*life span of proteins:
→ gloubular: seconds to minutes (very short)
→ fibrous: hours to months (long)

*lysosomal enzymes are responsible for the degradation of dead enzymes.

*axial ratio
(الطول / العرض)
→ globular protein: < 10
→ fibrous protein: > 10

Enzymology- An overview-1

– all enzymes are protein in nature except ribozymes
– enzymes are never consumed.

*type of catalyst:
→ organic → enzyme
→ inorganic → mostly metal ions
(ex. Ni in oil hydrogenation)

→ turn over number is the differnce between them, it's the amount of product produced by one unit of catalyst in one unit of time,
→ enzyme : $10^6 - 10^{12}$
→ metal ion: 10^3 only

*we can preforme any reaction anywhere in test tubes.

Enzymes- An introduction

enzyme activity must be regulated (activated when needed and deactivated when not needed)

- Biologic (organic catalysts) polymers that catalyze the chemical reactions.
  accelerate the reaction, occour in the shortest time
  * enzymes are gloubular protein

يستنزف

- Enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction.
  doesn't participate in the reaction, exit the first reaction to catalyse a second, third fourth reaction ..etc, as long as its life span is not done.

- With the exception of catalytic RNA molecules, or ribozymes, enzymes are proteins.
  9 types of RNA
  "not protein"
  Short streaches of RNA (90-300 neuclutide) they catalyse the processing of RNA
  turning the nonfunctioning immature RNA to functioning mature RNA

"كثير فعّالين" بيساعدوا بالتفاعل بقوى أكبر من انه يكون لحاله.

- In addition to being highly efficient, enzymes are also extremely selective catalysts.
  the enzyme is specific for its substrate and reaction
  the enzymes acting on the cell membrane are different from the ones acting in the nucleous and so on, each site of the cell have its spesific enzyme

"بيتأثر بالحرارة"

-Thermolabile, site specific, with a high turn over number compared to the inorganic catalysts.
  (organic) أكبر من (inorganic)
  – enzymes of the creb cycle are found in the mito condrea

Characteristics of the enzymes

Enzymes are both intracellular & extracellular catalysts

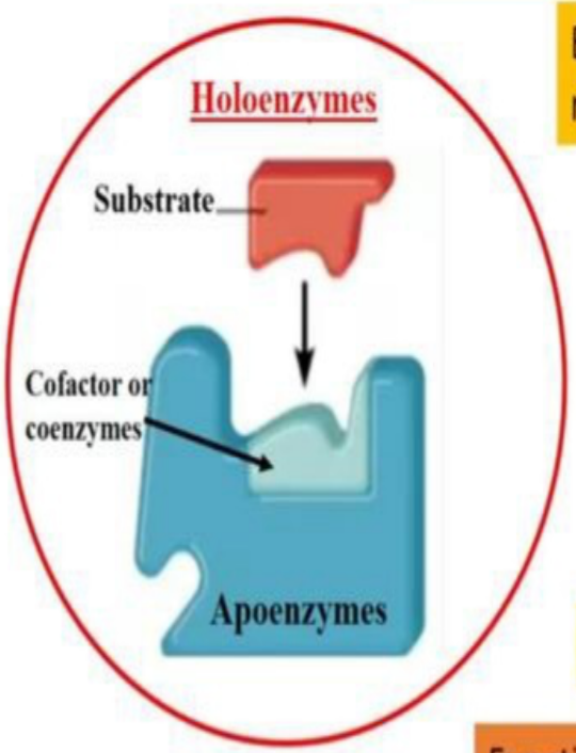
Some are **globular** proteins, and few are RNA-based molecules

Some enzymes need coenzymes or cofactors

Enhance the speed of biochemical reactions

Forms enzyme-substrate-complex

Lowers the activation energy



Active site contains less hydrophobic amino acids

Produces product using specific substrate

Sensitive to temperature, pH ,and substrate concentration

thermolabile

Required in very less amount compared to chemical catalyst

Active site contain 3 to 12 amino acids

Enzymes can be recycled or reused

Functions can be inhibited by inhibitors

Enzymes are larger than substrate

- Any enzymes requiring cofactor to act called apoenzyme (May functional with low efficacy or non-functional), when cofactor binds to it then it called Holoenzyme (active)
- Any enzymes not need cofactor to act called ACTIVE enzymes (100% functional)

Nomenclature of enzymes

ex. uric acid → uricase
urea → urease
histedine → histednase.

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

–the correct name must show the name of substrate and type of reaction.

-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 removal of H from lactase (oxidation)

Pyruvate decarboxylase removal of CO_2 from pyruvate.

Pyruvate sulfase: addition of sulfer

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

Catalase anti oxidant

Pepsin digest protein (stomach) Produced by Pancrease

Chymotrypsin protein digestion (intestine)

Trypsin protein digestion (intestine) Produced by Pancrease

* peptidases:

break the peptide bond

ex. Pepsin, trypsin, chymotrypsin.

protein digestion enzymes.

Classification of Enzymes

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

- Each enzyme was given 4 digit numbers [1.2.3.4]

Connection numerical code (CNC)

"type of reaction"

1st one of the 6 major classes of enzyme activity

Oxidoreductases, lyases, transfarase ... etc.

bond to be broken

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

عامل مساعد

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

ex. alkyl, carbonyl, hydroxyl ... etc.

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

date of addition to the enzyme group.

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

oxidation – reduction both should be done together

- Reductases
- Oxidases

the H taken from one substrate should be given to another substrate other wise it will shift the acidity of the blood, (from slightly basic to acidic)

– no oxidation reaction in any living organism take place without being combined with a reduction reaction.

– the oxidation of one substrate will cause the reduction of another substrate.

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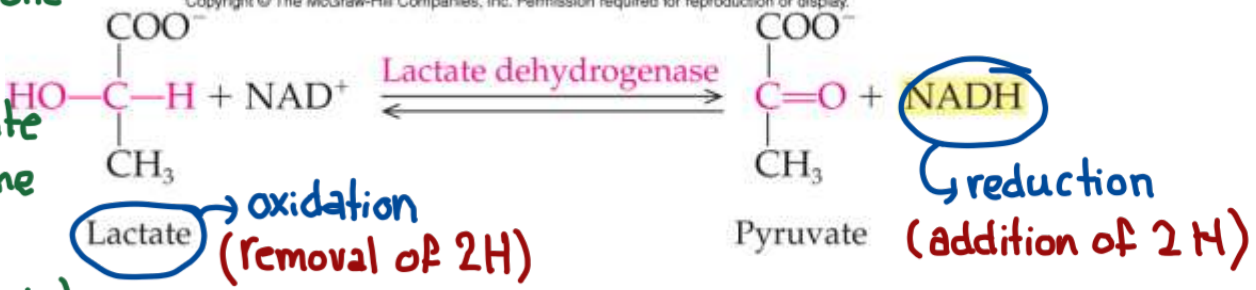

oxidation (removal of 2H)

reduction (addition of 2 H)

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

Phenylethanolamine N_methyl transferase

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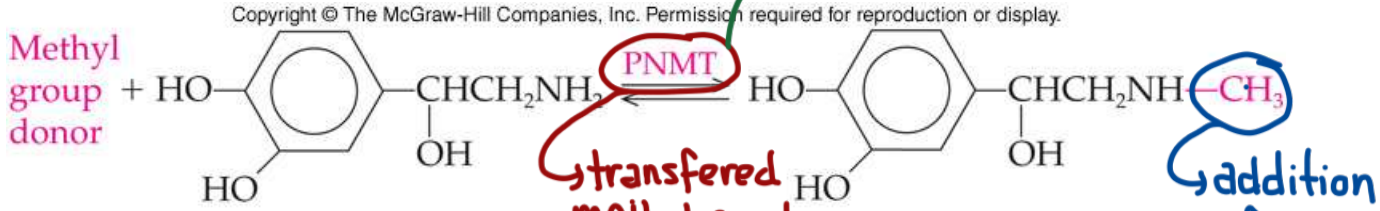

transfered methyl groub

addition of methyl

Norepinephrine

Secreted by the adrenal medula

Epinephrine

لـ نفسه الادرينالين

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

all digestive enzymes

- Phosphatases moves a phosphate groub from a protein.
- Peptidases (Proteins)
- Lipases (fats)
- glycosidase (carbs)

ex. amylase, pepsin

* all digestive enzymes starting from the salivary amylase in the mouth and ending with all the enzymes found in the intestine.

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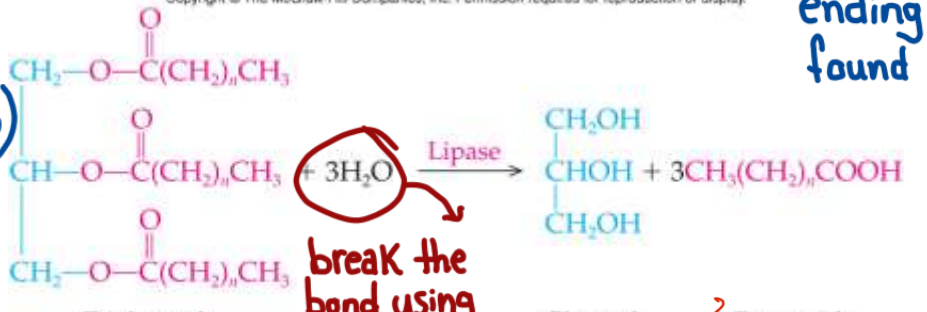

break the bond using water

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

forming or breaking down a double bond (even if water is used)

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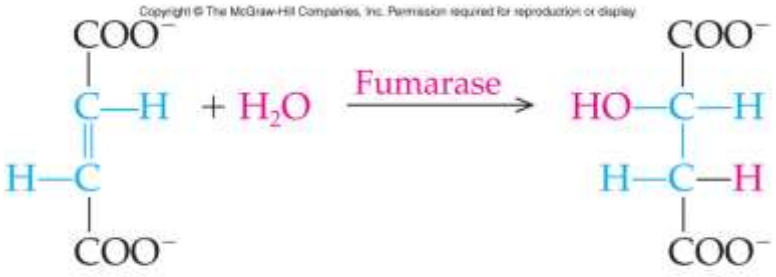
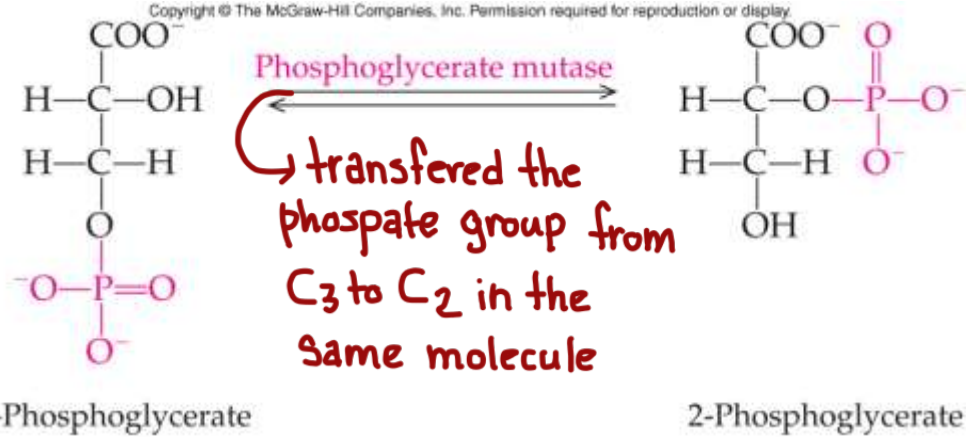


5- Isomerases (**EC.5**) catalyze intramolecular rearrangements, such as (Alanine racemase [EC 5.1.1.1])

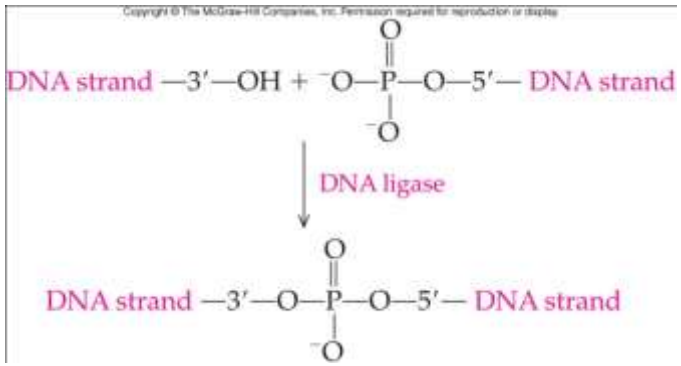
- Epimerases work on epimers.
- Mutases



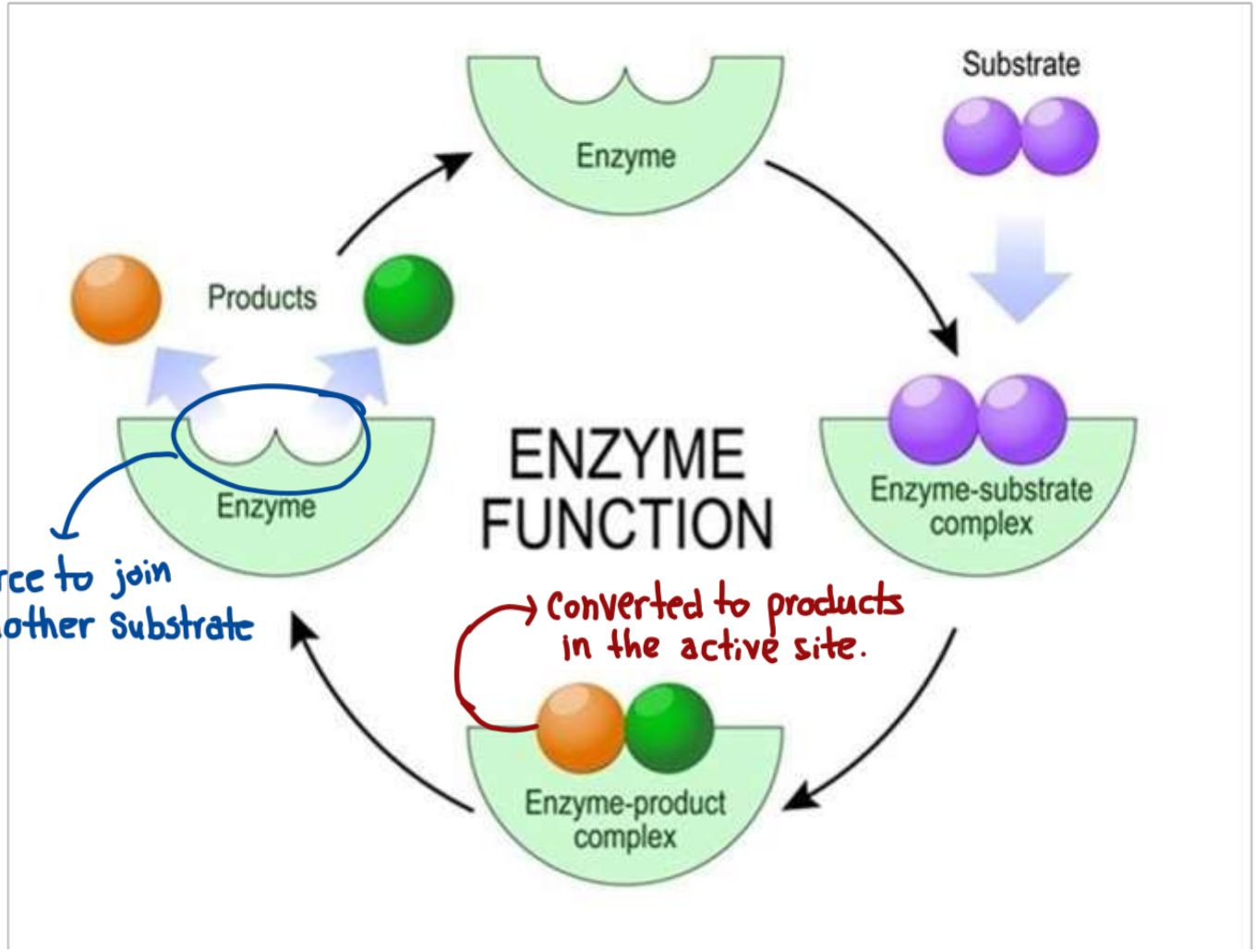
مجموعات رابطة

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 (Isoleucine-tRNA ligase [EC 6.1.1.5])

ligation (bonding) using a covalent bond (require energy) "from ATP"



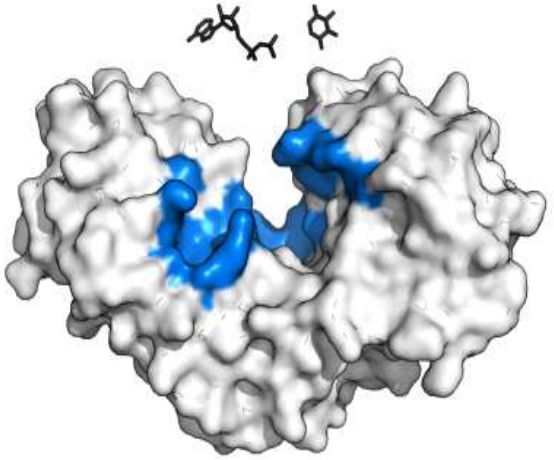
- the most common one is T4 DNA ligase, which connects the DNA fragments to give continous sequence of DNA


Substrate
Enzyme
Products
Enzyme
ENZYME FUNCTION
Enzyme-substrate complex
free to join another substrate
converted to products in the active site.
Enzyme-product complex

Active site

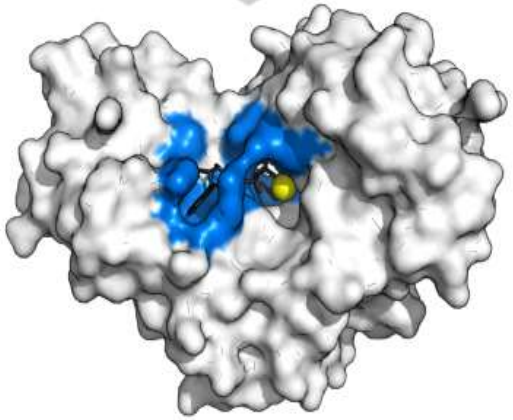
Contain spesific groub of amino acids which are highly reactive.

- 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



*the active site have 3D configuration (complementarity between the substrate and the enzyme to be will stablized) to allow the binding of the substrate (monomeric enzyme)

*multimeric enzyme (more than one polypeptide chain) the active site is located in the interphase between the sub units (recruting of more active sites as the active groub of the first chain will react with other active ones on the other chain (substrate will be highly stabalized)



* active site: region with specific 3D structure that binds to a substrate facilitating a biochemical reaction.

- contains amino acid residues that directly participate in catalysis (reactive)
- the complementarity of the shape of substrate and enzyme is important.

* types of functional amino acid groub in the active site:

→ hydroxyl groub (Ser, Thr, Tyr) + hydroxy proline.
→ imidazole ring (His)
→ thiol (sulfhedral) groub (ces)
→ carboxylate acid (Asp, Glu)
→ basic amino acid (Lys)

* the arrangment of the reactive groups on the active site is responsible for the specificty.

* in multimeric enzyme:

the active site is formed by the interaction of multiple subunits, where each subunit contributes amino acid residues for the formation of the active site.

the cooperative binding occurs, the binding of a substrate to one unit will influence the affinity of the other units for the substrate, the non covalent bonding (hydrogen, vander waal, electrostatic interaction) between the substrate and the amino acid groups from different subunits of the enzyme will help highly stabilizing of the enzyme-substrat complex.

the active site is located in the interphase between the subunits.

(recruting of more active sites as the active groub of the first chain will react with other active ones on the other chain

(Substrate will be highly stabalized)

Enzyme substrate binding

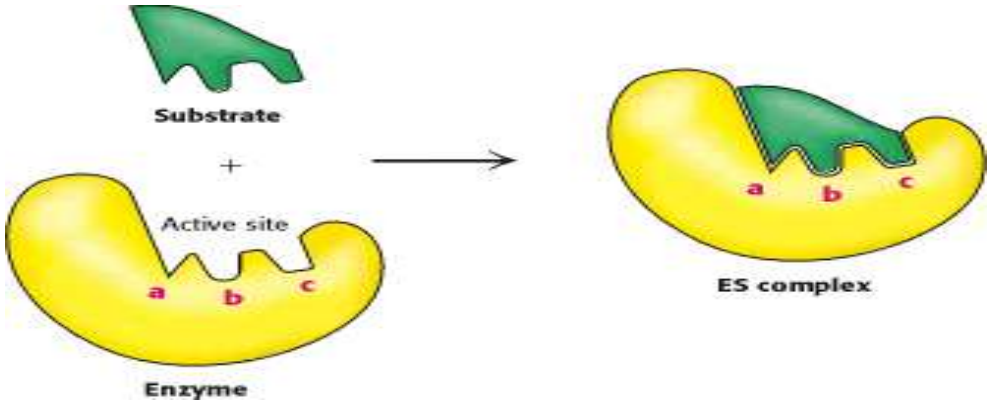
-Two models have been proposed to explain how an enzyme binds its substrate: the lock-and –key model and the induced-fit model.

↳ fixed rigid model, in there's no complementarity → no reaction (no change in the shape)

- 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.

↳ resposible for the specifity of the enzyme.



Induced-Fit Model of Enzyme-Substrate Binding

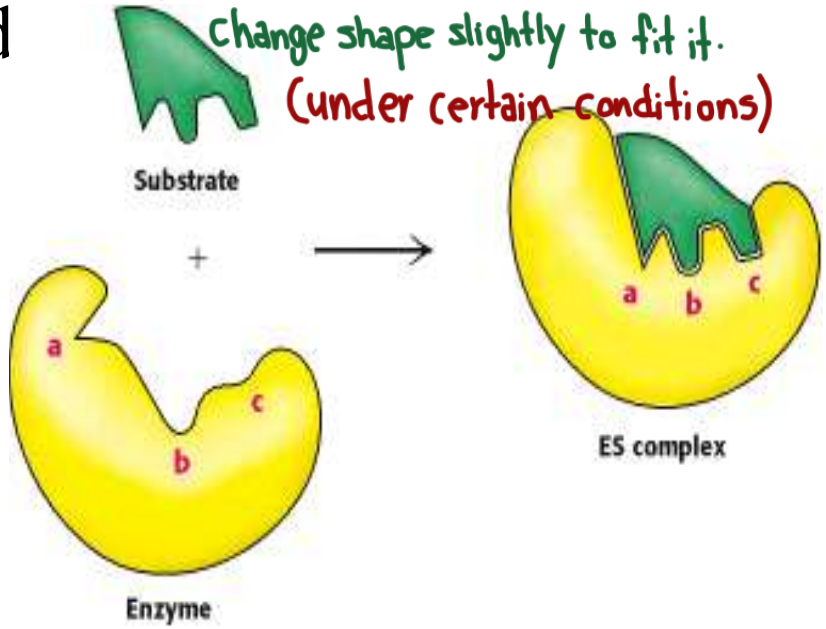
dinamic. flexible.

- In this model, the enzyme changes shape on substrate binding. (first, there's no complementary between the substrate and the active site, the moment of binding it won't be well stablized, then, the shape of the active site will change to fit the binding substrate)

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

Proximity: the substrate approaches the active site, in the begining it won't fit, but then the active site will change shape slightly to fit it. (under certain conditions)

- 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).



* when the reaction ends, the model return back to its original form.

the induced fit model describe how enzyme undergo conformational changes upon binding with a substrate, optimizing the fit between the enzyme's active site and the substrate

_this dynamic interaction enhances catalytic efficiency in biological reactions.

* the enzymes active site undergoes conformational changes when it interact with the substrate, initially the active site may not be an exact match for the substrate, as the substrate binds to the active site, the enzyme undergoes adjestments in its shape to accomodate the substrate more effectively.

the induced conformational change results in a tighter fit between the enzyme and the substrate, facilitating the formation of the enzyme-substrate complex.

_ bringing the reactive groupes into close proximity, lowering the activation energy required for the reaction to occur.

[the enzyme and the substrate undergo mutal adjustment to enhance the efficiency of the catalytic process]

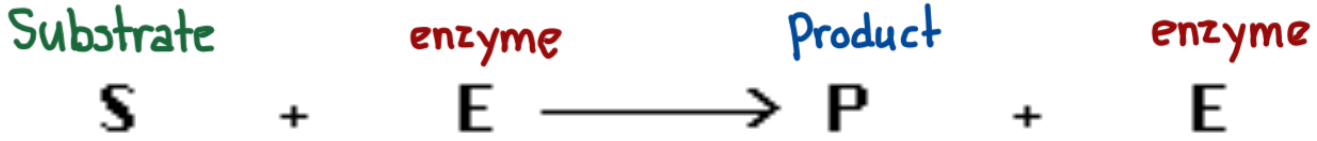
* this occur under certain conditions:

→ hormons

→ some neurological signals.

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:

1- Thermodynamic changes
2- Processes at the active site

→ inside the active site

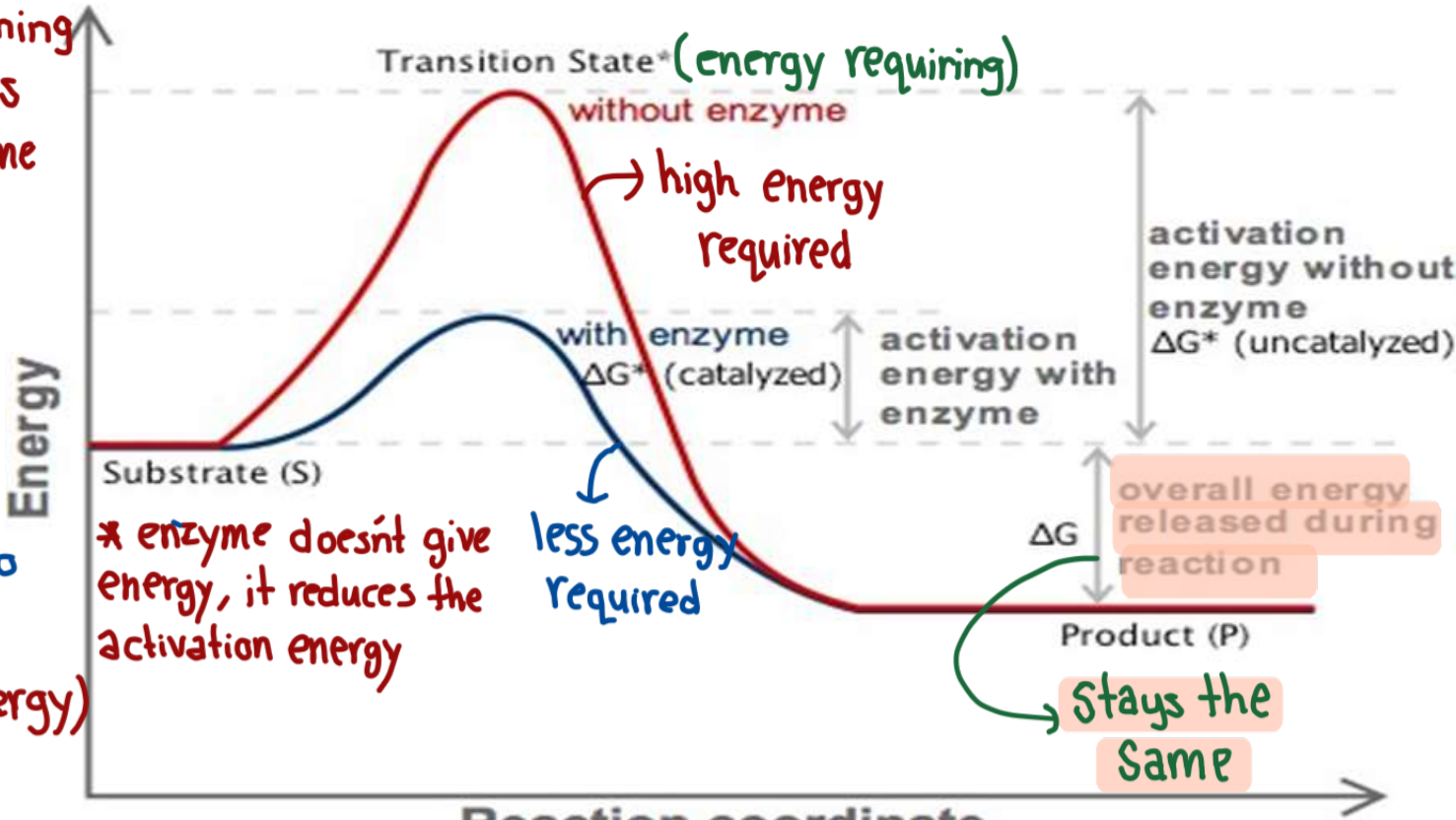
Thermodynamic changes

if we compare the catalysed reaction and the non catalysed reaction, the difference is the activation E

- All enzymes accelerate reaction rates by providing transition states with a lowered $\Delta G^{\ddagger}$ for formation of the transition states.
- The lower activation energy means that more molecules have the required energy to reach the transition state.

* two reactions happening in the same time, one is not catalysed by enzyme and the other is catalysed by enzyme

* for the reaction to occour, the enzyme-Substrate complex need energy to reach the transitional state (activation energy)



Processes at the active site

enzyme can use one of these methods, or a variety of them.

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. (proper arrangment and close approach)

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

for the reaction to happen, the ionization of groups is wanted

(most with acidic and basic groubs)

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

- 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.

1) Catalysis by proximity
   - very spesific orientation.
   - the substrate molecule is going to reach the active site

- the interaction between the active groub in the active site, and the reactive groubs in the binding site of substrate to form bonds (hydrophobic, electrostatic, hydrogen and sulfhedral bond)
  - to form these bonds, the substrate molecule should be in what we call, "the bond forming distance" which is the distance at which the reaction of the reactive groub in both substrate and active site takes place.
- we also have crowdness around the active site, more than one substrate molecule will gather around the enzyme to go in the active site, but only one at a time (high local substrate region around the active site)
  - → afterwards, there'll be orientation and modifying of the active groub in substate and active site to interact.

approach
↓
bond forming distance
↓
Crowdness of Substrate molecule
↓
orientation and modification.

* we have three important Keypoints:
  - → bond forming distance between substrate and the active site of the enzyme.
  - → region of high local Consentration of substrate (Crowdness)
  - → modification and orientation of the active site and substrate
    - for the groubs on both to meet and form bonds between them.

- the interaction bond between the reactive groub on both active site and Substrate should not be strong, because the substrate will eventually leave the enzyme as product, if the bond was strong, the substrate will be firmly bonded and will leave hardly.

* types of weak interactions:
  - → hydrogen
  - → Hydrophobic
  - → salt bridge

2) acid base catalysis:

- the presence of the ionizable groubs is important for these reactions to happen.
- enzymes use ionizable amino acid residues to donate or accept H^+
  - during a reaction to facilitate the conversion of substrates to products.
  - ex. amino acid like His, Asp, Glu, are often play a role in acid-base catalysis.
    - * for the reaction to happen, both reactive groubs should be ionized
      - ionization will be more with acid and base (Glu, Asp, His, ... etc.)

- general acid catalysis: Partial proton transfer from a doner (ionization of reactive groub) allowing the interaction of the binding site of substrate and the active site of enzyme, this will facilitate lowering of the activation energy. and the movement of molecules (result in bombarding movement energy inside the reaction → energy will increase for all substrate to facilitate the reaction)
  - [enzyme donates a proton to the substrate]
    - ex. Asp, Glu

- general base catalysis: Partial Proton acceptance,
  - [the basic groub of the enzyme accept protons from the substrate]
    - facilitate the chemical reaction
      - ex. His

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.

Stressing the bond to facilitate the breakage by binding unfavorably with enzyme

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

Three stages in covalent catalysis:

1- Nucleophilic reaction between enzyme and substrate

2- Electrophilic withdrawal of electrons from substrate

3- Elimination reaction (reverse of stage 1)

3) catalysis by strain:
(not for all enzymes)

→ lytic refers to the process where something is broken

→ Special for the enzymes preforming lytic reactions (reactions of the digestive enzymes that uses a water molecule for the cleavage of molecules (substrate)
_ we'll have binding of the substrate to the active site in unvavorable binding, the substrate binds in unfavourable configuration (slightly slanted) مائل
this will Put the boned to be cleaved (broKen) under stress,
being in this position will facilitate the function of the enzyme.
[facilitate the breakage of the bond]

→ this type of catalysis is also not for all enzymes.

4) Covalent Catalysis: (3 Stages)
through the formation of enzyme-substrate covalent bonding,
this covalent bond is temporarly (transient)
→ stage 1: this covalent bondig will create a nucleophilic reaction between the enzyme and substrate, there's give and take of protons to ionize more groups on the active site of enzyme and the binding site of the substrate,
→ stage 2: electrophilic interaction where the enzyme attrats electrons from the substrate.
→ stage 3: reverse of stage 1 to breaK the temporarely covalent bond.
to regenerate the original enzyme.

5- Metal Ion catalysis

these metal ion must be provided through the dietary intake specialy the ones acting on the energy production (ex Fe)

- Two classes of metal ion dependent enzymes:

1- Metalloenzymes contain tightly bound transition metal ions (Fe2+, Fe3+, Cu2+, Zn2+, Mn2+) can't work without it, metal ion is an essential part of the enzyme. if removed → no reaction

2- Metal-activated enzymes loosely bind metal ions (alkali or alkaline metal including Na+, K+, Mg2+ and Ca2+)
→ won't act effciently, not essential Part, if removed it will work but not effciently.

- Metal ions enhance catalysis in three major ways:

1- Binding to and orienting substrates for reaction as Mg2+ binding to ATP → different bridges mentioned below.

2- Mediating redox reaction through changes in oxidation state such as reduction of O2 to H2O through electron transfer → remove $\bar{e}$ from the substrate.

3- Electrostatic stabilization or shielding of negative charges as Mg2+ binding to ATP if ionization occured and the negative charges came back, there'll be no ionization, no reaction, cause there should be a limited ionization cause if the ionization rate ↑, the bonds will be broken between the enzyme and substrate, therefore there'll be no stabalization of the substrate. (prevent the (-) charges from reaching the ionization)

5) metal ion catalysis:

we have three types of connections:

→ first, the metal ion is in between the enzyme and substrate, attracts the substrate toward the enzyme [enzyme- metal-substrate bridge]

→ second, the metal ion is behind the substrate and enzyme, pushs the substrate toward the active site [metal-substrate-enzyme bridge]

→ third, the metal ion is after the enzyme, push the enzyme toward the substrate [substrate-enzyme-metal bridge]

* we can enhance this type of catalysis by controling the presence of ions

ex. for the crep cycle (ATP production), iron and copper are the most important metals in the electron transportage chain.

* metal ion catalysis refers to the use of metal ions to accelerate the chemical reaction, it's done by accepting electrons, facilitating electron transfer, stabilizing charged particles ... etc.

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 rearrangment of charged species in a certain way that enhances the reaction during the transition state.
- Electrostatic interactions are stronger
- 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

→ used as antioxidant (as soon as free radicals form inside our cells, it destroys it) this enzyme is abbriviated as SOD, it functions as antioxidant, guides polar substrates, maintains cellular redox.

6) electrostatic catalysis :

involves the use of electrostatic interaction (atraction and repulsions between charged particles) to facilitate chemical reactions.

- during the transition state, electrostatic catalysis stabalizes the transtion state by strategically placing charges to reduce repulsion or increase attraction between reacting species(facilitate the reaction)

-there's also an excluding of water from the active site to create low dielectric constant (reduced ability to insulate electrical fields, more conducive to the transmission of electric signals)

- we have an electron cloud of electrons surrounding the enzyme-substrate (doesn't belong to any) present in the feild all the time, forms some sort of interaction without the transfer of electrons (no donating, no gaining) but they're still present there to facilitate the ionization.

---

*enzyme specifity :

- Some reaction are being catalysed by unrelated enzymes, ex. in the Kreb cycle, the enzyme isocitrate dehydrogenase (dehydrogenation) ↪ oxidation.

this enzyme is found to catalyse another reaction in the citric acid cycle which is a decarboxylation reaction (results from the dehydrogenation reaction good leaving groub)

Enzyme Specificity

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

this enzyme is for this reaction, and it can't catalyse any other reaction.

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

ex. methylase: add methyl / decarboxylase: remove CO_2
deamylase: remove amine groub (more notes above)

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

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

breaking of a particular bond without carring what's around the bond, ex. ligase enzyme digest triacilglicerol using 3 H_2O molecules, (enzyme act on the bond)

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

acting on specific isomers.

- Some enzyme require cofactors to be active (another classification)
- Cofactors are a non-protein components of the enzyme. → vitamins temporarily attached
- Organic Molecules (Coenzymes)
- Inorganic ions e.g., Ca2+ , Zn2+

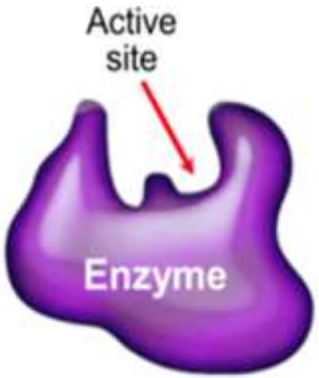
(Prosthetic group) Permanently attached

- Cofactors may be:

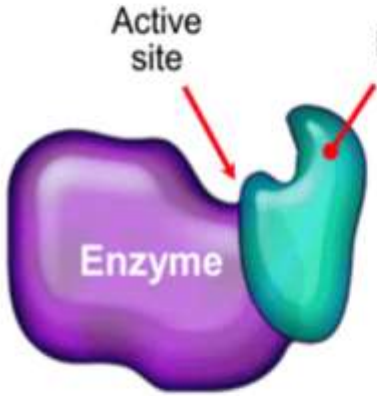
1- The Permanently attached cofactors, are called Prosthetic group (such as a vitamin, sugar, or lipid or inorganic such as a metal ion) if removed → no reaction

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

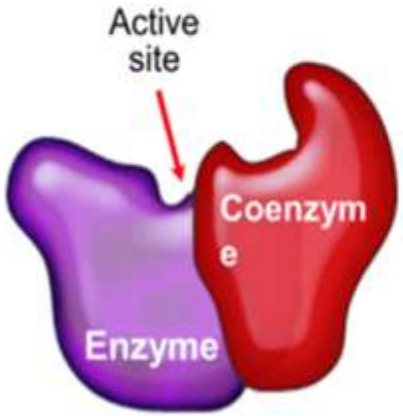
→ attached during catalysing the reaction, and after completing it will leave.



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.

coenzymes

- 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

linked only during the reaction.

ex. NAD with lactate dehydrogenaze, it acts as a H carrier, but NAD is not specific for this enzyme, it can work with any dehydrogenase enzyme (NAD derived from vit. B3)

Prosthetic groups

↳ mostly inorganic but could be organic (vit. derivatives)

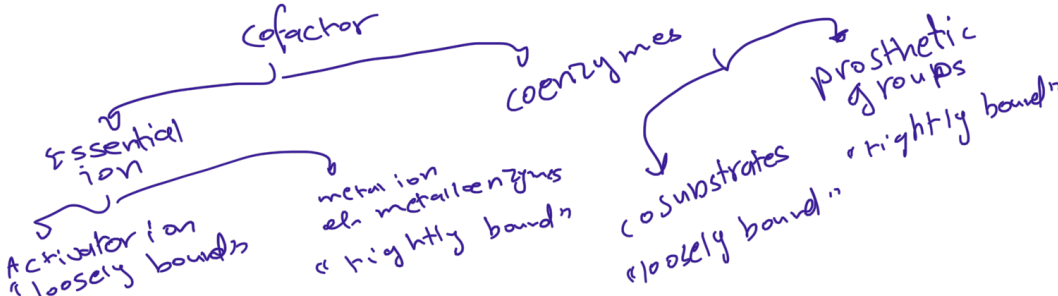
- Tightly integrated into the enzyme structure by covalent or non-covalent forces. e.g.;

Pyridoxal phosphate from vit. B6
Flavin mononucleotide (FMN) from vit B_2
Flavin adenine dinucleotide (FAD) from vit B_2
Thiamin pyrophosphate (TPP) from vit B_1
Biotin from vit B_7 (often called vit. H)

vitamin derivatives but prosthetic

Metal ions – Co, Cu, Mg, Mn, Zn

- Metals are the most common prosthetic groups



Coenzymes

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

play a role in trasfering groups from origin to final distenation.
ex. NAD transfer H to the electron transportage chain

- 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).

coenzymes are not specific to one reaction

Important Prosthetic Groups and Coenzymes

Prosthetic Group	Enzymes/ Proteins
Zn^{++}	Carbonic anhydrase , Alcohol dehydrogenase , Superoxide dismutase.
Fe^{+++} or Fe^{++}	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 ex. the main test for the diagnosis of diabetes is the glucose oxidase test (using enzyme to estimate the level of blood glucose)

Enzymes as diagnostic markers

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

- 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. found in high concentrations in the blood, if were found in high concentrations in the cells, there's leakage

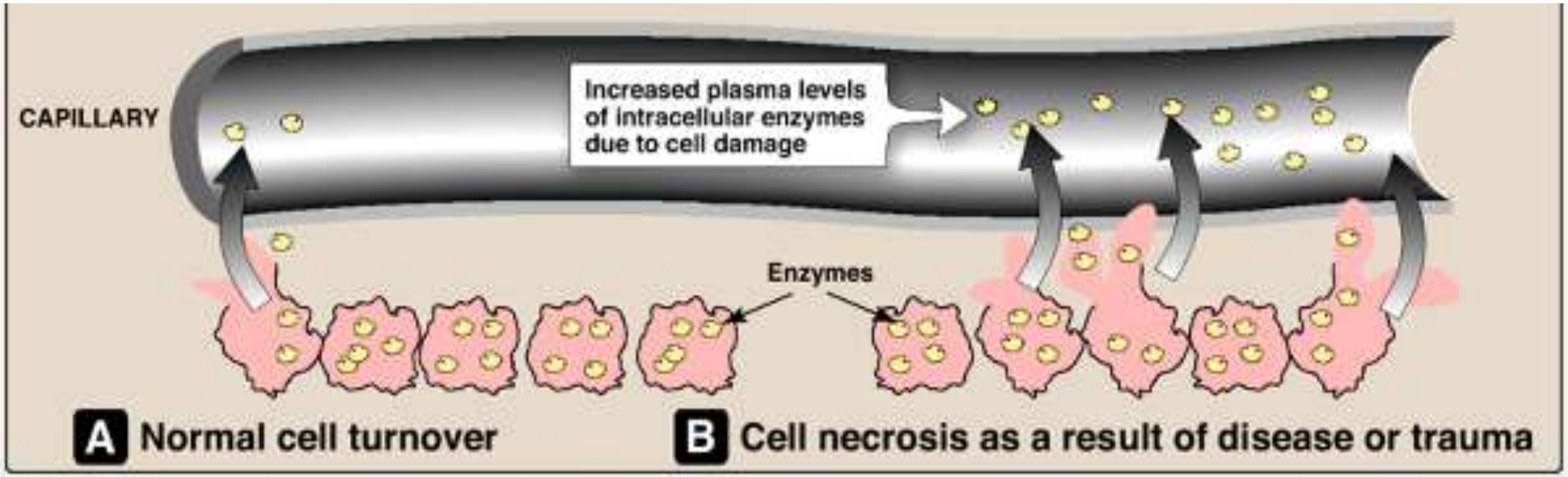
Examples of these functional plasma enzymes include **lipoprotein lipase**, **pseudo cholinesterase**, and the **proenzymes of blood coagulation and blood clot dissolution** .

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

found in high concentrations in the cells, low concentrations in blood
if were found in high concentrations in the blood there's leakage



Isoenzymes (Isoenzymes)

isomers.

- 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.
- 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.:

* متشابهين وظيفياً وتركيبياً.

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. also found in other cells.
- M is optimized to work under anaerobic conditions and H optimized to work under aerobic conditions.

* isoenzymes (isomers)

→ agree in three points (every thing related to the reaction):

- catalysing the same reaction
- acting on the same substrate
- giving the same product.

ex. some enzyme have 9 isoenzyme, all of them catalysing the same reaction, acting on the same substrate, giving the same product.

→ differ in three points:

- origin (ex. one secreted from the liver and one from kidney, pancrease ... etc)
- effect of inhibitors and activators, ex. some preform complete inhibition, some partialy, some are never inhibited by the inhibitors.
- physical character, due to the different migration rate under the effect of electristy (when we seperate them from eachother by electrophoresis, it's found that they're different from eachother, ex. the molecular weight)

↳ lighter migrate faster and vise versa.

ex. lactase dehydrogenase enzyme, one of its isoenzymes is used in diagnosis of many diseases.

molecular weight of the lactase dehydrogenase enzyme is 140 KDa (Kelo dalton) and it's a tetrameric molecule (consist of 4 poly peptide chains of two types only M and H)

- lactase dehydrogenase enzyme have (5) isozymes, could be seperated by electrophoresis (differ in their molecular weight)

-There are 5 different isoenzymes.
-The relative ratio of the isoenzymes depends on the location in the organism as well as the developmental stage.

Isoenzyme	Tissue origin
LDH1 (H4) Pure H	Cardiac and kidney
LDH2 (H3M) 3H,1M	Cardiac, kidney, brain and RBCs
LDH3 (H2M2) 2H,2M	Brain, lung and WBCs
LDH4 (HM3) 1H,3M	Lung, skeletal muscle
LDH5 (M4) Pure M	Skeletal muscle and liver

homotetrameric

hetro

homo

CK/CPK Isoenzymes

CK: creatine kinase

- 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%

mostly found in blood

most important

myocardial infarction

until now not used in any diagnosis related to the brain.

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.