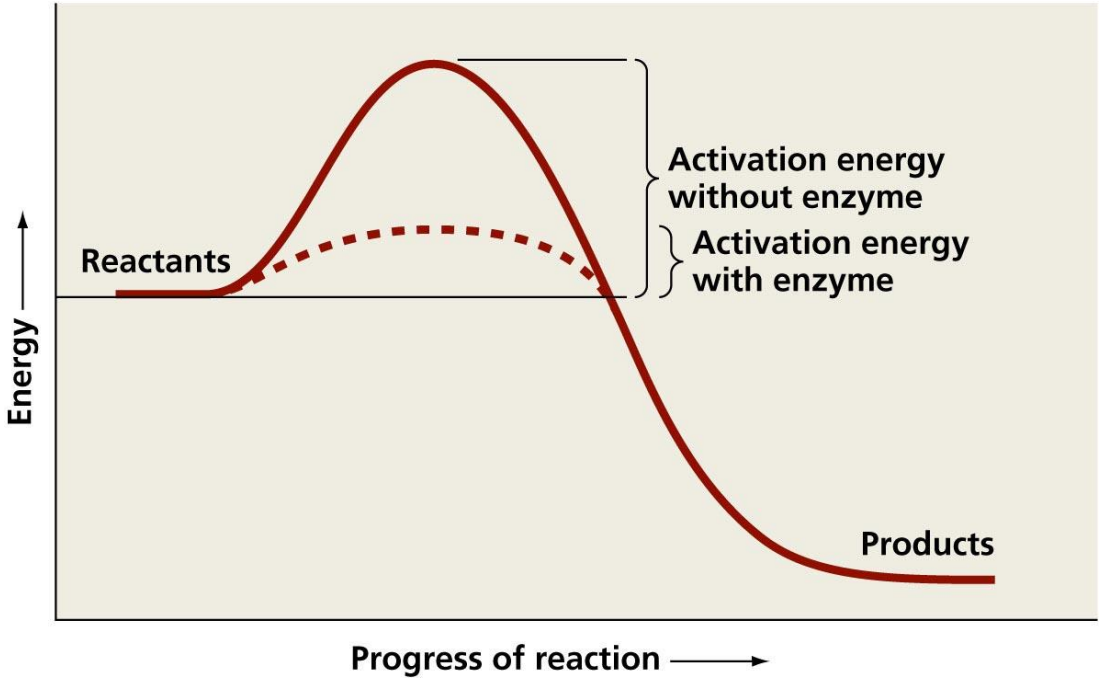
- Lactate Dehydrogenase (LDH)
- Creatine phosphokinase (CPK)

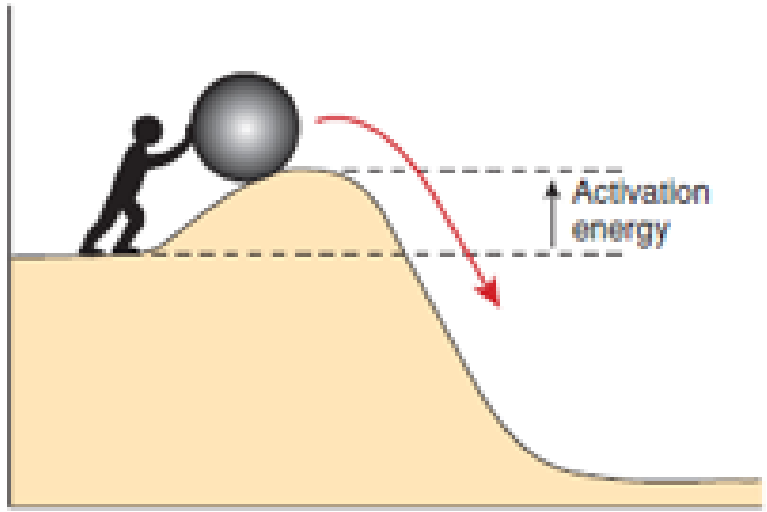
  LDH and CPK have valuable **diagnostic value.**

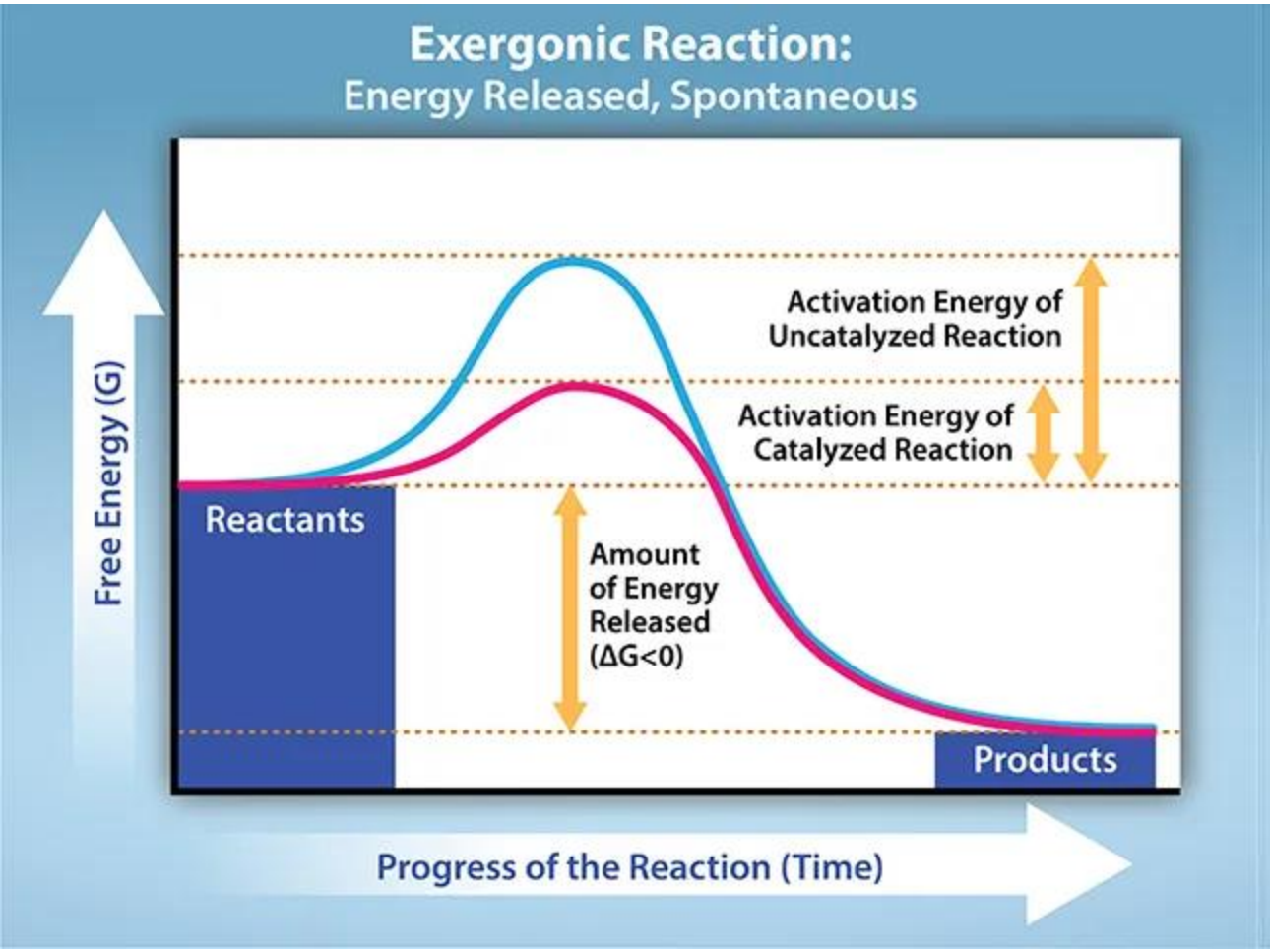


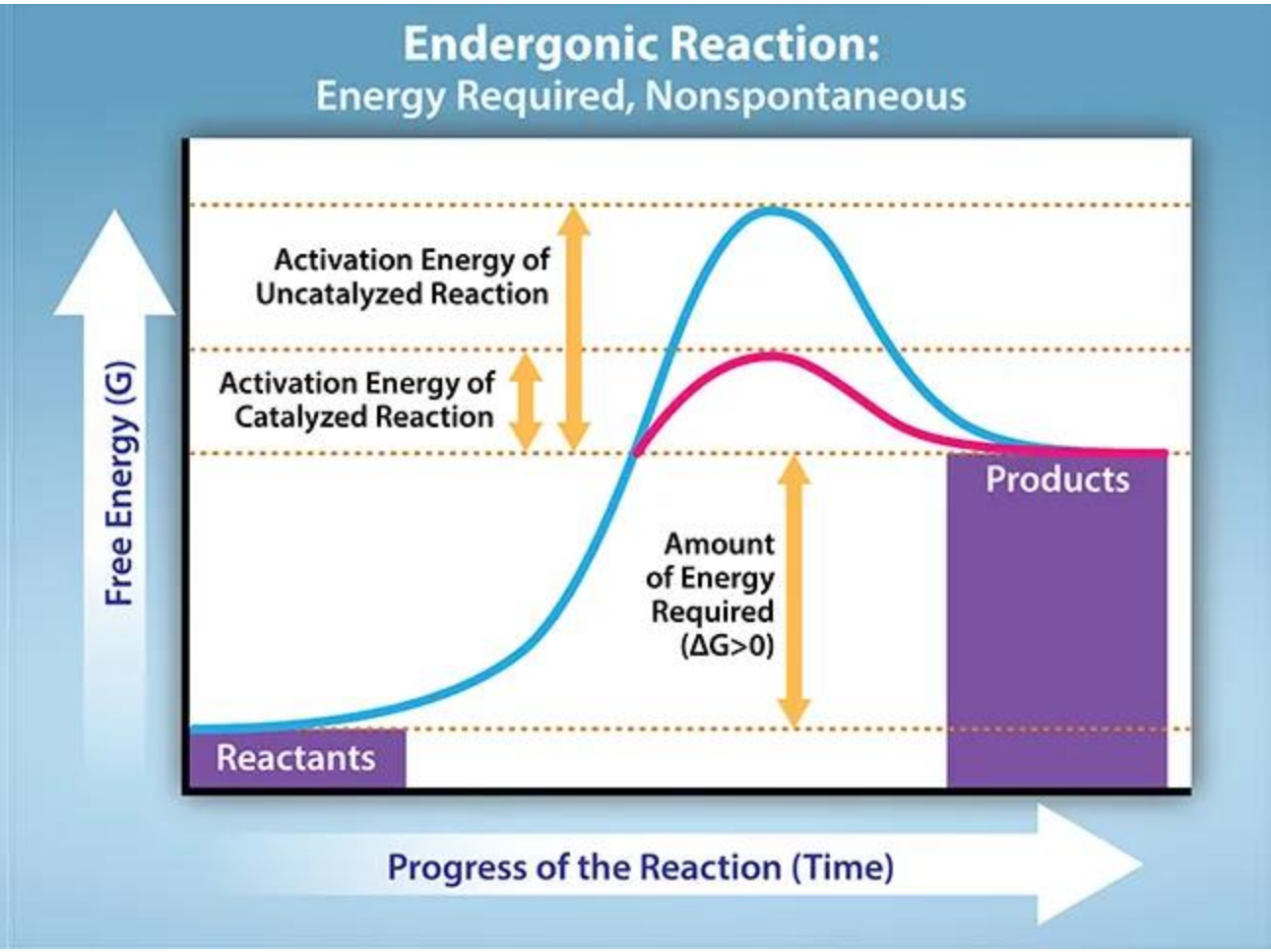
How do enzymes work?

Conversion of a substrate to a product requires an energy called activation energy.
Activation Energy: the minimum energy required for the reactants to start a chemical reaction.
Enzymes accelerate reactions by lowering this energy.






Exergonic Reaction:
Energy Released, Spontaneous
Free Energy (G)
Activation Energy of Uncatalyzed Reaction
Activation Energy of Catalyzed Reaction
Reactants
Amount of Energy Released (ΔG<0)
Products
Progress of the Reaction (Time)

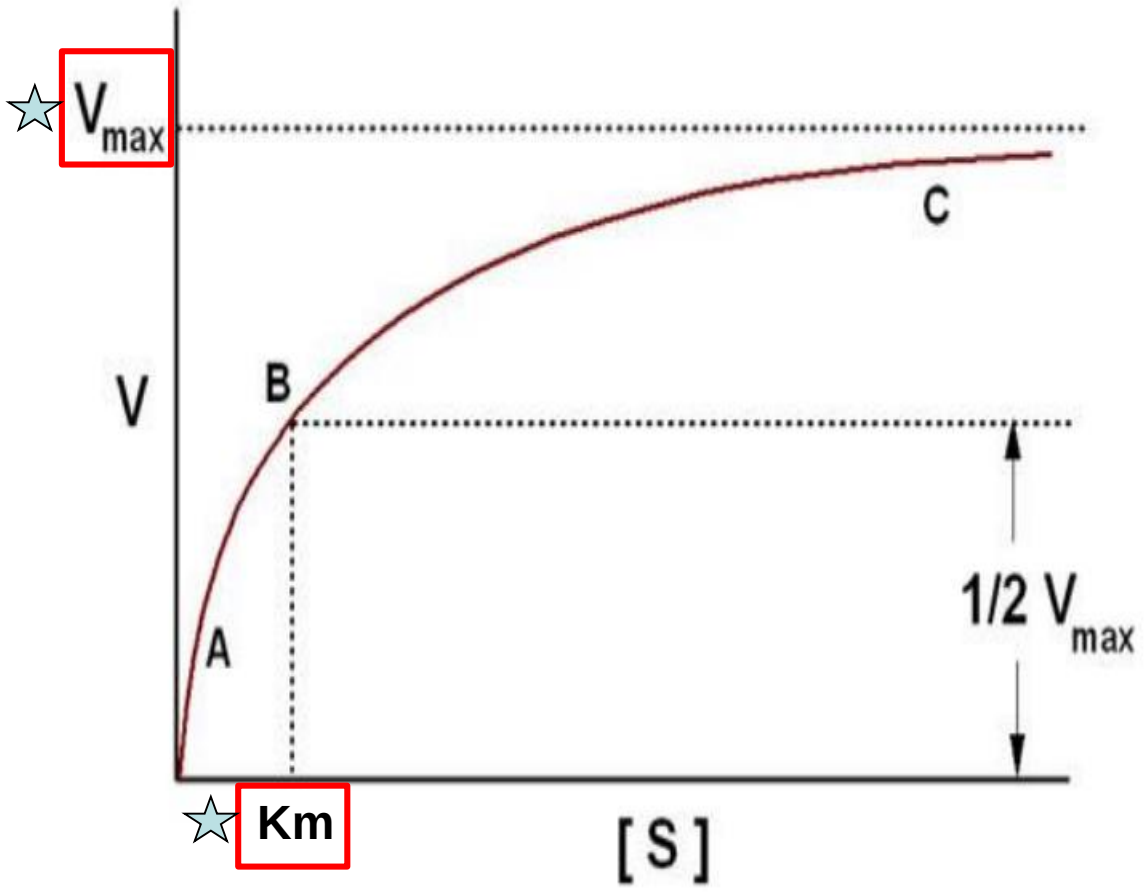

Endergonic Reaction:
Energy Required, Nonspontaneous
Free Energy (G)
Activation Energy of Uncatalyzed Reaction
Activation Energy of Catalyzed Reaction
Products
Amount of Energy Required (ΔG>0)
Reactants
Progress of the Reaction (Time)

Enzyme kinetics

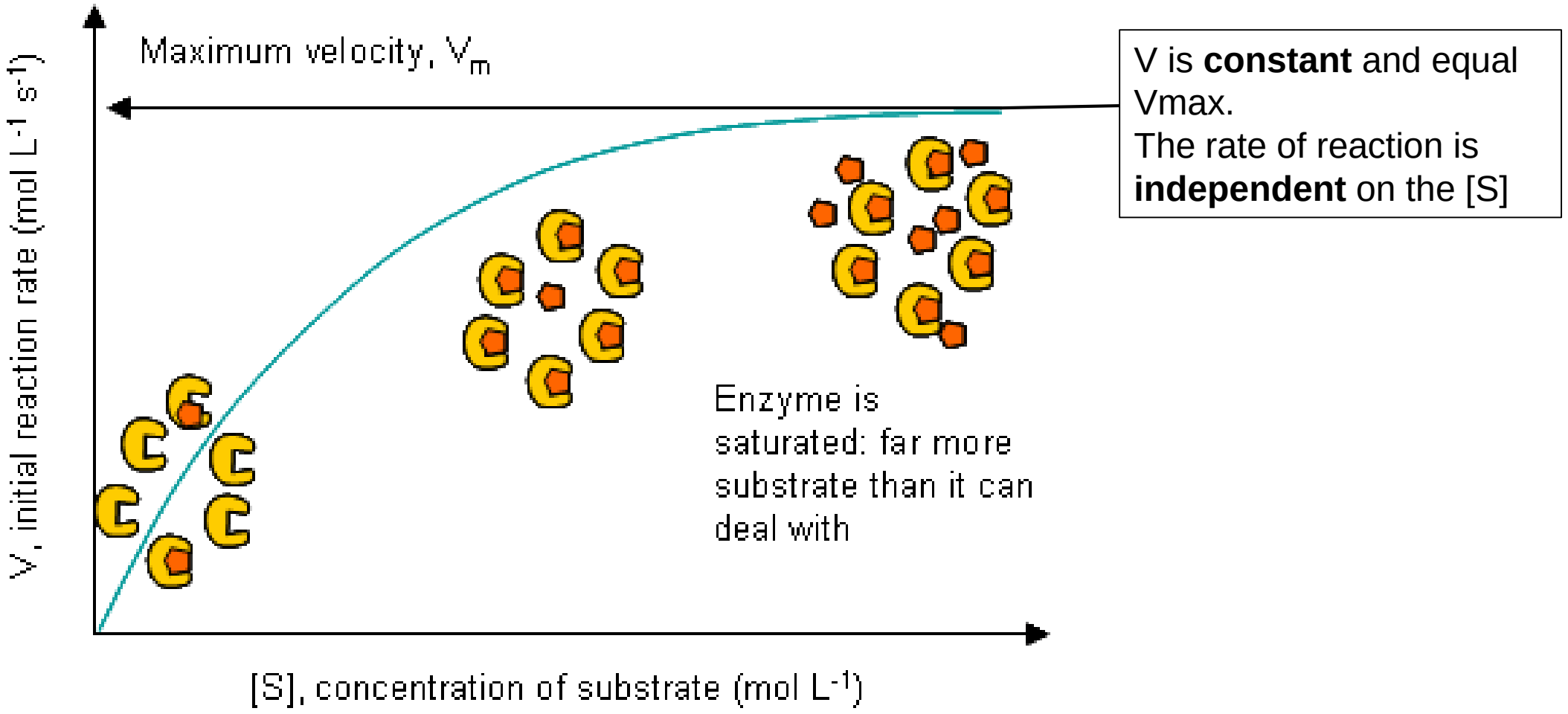
There are two factors related to the kinetics of an enzymatic reaction:

- Reaction velocity: each enzyme has a maximal reaction rate **(Vmax)**.
- Enzyme affinity to substrate **(Km):** the bigger the Km, the lower the affinity and *vice versa*.

<u>Vmax and Km are *characteristic* for each enzyme.</u>



- Reaction velocity: each enzyme has a maximal reaction rate **(Vmax)**.

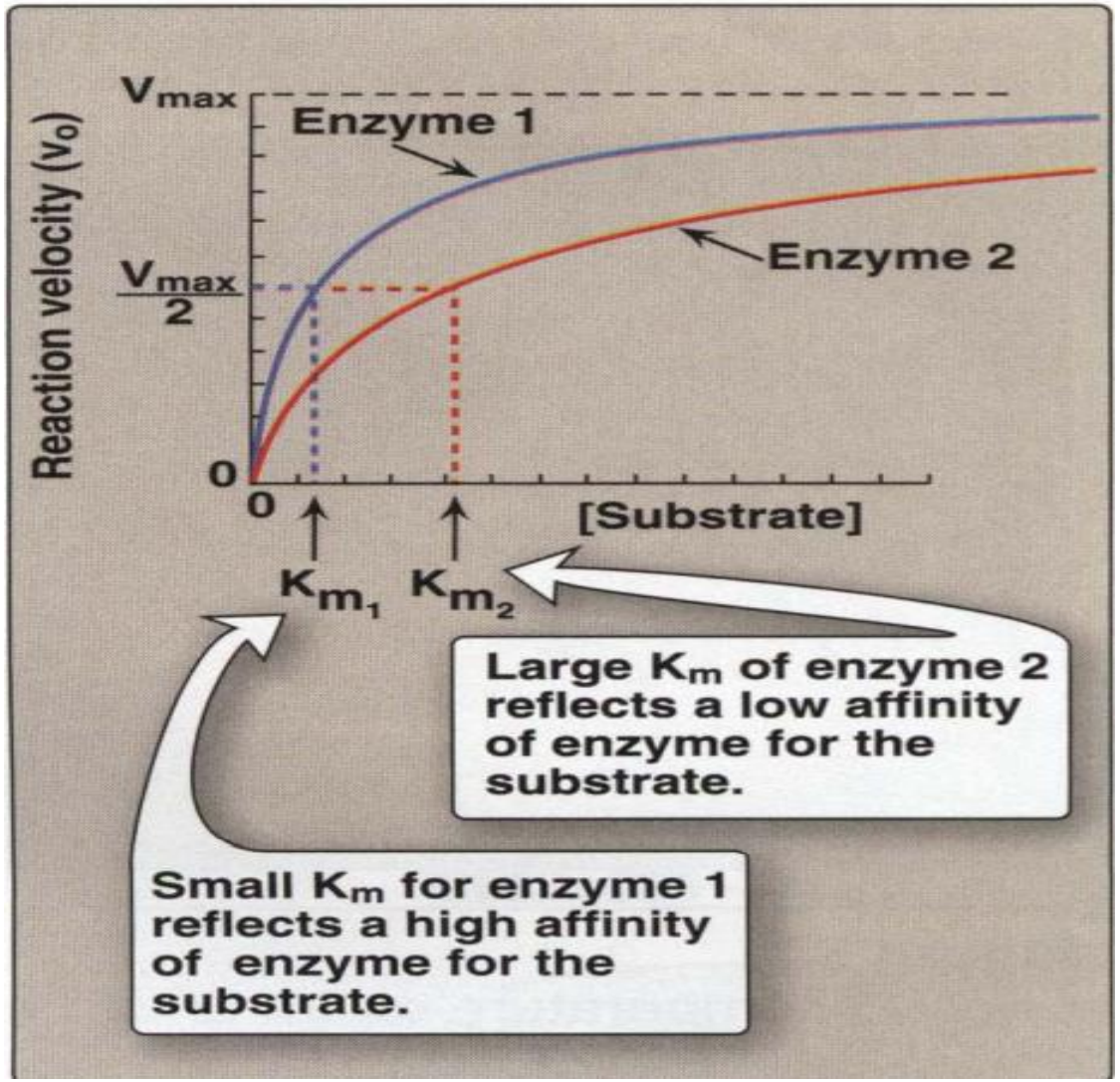


Enzyme affinity to substrate **(Km):** the bigger the Km, the lower the affinity and *vice versa*.

-Km numerically equal to the **concentration substrate at which the reaction velocity is equal to ½ Vmax.**

-Low Km: low [S] is needed to half-saturate the enzyme (to reach ½ Vmax)= **high affinity**

-Large Km: high [S] is needed to half-saturate the enzyme (to reach ½ Vmax)= **low affinity**

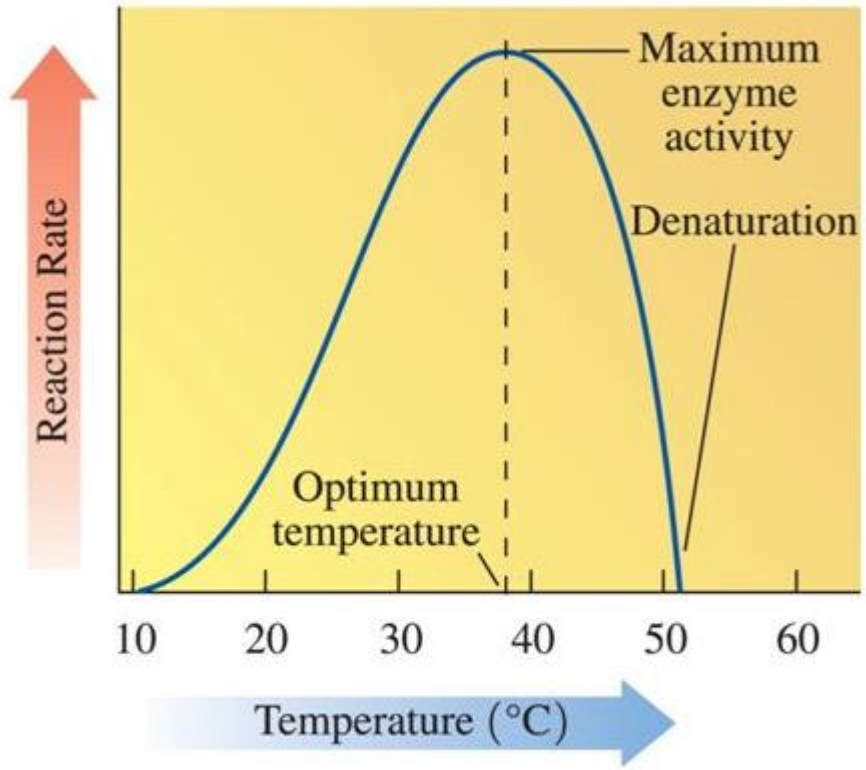


Four main factors affect the reaction rate:

1. **Temperature** changes
2. **pH** changes
3. Changes in **substrate concentration**
4. Changes in **enzyme concentration**

1. Temperature

1. **As the temperature rises**, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases.
2. There is a certain temperature at which an enzyme's catalytic activity is at its greatest. This **optimal temperature** is usually around human body temperature (37.5 °C) for the enzymes in human cells.
3. **Above this temperature** the enzyme structure begins to break down (denature) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.



The optimum temperature for most human enzymes is between 35 and 40°C. Human enzymes start to denature at temperatures above 40°C, but thermophilic bacteria found in the hot springs have optimum temperatures of 70°C.

2. pH

1.Effect of pH on the ionization of the active site:

The enzyme and substrate have specific groups in either an ionized or un-ionized state to interact. For example (-NH_3^+) of the enzyme protonated or deprotonated according pH.

2. Effect of pH on enzyme denaturation: Extremes of pH lead to denaturation of the enzyme, because active site depends on the ionic character of the amino acid side chains.

3. The pH optimum varies for different enzymes:

Different enzymes have different Optimum pH values.

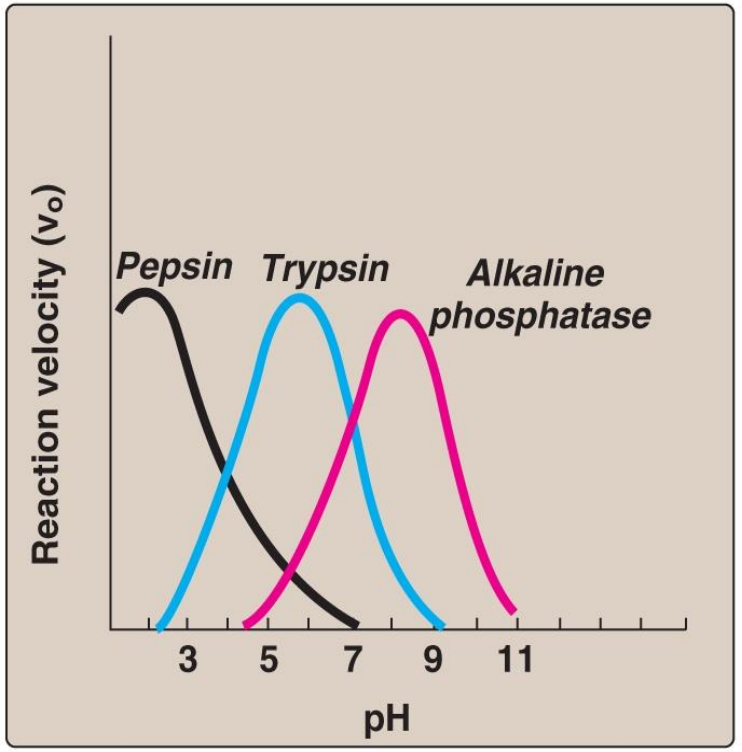


Figure 5.8
Effect of pH on enzyme-catalyzed reactions.

3. Concentration of Substrate

1. For a given enzyme concentration, as the substrate concentration increases, the reaction rate keeps increasing up to a certain point beyond which no further increase in the enzymatic reaction velocity is recognized (**plateau**).
2. Plateau reflects the **saturation** with a substrate of all available binding sites on the enzyme.
3. **Maximum activity** is reached when all of the enzymes combines with the substrate.

- So, the enzyme/substrate complex must dissociate before the active sites are free to accommodate more substrate.

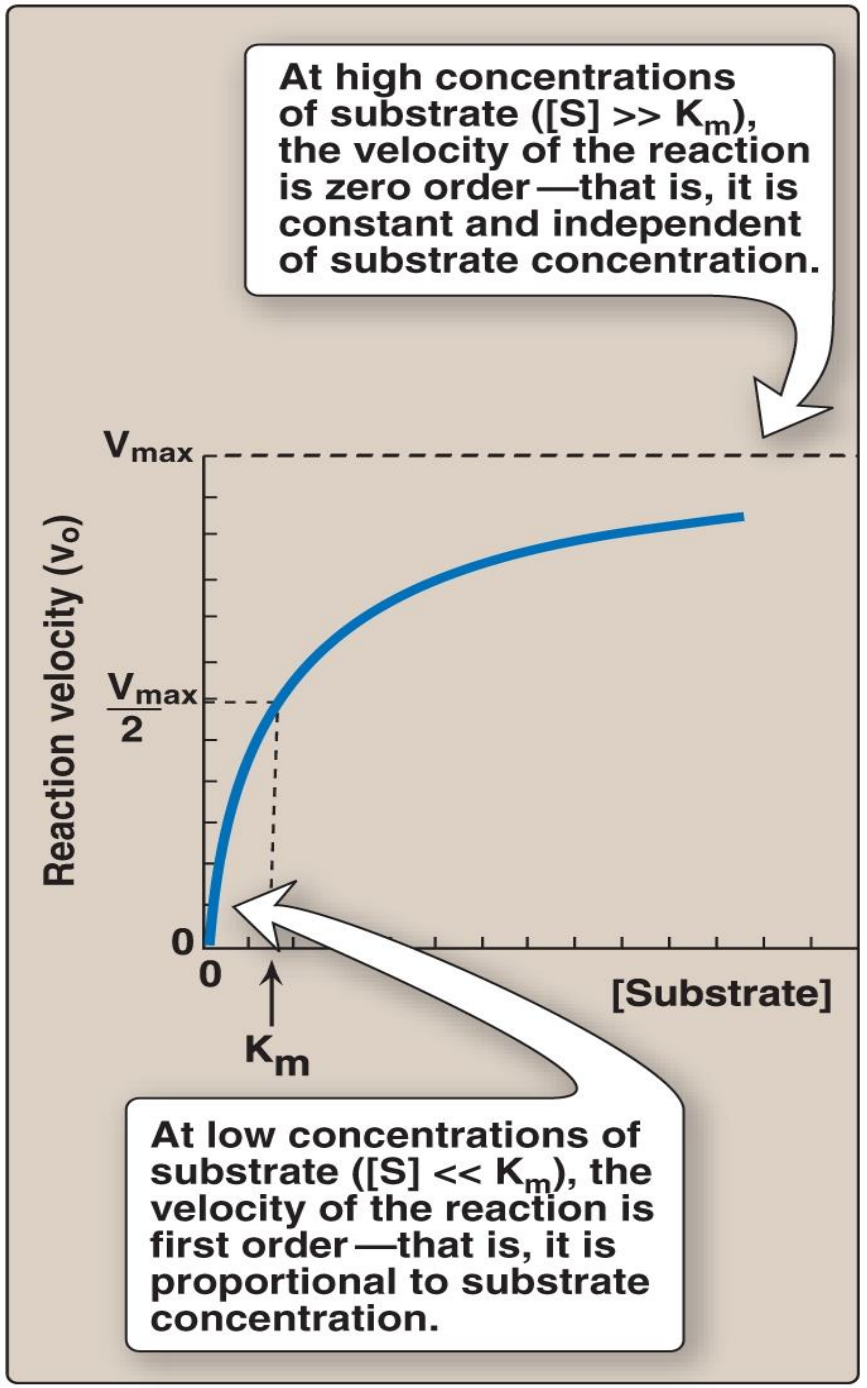
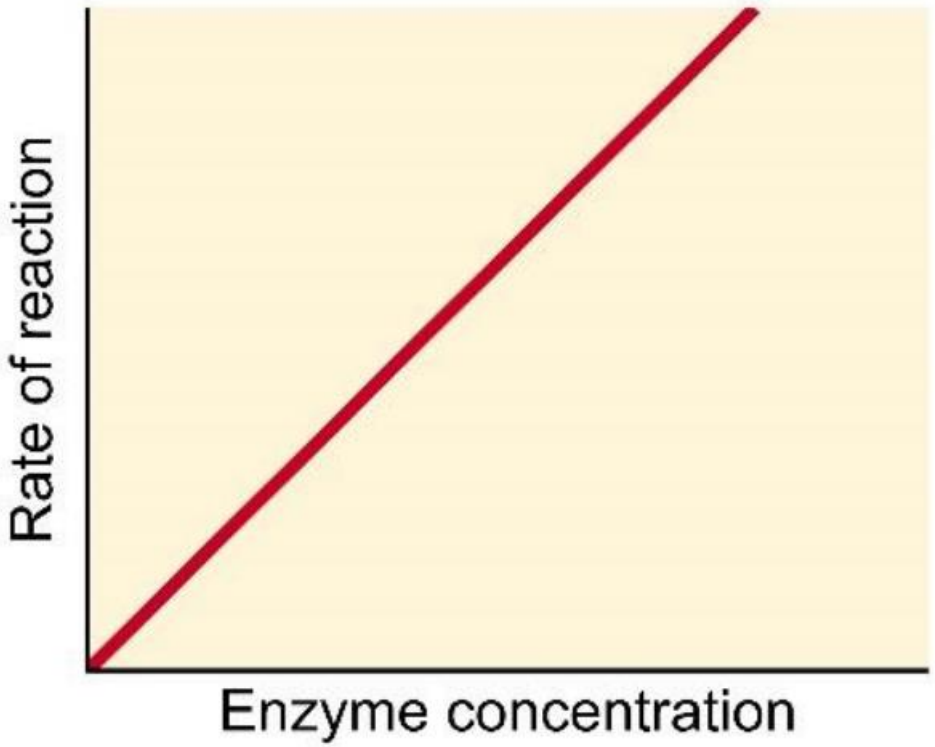


Figure 5.10
Effect of substrate concentration on reaction velocity for an enzyme-catalyzed reaction.

4. Concentration of Enzyme

Provided that the substrate concentration is high, and that temperature and pH are kept constant, the rate of reaction **is proportional to the enzyme concentration.**



Regulation of enzymes

Enzymes can be regulated so the chemical reactions they catalyze can adapt to different physiological needs.

There are three modes of regulation:

1. **Allosteric regulation**
2. **Covalent modification**
3. **Induction / Repression**

Note that the first two types of regulation affect enzyme activity, whereas the third type affects enzyme concentration.

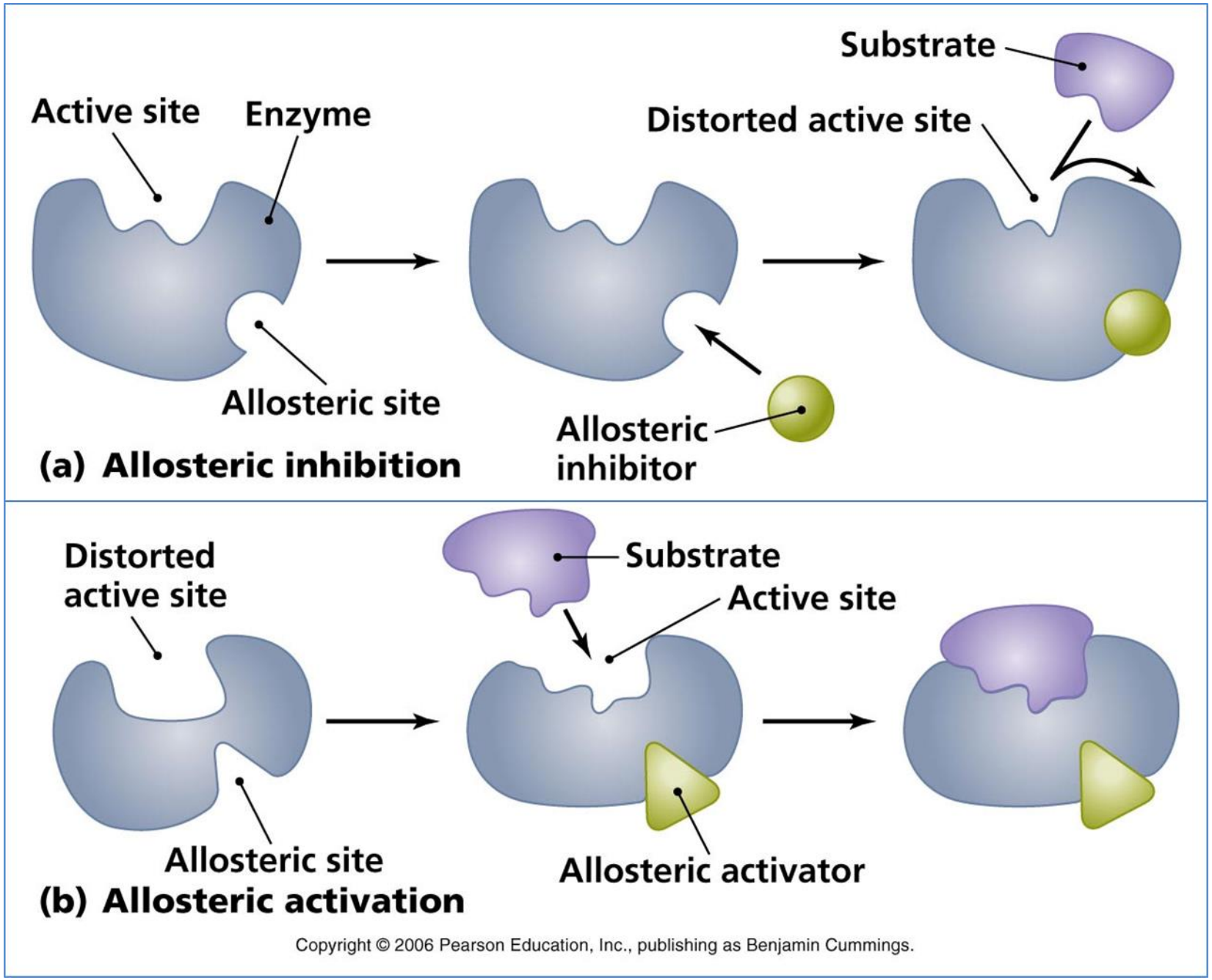
1. **Allosteric regulation**: this type of regulation is for *allosteric* enzymes only.

- Allosteric enzymes have an allosteric site where certain molecules bind and affect enzyme activity.
- Molecules that bind to these sites are called "**effectors**“
- Effectors can modulate enzyme activity either by activating the enzyme (positive effectors), or inhibiting the enzyme (negative effectors)
- Allosteric enzymes are usually **rate-limiting enzymes** in metabolic pathways, and undergo negative feedback inhibition.

Allosteric control

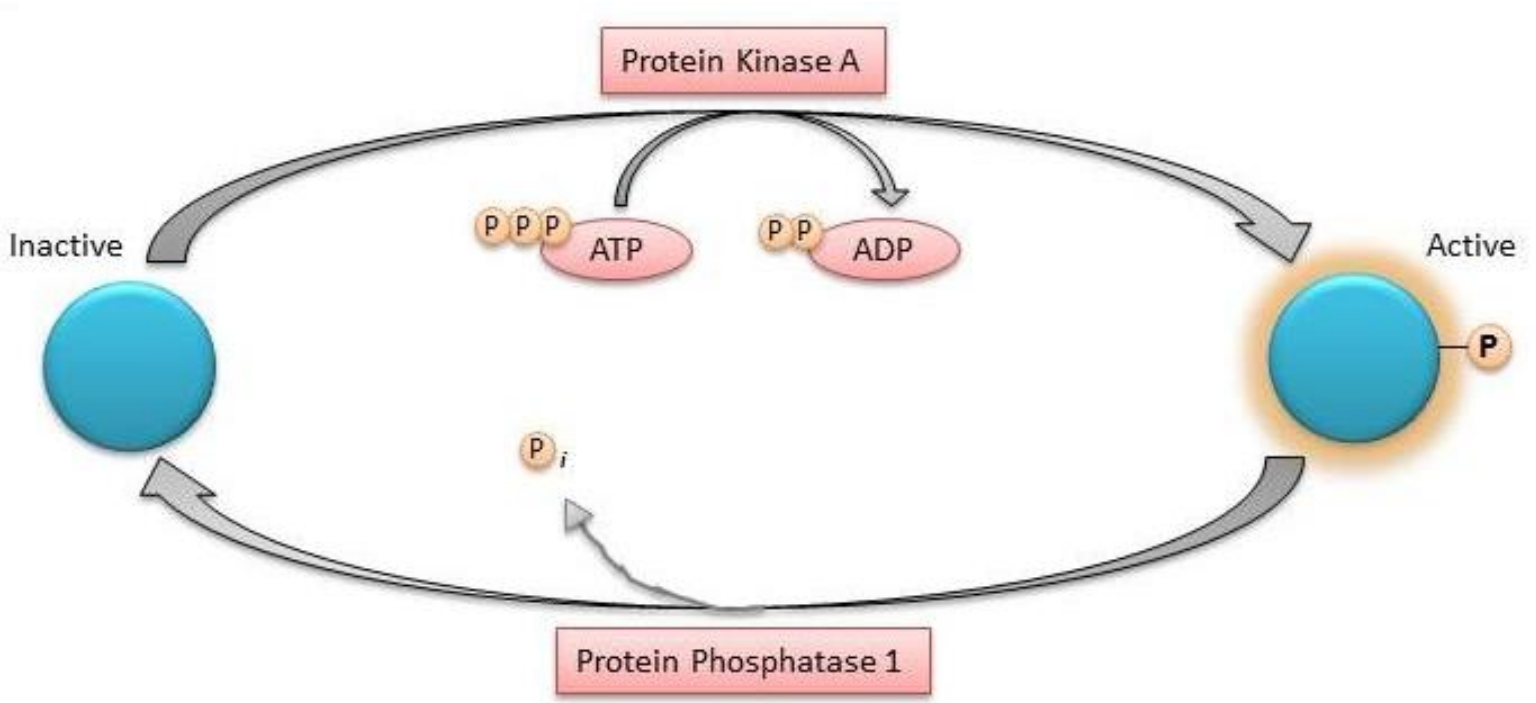
Negative allosteric regulation

Positive allosteric regulation



2. Covalent modification

- Certain molecules bind *covalently* to the enzyme changing its activity.
- The most important example of this type of regulation is "enzymatic phosphorylation".
- Some enzymes are active in the phosphorylated form whereas other enzymes are active in the dephosphorylated forms.
- Enzymes that catalyze phosphorylation are kinases, whereas enzymes that catalyze dephosphorylation are "phosphatases".

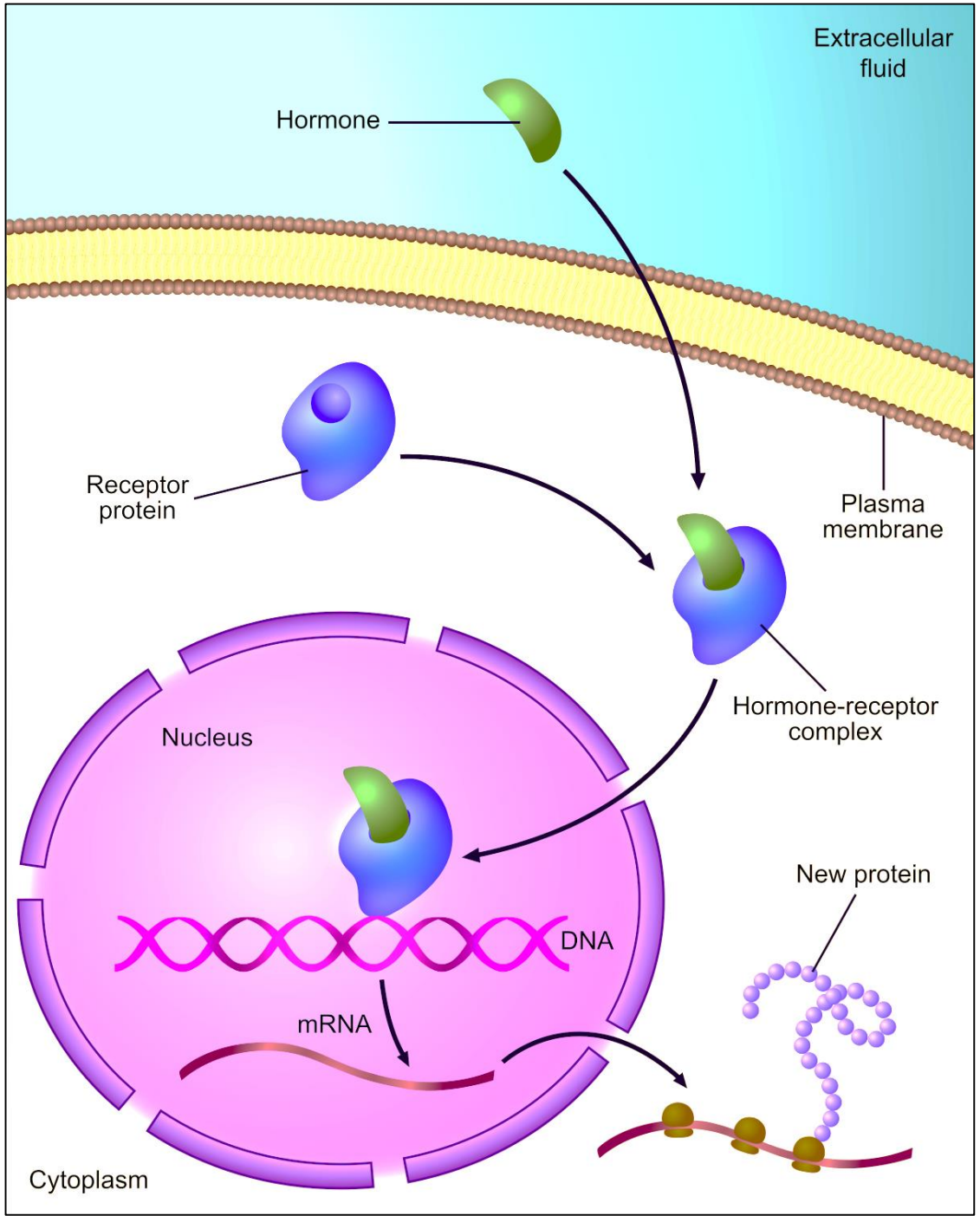


Regulation of Phosphorylation by Protein Kinase A and Protein Phosphatase 1

3.Induction / Repression

in this type of regulation the **synthesis of the enzyme** is either increased (induced) or decreased (repressed) according to physiological needs.

Metabolic enzymes are usually under this type of regulation that is mediated by **hormones** (e.g. insulin regulates synthesis of glycolytic enzymes).



Feedback inhibition

A common type of control occurs when an enzyme present early in a biochemical pathway is inhibited by a late product of pathway

This is known as feedback inhibition or negative feedback regulation

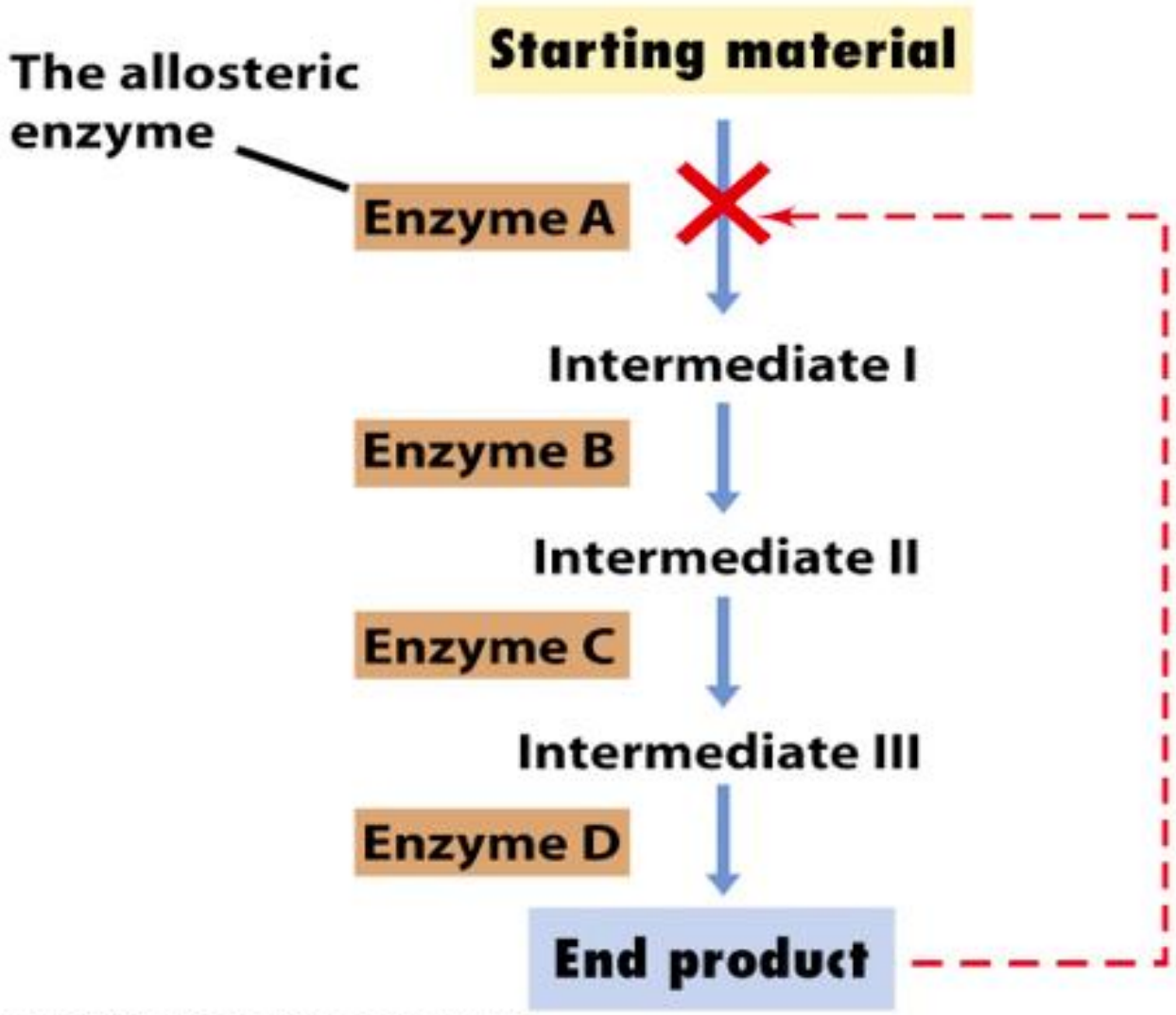


Figure 8-2 Brock Biology of Microorganisms 11/e

Inhibition of enzymes

Some substances reduce or even stop the catalytic activity of enzymes in biochemical reactions. They block or distort the active site **(temporary or permanent)**. These chemicals are called **inhibitors**, because they inhibit the reaction.

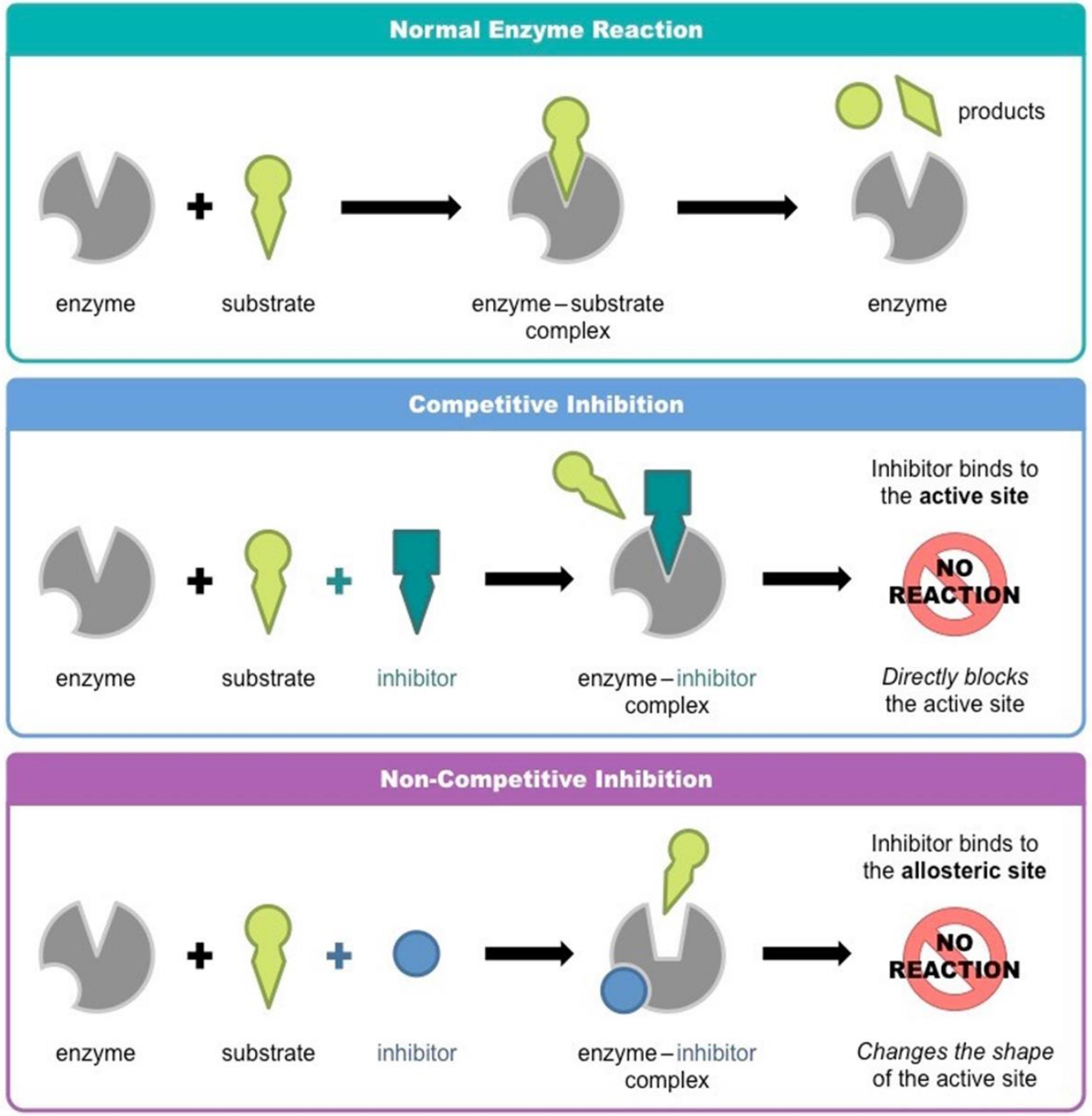
Three main types of enzyme inhibition:

1. **Competitive inhibition**
2. **Noncompetitive inhibition**
3. **Irreversible inhibition**

Reversible enzymes inhibition

- **Competitive:** Inhibitors that ***occupy the active site*** and prevent a substrate molecule from binding to the enzyme are said to be active site-directed (or **competitive**, as they 'compete' with the substrate for the active site).

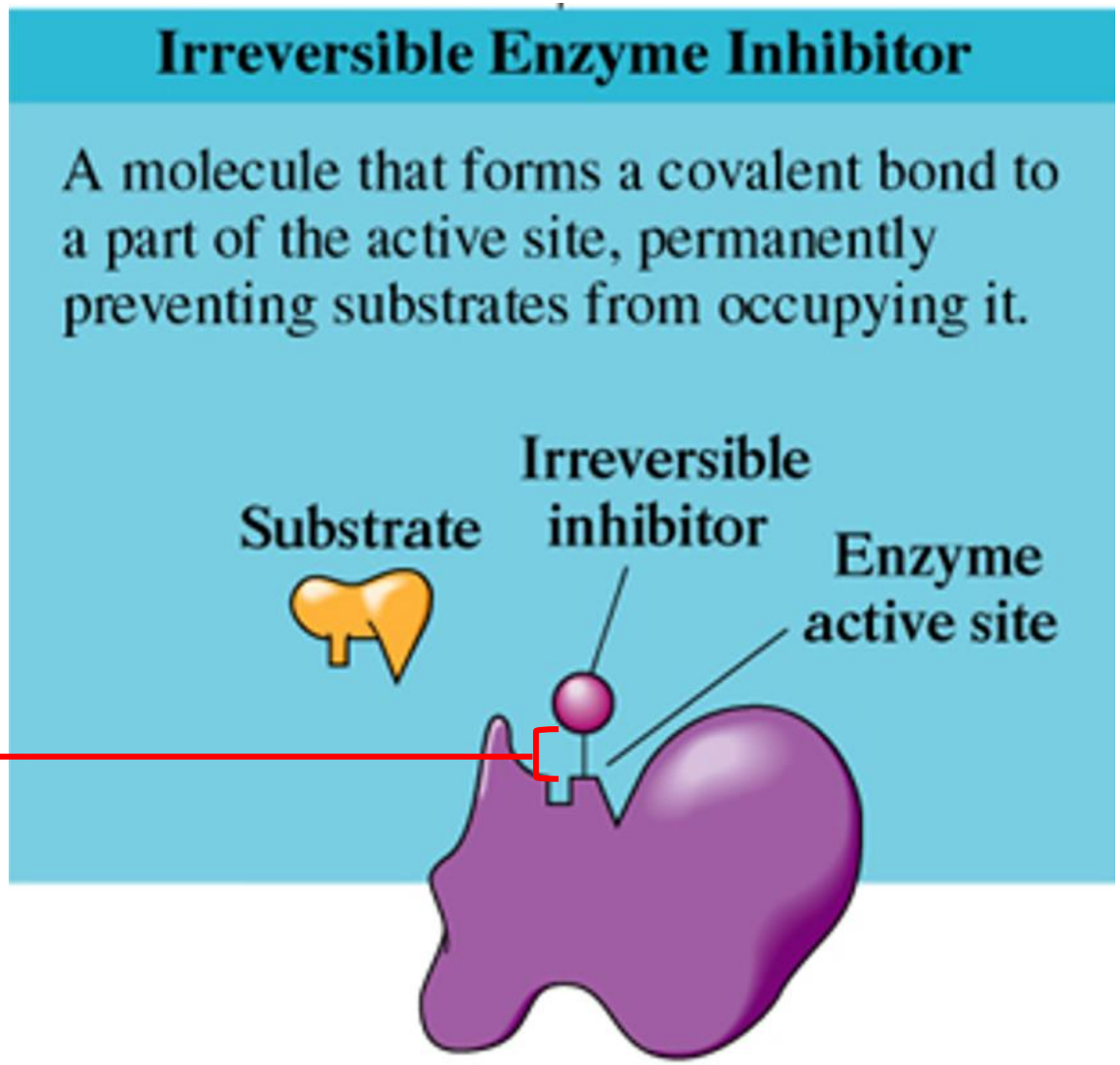
- **Non- competitive:** Inhibitors that **attach to *other* parts of the enzyme** molecule, perhaps distorting its shape, are said to be non-active site-directed (or **non-competitive**).



Irreversible inhibitor

An irreversible inhibitor forms a stable complex with the enzyme. As a result, the enzyme is **permanently** inactivated or, at best, is slowly reactivated (requiring hours or days for reversal).

Usually, the irreversible inhibitor forms a **covalent bond** with the enzyme.



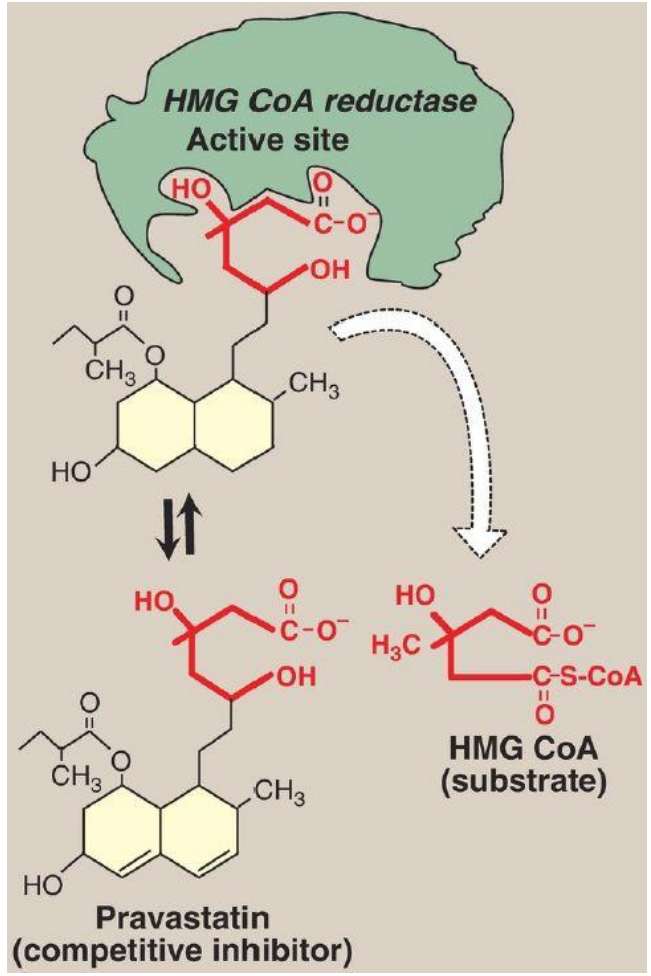
Compounds acting as enzyme inhibitors

Some Drugs as reversible inhibitors:

- Statins: anti-hyperlipidemic drugs that help in lowering cholesterol level in the blood.
- ACE (Angiotensin Converting Enzyme) inhibitors: anti-hypertensive drugs that help in lowering blood pressure in patients with hypertension.
- β- lactam antibiotics (such as penicillin and amoxicillin): this class of antibiotics inhibit certain enzymes involved in bacterial cell wall synthesis.

Toxins as irreversible inhibitors

- Heavy metals (e.g. lead "Pb"): inhibit ferrochelatase (an enzyme involved in heme synthesis) → Hemolysis & anemia.
- Cyanide (CN^-): cyanide inhibits cytochrome c oxidase enzyme; a key enzyme in the electron transport chain (ETS) → interferes with respiration.
- Insecticides: inhibit acetylcholinesterase (the enzyme that breaks down the neurotransmitter acetylcholine (Ach) → Neurotoxicity.



Pravastin (a statin) competes with the substrate of HMG CoA reductase for the enzyme binding site, inhibiting its activity.

Clinical correlations

Disease	Defective Enzyme or System	Symptoms	Treatment
Phenylketonuria (PKU)	phenylalanine hydroxylase	severe mental retardation	screening; dietary modification
Maple Syrup Urine disease	branched-chain ketoacid dehydrogenase complex	elevations of branched-chain amino acids, characteristic odor of the urine, episodes of ketoacidosis, death	thiamine; careful regulation of dietary intake of the essential branched-chain amino acids
Disorders of Fatty Acid Metabolism			
Familial Hyperlipoproteinemia (Type Ia & V)	Lipoprotein lipase (LPL)	cardiovascular disease	Type I: Diet control. Type V: Niacin, Fibrate.
Disorders of CHO Metabolism			
G6PD* deficiency	glucose-6-phosphate dehydrogenase (G6PD)	Blood hemolysis leading to fatigue, pallor, tachycardia, splenomegaly…	Avoidance of triggers (foods, drugs, chemicals…). Transfusion in severe cases.
Galactosemia	galactose-1-phosphate uridyl transferase	liver failure in infancy	newborn screening; milk avoidance

*Glucose-6-phosphate dehydrogenase (G6PD) is a hormone involved in a CHO metabolic pathway called Pentose Phosphate pathway (PPP). G6PD deficiency is a condition in which red blood cells break down (undergo hemolysis) when the body is exposed to certain foods, drugs or the stress of infection. It is a hereditary disease (X-linked recessive inborn error of metabolism).