Enzymes

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Enzymes

•**Enzymes** are biological catalysts means substances that accelerate the chemical reactions.

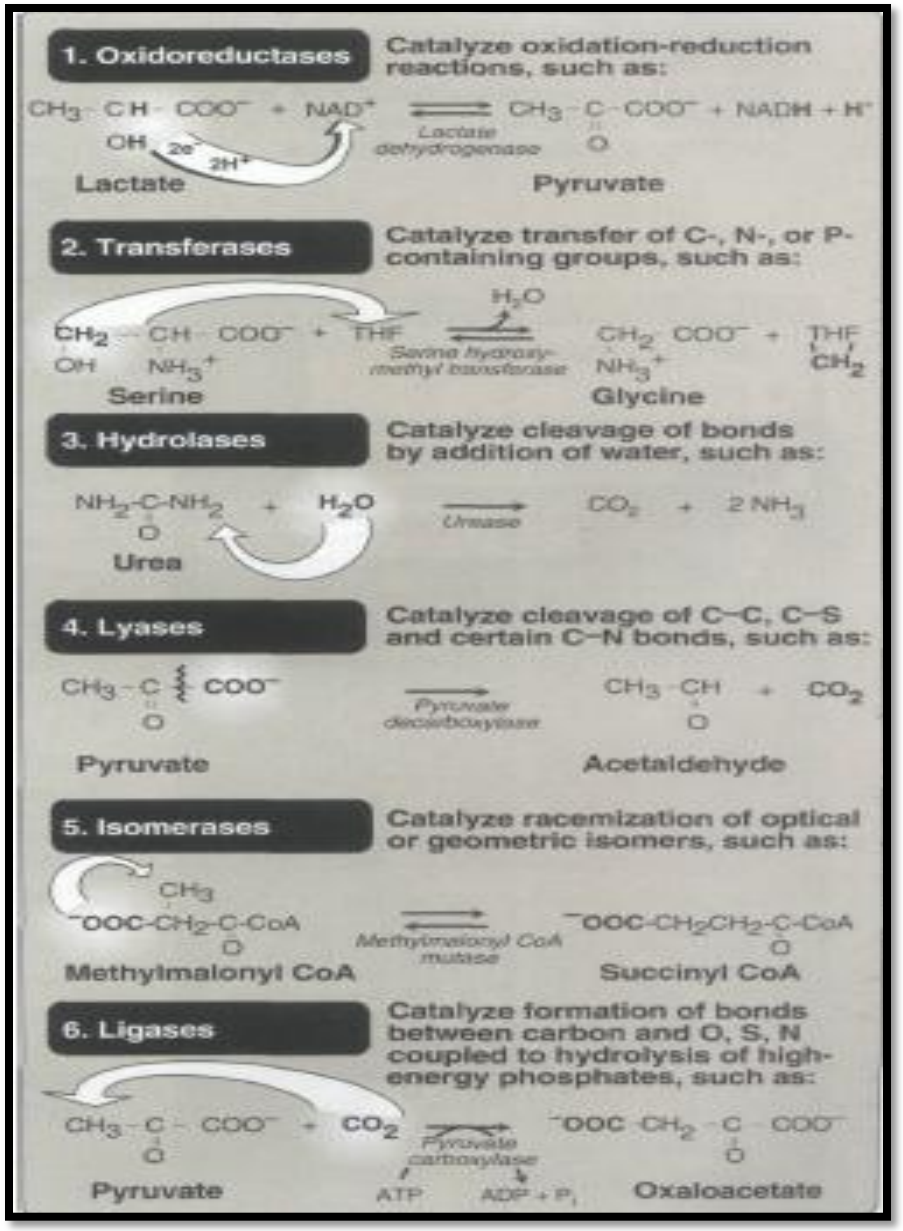
•Each cell is equipped with its own genetically determined set of enzymes which are involved in all metabolic pathways.

•Most of enzymes are proteins, although some of them are ribonucleic acids, the **ribozymes** which is an RNA molecule able to perform a specific chemical reaction.

Enzymes nomenclature

•The **recommended name** of the enzyme consits of the name of the **substrate** with suffex **ase** like *urease*, *glucosidase* etc**.**

• The **international union of Biochemistry** name depend consider the name depend on the **chemical reaction** being catalyzed like *pyruvate carboxylase*

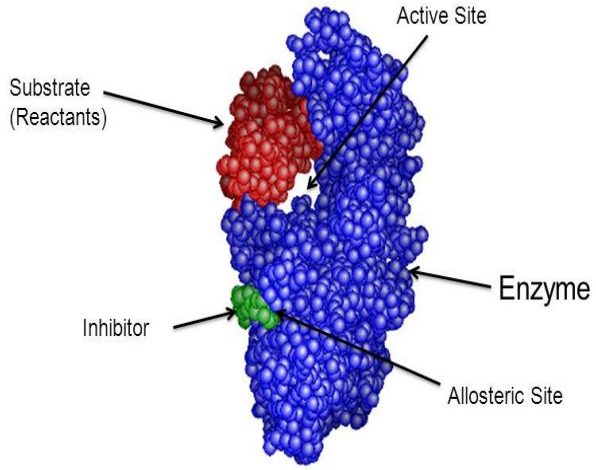


Other examples

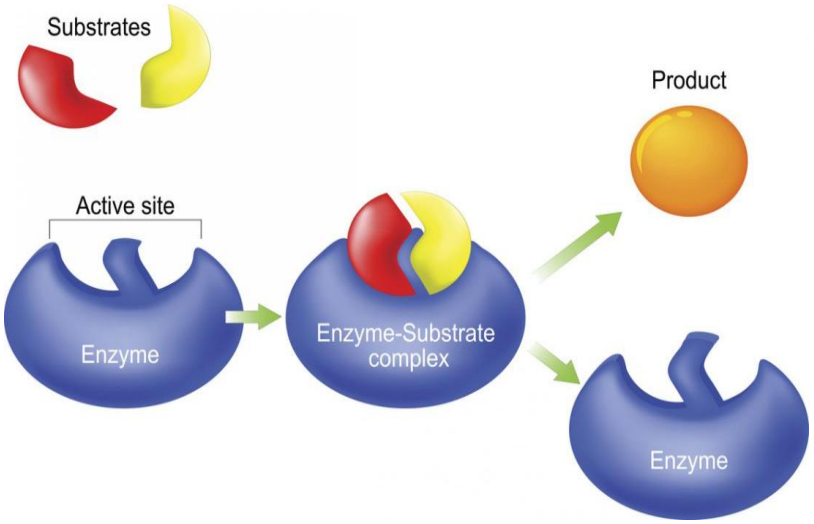
Class	Type of Reaction Catalyzed	Example
Hydrolase	Hydrolysis (catabolic)	Lipase—breaks down lipid molecules
Isomerase	Rearrangement of atoms within a molecule (neither catabolic nor anabolic)	Phosphoglucoisomerase—converts glucose 6-phosphate into fructose 6-phosphate during glycolysis
Ligase or polymerase	Joining two or more chemicals together (anabolic)	Acetyl-CoA synthetase—combines acetate and coenzyme A to form acetyl-CoA for the Krebs cycle
Lyase	Splitting a chemical into smaller parts without using water (catabolic)	Fructose 1,6-bisphosphate aldolase—splits fructose 1,6-bisphosphate into G3P and DHAP
Oxidoreductase	Transfer of electrons or hydrogen atoms from one molecule to another	Lactic acid dehydrogenase—oxidizes lactic acid to form pyruvic acid during fermentation
Transferase	Moving a functional group from one molecule to another (may be anabolic)	Hexokinase—transfers phosphate from ATP to glucose in the first step of glycolysis

Enzyme properties

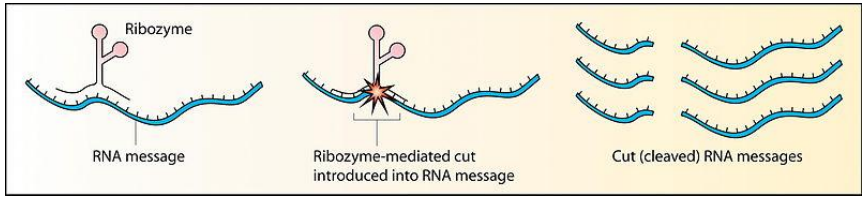
•**Active site** is a sequence of amino acids forms a three dimentional surface site complementary to the substrate.



• **S + E** produce **ES** complex which dissociate to E and product.



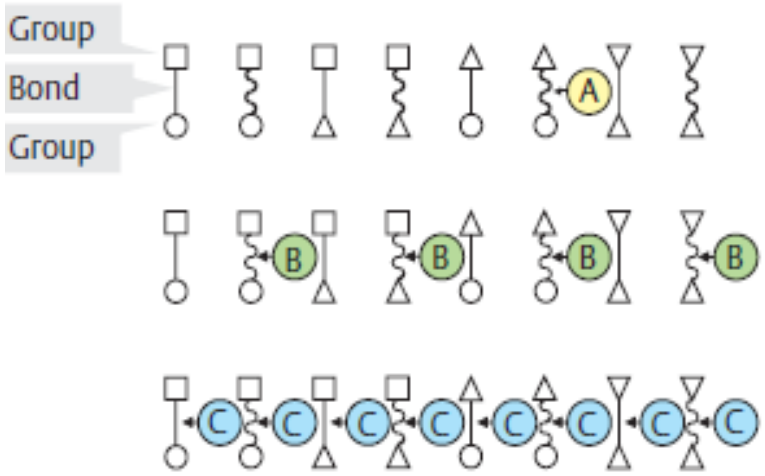
•**Catalytic activity of each enzyme** the catalyzed reaction is 10^3 -10^8 times faster than the uncatalyzed reaction, each enzyme capable of transforming 100-1000 substrate molecules each second. Which is called a **turnover number**



Reaction and substrate specificity

•The **enzyme** always catalyze one chemical reaction and is specific for this type of reaction, Interacting with one substrate which determine the reaction specificity.

•For example **bond breaking enzyme** could be Highly specific enzymes (**type A, top**) catalyze the cleavage of only *one type* of bond, and only when the structure of the substrate is the correct one. Other enzymes **(type B, middle)** have narrow reaction specificity, but broad substrate specificity. **Type C** enzymes (with low reaction specificity *and* low substrate specificity, bottom) are very rare.



	Reaction specificity	Substrate specificity
A	High	High
B	High	Low
C	Low	Low

Cofactors

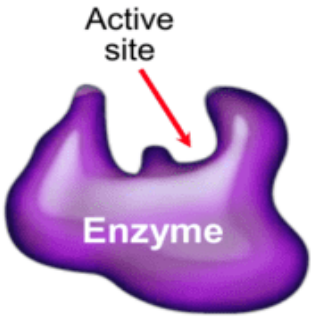
Some enzymes require **cofactors** to be active.

Cofactors are a **nonprotein** component of an enzyme. Cofactors can be:

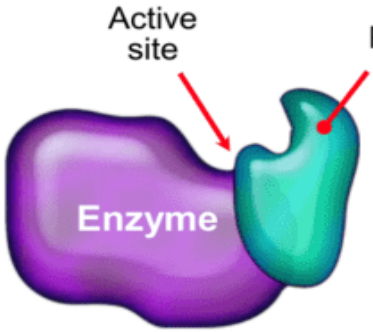
- **organic molecules** (coenzymes).
- **inorganic ions** (e.g. Ca^{2+}, Zn^{2+}).

Cofactors may be:

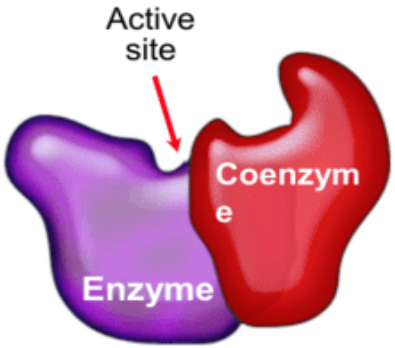
- **Permanently attached**, in which case they are called **prosthetic** groups.
- **Temporarily attached coenzymes**, which detach after a reaction, and may participate with another enzyme in other reactions.



Enzyme is protein only
Example: lysozyme



Enzyme + prosthetic group
Example:
flavoprotein + FAD



Enzyme + coenzyme
Example:
dehydrogenases + NAD

Cofactors

•**Prosthetic group** are tightly integrated to the enzyme protein's structure by covalent or noncovalent forces. Examples include pyridoxal phosphate, flavin mononucleotide (FMN), flavin dinucleotide (FAD), thiamin pyrophosphate, biotin, and the metal ions of Co, Cu, Mg, Mn, Se, and Zn. Metals are the most common prosthetic groups. The roughly one-third of all enzymes that contain tightly bound metal ions are termed **metalloenzymes.**

•**Cofactors** have similar functions of the prosthetic groups but transient bind either to the enzyme or to the substrate like ATP. have to be present in the medium of the reaction for catalysis to occur.

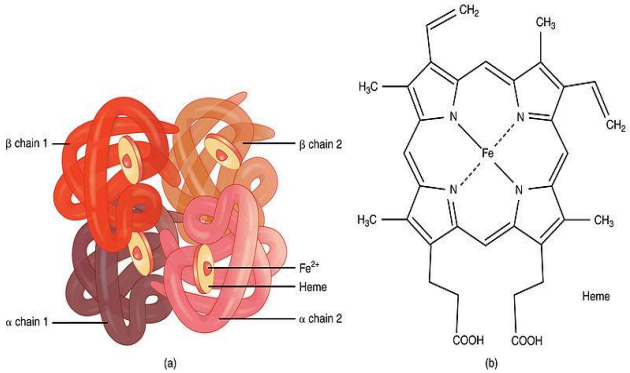
•**Enzyme** that need a metal ion cofactor called **metal activated enzyme**. metal ions such as Fe or Zn , and organic molecules.

• **Coenzymes**, are often derivatives of vitamins serve as recyclable shuttles or group transfer reagents. the **coenzyme NAD** contains niacin, **FAD** contains riboflavin, and coenzyme A contains pantothenic acid. also stabilizes substrates such as hydrogen atoms or hydride ions that are unstable in the aqueous environment of the cell.

•The enzyme activity can be regulated depend on the need of cell which can be activated or inhibited.

Prosthetic group Vs Coenzyme

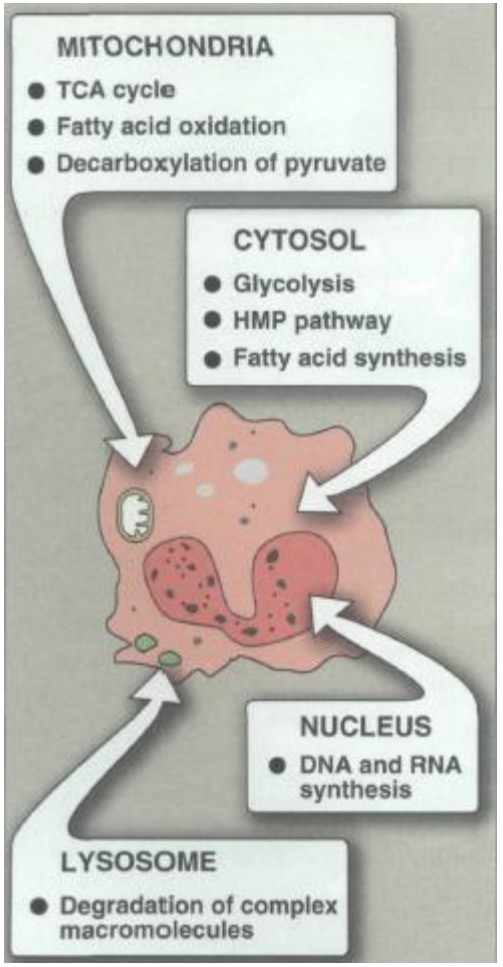
Prosthetic group	Coenzyme
Prosthetic group is a type of a helper molecule which is a nonproteinaceous compound that helps enzymes to perform their functions.	Coenzyme is a specific kind of cofactor molecule which is an organic molecule that helps enzymes to catalyze chemical reactions.
Bond with Enzymes	
They bind tightly or covalently with enzymes to aid enzymes.	They bind loosely with the active site of the enzyme to help catalytic function.
Composition	
Prosthetic groups are metal ions, vitamins, lipids, or sugars.	Coenzymes are vitamins, vitamin derivatives or nucleotides.
Main Function	
Prosthetic group mainly provides a structural property to the enzyme.	Coenzyme mainly provides a functional property to the enzyme.
Removal from the Enzyme	
Prosthetic groups cannot be easily removed from the enzymes.	Coenzymes can be easily removed from the enzymes.
Examples	
Examples include flavin nucleotides and heme.	Examples include AMP, ATP, coenzyme A, FAD, and NAD^+, S-adenosyl methionine



Hemoglobin

Enzyme localizaion in the cell

•Enzymes present in a specific organelles inside the cells serve to isolated the reaction substrate and product from other competing reaction.



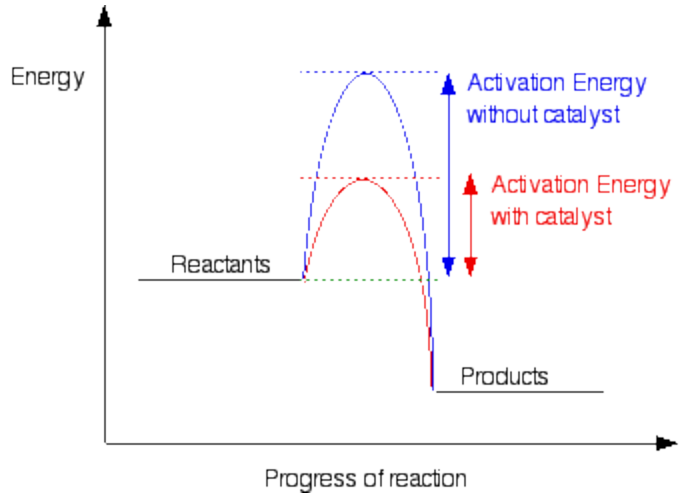
How enzyme works

•There are two aspects of the mechanism which enzyme works first the **energy changing** and second **the chemistry** of the **active site**.

•**Energy changing during the reaction**

The **energy barrier** in the chemical reaction is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product

$$A \rightleftarrows T^* \rightleftarrows B$$



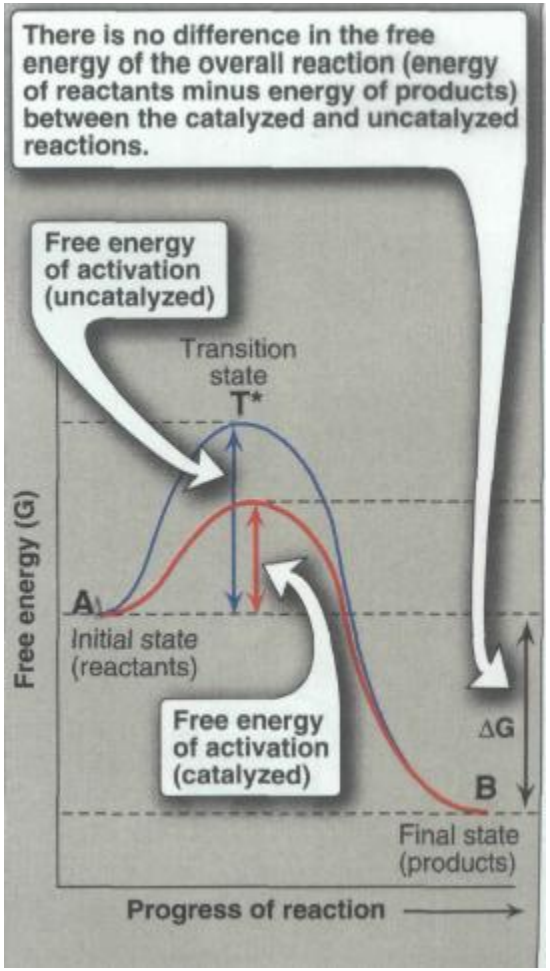
How enzyme works

•**Free energy of activation**

Is the difference in free energy between the reactant and T transition state (high energy intermediate) where the high-energy intermediate is formed during the conversion of reactant to product. Because of the high free energy of activation, the rates of uncatalyzed chemical reactions are often slow.

Rate of reaction

For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. In the absence of an enzyme, only a small proportion of a population of molecules may possess enough energy to achieve the transition state between reactant and product. The rate of reaction is determined by the number of such energized molecules. **the lower the free energy of activation, the more molecules have sufficient energy to pass through the transition state, and, thus, the faster the rate of the reaction**.



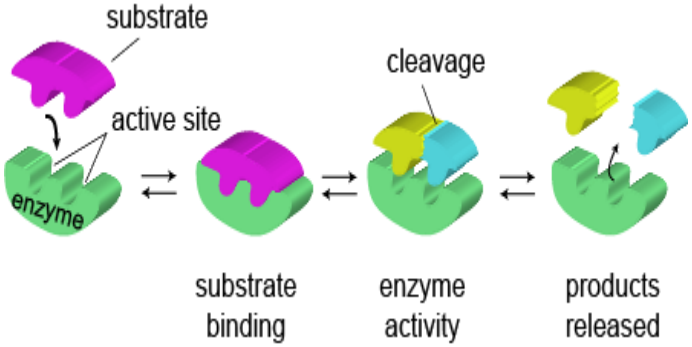
How enzyme works

•**Chemistry of the active site**

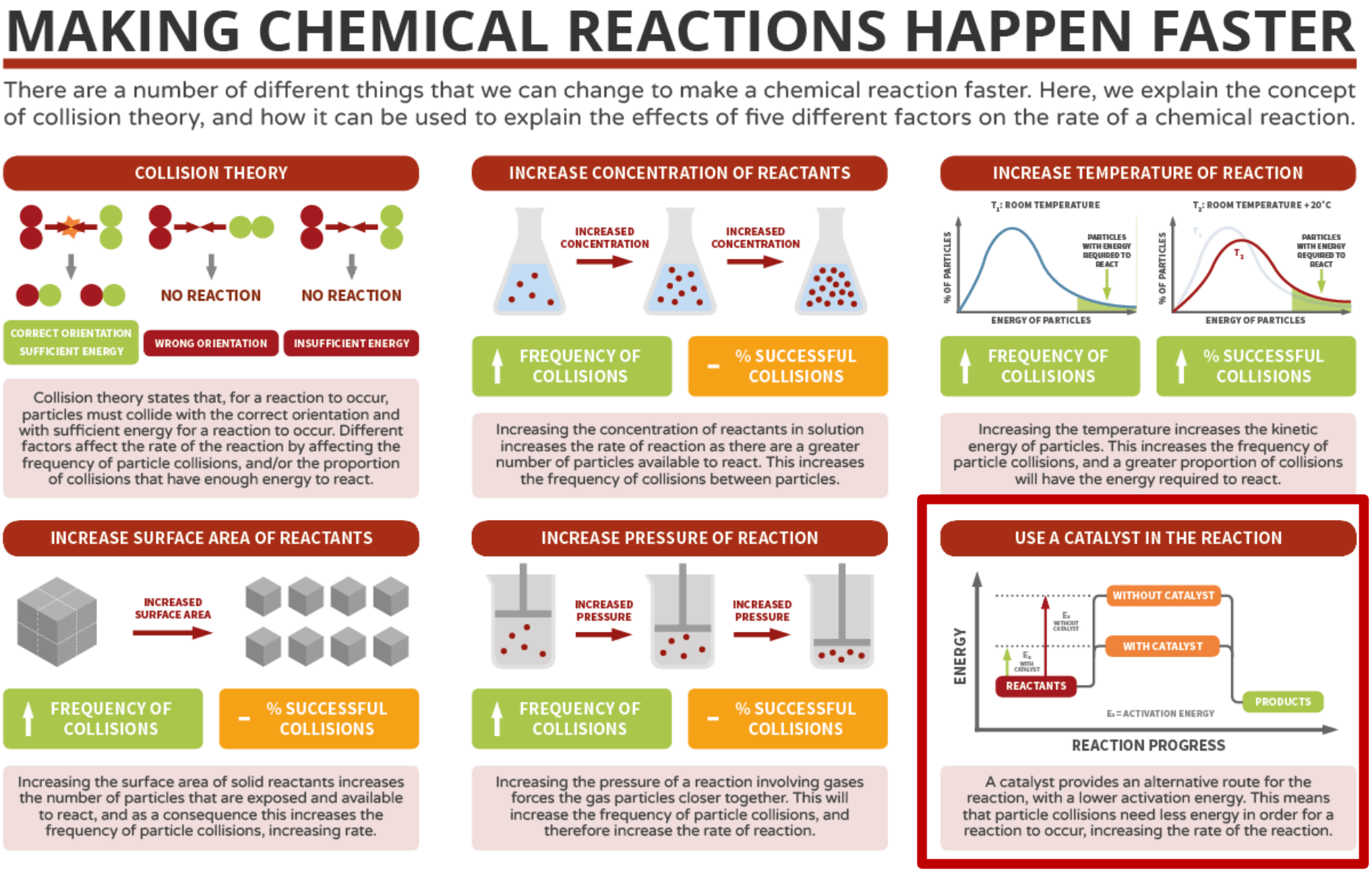
•The enzyme active site involve many chemical mechanism to convert the substrate to products .

1- stability of the substrate in transition state increase the concentration of the reactive intermediate that can be converted to product thus accelerate the reaction.

2-catalytic group provided by the active site like the amino acid residue which provide or accept proton in acid base catalysis reaction.



Factors influnce the chemical reaction velocity



Factors influnce the reaction velocity

- **Substrate concentration**
- **Temperature**
- **PH**

Factors influnce the reaction velocity

•**Substrate concentration**

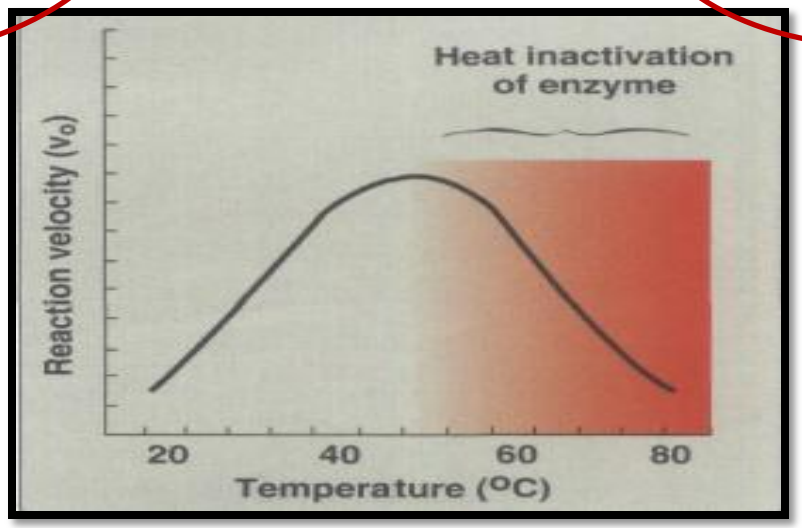
•The chemical reaction arrive to the maximal velocity (**V max)** when the all binding site of the available molecules is saturated with the substrate

•The **rate of velocity** is the number of substrate molecules converted to product per unit time; velocity is usually expressed as of **product formed per minute**.

•**Temperature**

Increased in number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction

decrease in reaction velocity as a result of temperature-induced **denaturation of the enzyme**



Factors influnce the reaction velocity

•**PH**

The concentration of the PH could affect the enzymatic reaction in two ways, first **ionization** of the active site, second enzyme **denaturation,** further more each enzyme have **optimum PH**

•**Ionization of the active site which depend on the H+ concentration**

The catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or unionized state in order to interact. For example the catalytic activity of some enzyme needs the amino group to be protonated NH3+ . In alkkaline PH the amino group is deprotonated and the reaction velocity decline .

•**PH effect on the enzyme denaturation**

•The extreme changes in the PH leads to changes in the protein structure because of changes in the amino acid ionic charecterizations.

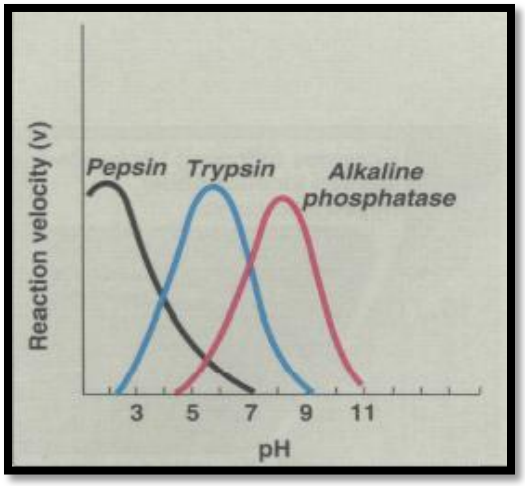
•**The optimum PH**

The ideal PH for each enzyme is different from each other like pepsin its ideal PH is 2. Wile alkaline phosphatase is optimally activated at alkaline PH environment.

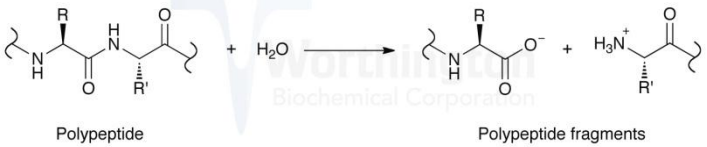
Example of Pepsin & Trypsin

Pepsin : which is an digestive enzyme produced in the stomach, breakdown protein into smaller peptides.

Trypsin: Another digestive enzyme produced from pancreas as an inactive form, being activated in the small intestine, where breakdown peptide into smaller peptide proteins.



Pepsin



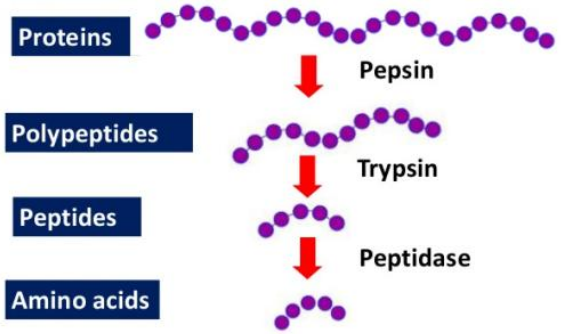
Polypeptide — Polypeptide fragments

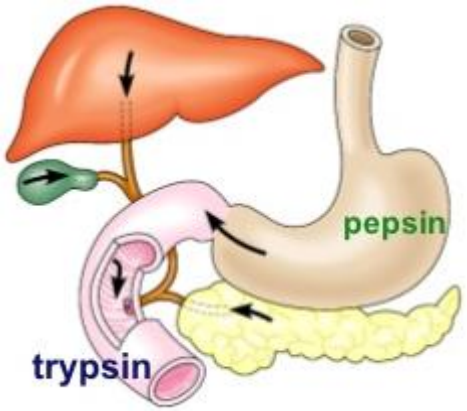
R and R' = Leu, Phe, Trp, and Tyr (preferred); also hydrolyzes esters

Trypsin

Polypeptide — Polypeptide fragments

R = Arg and Lys





Michalis – Menten Equation

•**The equation** The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:

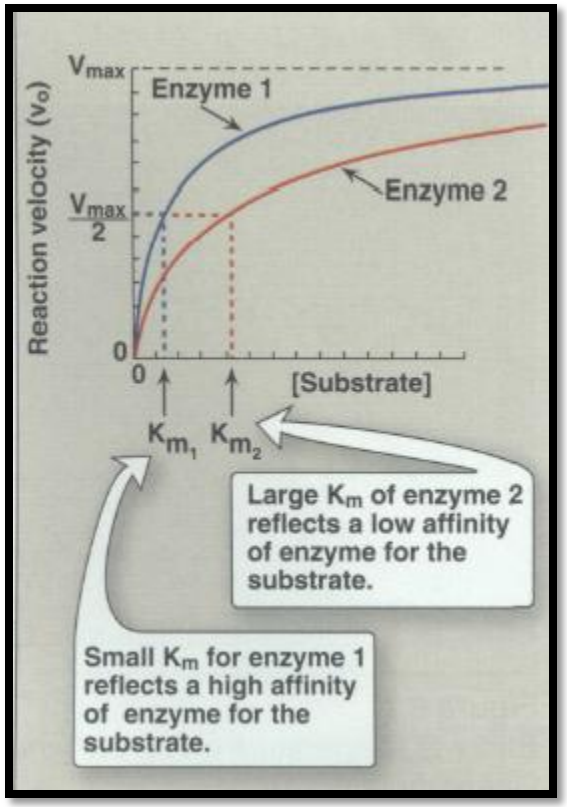
$$Vo = \frac{V_{max}[S]}{Km + [S]}$$

V0 = Initial velocity (moles/times)

[S] = substrate concentration (molar)

V_{max}= maximum velocity

K_m = substrate concentration at half V_{max}



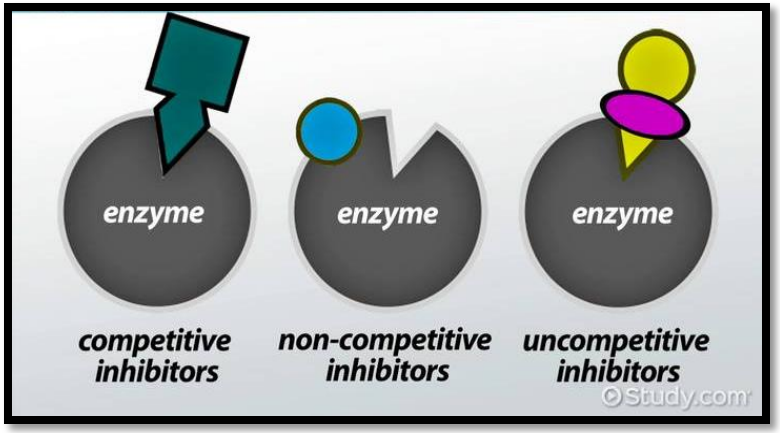
Enzyme activity inhibition

•**The inhibitor** a substance that decrease the velocity of the enzyme catalyzed reaction. There are two types of inhibitors.

•**Reversible inhibitors** make a noncovalent bond with the enzyme which by dilution of the complex dissociate the inhibitory bond

•**Irreversible inhibitors** when the dilution doesn't reverse the inhibition activity.

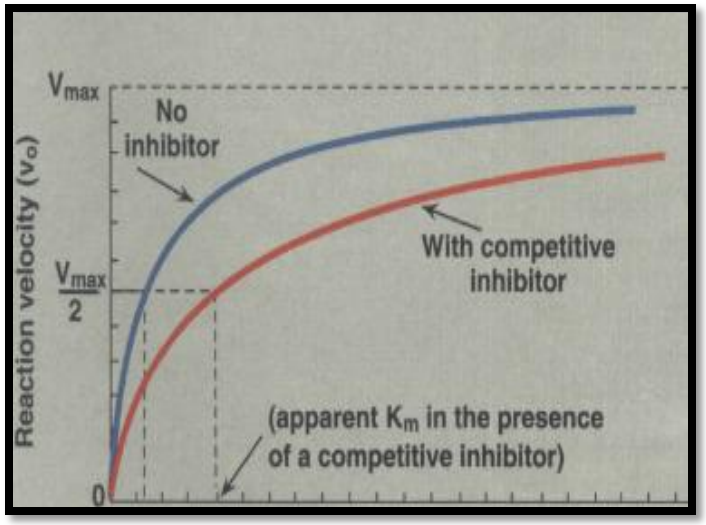
•The enzyme inhibition could be **competitive** or **non competitive**



Enzyme competitive inhibition

•**Occurs when the inhibitor binds to same site of the substrate.**

•There are many factors could influence this inhibition reaction:



•**Vmax**: increasing the substrate concentration reverse the inhibitor effect.

•**Km**: the Km is increased by the competitive inhibitors means more substrate is needed to achieve the ½ of Vmax.

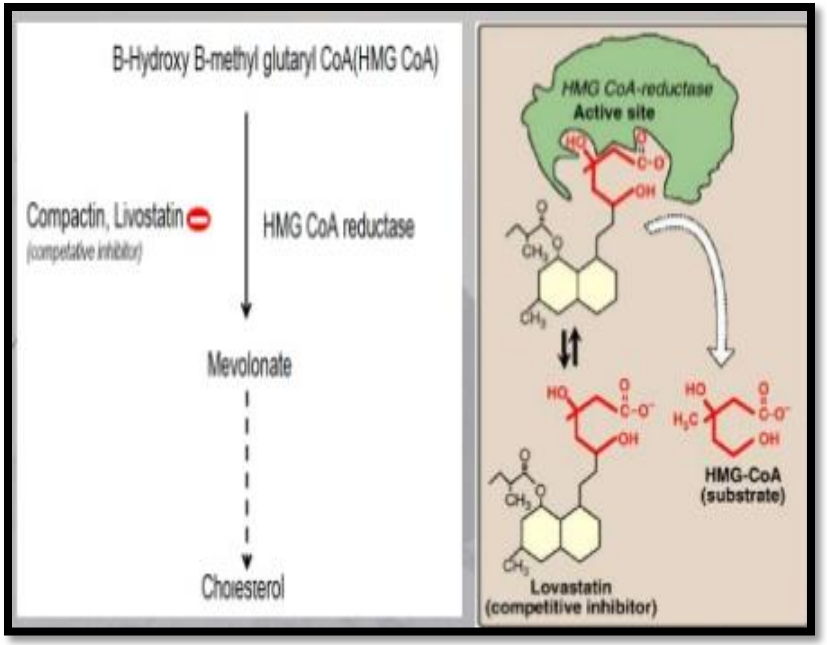
•**Satins drugs are example of the enzyme competitive inhibitors.**

Enzyme competitive inhibition

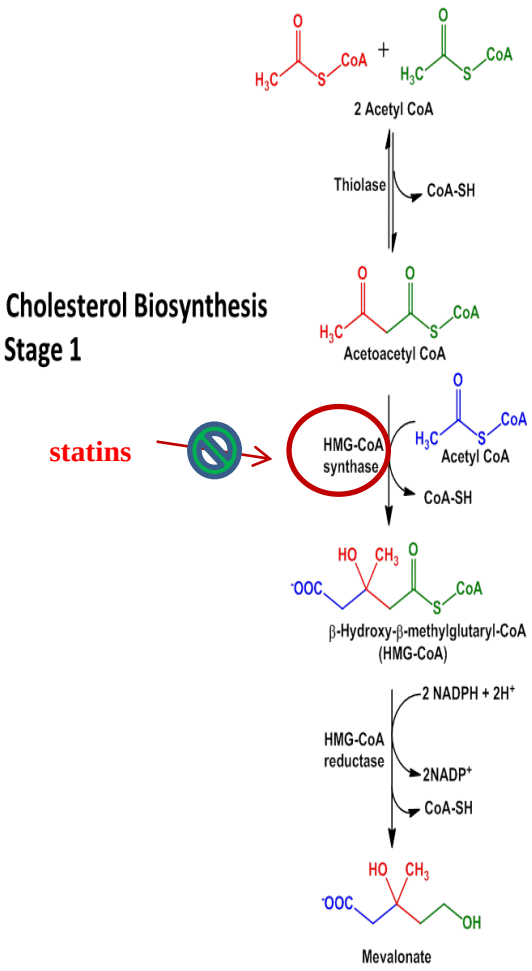
•Statin drugs is an important anti hyperlipidemic group and its mechanism of action is an example of enzyme competitive inhibition.

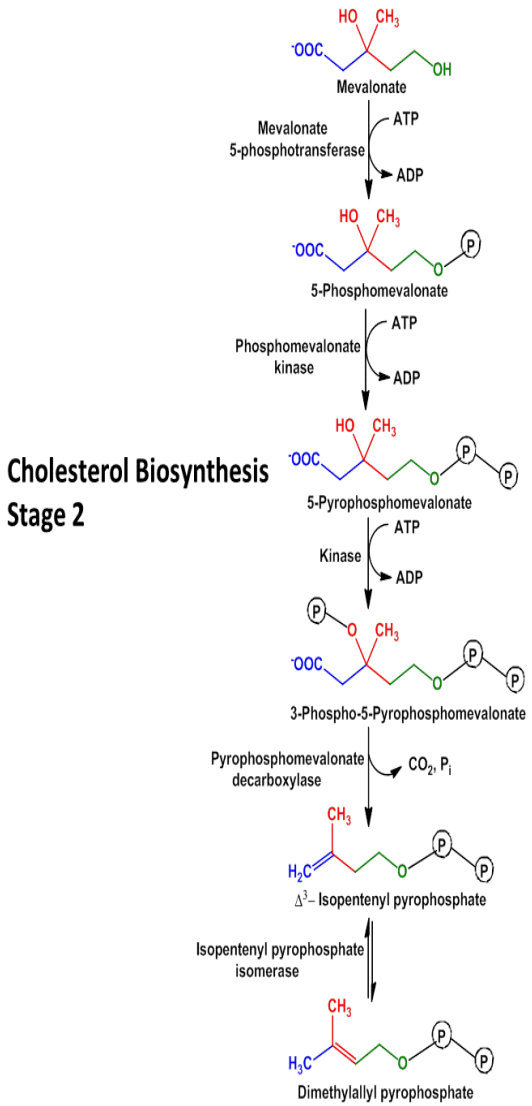
•The first step in cholesterol synthesis require HMG CoA (hydroxymethylglutaryl CoA reductase enzyme reductas).

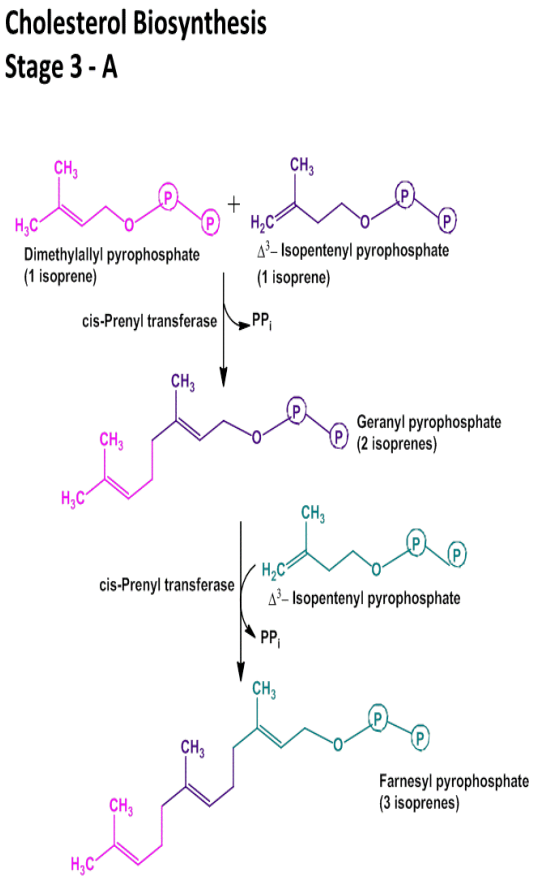
•Statins drugs like atorvastatin and simvastatin are analogue of the HMG CoA reductase and compete effectively to the active site



Cholesterol Biosynthesis

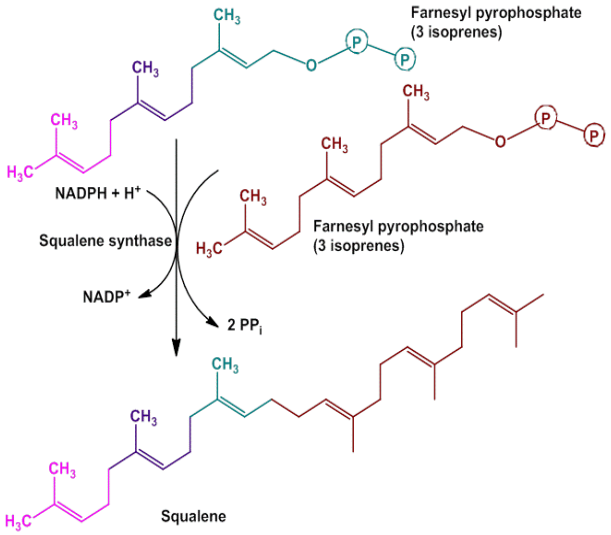




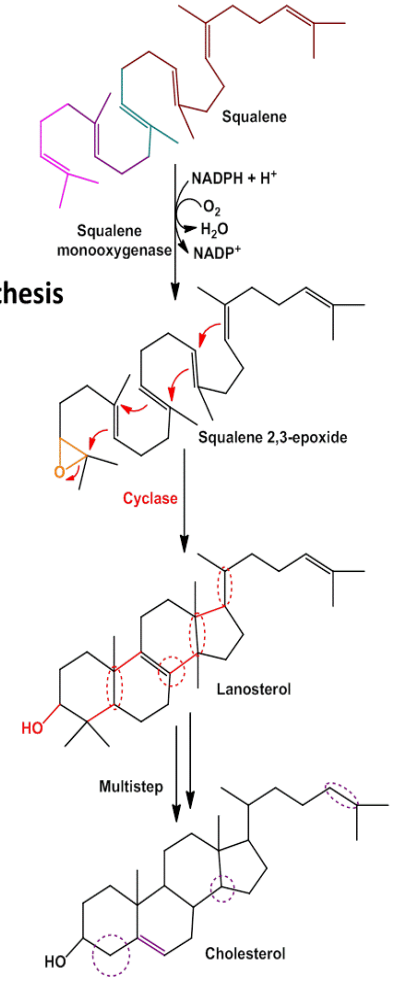


Cholesterol Biosynthesis

Cholesterol Biosynthesis
Stage 3 - B



Cholesterol Biosynthesis
Stage 4



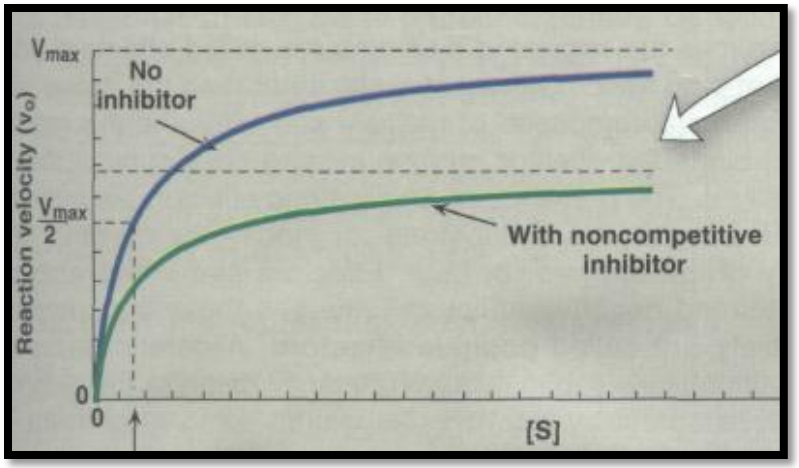
Enzyme non competitive inhibition

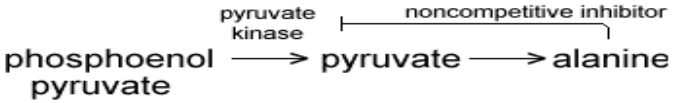
•The non competitive inhibition occurs when the inhibitor and substrate bind at different site unlike the competitive one.

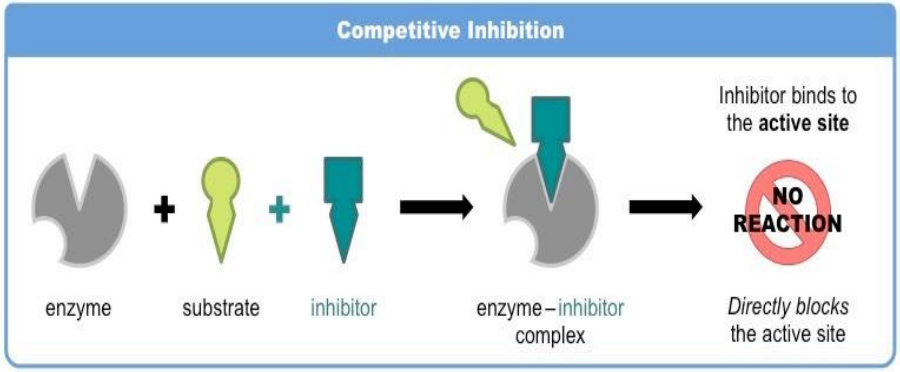
•The enzyme inhibitor could bind to the enzyme alone or to the complex enzyme substrate.

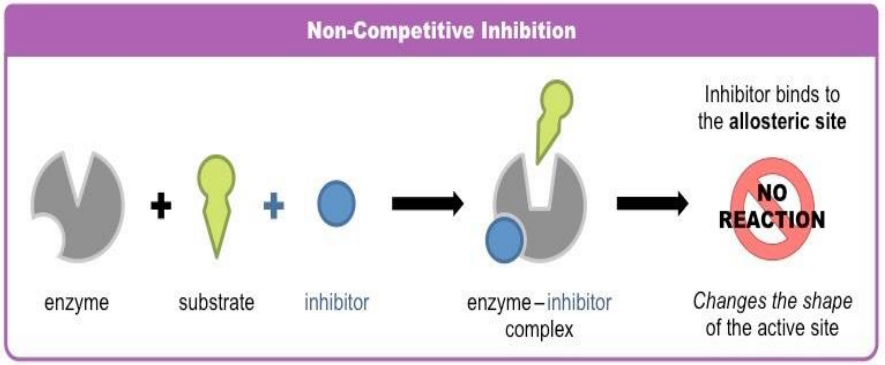
•**V max :** the non competitive inhibitor decreases the V max, but the inhibition reaction could not be reversed by increasing the concentration of the substrate.

•**Km :** the enzyme show the same KM with or without the inhibitor.









Regulation of enzyme activity

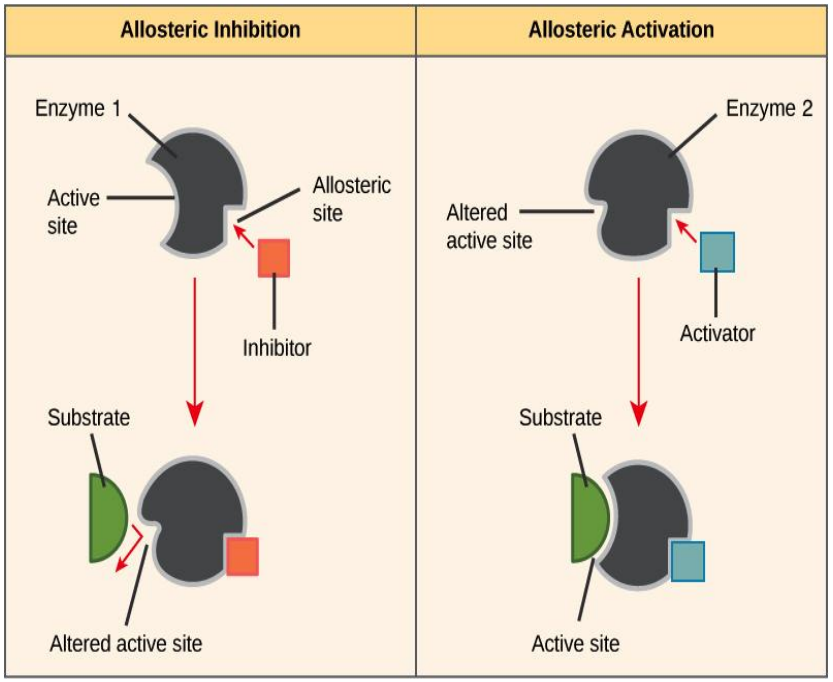
- **Enzyme activity (**allosteric regulation, covalent modification)

- **Enzyme quantity** (regulation of gene expression)

Allosteric regulation

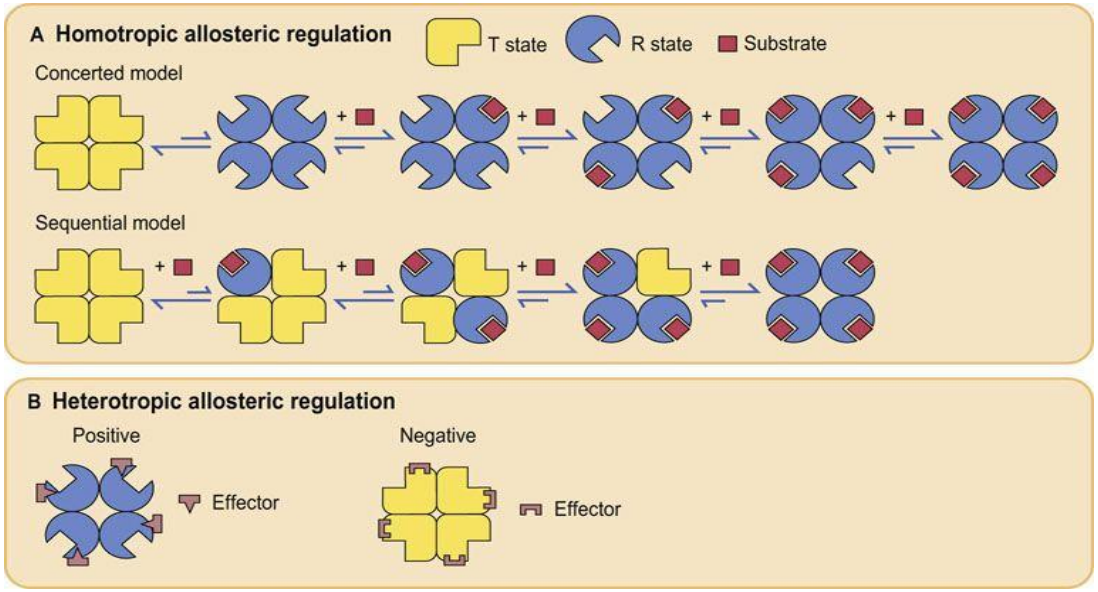
- **Allosteric** enzyme are regulated by molecules called effectors (also modifiers) that bind noncovalently at a site other than the active site. This enzyme consist of multiple subunits.

- Their catalytic activity can be modified even its affinity to the substrate by the effectors (positive and negative effecots)



Allosteric regulation

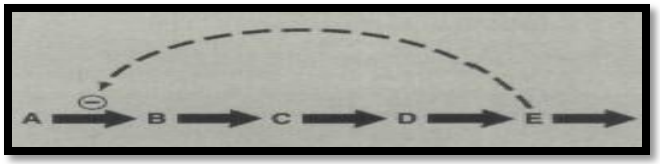
- **Homotropic effectors** when the substrate itself serve as an effector and the effect called homotropic.
- **Heterotropic effector** the effector is different from the substrate.

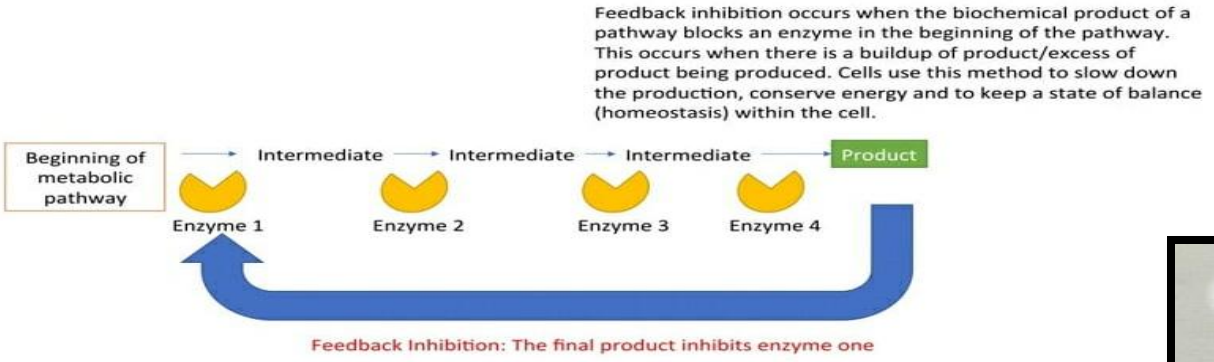


- **(A)** In homotropic regulation, the substrate acts as an allosteric effector. Two models are presented. In the concerted model, all of the subunits convert from the T (tense; low affinity for substrate) – into the R (relaxed; high affinity for substrate) – state at the same time; in the sequential model, they change one by one, with each substrate binding reaction. **(B)** In heterotropic regulation, the effector is distinct from the substrate, and binds at a structurally different site on the enzyme. Positive and negative effectors stabilize the enzyme in R and T state, respectively

Allosteric regulation

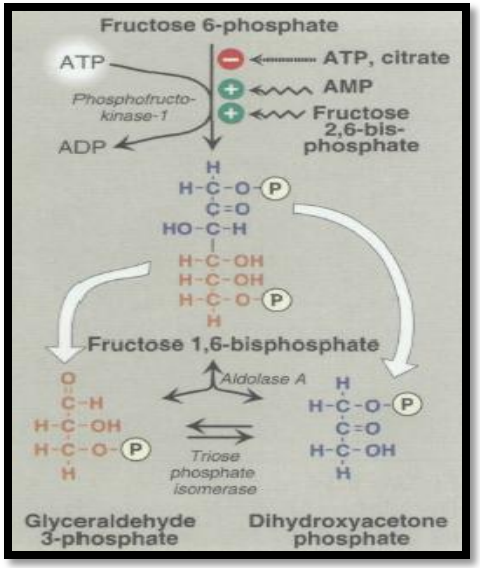
- **Heterotropic effector**
- For example the feedback inhibition





- Example : cholesterol production, glycolytic enzyme.

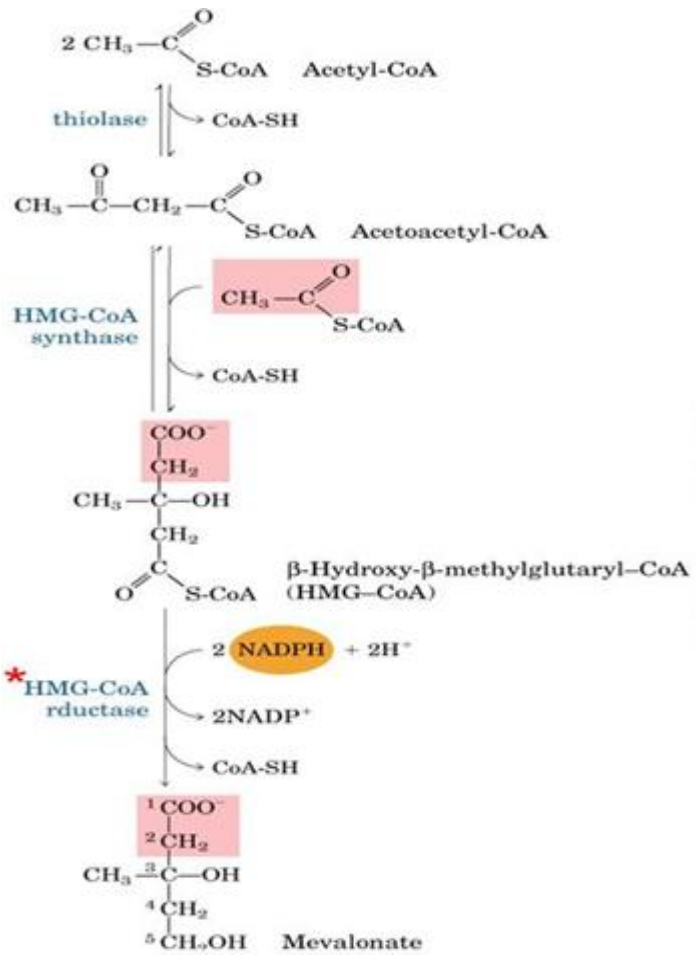
- **Glycolytic enzyme** phosphofructokinases is allosterically inhibited by citrate which is not substrate for this enzyme.



Allosteric regulation

- **Regulation of cholesterol synthesis is an example of allosteric regulation**
  - HMG CoA reductase is allosteric enzyme.
  - Feedback-inhibited by free cholesterol.
  - Insulin stimulate HMG CoA reductase.

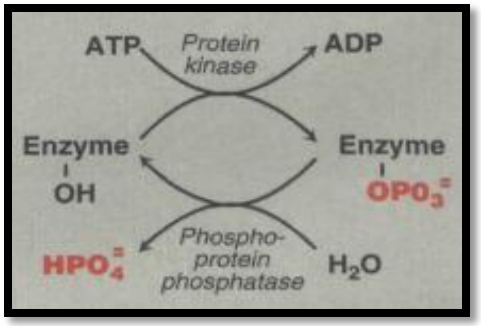
- **long term regulation** is by transcription factor that leads to more cholesterol-producing enzyme being made. When a lot of cholesterol is present in the blood, no new cholesterol-producing enzyme is made, which leads to a fall in cholesterol over time feedback regulation



Covalent modification

- Protein phosphorylation is an example of the covalent modification of the enzyme
- **Phosphorylation and dephosphorylation:**

  Phosphorylation reactions are catalyzed by a family of enzymes called protein kinases that use adenosine triphosphate (ATP) as a phosphate donor.Phosphate groups are cleaved from phosphorylated enzymes by the action of phosphoprotein phosphatases.

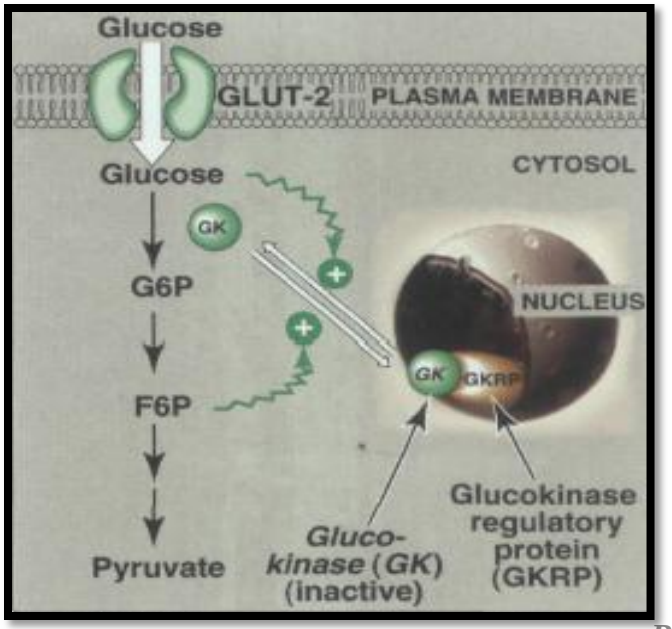


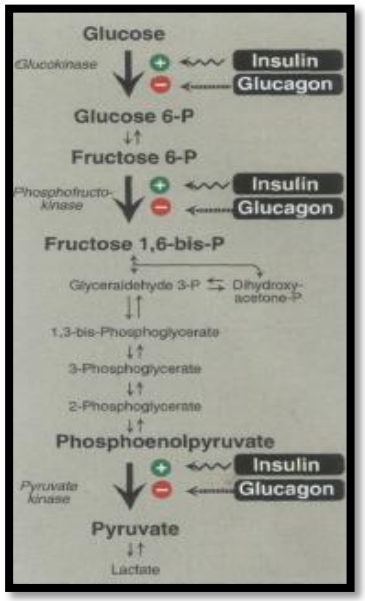
- **Response of enzyme to phosphorylation:**

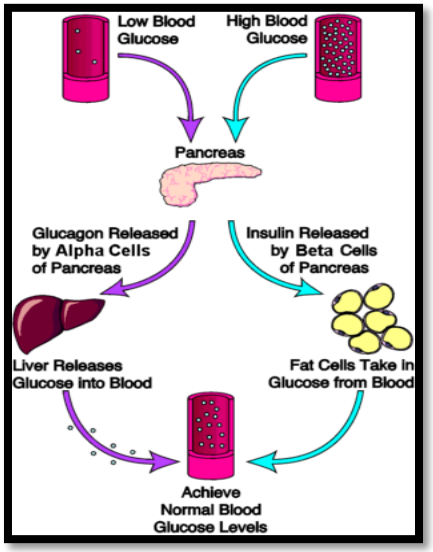
  depending on the enzyme the phosphorylated form could be more or less effective than the unphophorylated one. For example phosphorylation of glycogen phosphorylase (an enzyme that degrades glycogen) increases activity, whereas the addition of phosphate toglycogen synthase (an enzyme that synthesizes glycogen)decreases activity

Induction and repression of enzyme synthesis

- While allosteric regulation can occur in few minutes the enzyme regulation activity by inhibition or repression needs hours to days to happen.
- Activation or inhibition the enzyme synthesis alters the enzyme quantity.
- Under certain physiological conditions the cells can regulate the enzyme synthesis.
- **For example**: the increased levels of insulin can increase the synthesis of enzyme involved in glucose metabolism.
- **long term regulation of cholesterol synthesis** is by transcription factor that leads to more cholesterol-producing enzyme being made. When a lot of cholesterol is present in the blood, no new cholesterol-producing enzyme is made, which leads to a fall in cholesterol over time feedback regulation

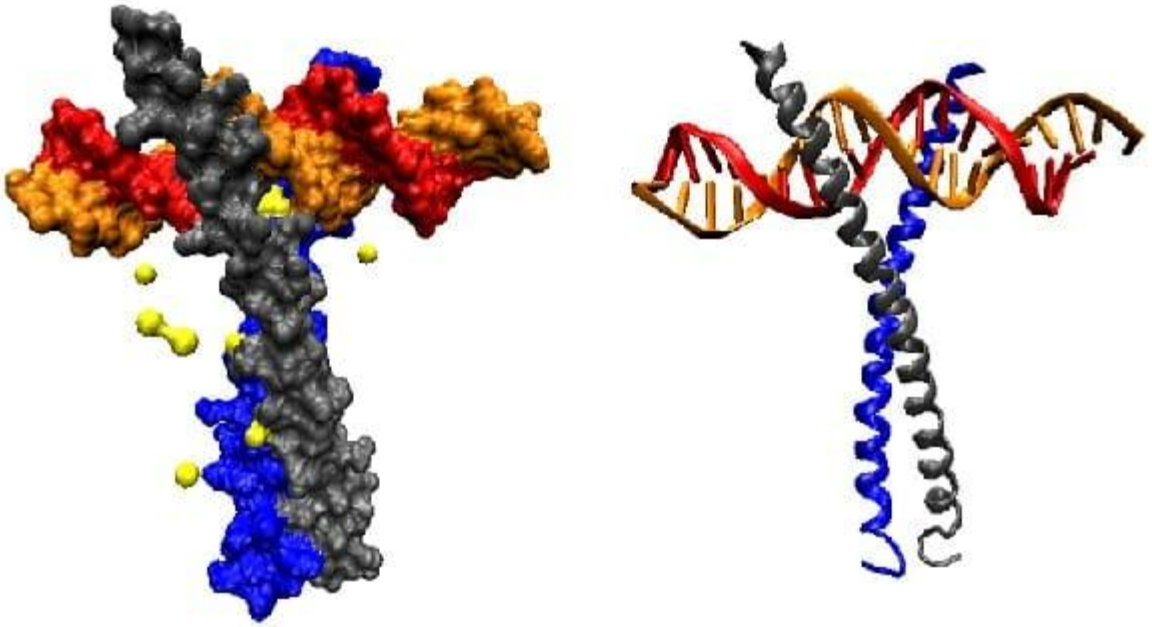






What is a transcription factor

- **Transcription factors** are DNA-binding proteins that play a key role in gene transcription. They are modular in structure and heterodimeric. Built within the transcription factor is a DNA-binding domain and several sites for the other transcription co-regulators to bind. Transcription factors bind to short conserved sequences located within each promoter along the strands of DNA.



Scheme of enzyme regulation activity

REGULATOR EVENT	TYPICAL EFFECTOR	RESULTS	TIME REQUIRED FOR CHANGE
Substrate availability	Substrate	Change in velocity (v_o)	Immediate
Product inhibition	Reaction product	Change in V_{max} and/or K_m	Immediate
Allosteric control	Pathway end product	Change in V_{max} and/or $K_{0.5}$	Immediate
Covalent modification	Another enzyme	Change in V_{max} and/or K_m	Immediate to minutes
Synthesis or degradation of enzyme	Hormone or metabolite	Change in the amount of enzyme	Hours to days

Enzymes and clinical diagnosis

- **Isoenzymes** differ in the amino acid sequence but catalyze the same chimical reaction.

- some enzyme essential for cell vitality and present always in the body tissue, while other being expressed in a specific cell types during certain period of development or in response to a specific physiologic or pathophysiologic changes.

- Analysis of the presence and distribution of enzyme expression is normally tissue-, time-, or circumstance-specific—often aid diagnosis.

Non functional plasma enzyme aids diagnosis

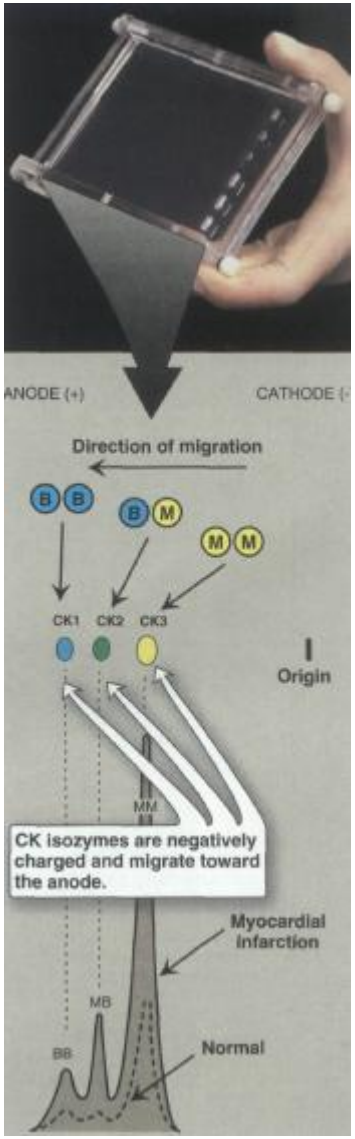
- **Functional plasma** enzyme present at all times in the circulation of normal individuals and perform a physiologic function in the blood like lipoprotein lipase, pseudocholinesterase, and the proenzymes of blood coagulation and blood clot dissolution. Most of them synthesized in the liver.

- **Non functional enzyme** have no activity in the plasma arise form normal routinely destruction of red, white blood cells and other body cells. In cases of tissue damages or necrosis arise from injury of a diseases results in increase the plasma concentration of the correspondent non functional plasma enzyme.

Serum Enzyme	Major Diagnostic Use
Aminotransferases	
Aspartate aminotransfer-ase (AST, or SGOT)	Myocardial infarction
Alanine aminotransferase (ALT, or SGPT)	Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson's disease)
Creatine kinase	Muscle disorders and myocar-dial infarction
γ-Glutamyl transpeptidase	Various liver diseases
Lactate dehydrogenase (isozymes)	Myocardial infarction
Lipase	Acute pancreatitis
Phosphatase, acid	Metastatic carcinoma of the prostate
Phosphatase, alkaline (isozymes)	Various bone disorders, ob-structive liver diseases

Isoenzyme and disease of heart

- **Isoenzyme or isozyme** are enzyme that catalyze the same reaction.
- Because of these enzyme could differ in the amino acids sequence so have a different physical properties and separate differently by electrophoresis.
- **For example** the plasma levels of creatine kinase (CK) and LDH lactatedehydrogenase are commonly determined in the diagnosis of myocardial infarction. They are particularly useful when the electrocardiogram is difficult to interpret, such as when there have been previous episodes of heart disease.



Lactate Dehydrogenase (LDH)

- **Lactate Dehydrogenase (LDH)** It is formed by the association of five peptide chains of two different kinds of monomers: M and H
- The variants seen in humans are:
- **LDH1: H H H H** (abundant in heart, brain erythrocytes; around 33% of serum LDH)
- **LDH2: H H H M** (abundant in heart, brain erythrocytes; around 45% of serum LDH)
- **LDH3: M M H H** (abundant in brain, kidneys, lung; around 18 % of serum LDH)
- **LDH4: H M M M** (abundant in liver, skeletal muscle, kidney; around 3% of serum LDH)
- **LDH5: M M M M** (abundant in liver, skeletal muscle, ileum; around 1 % of serum LDH)

- In **myocardial infarction**, **Total LDH** increases, and since heart muscle contains more LDH1 than LDH2, LDH1 becomes greater than LDH2 between 12 and 24 hours, after the infarction, so the ratio LDH1/LDH2 becomes higher than 1 and will stay flipped for several days. An increase of **LDH 5** in serum is seen in different hepatic pathologies: **cirrhosis, hepatitis** and others. An increase of LDH5 in heart diseases usually indicates secondary congestive liver involvement.

Creatine Kinase (CK)

- **Creatine Kinase** :creatinekinase occurs as three isoenzymes. Each isoenzyme is acomposed of two polypeptides

- Creatine Kinase (CK) or Creatine phosphokinase (CPK) is a similar example: three isoenzymes formed by combinations of different subunits:

- **CK1** (BB) is abundant in brain and smooth muscle (practically absent form serum)
- **CK2** (MB) is abundant in cardiac muscle, some in skeletal muscle (practically absent from serum)
- **CK3** (MM) is abundant in skeletal muscle and cardiac muscle (practically 100 % of serum CK)

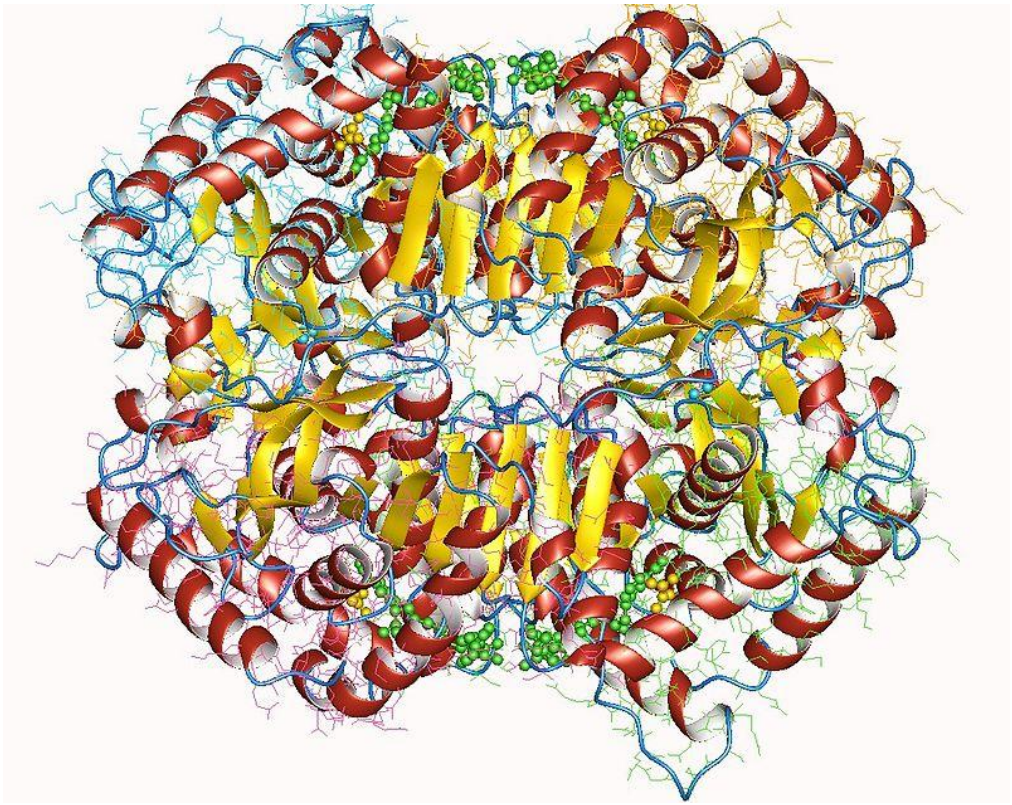
Creatine Kinase (CK)

- They can be differentiated based on their different electrophoretic mobility.

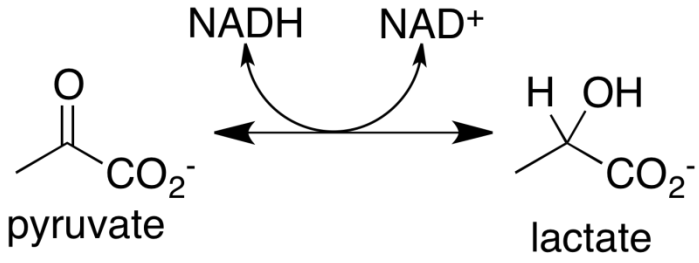
- The primary clinical use of CK studies is the diagnosis of Myocardial Infarction, (increased in the MB variant), but CK is also increased in different conditions as muscular diseases and traumas (MM and MB) and brain trauma and brain surgery (BB).

- CK2 appears in serum within 6 hours after the myocardial infarction and is cleared after 24 to 48 hours. A persistence of CK2 in serum indicates extension of the infarction to other areas or another infarction.

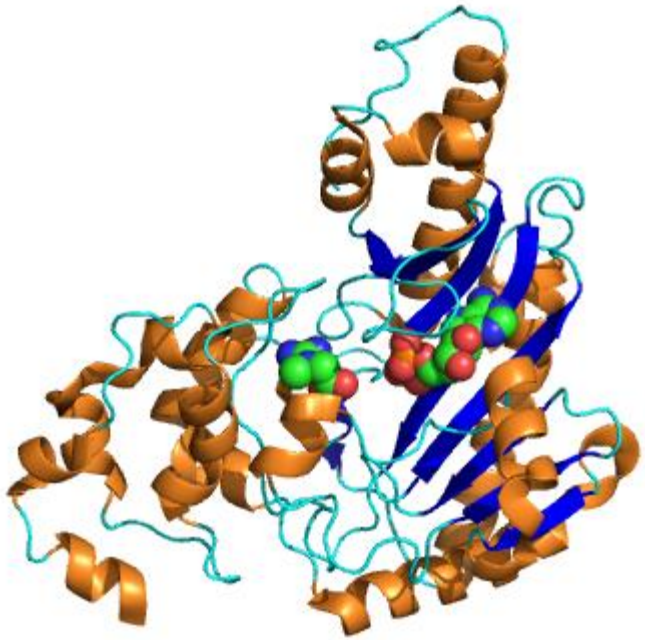
Quaternary structure of isoenzyme

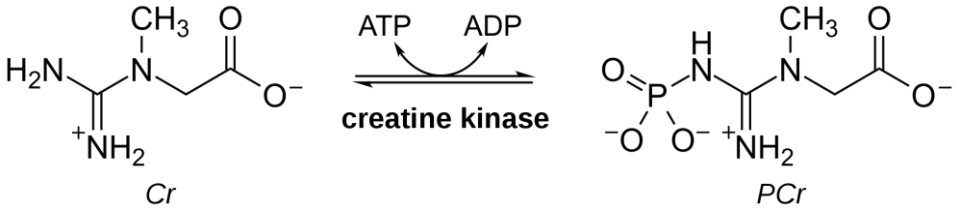


Lactate dehydrogenase M tetramer (LDH5) + 4 NADH (green)



Quaternary structure of isoenzyme

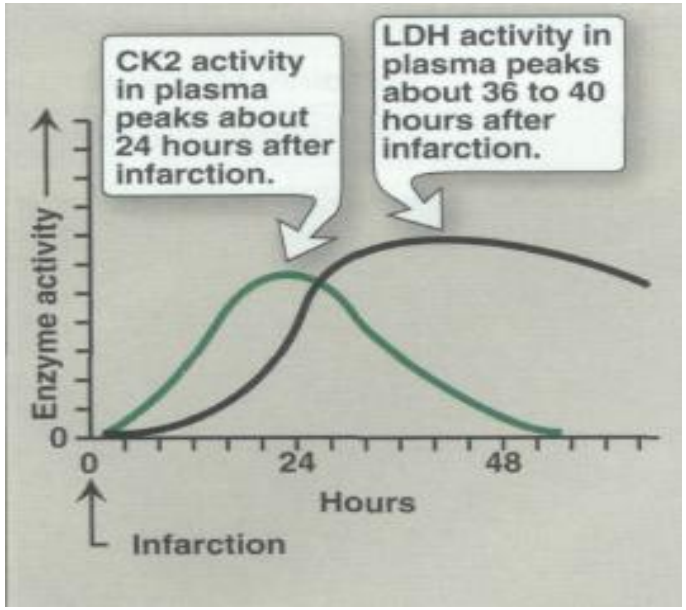


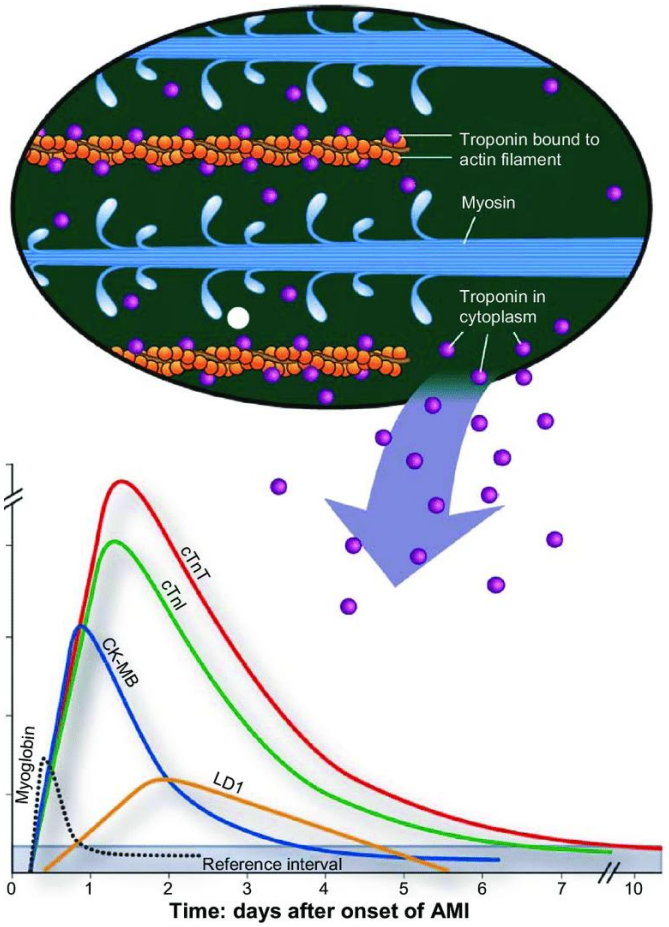


Crystal structure of Human Brain-type Creatine Kinase CK1 with ADP

Troponin T and TroponinI

- **Troponin T and troponin I** are two proteins involved in myocardial contractility , released into plasma in response to the cardiac damage , the elevated serum value are more predictive in un stable angina and myocardial infarction than CK2





Thank you