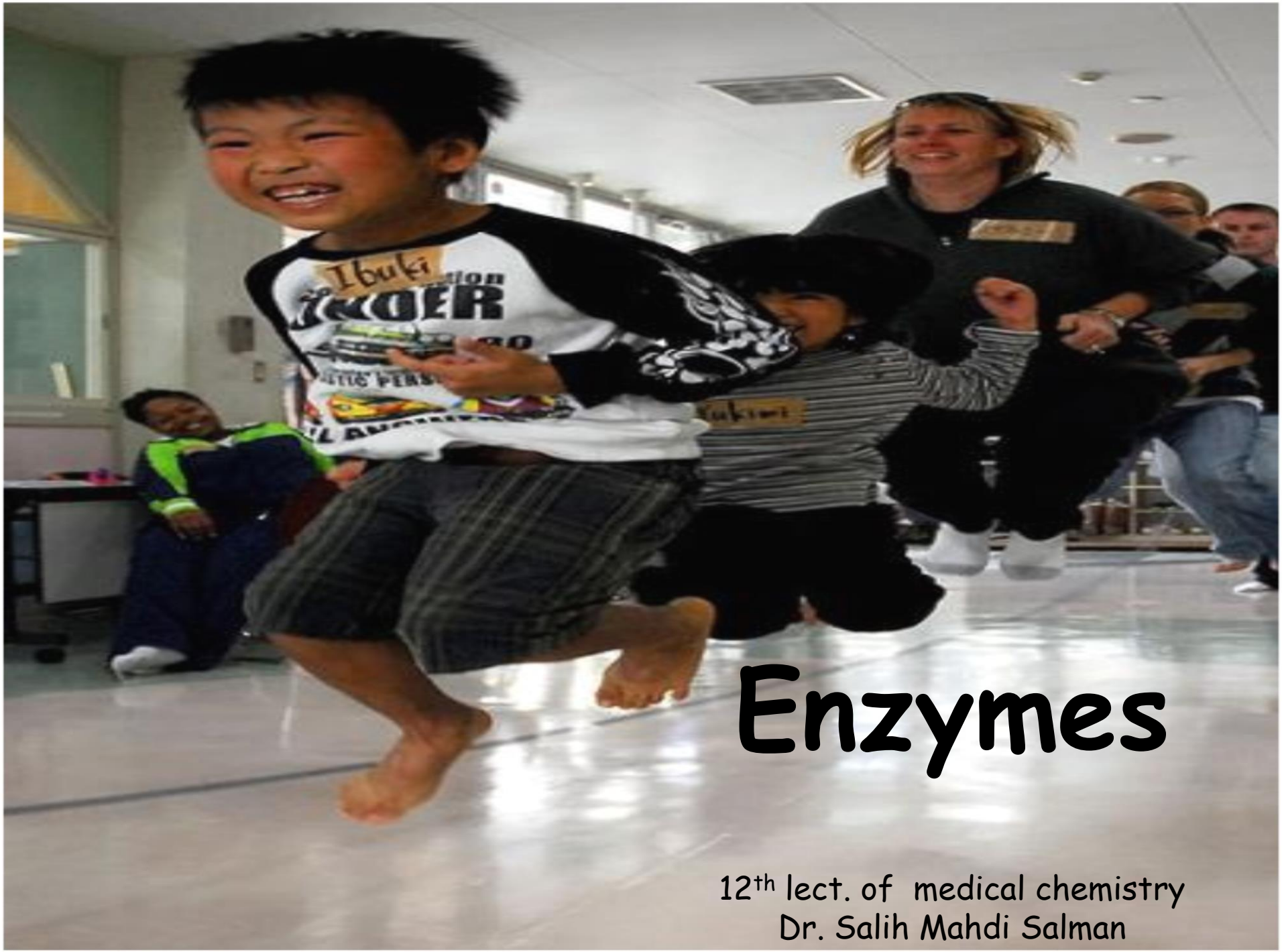
Enzymes

12th lect. of medical chemistry

Dr. Salih Mahdi Salman



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Introduction

Enzymes are biological catalysts which speed up the rate of a chemical reaction without being itself changed in the process."

With the exception of some Ribozymes that catalyze their own splicing, all enzymes are proteins.

Enzymes can increase the rate of a reaction by a factor of up to 10^{20} over an uncatalyzed reaction.

Naming Enzymes

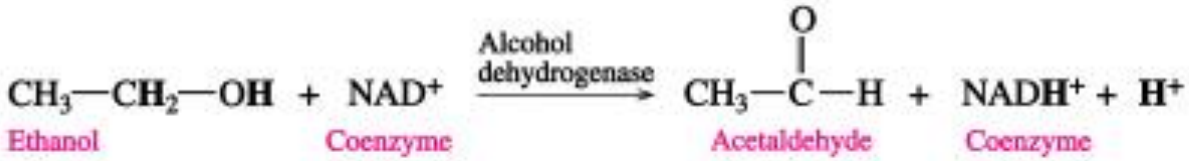
Enzymes names can be formed in Four ways:-

1. **Depend of the reacting substance (substract)** usually ends in *-ase add to the substract*
   Ex: *sucrase* catalyzes the hydrolysis of sucrose
2. **Depend on the function (reaction) of the enzyme**
   Ex: *oxidases* catalyze oxidation reactions.
3. **Depend on both the substrate and the function**
   Ex: *alcohol dehydrogenase* oxides ethanol
4. **Sometimes common names are used**, particularly for the digestion enzymes such as *pepsin* and *trypsin*

Classification of Enzymes

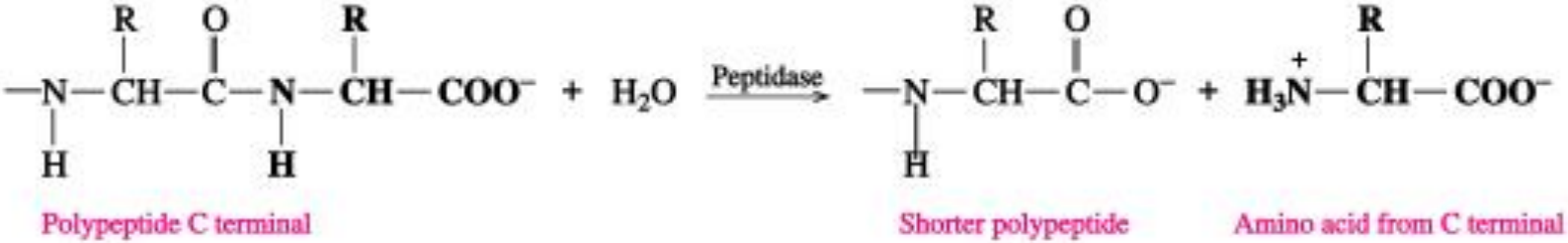
Enzymes are classified according to the type of reaction they catalyze:

Class	General Reactions Catalyzed	Typical Subclasses	Function
1. Oxidoreductases	Oxidation–reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Remove 2H to form double bonds

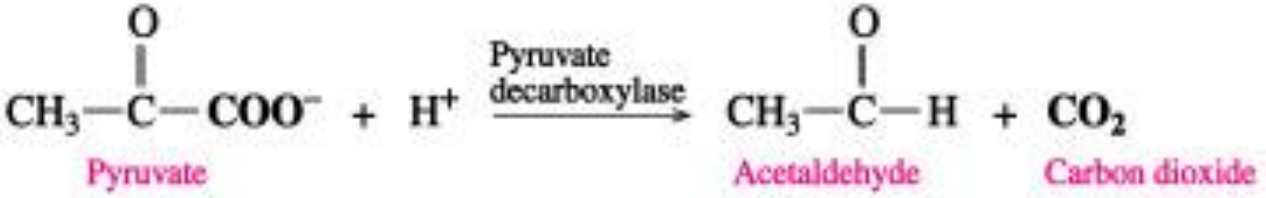


2. Transferases	Transfer of functional groups	Transaminases Kinases	Transfer amino groups Transfer phosphate groups

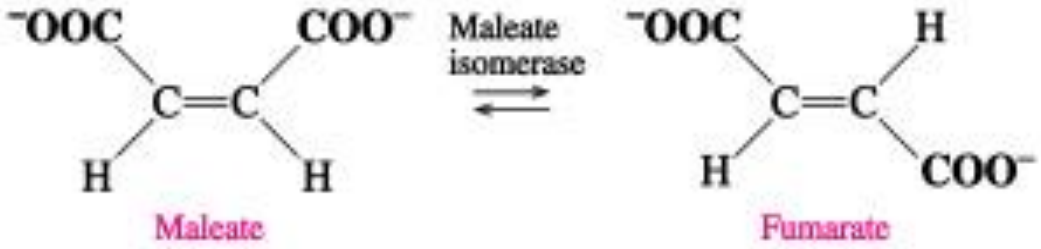
3. Hydrolases	Hydrolysis reactions	Peptidases Lipases Amylases	Hydrolyze peptide bonds Hydrolyze ester bonds in lipids Hydrolyze 1,4-glycosidic bonds in amylose



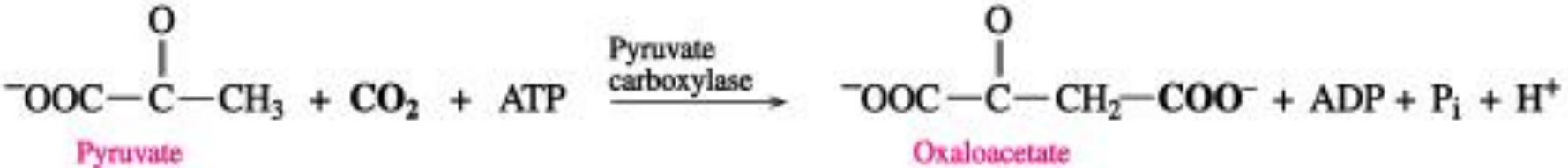
Class	General Reactions Catalyzed	Typical Subclasses	Function
4. Lyases	Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation	Decarboxylases Dehydrases Deaminases	Remove CO_2 Remove H_2O Remove NH_3



5. Isomerases	Rearrangement of atoms to form isomers	Isomerases Epimerases	Convert cis and trans Convert D and L isomers



6. Ligases	Bonding of molecules using ATP energy	Synthetases Carboxylases	Combine molecules Add CO_2



Types of enzymes

1. Endoenzymes

Enzymes that function within the cells. Most of the enzymes are these types. Eg. metabolic enzymes (cytochrome oxidase).

2. Exoenzymes

Enzymes that are liberated by cells and catalyse reactions outside the cell. Eg. digestive enzymes (amylase, lipase, protease)

Chemical composition of enzymes

1. **Simple enzymes** – only protein structure
2. **Complex enzymes** = protein structure + cofactor

Cofactors are nonprotein compounds. Cofactor can be:

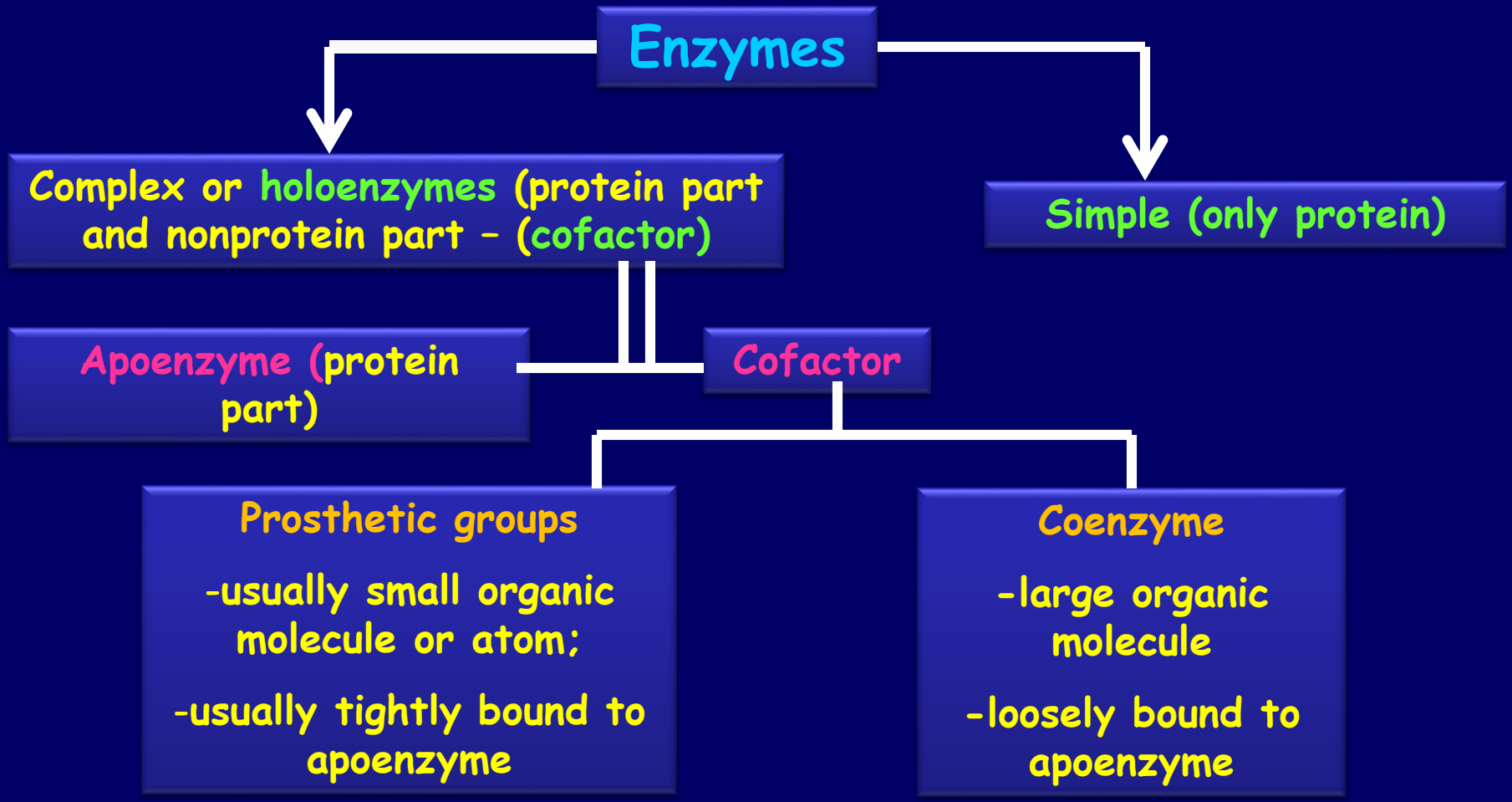
1) **inorganic element**: Zn^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+},

2) **organic molecule**

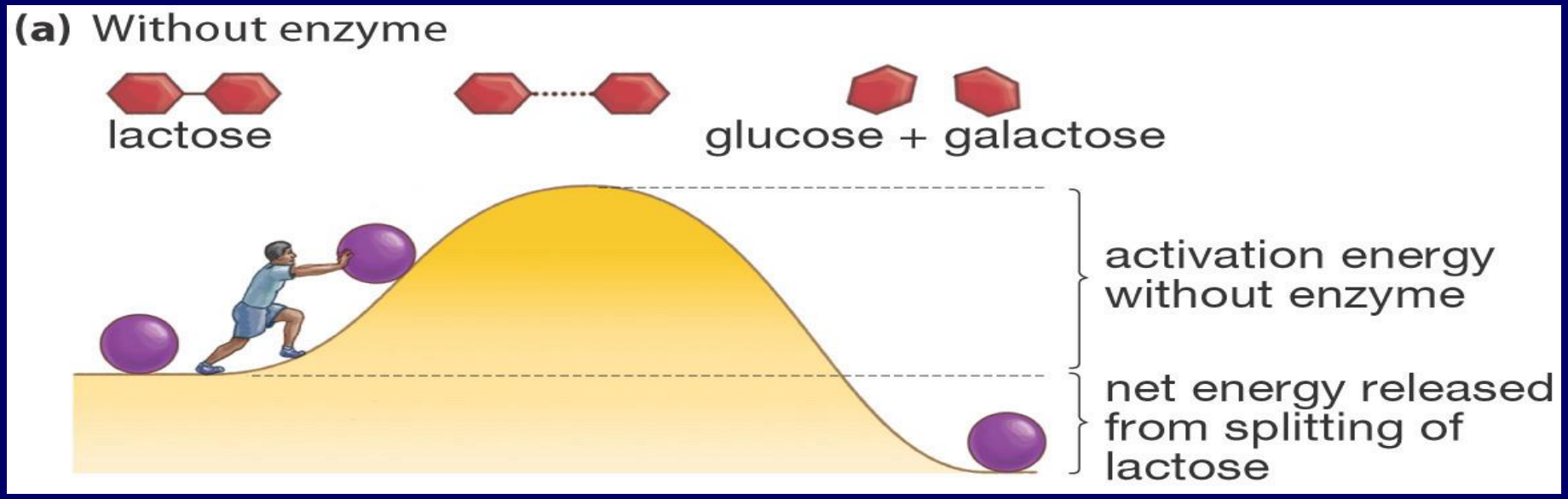
a) **coenzymes** are small non-proteins **slightly** bound to the enzyme, undergo a chemical change and are released: Coenzyme A (acyl transfer),Flavins (redox reaction) NAD^+ ($NADP^+$)(redox reactions), Vitamins: derivatives of B vitamins (B1, B2, B6, B12), niacin, folic acid, riboflavin

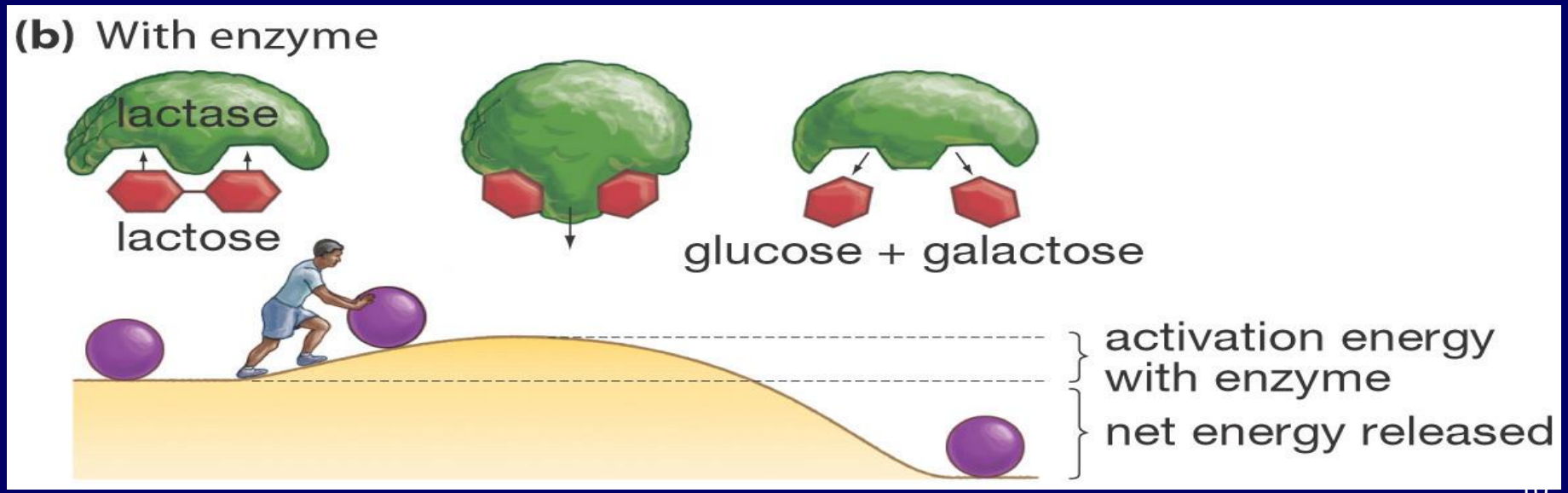
b) **prosthetic groups** are large complex **tightly** bound to the enzyme and remain associated with enzyme during reaction: heme, …

Structure of enzymes



Mechanism of enzymes work

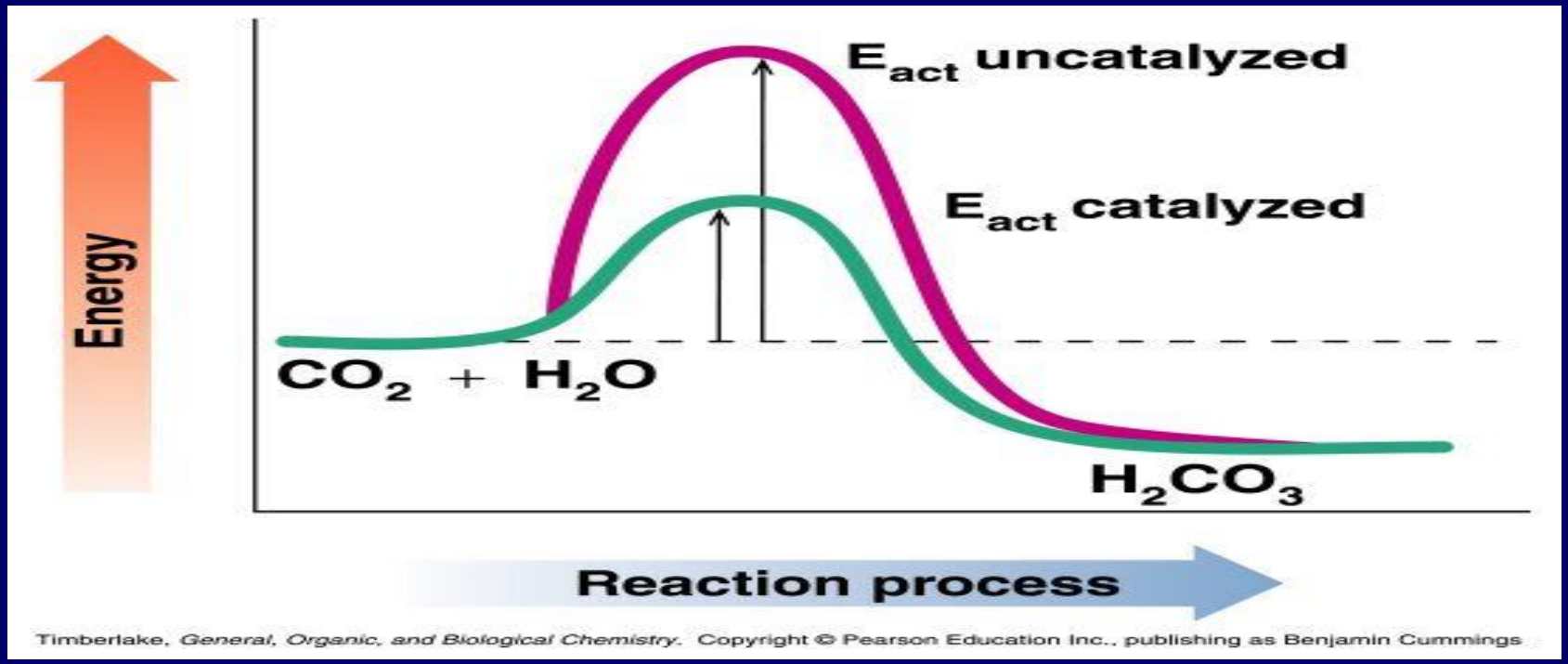




All chemical reactions have an energy barrier, called the **activation energy**

Activation energy: amount of energy needed to disrupt stable molecule so that reaction can take place.

Enzymes are proteins that increase the rate of reaction by lowering the energy of activation



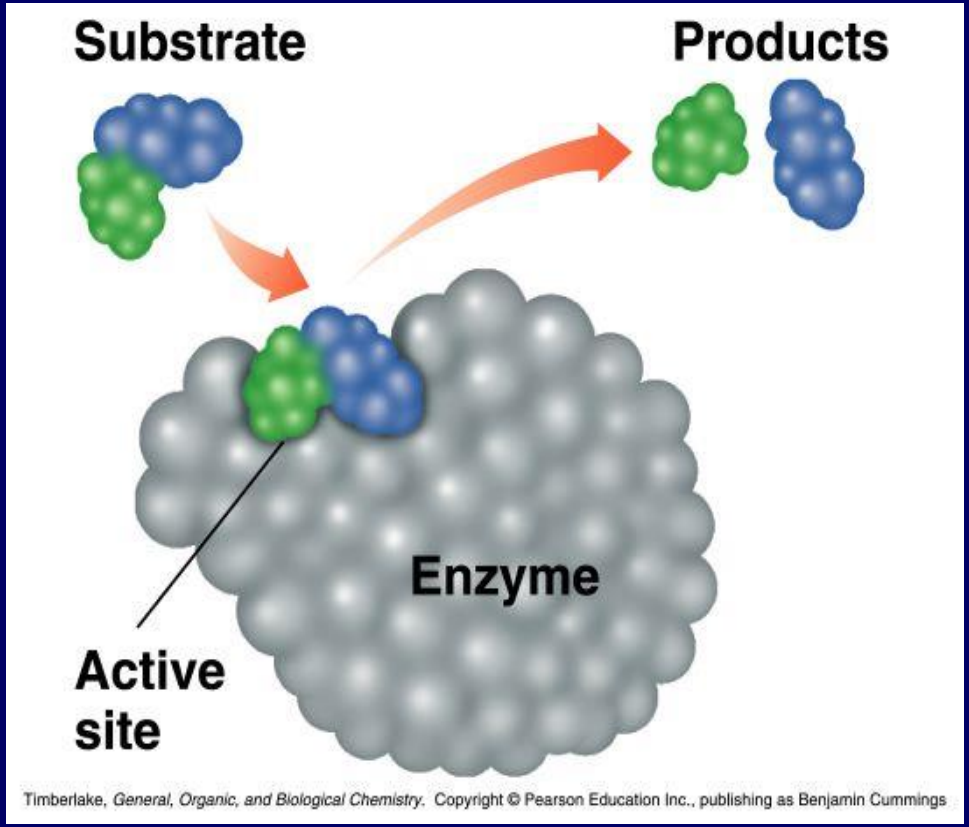
An enzyme is a biological catalyst.
The pockets formed by tertiary and quaternary structure can hold specific substances (SUBSTRATES).
These pockets are called ACTIVE SITES.
When all the proper substrates are nestled in a particular enzyme's active sites, the enzyme can cause them to react quickly.
Once the reaction is complete, the enzyme releases the finished products and goes back to work on more substrate.

Active Site of an Enzyme

✓The active site is a region within an enzyme that fits the shape of substrate molecules.

✓Amino acid side-chains align to bind the substrate through H-bonding, salt-bridges, hydrophobic interactions, etc.

✓Products are released when the reaction is complete (they no longer fit well in the active site)



Enzyme Catalyzed Reactions

When a substrate (S) fits properly in an active site, an **enzyme-substrate (ES) complex** is formed:

$$E + S \leftrightarrows ES$$

Within the active site of the ES complex, the reaction occurs to convert substrate to product (P):

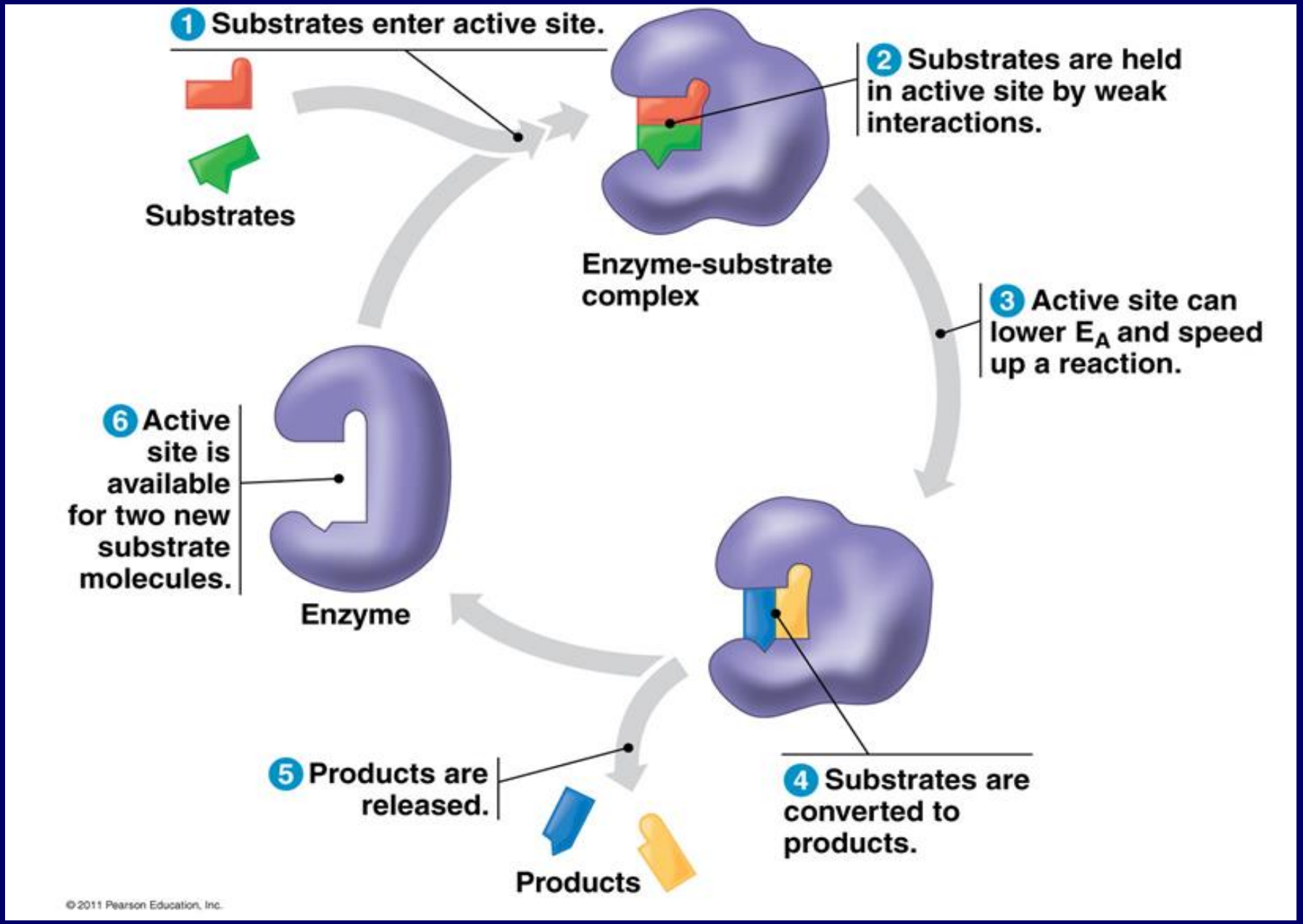
$$ES \rightarrow E + P$$

The products are then released, allowing another substrate molecule to bind the enzyme

- this cycle can be repeated millions (or even more) times per minute

The overall reaction for the conversion of substrate to product can be written as follows:

$$E + S \leftrightarrows ES \rightarrow E + P$$


1 Substrates enter active site.
Substrates
2 Substrates are held in active site by weak interactions.
Enzyme-substrate complex
3 Active site can lower E_A and speed up a reaction.
4 Substrates are converted to products.
5 Products are released.
Products
6 Active site is available for two new substrate molecules.
Enzyme
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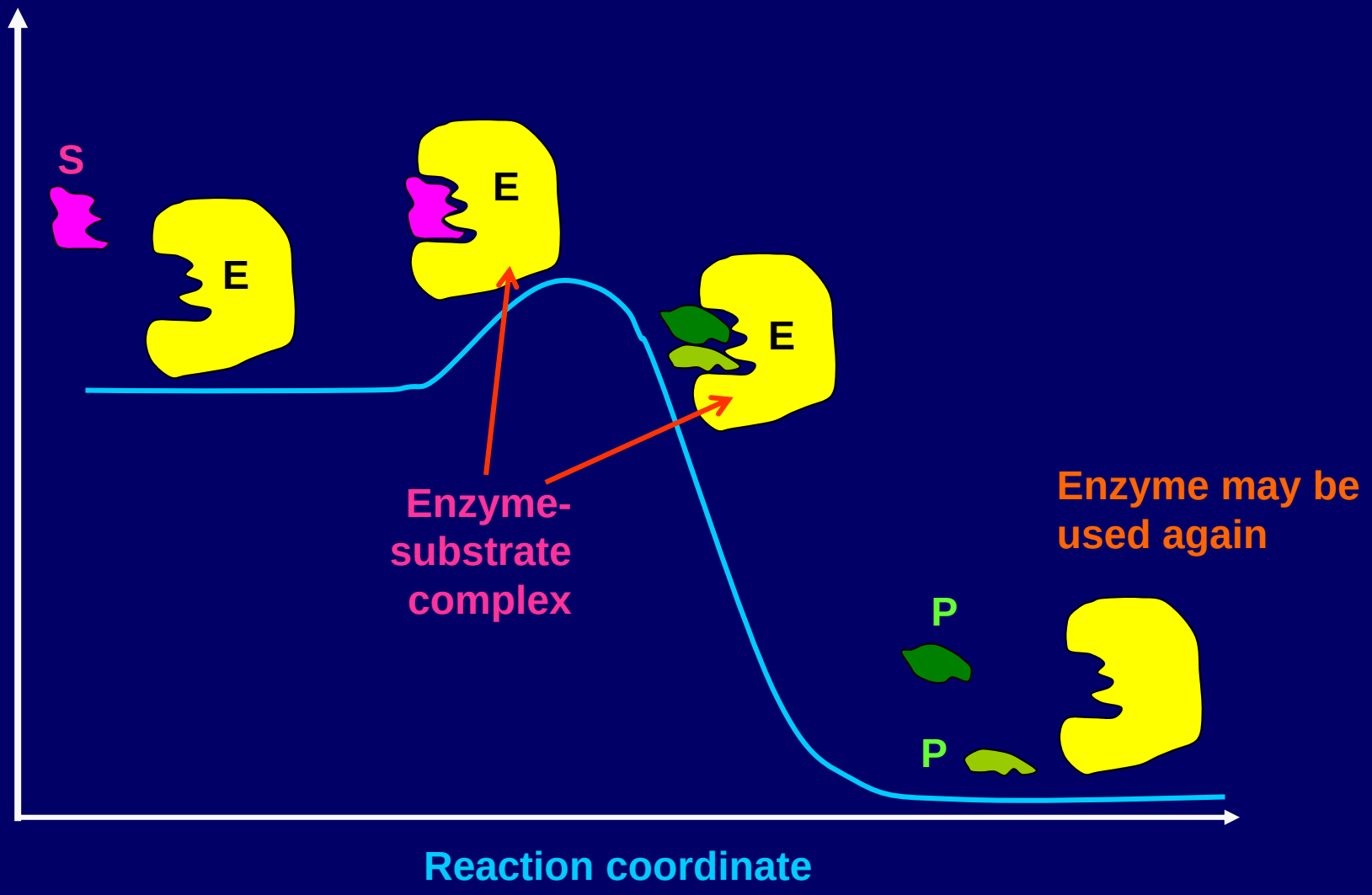
Theories of active site-substrate interaction

1. Fischer theory (lock and key model)

Fit between the substrate and the active site of the enzyme is exact.
Like a key fits into a lock very precisely.
The key is analogous to the enzyme and the substrate analogous to the lock.
Temporary structure called the enzyme-substrate complex formed
Products have a different shape from the substrate
Once formed, they are released from the active site
Leaving it free to become attached to another substrate


S
E
E
E
Enzyme-
substrate
complex
Enzyme may be
used again
P
P
Reaction coordinate

2. The Induced Fit Hypothesis

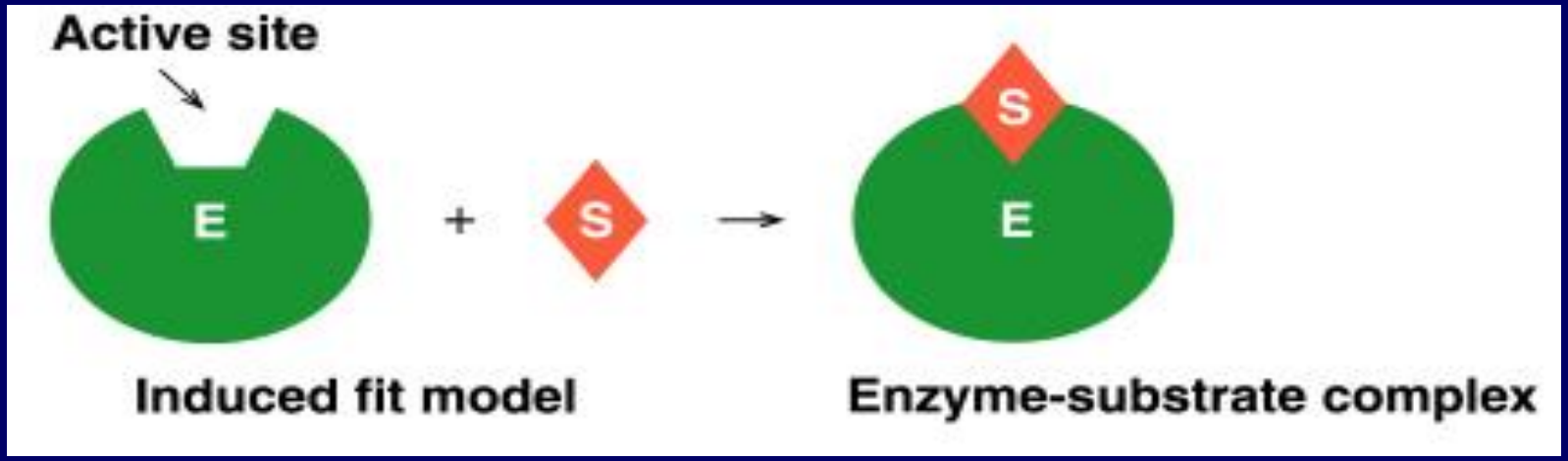
Some proteins can change their shape (conformation)

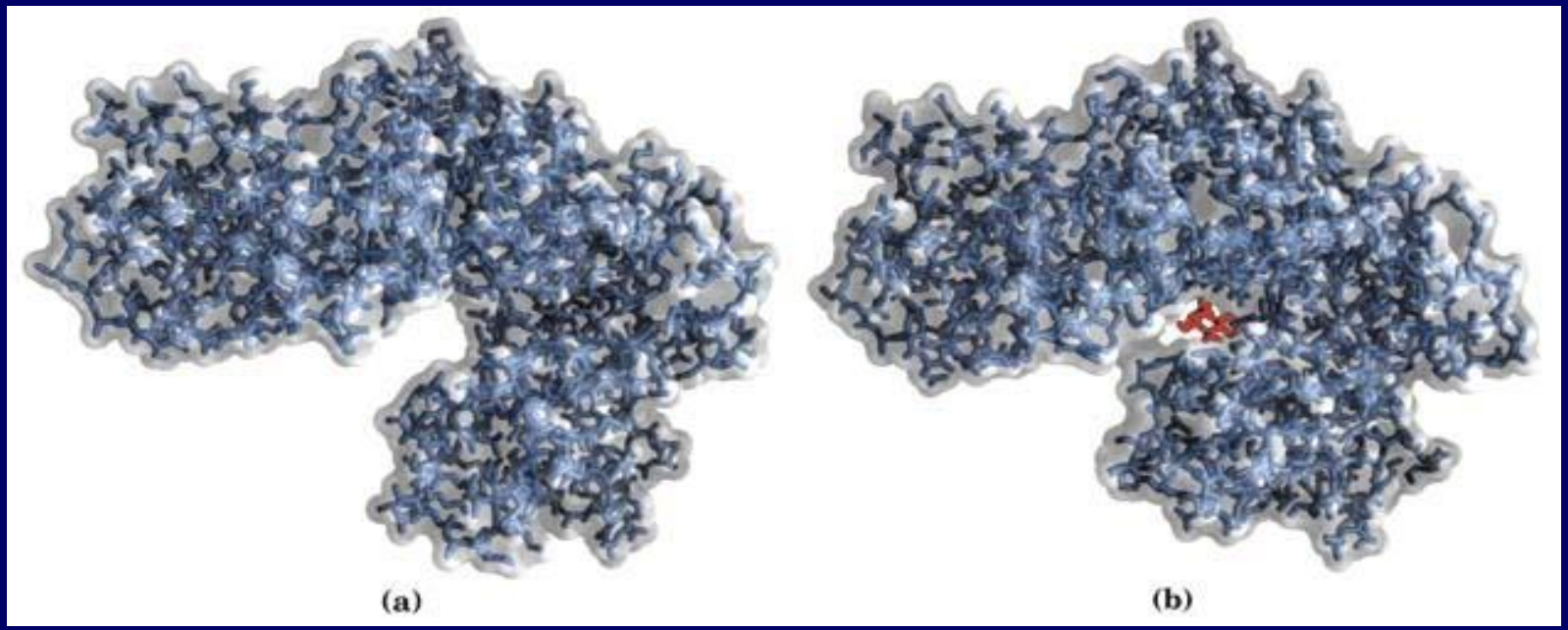
When a substrate combines with an enzyme, it induces a change in the enzyme's conformation

The active site is then moulded into a precise conformation.

Making the chemical environment suitable for the reaction

The bonds of the substrate are stretched to make the reaction easier (lowers activation energy)





Hexokinase (a) without (b) with glucose substrate

http://www.biochem.arizona.edu/classes/bioc462/462a/NOTES/ENZYMES/enzyme_mechanism.html

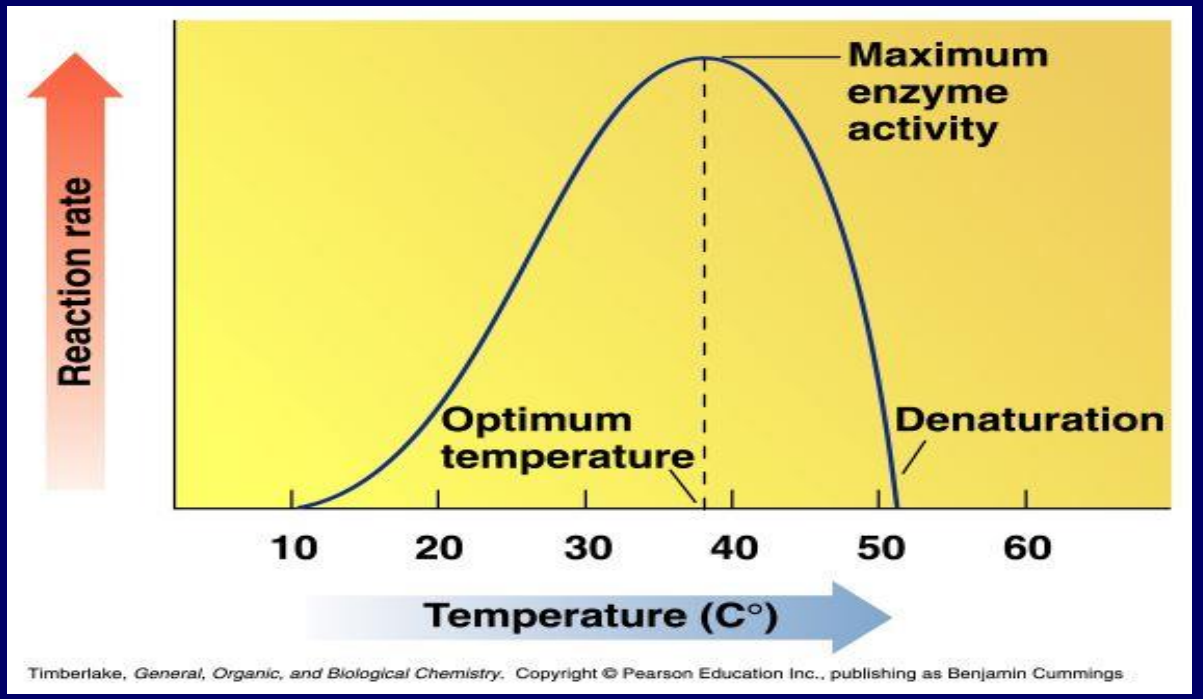
Factor affect Enzymes

1.Temperature

Enzymes are most active at an optimum temperature (usually 37°C in humans).

They show little activity at low temperatures.

Activity is lost at high temperatures as denaturation occurs.

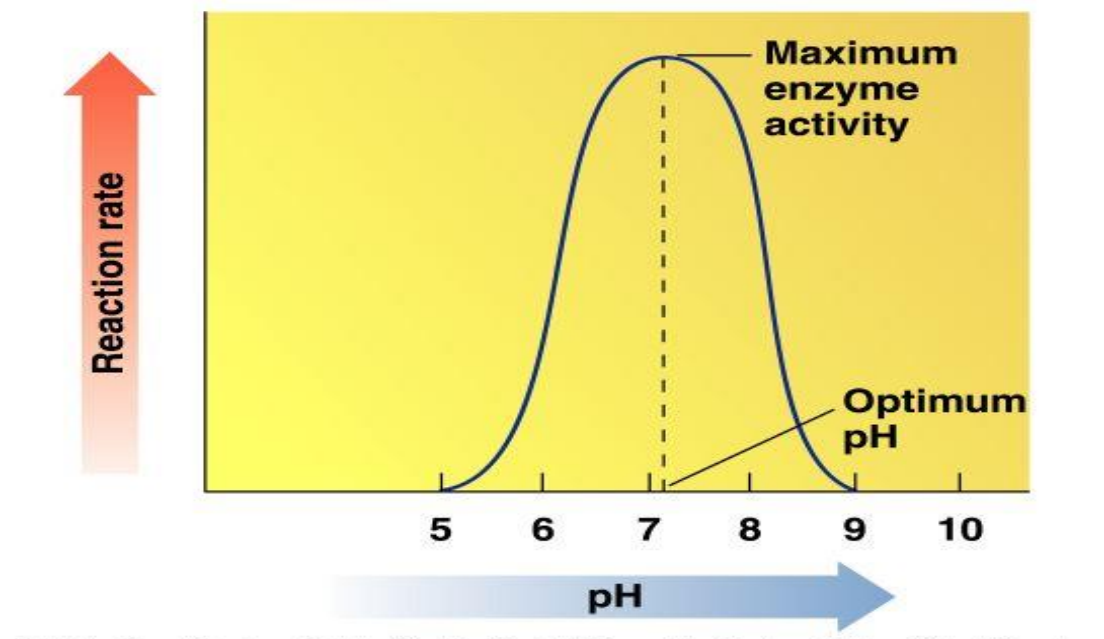


2. pH

Enzymes are most active at optimum pH.

Amino acids with acidic or basic side-chains have the proper charges when the pH is optimum.

Activity is lost at low or high pH as tertiary structure is disrupted.



Optimum pH for Selected Enzymes

Most enzymes of the body have an optimum pH of about 7.4

However, in certain organs, enzymes operate at lower and higher optimum pH values

Optimum pH for Selected Enzymes

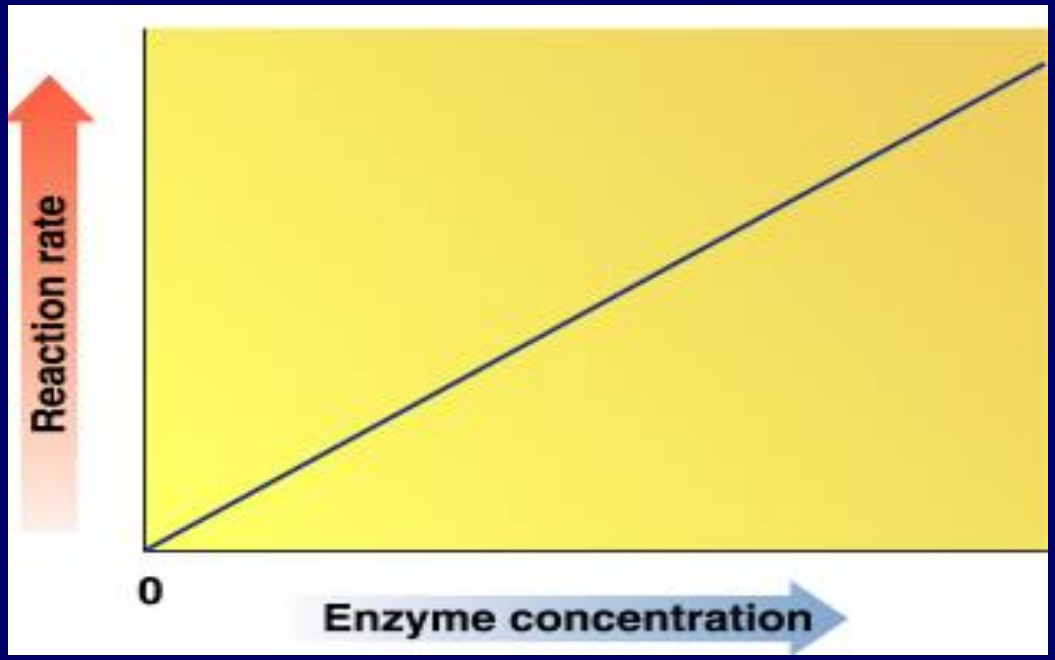
Enzyme	Location	Substrate	Optimum pH
Pepsin	Stomach	Peptide bonds	2
Urease	Liver	Urea	5
Sucrase	Small intestine	Sucrose	6.2
Pancreatic amylase	Pancreas	Amylose	7
Trypsin	Small intestine	Peptide bonds	8
Arginase	Liver	Arginine	9.7

3. Enzyme Concentration

The rate of reaction increases as enzyme concentration increases (at constant substrate concentration)

At higher enzyme concentrations, more enzymes are available to catalyze the reaction (more reactions at once)

There is a linear relationship between reaction rate and enzyme concentration (at constant substrate concentration

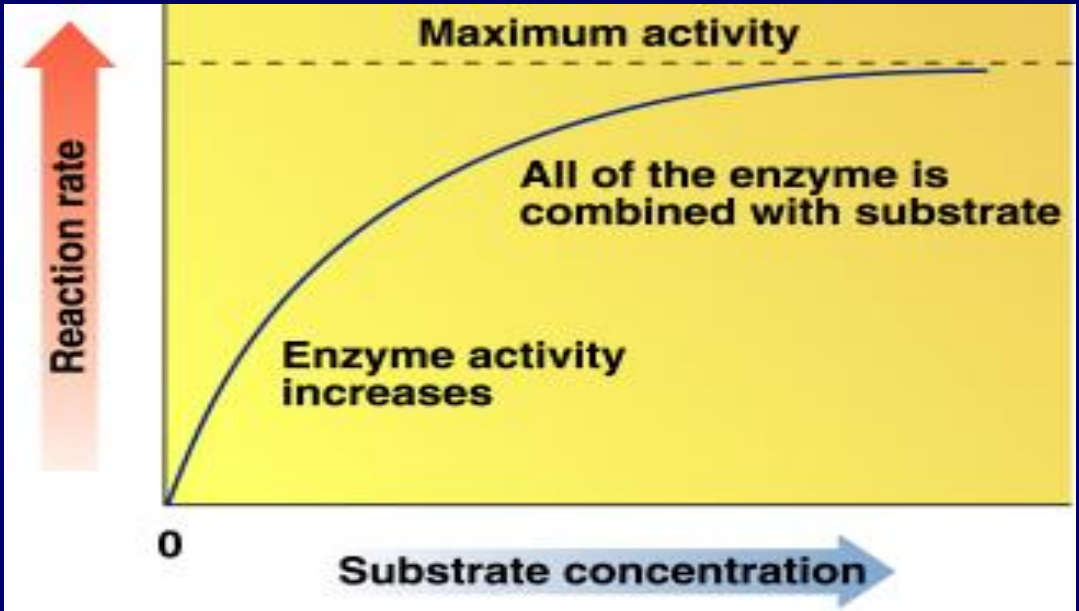


4. Substrate Concentration

The rate of reaction increases as substrate concentration increases (at constant enzyme concentration)

Maximum activity occurs when the enzyme is saturated (when all enzymes are binding substrate)

The relationship between reaction rate and substrate concentration is exponential, and asymptotes (levels off) when the enzyme is saturated.



The Michaelis-Menten Equation

The basic equation derived by Michaelis and Menten to explain enzyme-catalyzed reactions is

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

K_m - Michaelis constant;

Vo – initial velocity caused by substrate concentration, [S];

V_{max} – maximum velocity

Enzyme Specificity

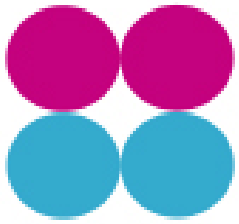
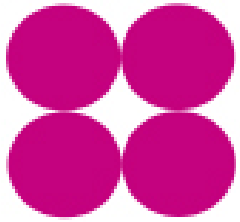
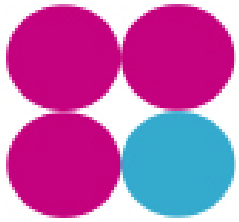
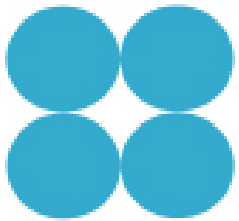
Enzymes have varying degrees of **specificity** for substrates .Enzymes may recognize and catalyze:a

1. Absolute : catalyze one type of reaction for a single substrate .Ex. urease catalyzes only the hydrolysis of urea.
2. Group : catalyze one type of reaction for similar substrates. Ex. Hexokinase adds a phosphate group to hexoses.
3. Linkage: catalyze one type of reaction for a specific type of bond. Ex. Chymotrysin catalyzes the hydrolysis of peptide bonds.
4. Stereospecificity: Ex. Phenylalanine hydroxylase uses L-Phe not D-Phe

Isoenzymese

Enzymes isolated from different organisms , catalysing same reactions but have same number amino acid with different sequence. Even within a single species, there may exist different forms of enzyme catalysing the same reaction. Differences may be:

1. Amino acid sequence
2. Some covalent modification
3. 3-D structure

Isoenzymes of lactate dehydrogenase	Highest levels found in the following:	Isoenzymes of lactate dehydrogenase	Highest levels found in the following:
		 H_2M_2 (LDH_3)	Brain, lung, white blood cells
 H_4 (LDH_1)	Heart, kidneys		
 H_3M (LDH_2)	Red blood cells, heart, kidney, brain	 HM_3 (LDH_4)	Lung, skeletal muscle
		 M_4 (LDH_5)	Skeletal muscle, liver

Enzyme inhibition

Inhibitor (I) are molecules that cause a loss of enzyme activity when binds to an enzyme and prevents the formation of ES complex or breakdown it to E + P.

In a tissue and cell there are different chemical agents (**metabolites, substrate analogs, toxins, drugs, metal complexes** etc) can inhibit the enzyme activity.

There are two types of Inhibitors:-

1. Reversible Inhibitors

Combining with enzyme (EI complex is formed) can rapidly dissociate

Enzyme is inactive only when bound to inhibitor

EI complex is held together by weak, noncovalent interaction

Three basic types of reversible inhibition:

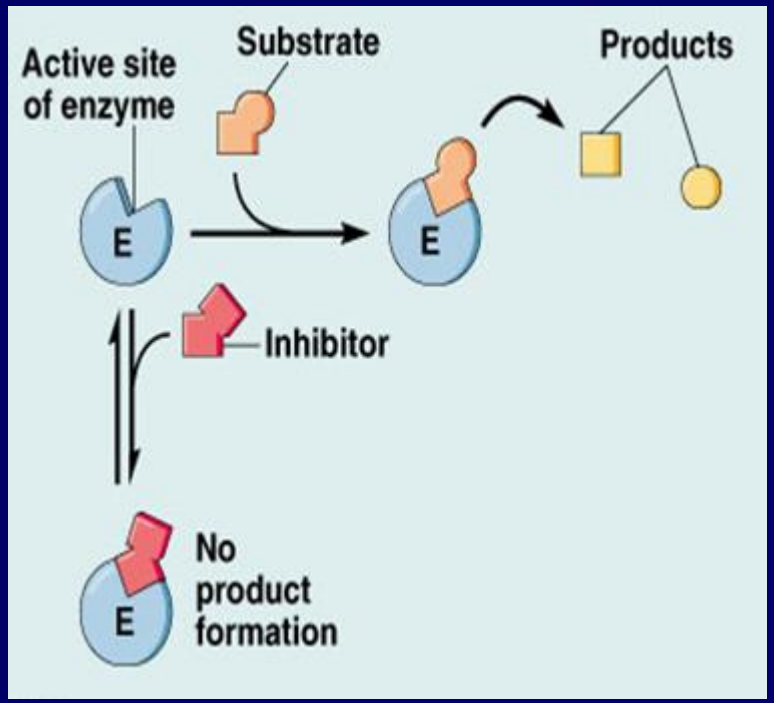
A. Competitive
B. Uncompetitive
C. Noncompetitive

A. Competitive

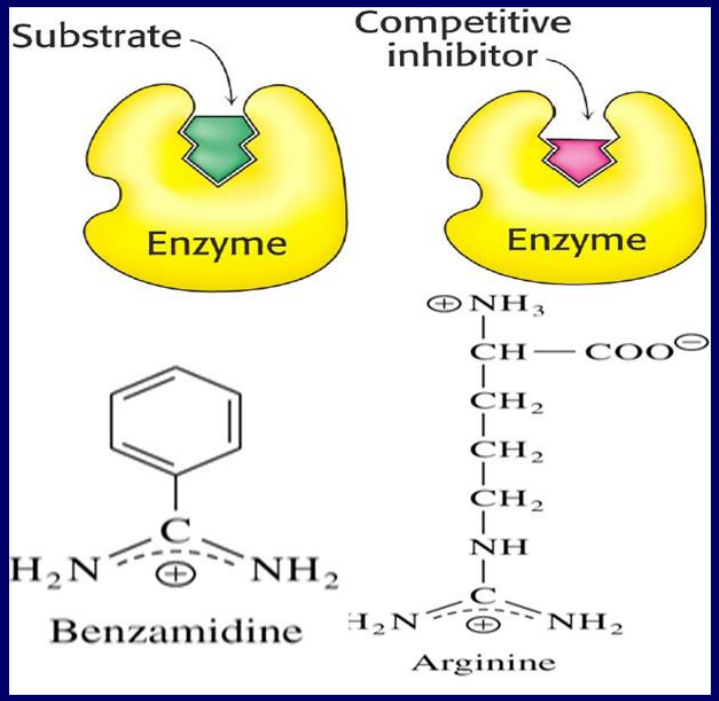
Inhibitor has a structure similar to the substrate thus can bind to the same active site, the enzyme cannot differentiate between the two compounds

When inhibitor binds, prevents the substrate from binding

Inhibitor can be released by increasing substrate concentration



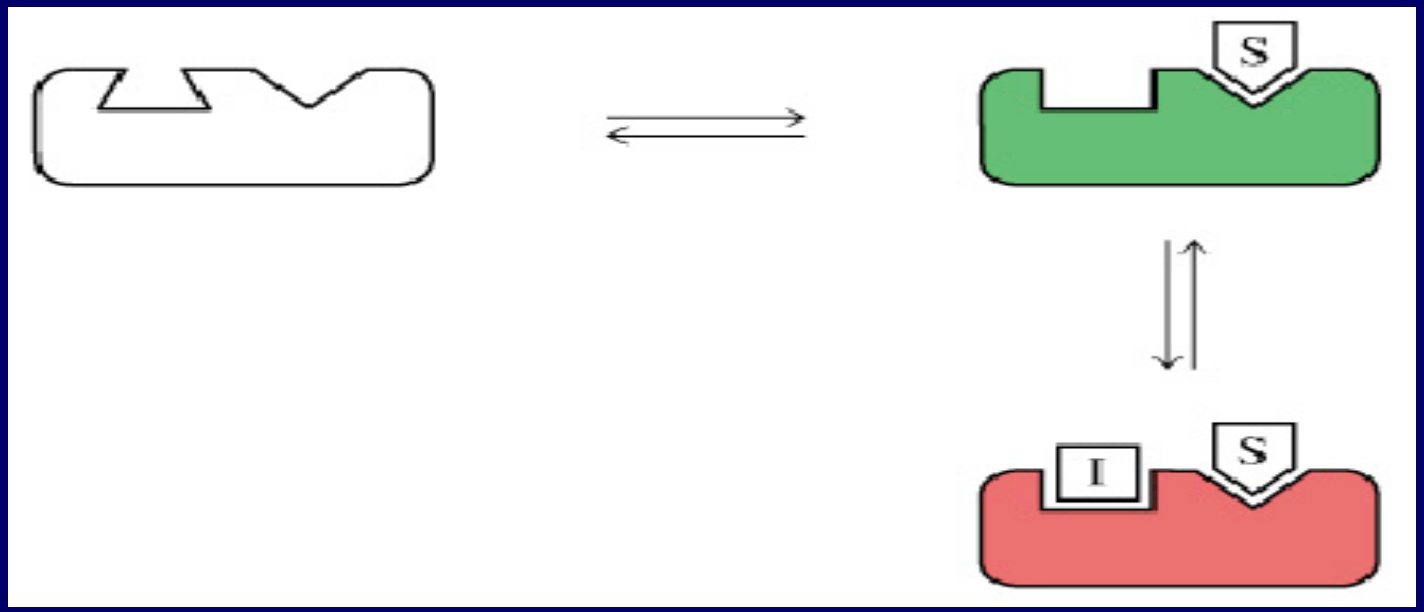
Ex. Benzamidine competes with arginine for binding to trypsin



B. Uncompetitive

Uncompetitive inhibitors bind to ES not to free E

This type of inhibition usually occurs in multisubstrate reaction only

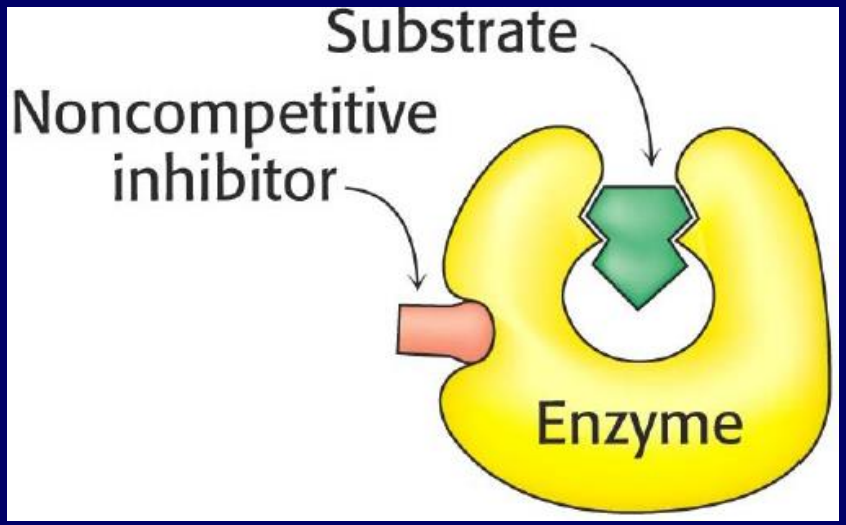


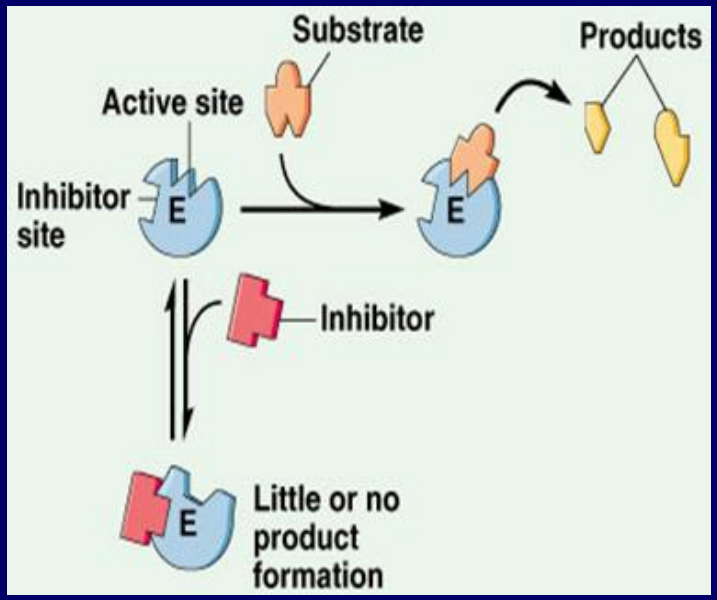
C. Noncompetitive

Binds to an enzyme site different from the active site.

Inhibitor and substrate can bind enzyme at the same time.

Cannot be overcome by increasing the substrate concentration.





2.Irreversible Enzyme Inhibition

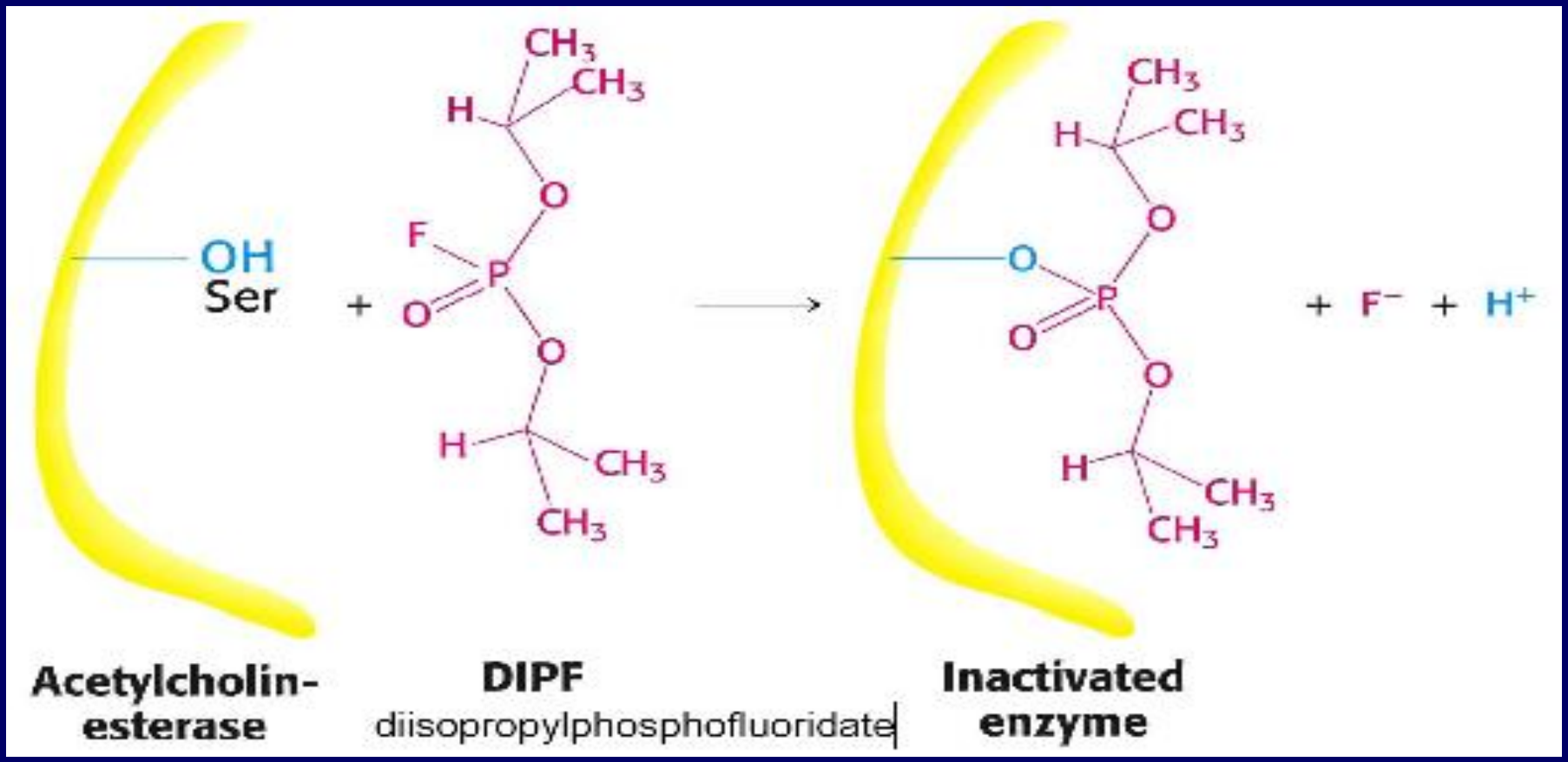
Irreversible inhibitors: binding with the functional groups of the amino acids in the active site.

This binding may block the active site binding groups so that the enzyme-substrate complex cannot form.

Example of irreversible inhibitors Snake venom nerve gas and cyanide

cyanide irreversibly inhibits the enzyme cytochrome oxidase found in the electron transport chain used in respiration. If this cannot be used, death will occur.

Nerve gases and pesticides, containing organophosphorus, combine with serine residues in the enzyme acetylcholine esterase.



Regulation of enzyme activity

Regulation means controlling the activity of enzymes.
Regulation of enzyme activity can be achieved by two general mechanisms:

1- Control of enzyme quantity

As enzymes are protein in nature, they are synthesized from amino acids under gene control and degraded again to amino acids after doing its work.

For example, the quantity of liver arginase enzyme increases after protein rich meal due to an increase in the rate of its synthesis; also it increases in starved animals due to a decrease in the rate of its degradation.

2. Altering the catalytic efficiency of the enzyme

Catalytic efficiency of enzymes is controlled by:

A-Allosteric regulation

B. covalent modification

C. Isozymes (isoenzymes) (see slide 25&26)

D, Proteolytic activation

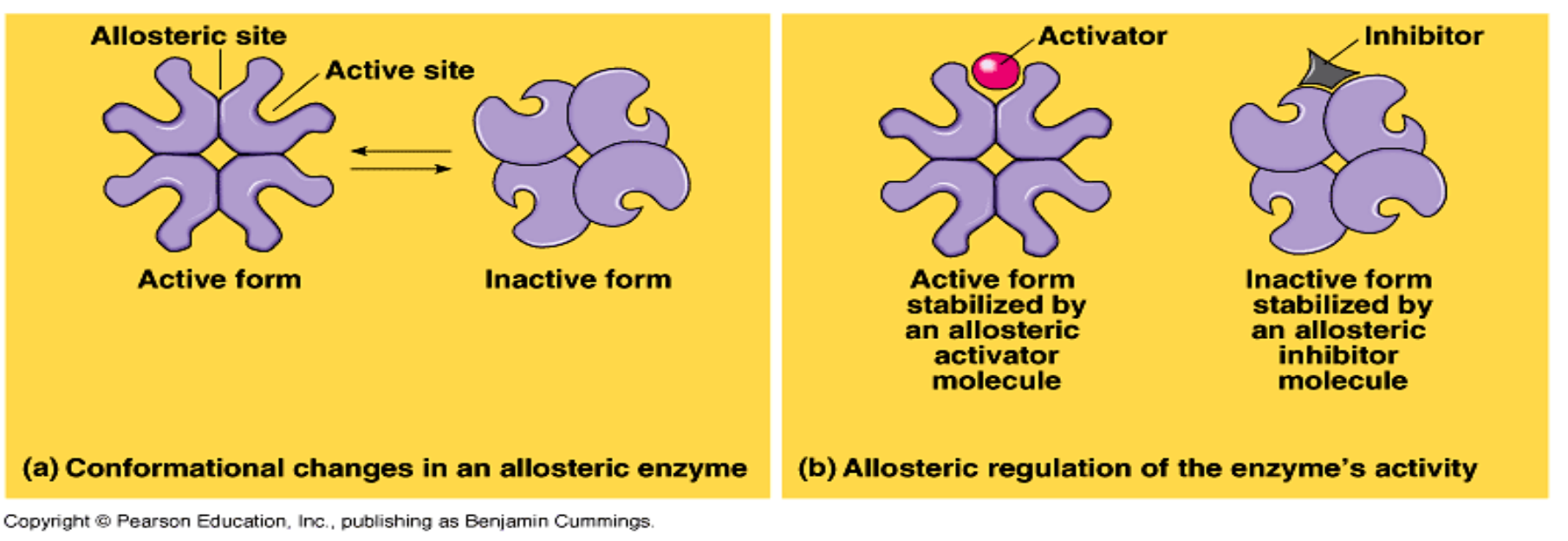
A-Allosteric regulation

Action at "another site" then the active site

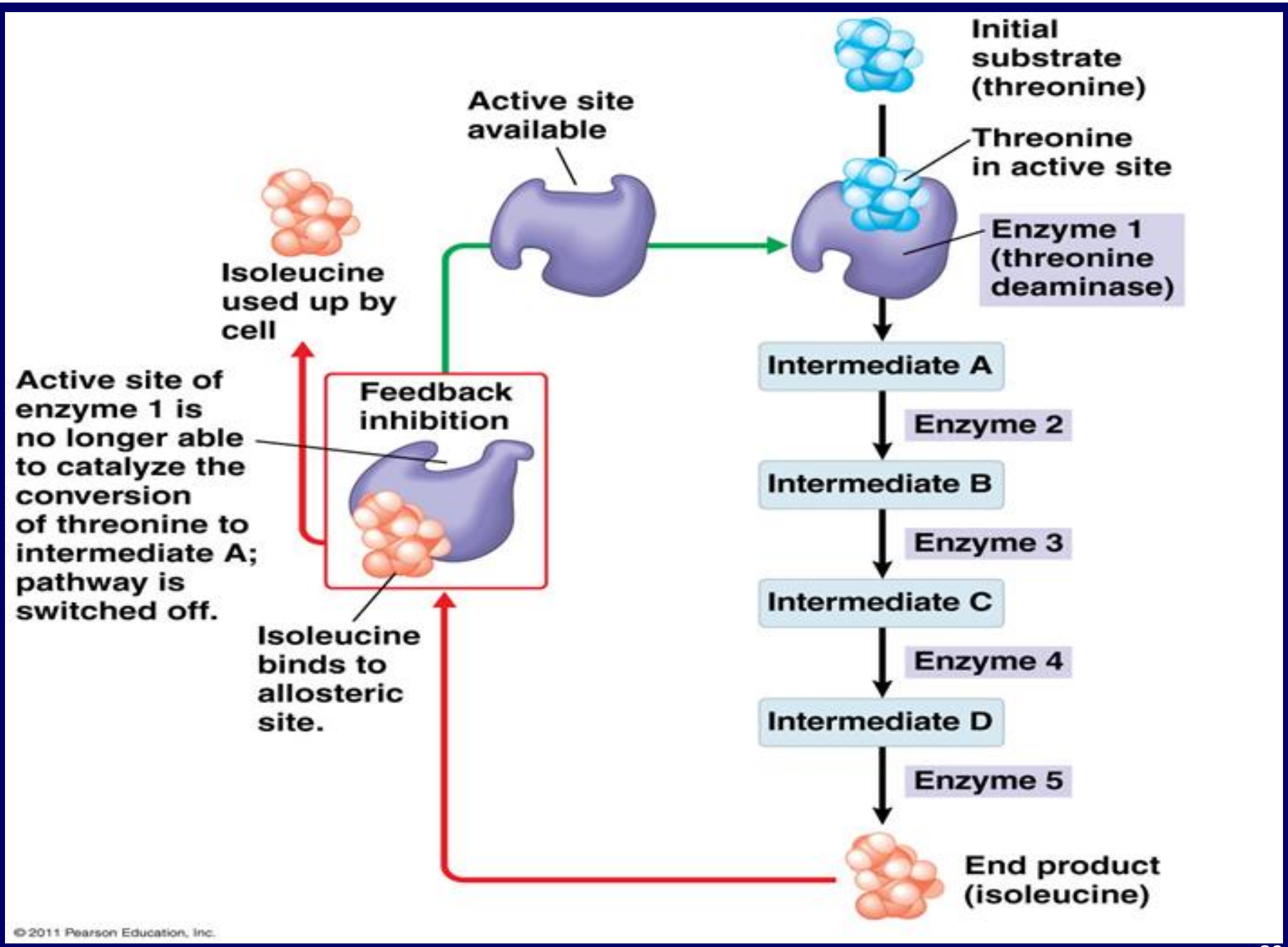
The binding of an effecter to the regulatory site causes conformational change of the protein and influences the activity of the catalytic site.

Positive effectors (activator)

Negative effectors (inhibitor)



allosteric site: site other than the active site


Initial substrate (threonine)
Active site available
Threonine in active site
Enzyme 1 (threonine deaminase)
Isoleucine used up by cell
Intermediate A
Enzyme 2
Intermediate B
Enzyme 3
Intermediate C
Enzyme 4
Intermediate D
Enzyme 5
End product (isoleucine)
Feedback inhibition
Active site of enzyme 1 is no longer able to catalyze the conversion of threonine to intermediate A; pathway is switched off.
Isoleucine binds to allosteric site.
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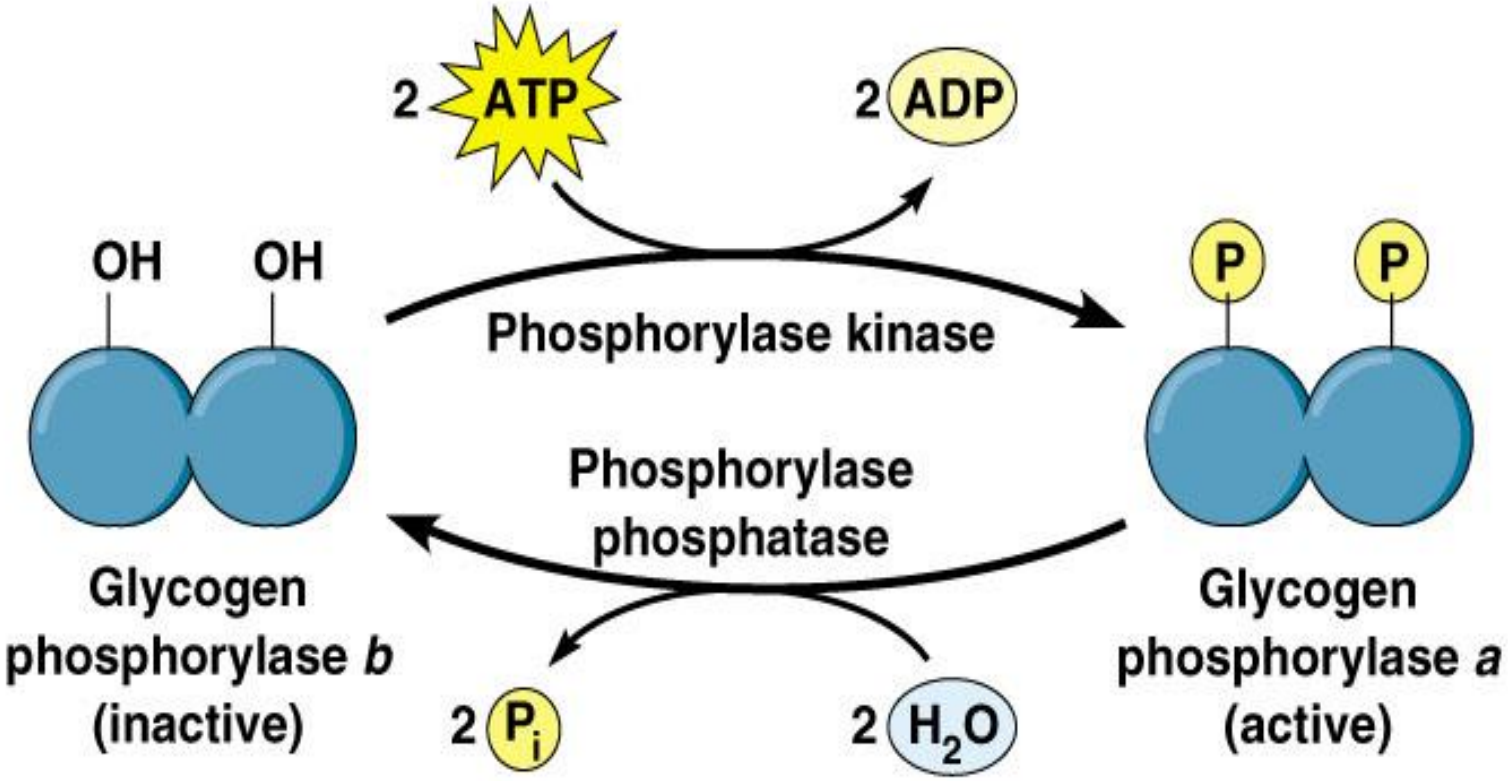
B-Covalent modification

Covalent attachment of a molecule to an amino acid side chain of a protein can modify activity of enzyme



(b) Regulation of glycogen phosphorylase

Activation by proteolytic cleavage

Many enzymes are synthesized as **inactive precursors (zymogens)** that are activated by proteolytic cleavage.

Proteolytic activation only occurs once in the life of an enzyme molecule

an example is trypsin, a digestive enzyme
it is synthesized and stored as trypsinogen, which has no enzyme activity.
It becomes active only after a six-amino acid fragment is hydrolyzed from the N-terminal end of its chain removal of This small fragment changes allow the molecule to achieve its active form

Examples of enzymes commonly assayed for diagnostic purposes

Enzyme	Location	Cause of elevated plasma level
Acid phosphatase - ACP	Prostate	Prostatic cancer
Alkaline phosphatase – ALP	Bone, liver	Rickets, hypoparathyroidism, osteomalacia, obstructive jaundice, cancer of bone/liver
Alanine aminotransferase – ALT	Liver (muscle, heart, kidney)	Hepatitis, jaundice, circulatory faillure with liver congestion
Aspartate aminotransferase – AST	Heart, muscle, red cells, liver	Myocardial infarction, muscle damage, anemia, hepatitis, circulatory faillure with liver congestion
Amylase - AM	Pancres	Acute pancreatitis, peptic ulcer
γ-Glutamyl transferase – GMT	Liver, kidney, pancreas	Hepatitis, alcoholic liver damage, cholestasis