Enzymology- An overview-1

* البترول تشكل من موت اشجار وكائنات حتى ضغط عالي without enzymes
this is why the prodection took a very long time

* Ni عنصر مستخدم لعملية الزيوت

Catalysts
- organic (enzyme) 10^6 - 10^{12}
- unorganic (metal) mostly 1000

turn over number
عدد المواد المتفاعلة التي تحول الى مواد ناتجة

proteins
- Globular
  life span very short
  seconds to minutes
  (enzymes, hormones)
- Fibrous
  very long
  hours to months
  (Structural proteins)

Enzymes- An introduction (regulation)

- Biologic (organic catalysts) polymers that catalyze the chemical reactions.

↳ acceleration of the reaction

The enzyme is acting طول مدة الحياة (لا يدخل بأكثر من تفاعلات) لا تستهلك

- Enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction.

9 types

- With the exception of catalytic RNA molecules, or ribozymes, enzymes are proteins.

→ بشتغل كإنزيم لكن لا يعد بروتين

معالجة RNA (قطع من RNA) طوله (90-300

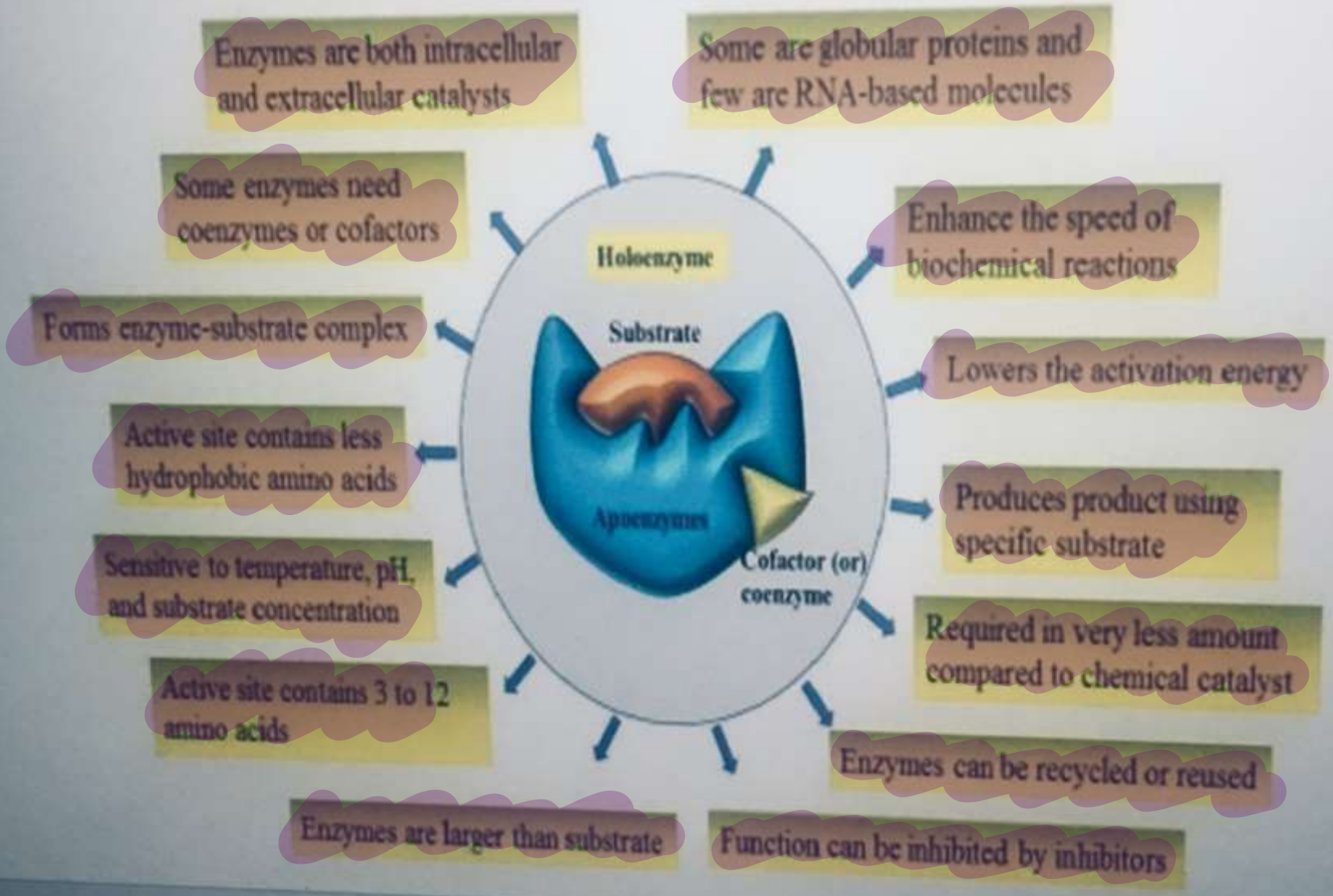
- In addition to being highly efficient, enzymes are also extremely selective catalysts.

Each site of the cell has its specific enzyme

- Thermolabile, site specific, with a high turn over number compared to the inorganic catalysts.

(Fibrous اكثر من 10 بكونت
globular اقل من 10 بكونت) Axial ratio ✱ النسبة ما بين كبري لصغير بال

Characteristics of the enzymes



Nomenclature of enzymes

-In most cases, enzyme names end in **–ase**

-The common name for a hydrolase is derived from the substrate

Urea: remove -a, replace with **-ase** = urease
Lactose: remove **- ose**, replace with **- ase** = lactase

- Other enzymes are named for the substrate and the reaction catalyzed

Lactate dehydrogenase (oxidation)
Pyruvate decarboxylase (removal)

(name of the substances) (the type of the reaction)

- Some names are historical - no direct relationship to substrate or reaction type

Catalase
Pepsin => Stomach
Chymotrypsin
Trypsin

breaking of the peptide bond on the protein molecul to give amino acids

peptidases

intestine

Classification of Enzymes

- Enzyme Commission (EC) – according to **International Union of Biochemistry and Molecular Biology (IUBMB)**

Commission numerical code (CNC)

- Each enzyme was given 4 digit numbers [1.2.3.4]

1st one of the 6 major classes of enzyme activity

نوع المركب أو الرابطة التي تتكسر

2nd the subclass (type of substrate or bond cleaved)

functional groups

3rd the sub-subclass (group acted upon, cofactor required, etc...)

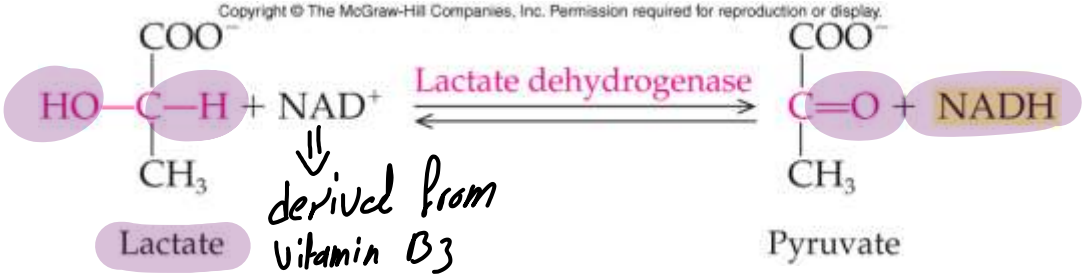
4th a serial number… (order in which enzyme was added to list)

متى تم إضافته إلى القائمة

1- Oxidoreductases (**EC.1**) catalyze redox reactions, such as (Alcohol dehydrogenase [EC 1.1.1.1])

- Reductases
- Oxidases

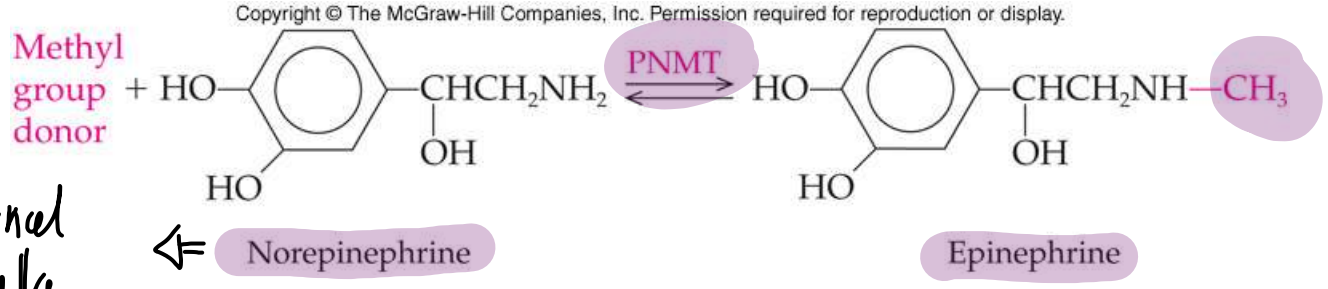
pH in blood (7.35-7.45) basic



2- Transferases (**EC.2**) transfer a group from one molecule to another, such as (Hexokinase [EC 2.7.1.2])

-Transaminases catalyze transfer of an amino group

- Kinases transfer a phosphate group

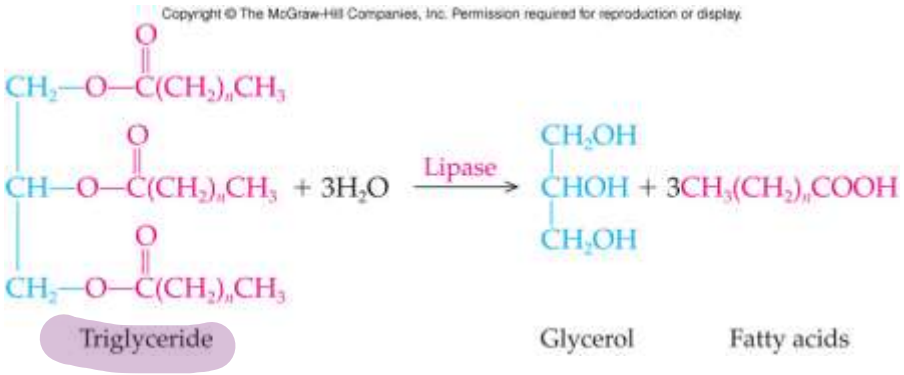


Adrenal Medulla

3- Hydrolases (**EC.3**) cleave bonds by adding water, such as (Alkaline phosphatase [EC 3.1.3.1]) ((Digestive enzymes))

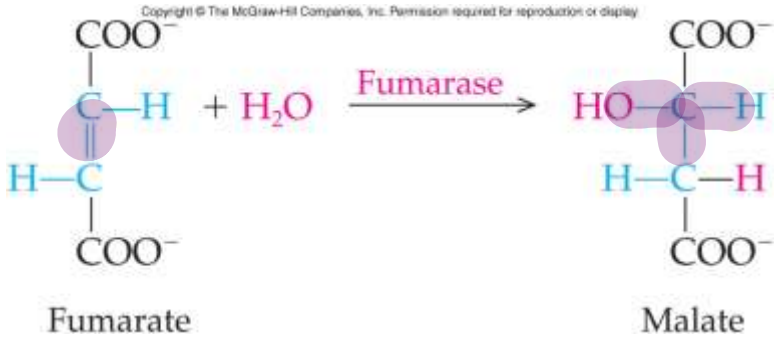
- Phosphatases
- Peptidases
- Lipases

3 Fatty acids



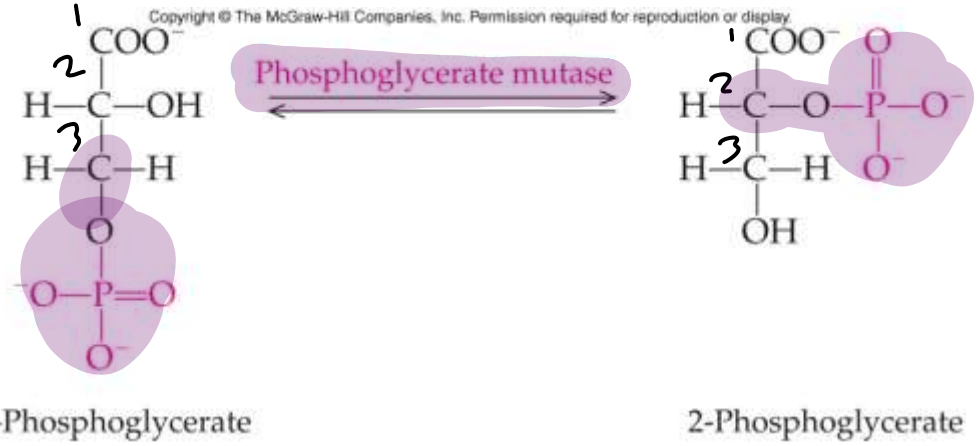
4- Lyases (**EC.4**) catalyze removal of groups to form double bonds or the reverse break double bonds, such as (Pyruvate decarboxylase [EC 4.1.1.1])

- Decarboxylases
- Synthases



5- Isomerases (**EC.5**) catalyze intramolecular rearrangements, such as (Alanine racemase [EC 5.1.1.1]) اعادة ترتيب داخل المركب

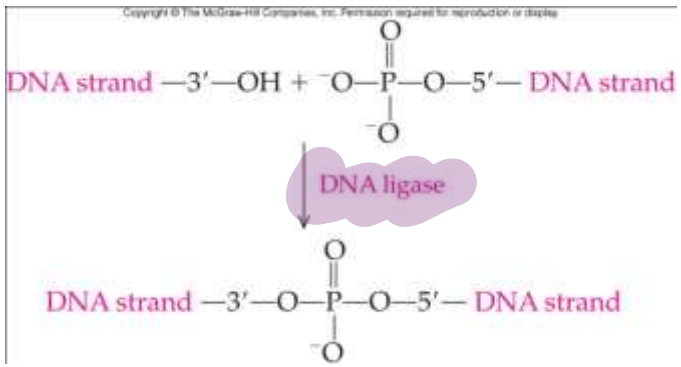
- Epimerases
- Mutases

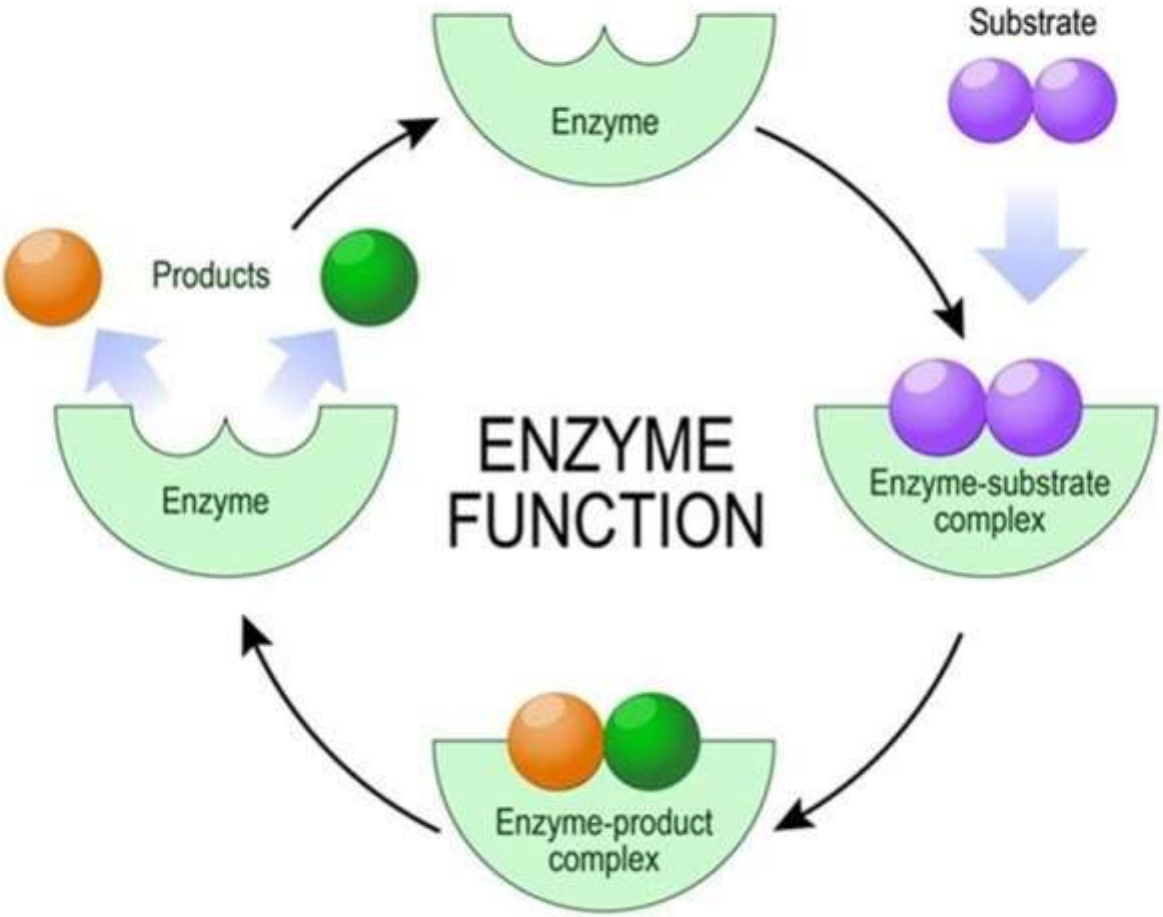


6- Ligases (**EC.6**) catalyze a reaction in which a C-C, C-S, C-O, or C-N bond is made or broken, such as Using energy

(Isoleucine-tRNA ligase [EC 6.1.1.5])

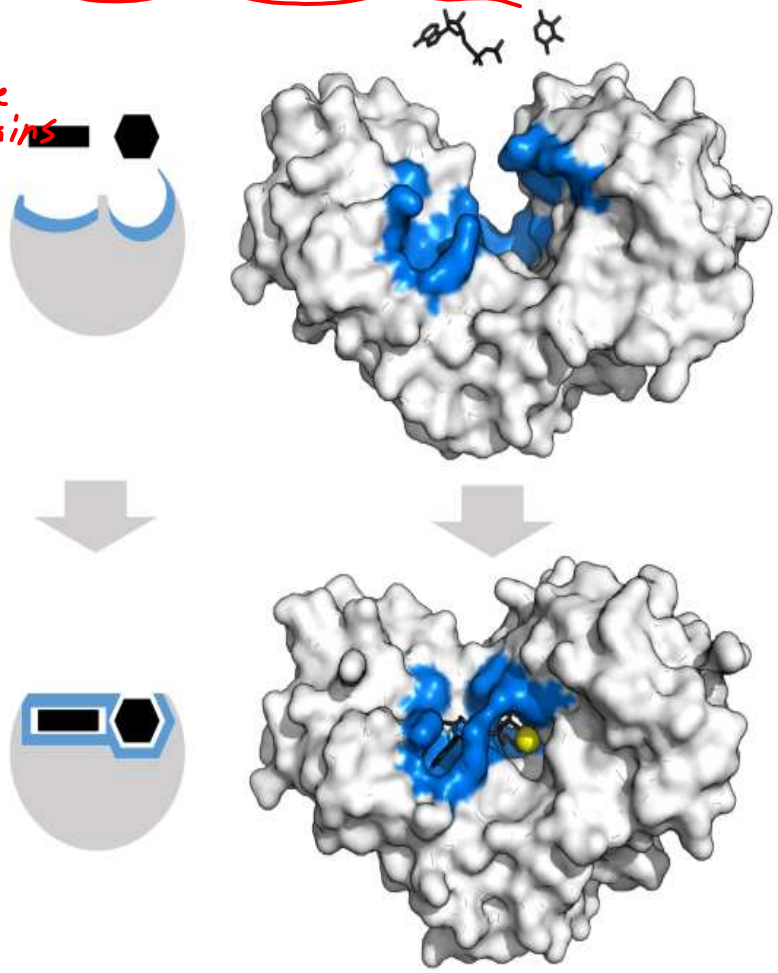
ربط قطع ال DNA to produce a continuous sequence of DNA




Substrate
Enzyme
Products
Enzyme
ENZYME
FUNCTION
Enzyme-substrate
complex
Enzyme-product
complex

Active site

- Takes the form of a cleft or pocket
- Takes up a relatively small part of the total volume of an enzyme
- Substrates are bound to enzymes by multiple weak attractions
- The specificity of binding depends on the precisely defined arrangement of atoms in an active site
- The active sites of multimeric enzymes are located at the interface between subunits and recruit residues from more than one monomer



Enzyme substrate binding

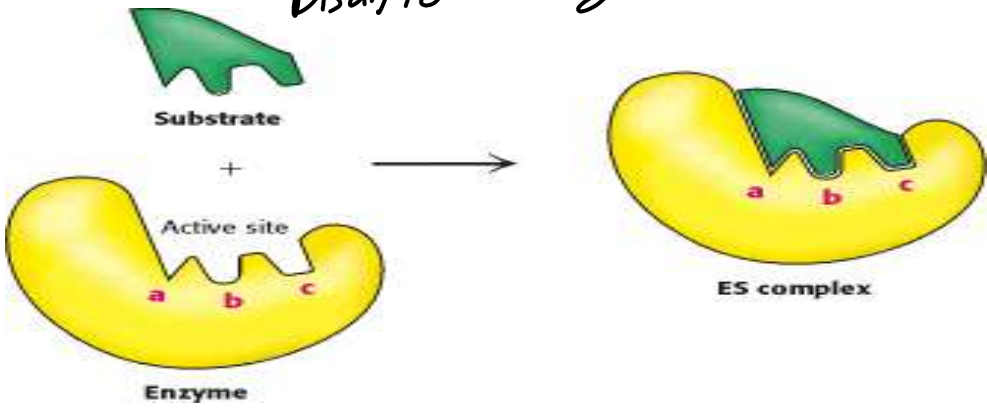
-Two models have been proposed to explain how an enzyme binds its substrate: the lock-and –key model and the induced-fit model.

ما في تغيير بالموقع لانه شكله ثابت ومخصص لمادة واحدة

- Lock-and-Key Model of Enzyme-Substrate Binding, in this model, the active site of the unbound enzyme is complementary in shape to the substrate.

-"lock and key model" **accounted for the exquisite specificity of enzyme-substrate interactions**, the implied rigidity of the enzyme's active site failed to account for the dynamic changes that accompany catalysis.

الربط ليست حرية
hydrogenic electrostatic
hydrophobic
Disulfid <= strong



Induced-Fit Model of Enzyme-Substrate Binding

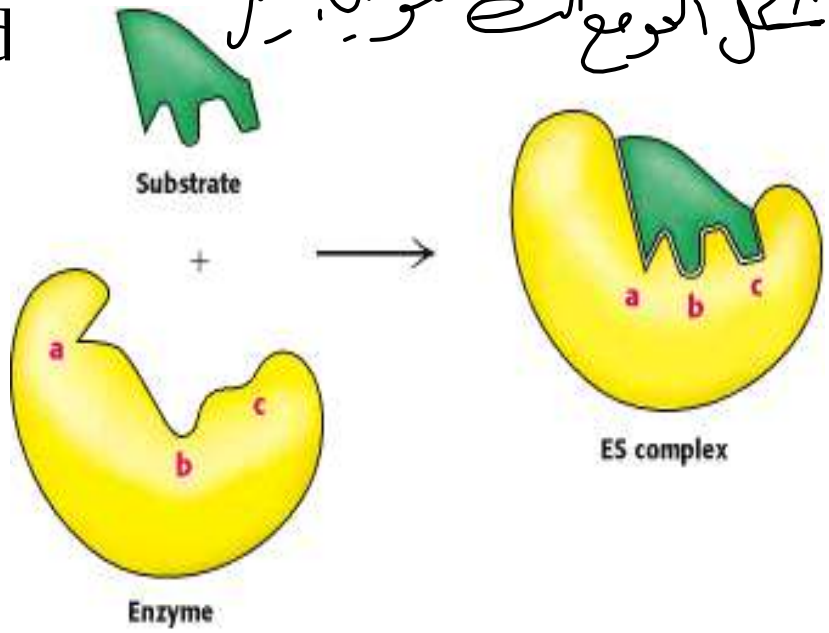
- In this model, the enzyme changes shape on substrate binding. flexibility

-The active site forms a shape complementary to the substrate only after the substrate has been bound.

Substrate بس بيترتب ⇐ according to the signals

- When a substrate approaches and binds to an enzyme they induce a conformational change, a change analogous to placing a hand (substrate) into a glove (enzyme).

شكل الموقع النشط حوالي بتغير



Mechanism of Action of Enzymes

- Enzymes are catalysts and increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are neither used up in the reaction nor do they appear as reaction products.
- The basic enzymatic reaction can be represented as follows:

- Where E represents the enzyme catalyzing the reaction, S the substrate, the substance being changed, and P the product of the reaction.
- The mechanism of action of enzymes can be explained by two perspectives: (imp) (وقفات)
  1- Thermodynamic changes
  2- Processes at the active site

Thermodynamic changes

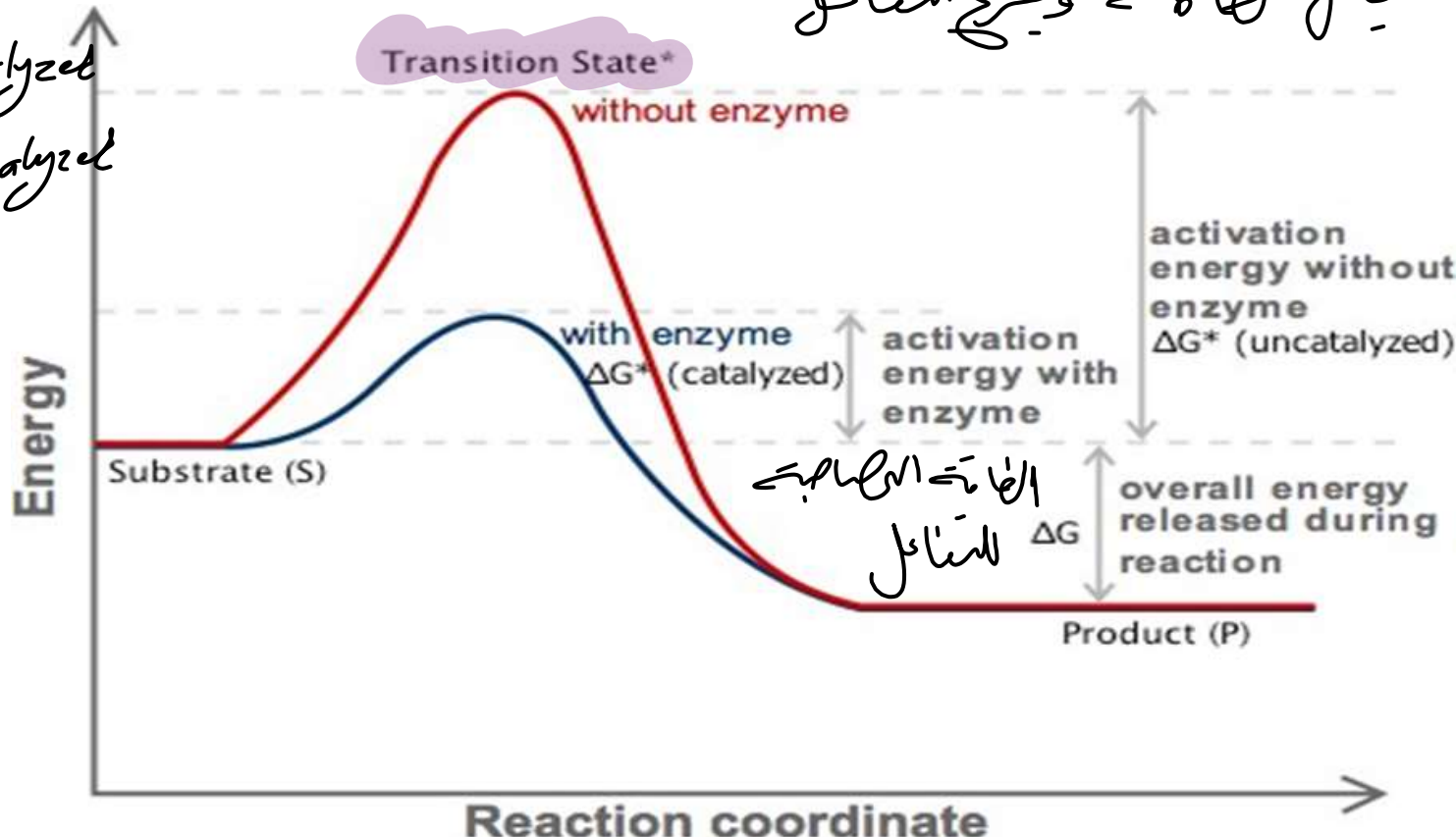
- All enzymes accelerate reaction rates by providing transition states with a lowered $\Delta G F$ for formation of the transition states.

طاقة التنشيط — الطاقة اللازمة ليصل المتفاعل لحالة transition state

- The lower activation energy means that more molecules have the required energy to reach the transition state.

يقلل الطاقة ويسرع التفاعل

Tow reactions one of them is non-catalyzed reaction and another is catalyzed by enzyme



Processes at the active site

1- **Catalysis by proximity**: for the molecules to react they must come within bond-forming distance of one another.

When an enzyme binds substrate molecules at its active site, it creates a region of high local substrate concentration.

Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another.

2- **Acid base catalysis**: the **ionizable functional groups** of aminoacyl side chains of prosthetic groups contribute to catalysis by acting as acids or bases.

- General acid catalysis involves **partial proton transfer** from **a donor** to lower the free energy of the transition state.
- General base catalysis involves **partial proton abstraction** from **an acceptor** to lower the free energy of the transition state.

3- **Catalysis by strain**: enzymes that catalyze the **lytic reactions** involve breaking a covalent bond typically bind their substrates in **a configuration slightly unfavorable** for the bond that will undergo cleavage. (Under Stress)

ترتبط بوضعية غير مريحة لتسهيل كسر الرابط

4- **Covalent catalysis**: accelerates reaction rates through **transient** formation of enzyme-substrate covalent bond.

Three stages in covalent catalysis: not for all enzymes

1- Nucleophilic reaction (نقل إلكترونات) between enzyme and substrate
2- Electrophilic withdrawal of electrons from substrate
3- Elimination reaction (reverse of stage 1)

5- Metal Ion catalysis

imp ⇐ enhancing نشجع فعل

- Two classes of metal ion dependent enzymes:

1- Metalloenzymes contain tightly bound transition metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}) essential for the reaction

2- Metal-activated enzymes loosely bind metal ions (alkali or alkaline metal including Na^{+}, K^{+}, Mg^{2+} and Ca^{2+})

not essential for the reaction

efficiently يشجع ما

- Metal ions enhance catalysis in three major ways:

1- Binding to and orienting substrates for reaction as Mg^{2+} binding to ATP

* Metal ions which are required for the enzyme acting an energy production exp// Air deficiency anemia

2- Mediating redox reaction through changes in oxidation state such as reduction of O_2 to H_2O through electron transfer

3- Electrostatic stabilization or shielding of negative charges as Mg^{2+} binding to ATP

ionization limited

يجذب المادة المتفاعلة نحو الانزيم

يضع المادة المتفاعلة على الانزيم

1) Metal between the enzyme and substrate ((Enzyme-metal-substrate bridge))
2) Metal before the substrate ((Metal-substrate-enzyme bridge))
3) Metal after enzyme ((substrate-enzyme-metal ion bridge)) يضع الانزيم على المادة المتفاعلة

6- Electrostatic catalysis

- Enzymes seem to arrange **active site charge distributions** to stabilize the **transition states** of catalyzed reactions
- Substrate binding generally **excludes water from an enzyme active site** generating **a low dielectric constant** within the active site
- Electrostatic interactions are stronger (Cloud of electrons around the enzyme and substrate with out transfer of electrons)
- pka's can vary by several pH units due to proximity of charged groups

- Alternative form of electrostatic catalysis: several enzymes as **superoxide dismutase** apparently **use charge distributions** to guide **polar substrates** to their active sites

anti oxidize

* يوجد عوامل اكسدة تكون داخل الخلايا تسبب امراض مثل cancer
* مهمة جزء من الانزيمات كل ما تكون free radical in our cells تحطمها

Enzyme Specificity

- In general, there are four distinct types of specificity:

⇐ بعض الانزيمات تشارك بتفاعلات تختلف عن اسمها

1- Absolute specificity: the enzyme will catalyze only one reaction.

2- Group specificity: the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups

3- Linkage specificity: the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure

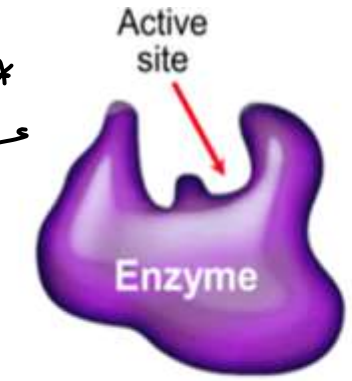
4- Stereo chemical specificity: the enzyme will act on a particular steric or optical isomer.

* تصنيفات الانزيم = 6 major class
Metal ions Catalysis

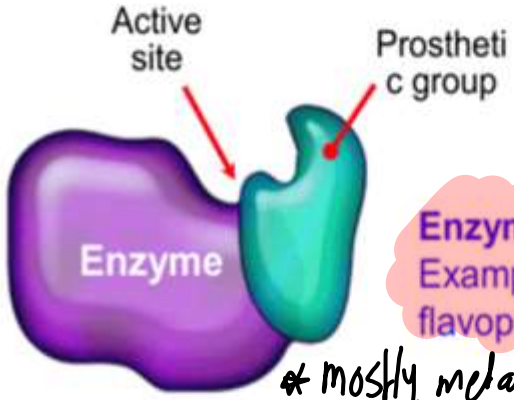
- Some enzyme require cofactors to be active.
- Cofactors are a non-protein components of the enzyme.
- Organic Molecules (Coenzymes)
- Inorganic ions e.g., Ca2+ , Zn2+ (Prosthetic group)
- Cofactors may be:

1- The Permanently attached cofactors, are called Prosthetic group (such as a vitamin, sugar, or lipid or inorganic such as a metal ion)

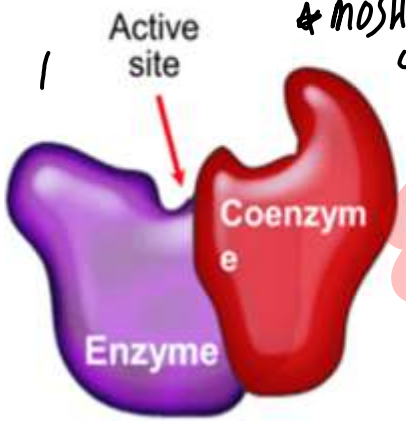
2- Temporarily attached cofactors are called coenzyme, its detach after a reaction and may participate in the reaction with other enzyme.



Enzyme is protein only
Example: lysozyme



Enzyme + prosthetic group
Example:
flavoprotein + FAD



Enzyme + coenzyme
Example:
dehydrogenases + NAD

Cofactors

- Cofactors can be subdivided into two groups: metals and small organic molecules

- Cofactors that are small organic molecules are called coenzymes.

- Most common cofactor are also metal ions.

- If tightly bound, the cofactors are called prosthetic groups.

- Loosely bound Cofactors serve functions similar to those of prosthetic groups but bind in a transient, dissociable manner either to the enzyme or to a substrate

Prosthetic groups

- Tightly integrated into the enzyme structure by covalent or non-covalent forces. e.g.;
  Pyridoxal phosphate B_6
  Flavin mononucleotide (FMN) B_2
  Flavin adenine dinucleotide (FAD) B_2
  Thiamin pyrophosphate (TPP) B_1
  Biotin $B_7 (H)$
  Metal ions – Co, Cu, Mg, Mn, Zn

- Metals are the most common prosthetic groups

Coenzymes

- Very often vitamins
- They serve as recyclable shuttles—or group transfer agents—that transport many substrates from their point of generation to their point of utilization.

- The water-soluble B vitamins supply important components of numerous coenzymes.

- Chemical moieties transported by coenzymes include hydrogen atoms or hydride ions, methyl groups (folates), acyl groups (coenzyme A), and oligosaccharides (dolichol).

Important Prosthetic Groups and Coenzymes

Prosthetic Group	Enzymes/ Proteins
Zn^{++}	Carbonic anhydrase , Alcohol dehydrogenase
Fe^{+++} or Fe^{++} Myoglobin(store O_2)	Hemoglobin, Cytochromes, ferrodoxin
Cu^{++} or Cu^{+++}	Cytochrome oxidase
K^{+} and Mg^{++}	Pyruvate Phosphokinase

Coenzymes	Vitamins
Nicotinamide adenine dinucleotide (NAD^{+}) or nicotinamide adenine dinucleotide phosphate ($NADP^{+}$)	vitamin B_3 (niacin)
Flavin mononucleotide (FMN^{+}) or flavin adenine dinucleotide(FAD^{+})	vitamin B_2 (riboflavin)
Pyridoxal phosphate	vitamin B_6 (pyridoxine)
Coenzyme A	Pantothenic Acid

Diagnostic significance of enzymes

1- Enzymes can act as diagnostic markers of underlying diseases .

2- Enzymes can also act as reagents for various biochemical estimations and detections

Glucos oxidase test ⇒ for diabetes mellitus

Enzymes as diagnostic markers

Another classification of enzymes

1- **Functional plasma enzymes** (Plasma derived enzymes):

موجوده في الدم

- Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiologic function in the blood.

Upnormal Condition <= high concentration in cells ← إذا كانت

Examples of these functional plasma enzymes include **lipoprotein lipase**, **pseudo cholinesterase**, and the **proenzymes of blood coagulation and blood clot dissolution** . inactive form

The majority of them are synthesized in and secreted by liver.

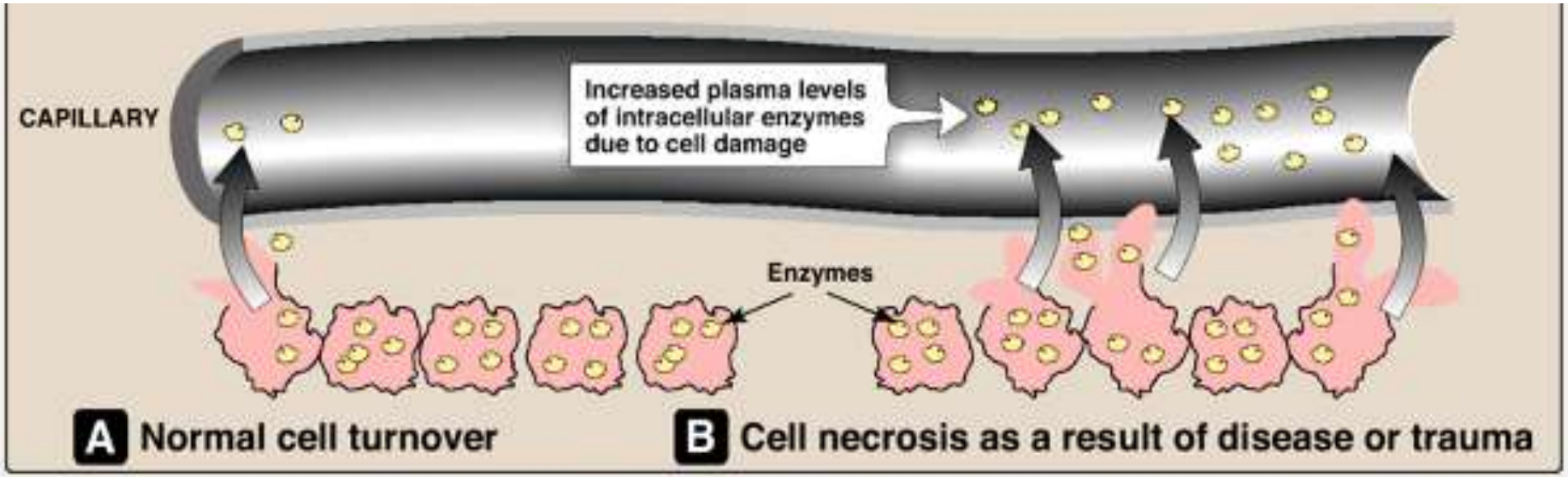
2- **Nonfunctional plasma enzymes** (Cell derived enzymes):

- Plasma also contains numerous other enzymes that perform no known physiologic function in blood.

-These apparently nonfunctional plasma enzymes arise from the routine normal destruction of erythrocytes, leukocytes, and other cells.

leakage

-Tissue damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several nonfunctional plasma enzymes.



Isoenzymes (Isoenzymes)

- Are homologous enzymes that catalyze the same reaction but have differences in enzymatic properties.
- Often different isoenzymes are found in different locations in a cell or in different organs/tissues of an organism. origin كتلف.
- They are from different polypeptide chains that coded by different genes and so, they are affected by different activators and different inhibitors in different tissues.

e.g.: different physical properities ((heavy, light molecular weight)

Lactate dehydrogenase isoenzymes,

- The enzyme interconverts lactate and pyruvate (LDH)
- Humans have two isoenzymic chains for lactate dehydrogenase: LDH (M) found in muscle and LDH (H) found in heart.

Skeletal

- M is optimized to work under anaerobic conditions and H optimized to work under aerobic conditions.

-There are 5 different isoenzymes. electrophoresis بعرف الفرق بين

-The relative ratio of the isoenzymes depends on the location in the organism as well as the developmental stage.

Isoenzyme	Tissue origin
LDH1 (H4)	Cardiac and kidney
LDH2 (H3M)	Cardiac, kidney, brain and RBCs
LDH3 (H2M2)	Brain, lung and WBCs
LDH4 (HM3)	Lung, skeletal muscle
LDH5 (M4)	Skeletal muscle and liver

CK/CPK Isoenzymes

- There are three Isoenzymes.
- Measuring them is of value in the presence of elevated levels of CK or CPK to determine the source of the elevation.
- Each isoenzyme is a dimer composed of two protomers 'M' (for muscles) and 'B'(for Brain).

-These isoenzymes can be separated by, electrophoresis or by ion exchange chromatography.

Isoenzyme	Electrophoretic mobility	Tissue of origin	Mean % in blood
MM(CK3)	Least	Skeletal muscle Heart muscle	97-100%
MB(CK2)	Intermediate	Heart muscle	0-3%
BB(CK1)	Maximum	Brain	0%

Enzyme Kinetics

- It is the field of biochemistry concerned with the quantitative measurement of the rates of enzyme-catalyzed reactions and the study of the factors affecting these rates.
- The rate of a chemical reaction is described by the number of molecules of reactant(s) to be converted into product(s) in a specified time period which is dependent on the concentration of the chemicals involved in the process and on rate constants that are characteristic of the reaction.