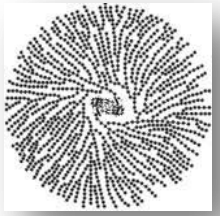
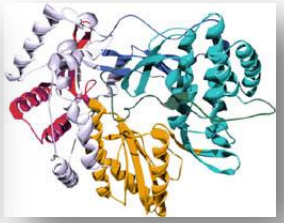
UNIVERSITY OF KWAZULU-NATAL

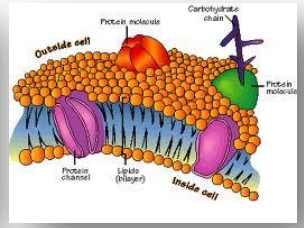
INYUVESI YAKWAZULU-NATALI

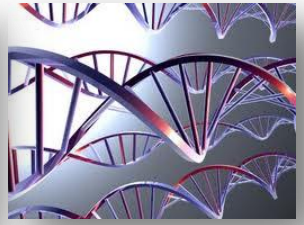
BIOC203W1

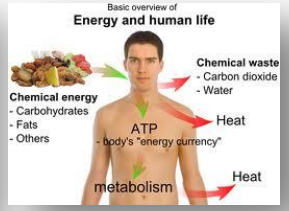
Biochemistry for Biologists











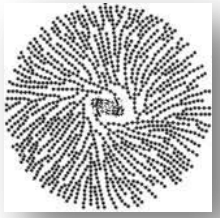
Dr MS Islam

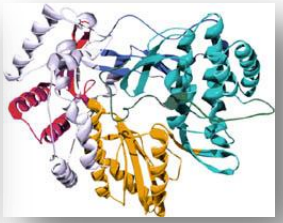
Sr. Lecturer of Biochemistry

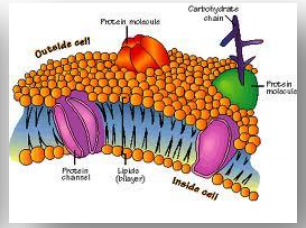
School of Life Sciences, Westville Campus

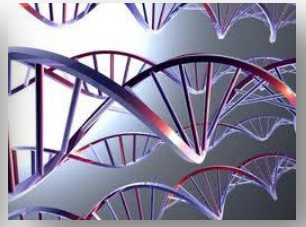
Enzymes:

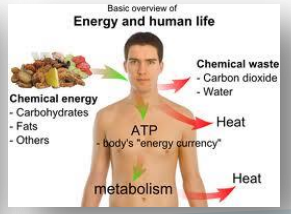
Biological catalysts



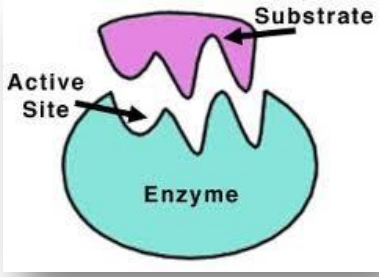






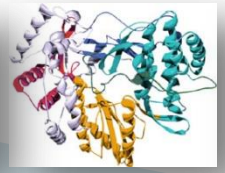


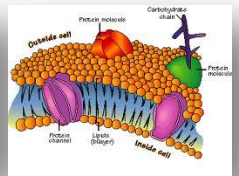
History of enzymes

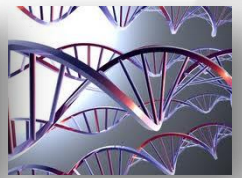


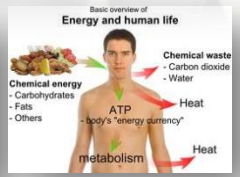
Nomenclature and discovery of ENZYME?

- The name enzyme derived from two Greek terms: 'en' means 'in' and 'zyme' means yeast
- Hence, the enzyme means something 'in yeast' which catalyzes the reaction of fermentation.
- **In 1878:**
  Fredrich Wilhelm Kuhne first created the name of enzyme.
- **In 1897:**
  Eduard Buchner obtained a cell-free extract that could catalyze the synthesis of ethanol from glucose.
- **In 1926:**
  James Sumner for the first time crystallized the enzyme from jack beans and demonstrated that this crystal consist of protein.

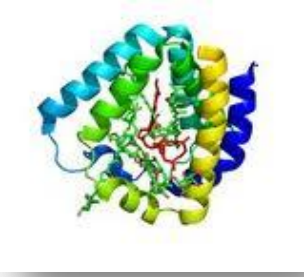






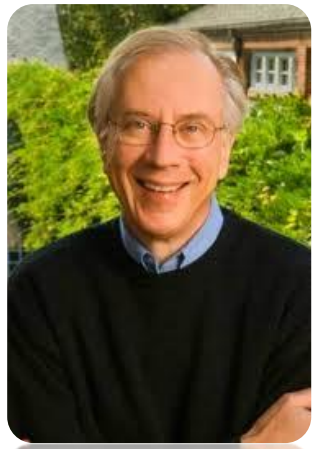


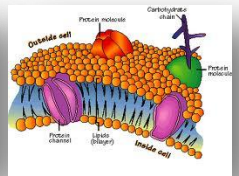
History of enzymes

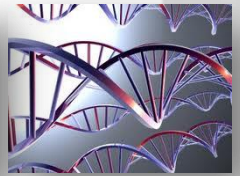


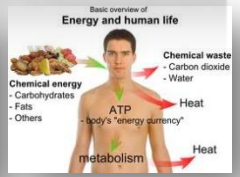
Nomenclature and discovery of ENZYME? Contd…

- **Mid 1930: John Northrop and Moses Kunitz for the first time confirmed the protein nature of crystalline enzymes such as- trypsin, chymotrypsin, pepsin etc.**
- **They found the direct correlation between the activities of above enzymes and the amount of proteins in the reaction mixtures.**
- **In 1963: the first amino acid sequence of an enzyme (bovine pancreatic ribonuclease) was reported.**
- **In 1965: the first X-ray structure of an enzyme (hen egg white lysozyme) was elucidated.**
- **In 1986: Thomas Cech has demonstrated that some Ribonucleic Acid (RNA) species also have activities like enzymes.**
- **In 2011: Thousands of purified and characterized enzymes are available.**

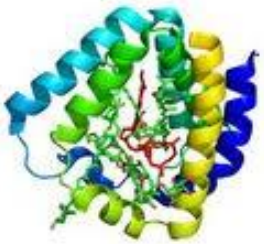




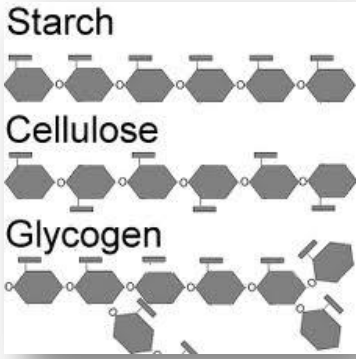


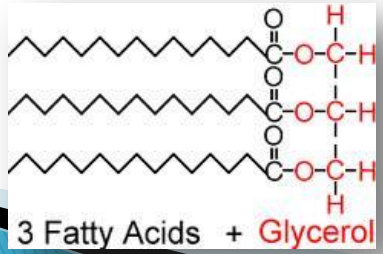


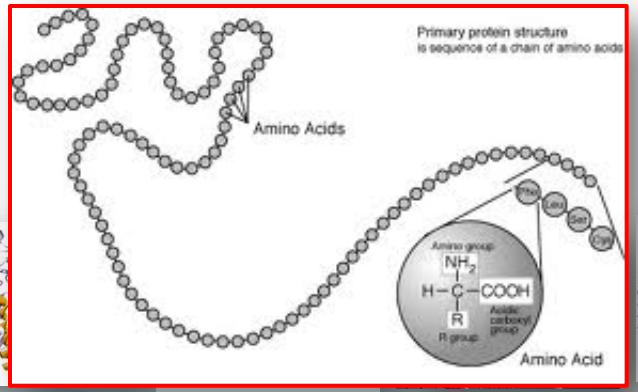
Which are enzymes?

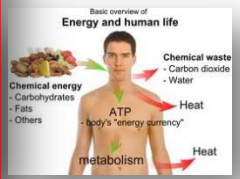


- Enzymes are biological catalyst which **increase, decrease or regulate** the rate of biological reaction
- The proteins which have **biological activity and can increase, decrease or regulate** the rate of biological reaction are called **ENZYME.**
- Almost all enzymes are PROTEIN but all proteins are NOT enzymes
- For example, food starch is converted to smaller carbohydrate units by an enzyme called ***Amylase***.
- **Proteins are converted to amino acids** by enzymes called *trypsin, chymotrypsin* and *pepsin*.
- **Fat is converted to free fatty acid and glycerol** by and enzyme called ***lipase***.





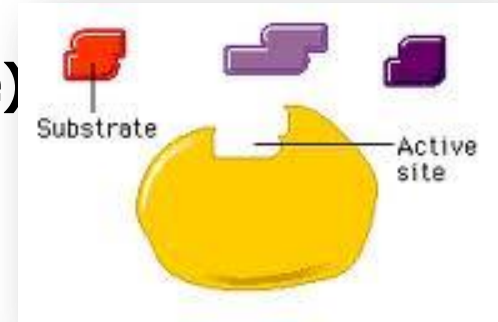


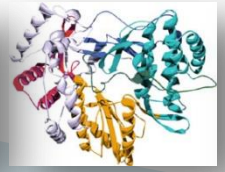


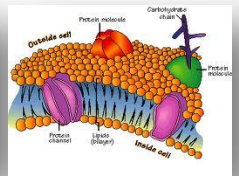
Properties of enzymes?

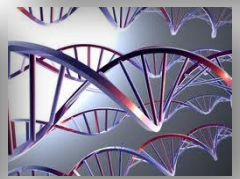
- Living process completely dependent on the numerous biochemical reactions.
- The primary role of enzyme is to **INCREASE** the rate of these biochemical reactions.
- Enzyme catalyzed reactions are typically 10^3 to 10^{20} times faster than corresponding uncatalyzed reactions.
- Enzymes are usually unchanged or can be temporarily changed during the reactions, but finally stay unchanged at the end of the reactions.
- Enzymes highly specific to substrates or reactants such as-

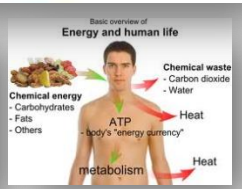
Glucose to Glucose-6-phosphate (Glucokinase)
Hexose to Hexose-6-phosphate (Hexokinase)











Catalysts vs. enzymes?

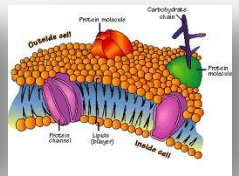
Difference between chemical and biological catalyst (enzyme):

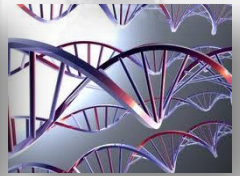
Reaction rates:

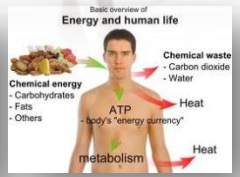
- Rates of enzymatically-catalyzed reactions are **significantly higher** than the chemical reactions
- Enzymes can **regulate the rate of biological reactions at several magnitude** compared to chemical catalysts.

Reaction conditions:

- Enzymatic reactions occur relatively **mild conditions and usually under physiological conditions** such as-
  - At 37°C (body temperature)
  - pH 7 (neutral pH)
  - Atmospheric pressure
- Chemically catalyzed reactions usually require **significantly higher environmental conditions.**





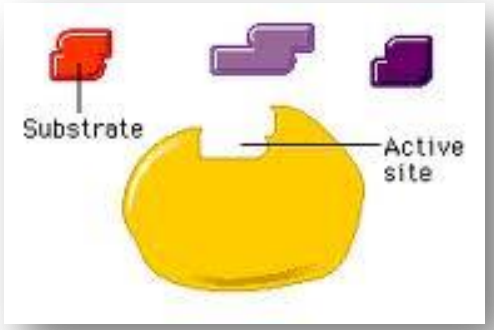


Catalysts vs. enzymes?

Difference between chemical and biological catalyst (enzyme):

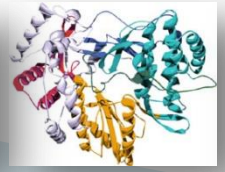
Reaction specificity:

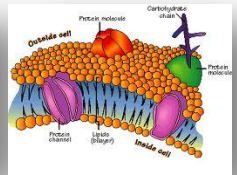
- Enzymes are **highly specific to their substrates** or reactants while chemical catalysts are not.

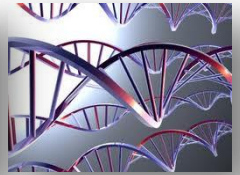


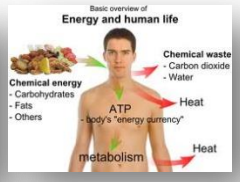
Capacity of regulation or control:

- Enzymes can **regulate the reactions rates in many different ways at several levels** while chemical catalyst can't do the same job.
- Physiological system is **AUTOMATICALLY ECONOMIC** while chemical system is not.

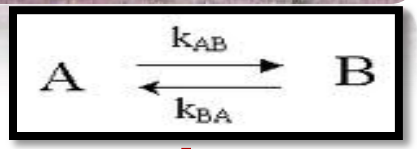




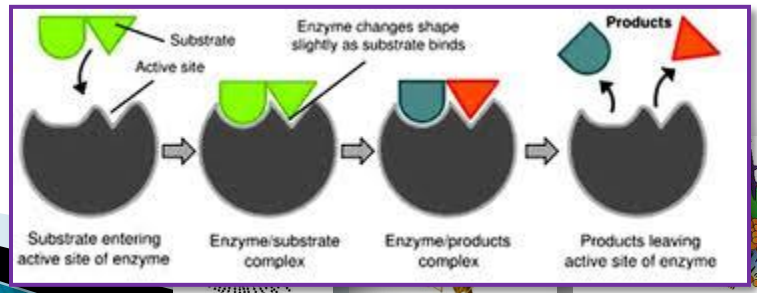


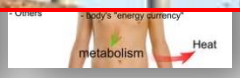


How do catalysts work?



- A catalyst either **INCREASES** or **DECREASES** the rate or velocity of a chemical reaction in **both forward and backward** directions.
- A catalyst may be **temporarily changed during the reaction, but it is remain UNCHANGED** in the overall process.
- Most biological catalysts are enzymes and they also regulate the rate of the biological reactions.
- An un-catalyzed reaction **requires more energy** compared to a catalyzed reaction.
- A catalyst ENHANCES the rate of reaction but DECREASES the required energy of the reaction.
- Sometimes catalysts provide an **ALTERNATIVE reaction pathway** that requires LESS energy.

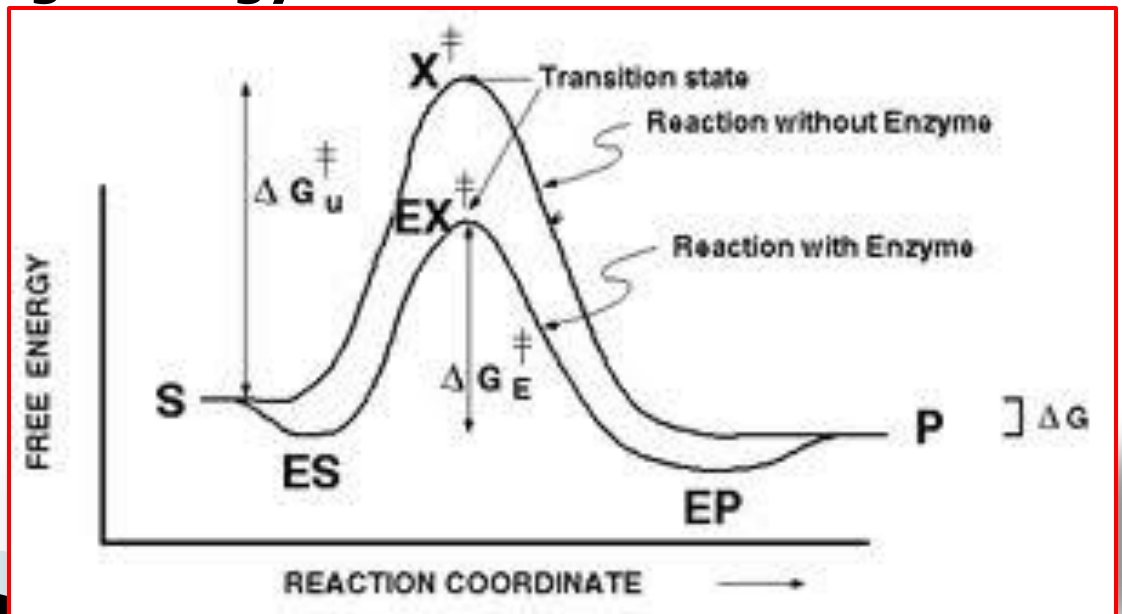




Transition state theory?

- Transition state is a state between the reaction of reactants with or without enzymes and the formation of products.
- Transition state is an UNSTABLE but HIGH ENERGY state and usually shown by a square [ ] bracket.
- Highest amount of energy requires at this state compared to any other state of the whole reaction process.
- Major reactions between the reactants happen at this state
- Bond breaking and bond formation to form a product also occur at this state by high energy

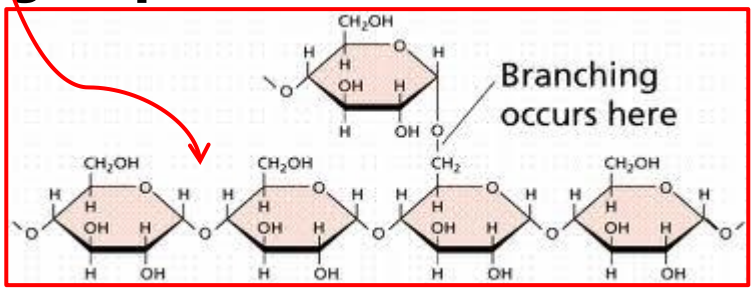
ΔG^o = Difference between the free energy of product and reactants
-ve ΔG^o = Reaction spontaneous
+ve ΔG^o = reaction non-spontaneous
Zero ΔG^o = Reaction equilibrium

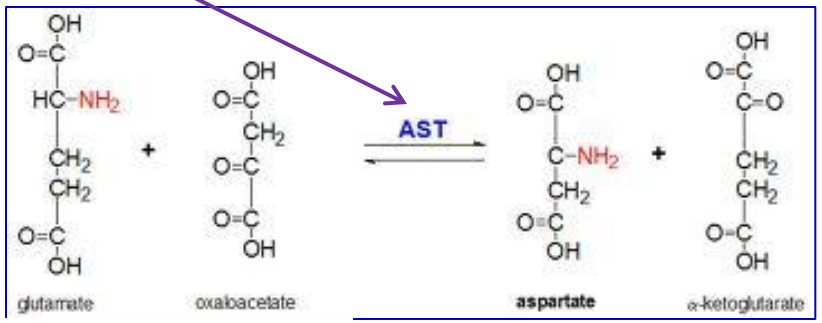


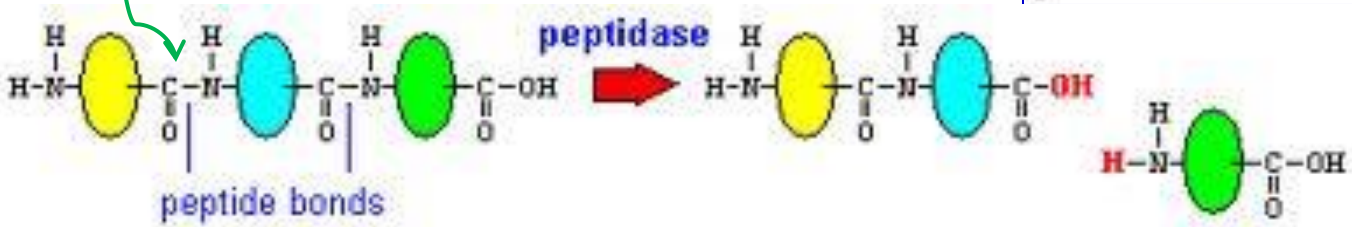
Page 2 in Handouts

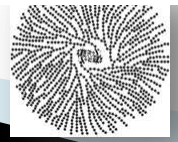
Terminology/ nomenclature?

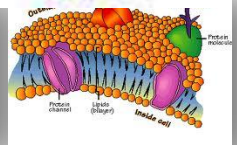
- Enzymes are often named by adding the suffix – *ase* to the substrate/ reactants such as-
- **Urease:** [Urea ⟶ Ammonia (NH_3) + CO_2]
- **Peptidase:** Which hydrolyze the peptide bonds in a protein
- **Amylase:** Which hydrolyze amylase and amylopectin in starch)
- **Transaminase or aminotransferase:** Which transfer amino group from amino acids to keto acids

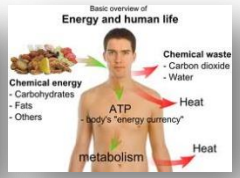






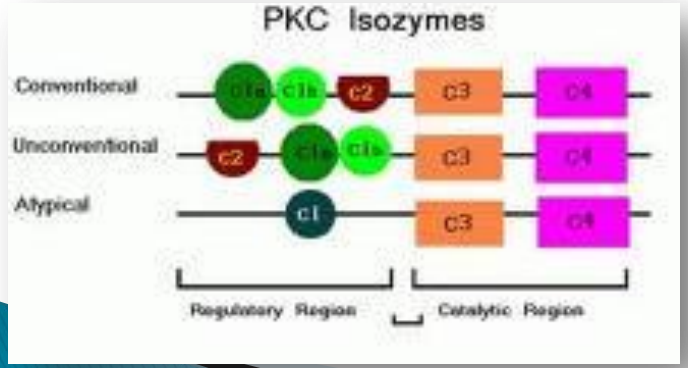




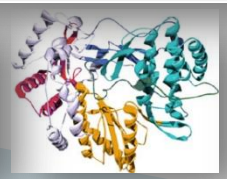


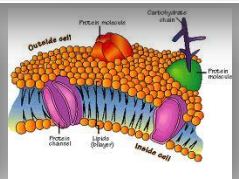
Terminology/ nomenclature?

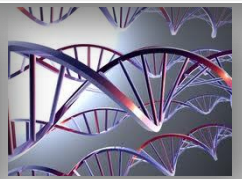
- There are also some trivial names of enzymes are still used as they were such as- **Trypsin, Chymotrypsin, pepsin** etc.
- The above nomenclature was confusing considering the vast number of enzymes.
- Finally, the **Enzyme Commission (EC) of the International Union of Biochemistry (IUB)** developed a new naming system.
- Each enzyme will have a code and a systemic name comprises of two parts. For example- an enzyme oxidizes alcohol is called "*Alcohol dehydrogenase*" which coding name is **E.C.1.1.1.1.**

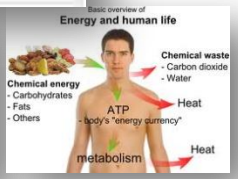


EC Number	Recommended Name	Synonyms
3.6.1.46	heterotrimeric G-protein GTPase	GTPase
3.6.1.47	small monomeric GTPase	GTPase
3.6.1.48	protein-synthesizing GTPase	GTPase
3.6.1.49	signal-recognition-particle GTPase	GTPase
3.6.1.50	dynamine GTPase	GTPase
3.6.1.51	tubulin GTPase	GTPase





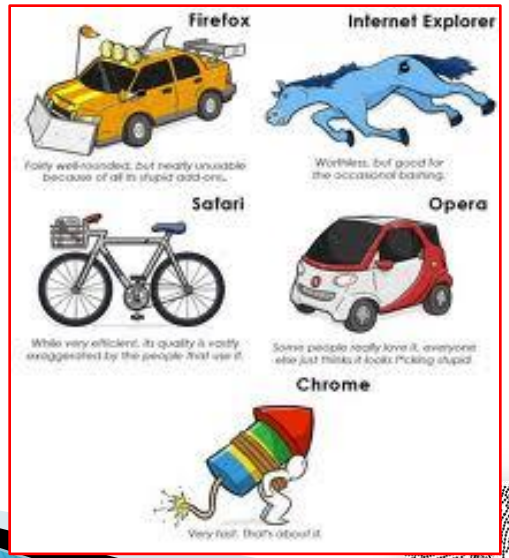


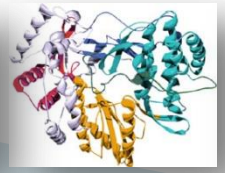


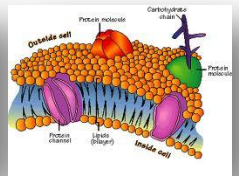
Classification of enzymes?

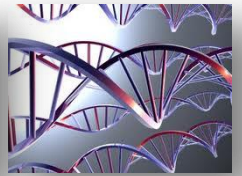
Basis on the nature of the functions of enzymes in biochemical reactions they are classified into 6 major classes:

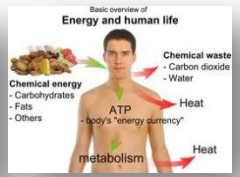
1. **Oxidoreductase**
2. **Transferases**
3. **Hydrolases**
4. **Lyases**
5. **Isomerase and**
6. **Ligases**











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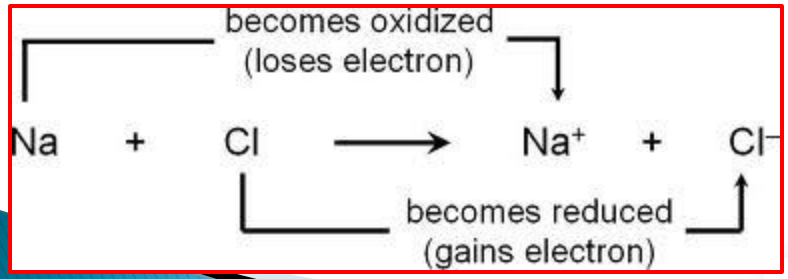
Class of enzymes?

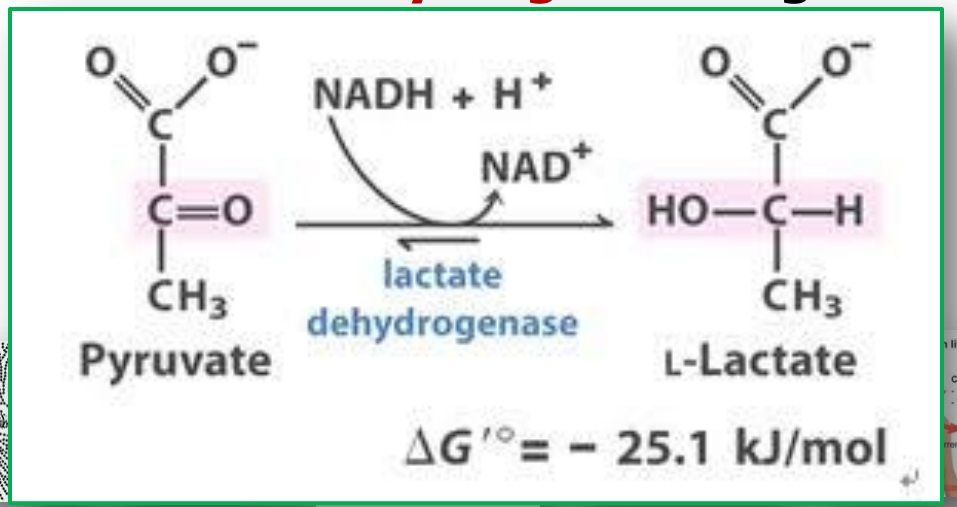
1. Oxidoreductase:

- The enzymes which catalyze the **oxidation-reduction reactions** are called oxidoreductase. For example-
  - Dehydrogenases e.g. alcohol dehydrogenase
  - Oxidases e.g. Xanthine oxidase
  - Oxygenases e.g. Cyclooxygenase (COX)
  - Reductases e.g. Glutathion reductase
  - Peroxidases e.g. Glucose peroxidase
  - Hydroxylases e.g. Tryptophan hydroxylase

$$A^- + B \longleftrightarrow A + B^-$$

- Most of the enzymes in this class are **dehydrogenase** e.g. ***Lactate dehydrogenase***





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Class of enzymes?

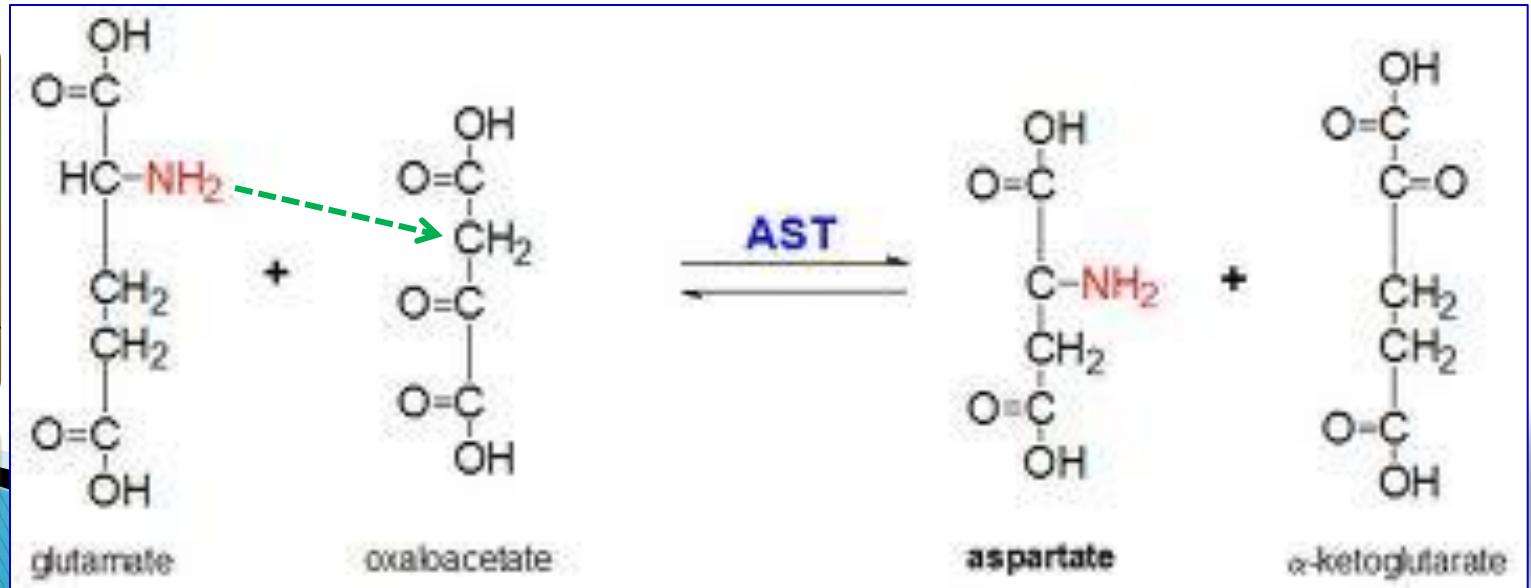
2. Transferases:

- Catalyze group transfer reactions e.g. **–NH_2 group from amino acid to keto acid, phosphate group from ATP** etc.
- Many of them require **co-enzymes** to work.
- Enzymes include in this group are amino **transferases** (or transaminases), **Kinases** (or phosphotransferase) etc.

General example-

$$A\text{-}B + C \longleftrightarrow A\text{-}C + B$$

Specific example-

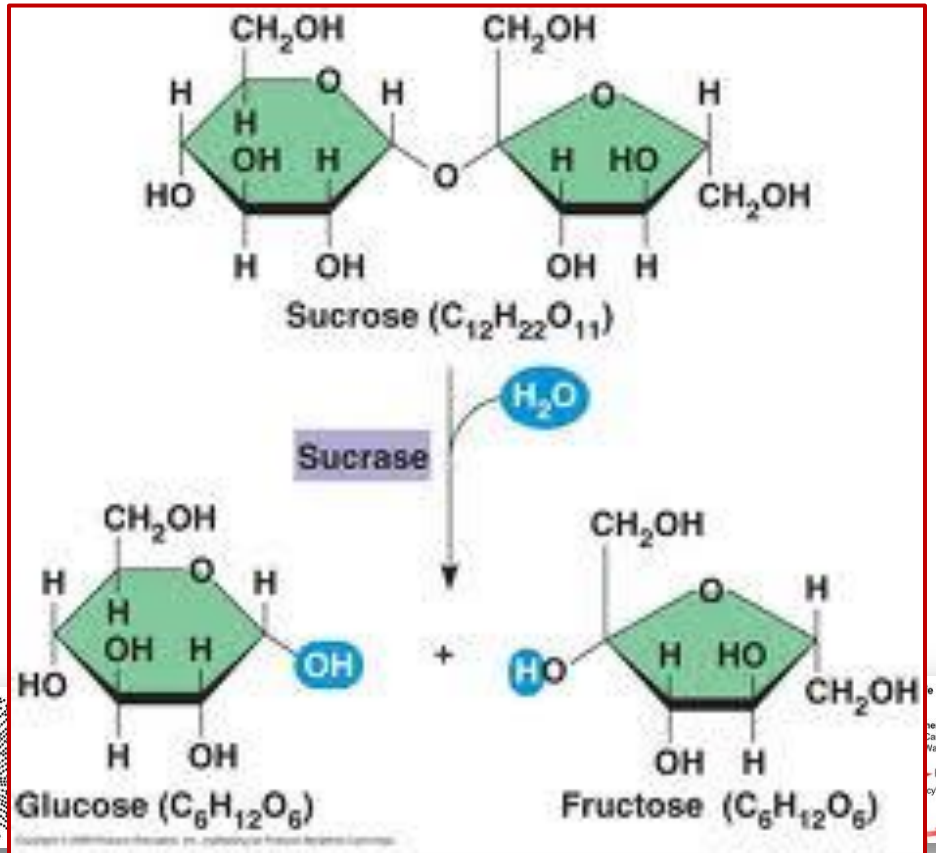


Class of enzymes?

3. Hydrolases:

- Catalyze **hydrolysis** reaction
- Water used in these enzymes act as an acceptor of the transferred chemical group

$$A\text{-}B + H\text{-}O\text{-}H \longleftrightarrow A\text{-}H + B\text{-}OH$$



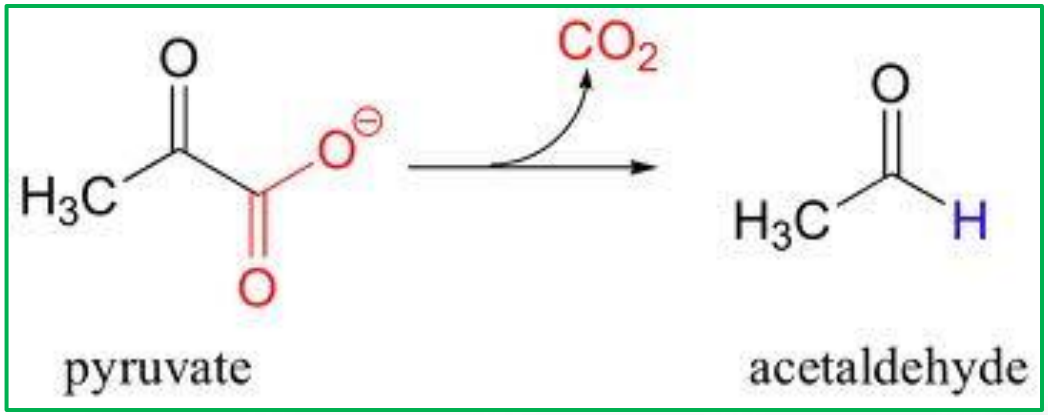
Class of enzymes?

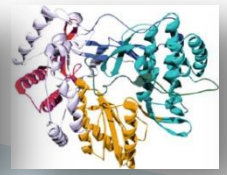
4. Lyases:

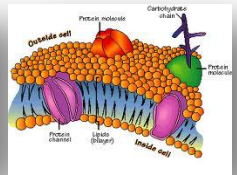
- Catalyze reaction in which H_2O, CO_2, NH_3 etc are removed from or added to a double bond
- These include decarboxylases, hydratases, dehydratases, deaminases and synthases.

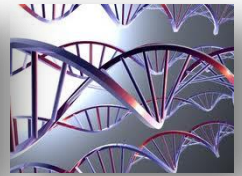
General example-

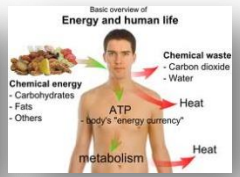
Specific example-







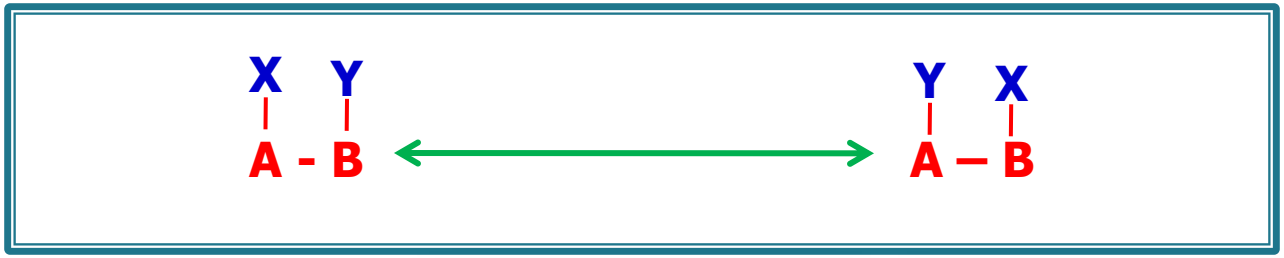


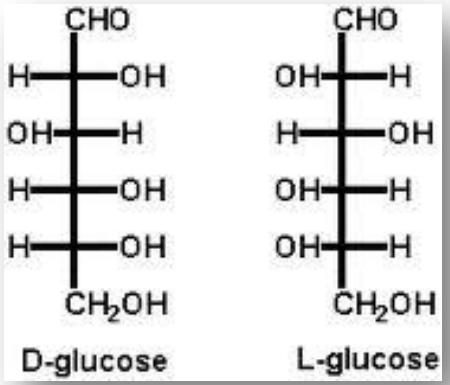


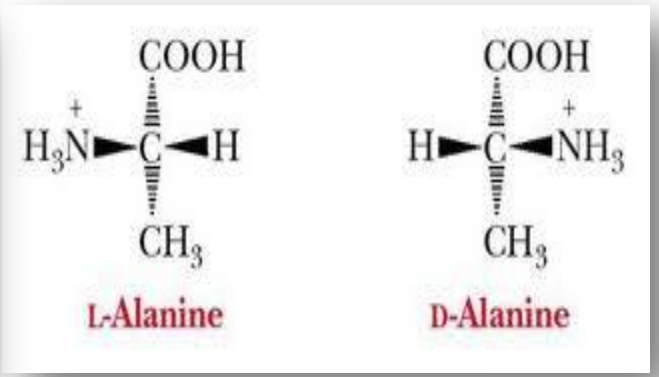
Class of enzymes?

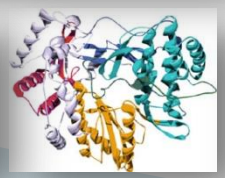
5. Isomerases:

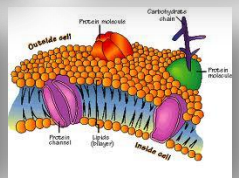
- Reaction of a single substrate and a single product
- Catalyze intramolecular change within a single molecule and form a single product
- These include epimerases, mutases and recemases

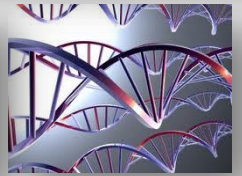


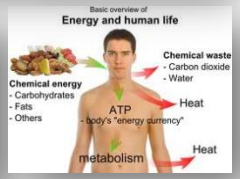












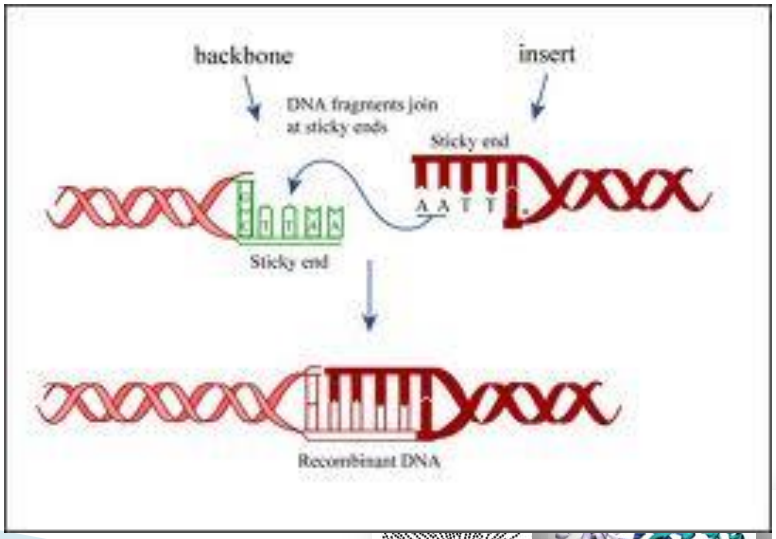
Class of enzymes?

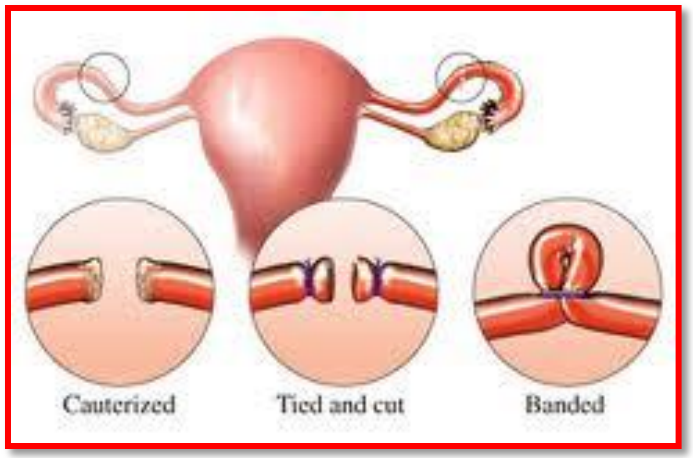
6. Ligases:

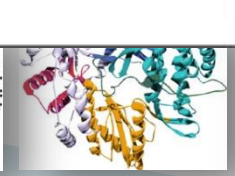
- Catalyze **ligation, joining or bond formation** between two substrates
- This is an energy requiring process which always supplied by the hydrolysis of ATP
- These include mostly **synthetases and carboxylases** etc.

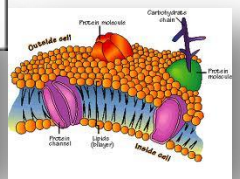
Example-

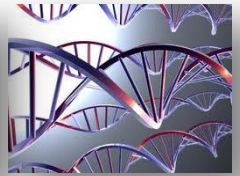
A + B = A-B

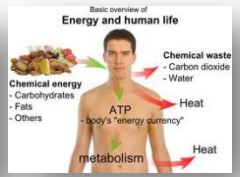






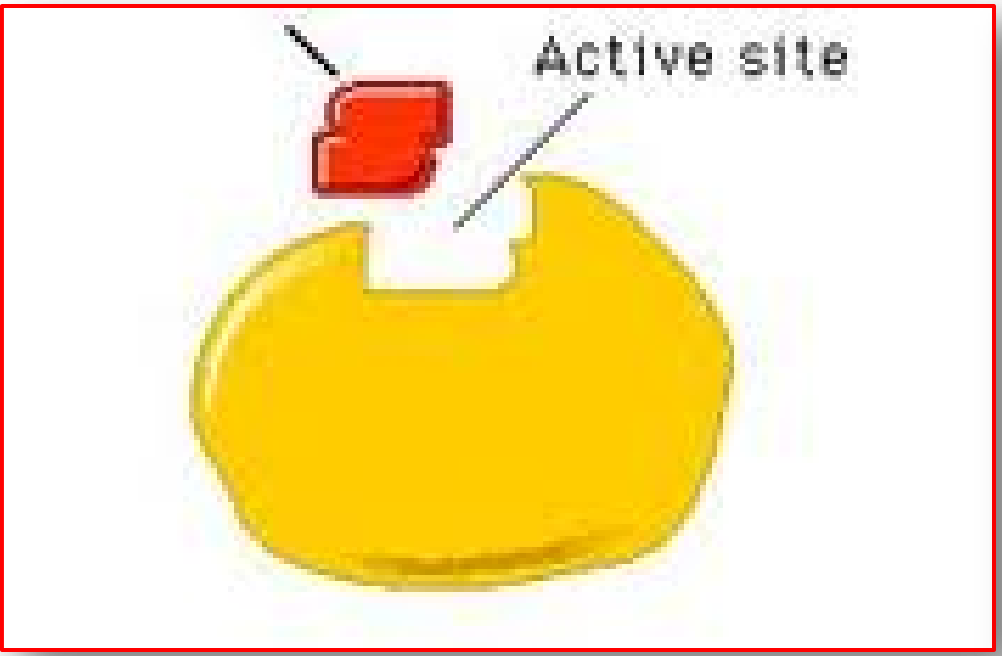


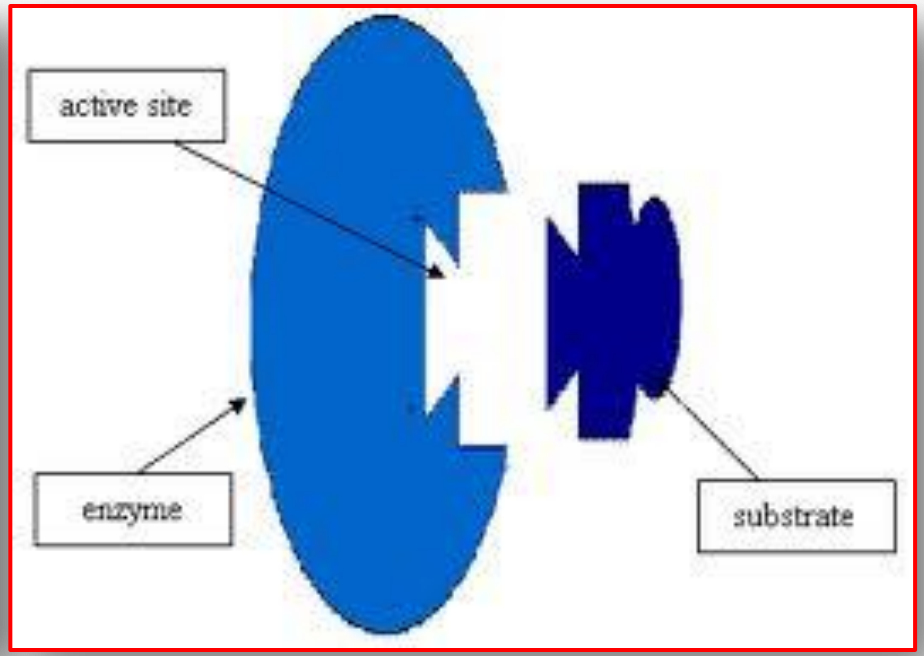


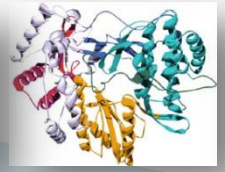
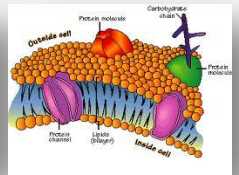
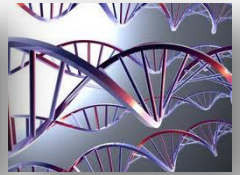
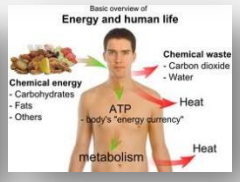


Active site of enzymes?

- Active site is a location or part in an enzyme where substrate molecule binds non-covalently
- Only specific substrate can fit into a specific enzyme or specific active site

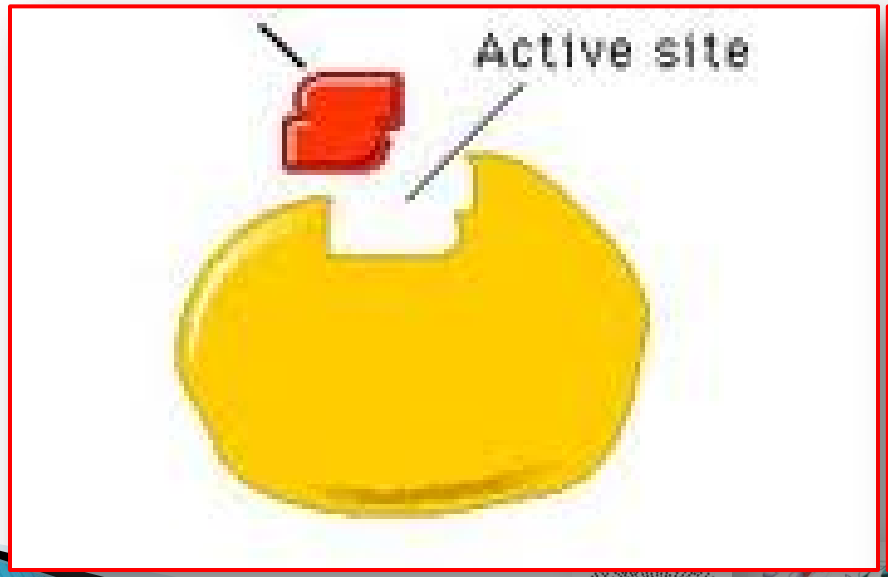


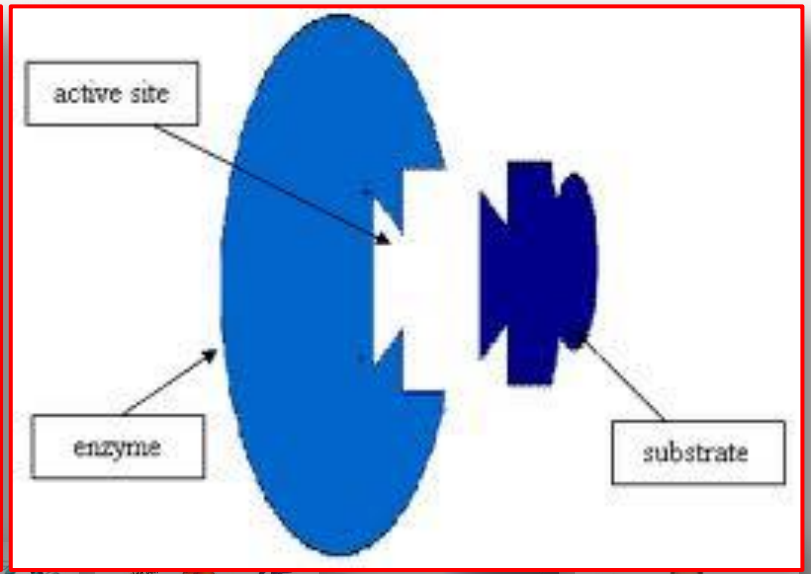


Active site of enzymes?

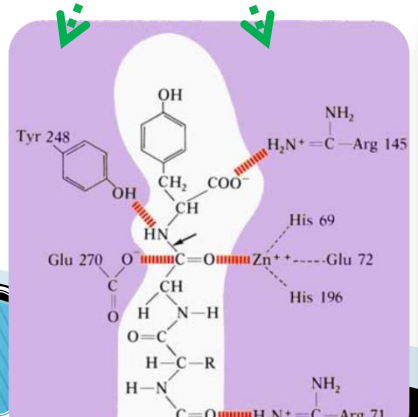
- Active site contains the amino acid residues (catalytic amino acid) that directly participate in the making and breaking of bonds during biochemical reactions.
- Although enzymes differ widely in terms of their mode of actions but there are a number of generalizations regarding their active sites.

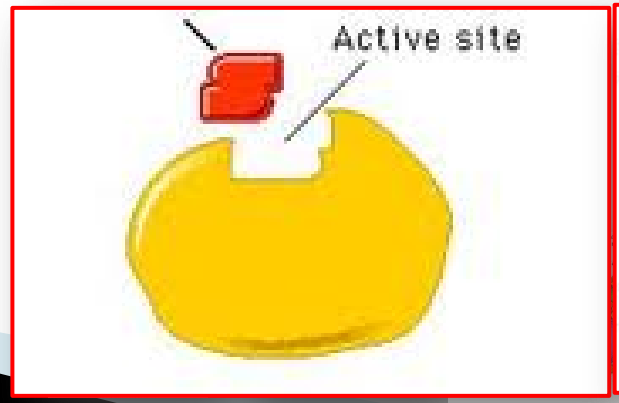


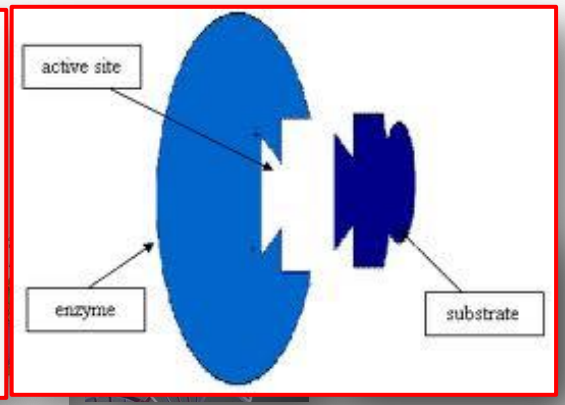


How big the active site is?

- Active site takes up a relatively small part of the total volume/surface of an enzyme which is about 5% of the total molecular surface of an enzyme
- Nearly all enzymes are made of more than 100 amino acid residues e.g. lysozyme has 129 amino acids when only 5 amino acids are involved in the active site.
- Chymotrypsin composed of 245 amino acid residues but only 3 amino acids are participate in the active site
- The small number of amino acids participate in the active site are called catalytic amino acids which bind non-covalently with substrate molecule during reactions.
- Then why enzymes are so BIG in structure?







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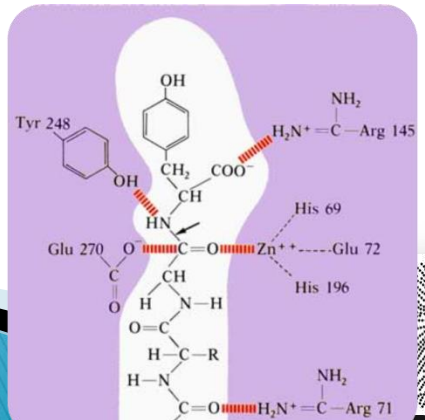
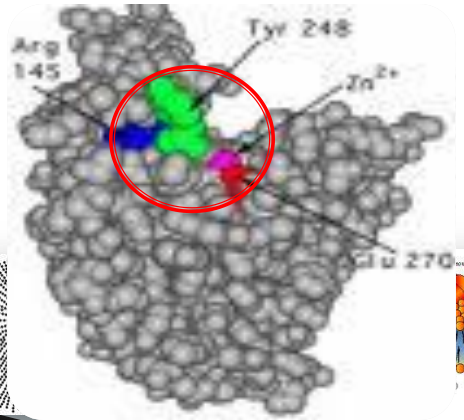
3D structure of active site

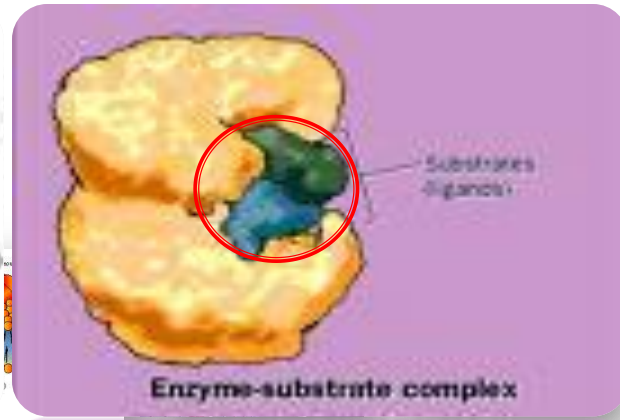
- **Active site is a 3-dimensional entity formed by binding**
  - **- catalytic amino acid residues and**
  - **- structural amino acid residues**

  in the polypeptide primary structure.
- **The R-group of amino acids forms the active site occupy distant position along the polypeptide chain and are brought together by many**
  - **- Folds**
  - **- Twists and**
  - **- Bends (secondary and tertiary structure)**

  of the polypeptide structures.







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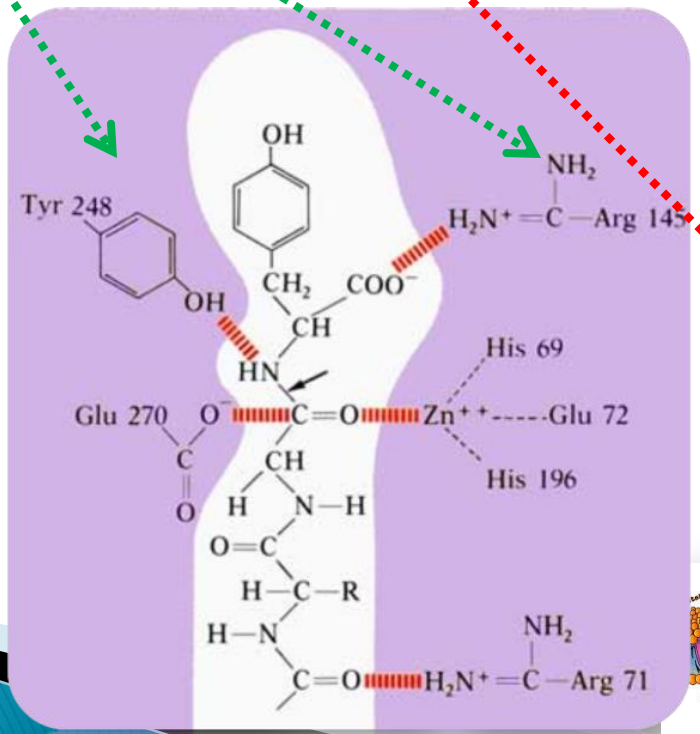
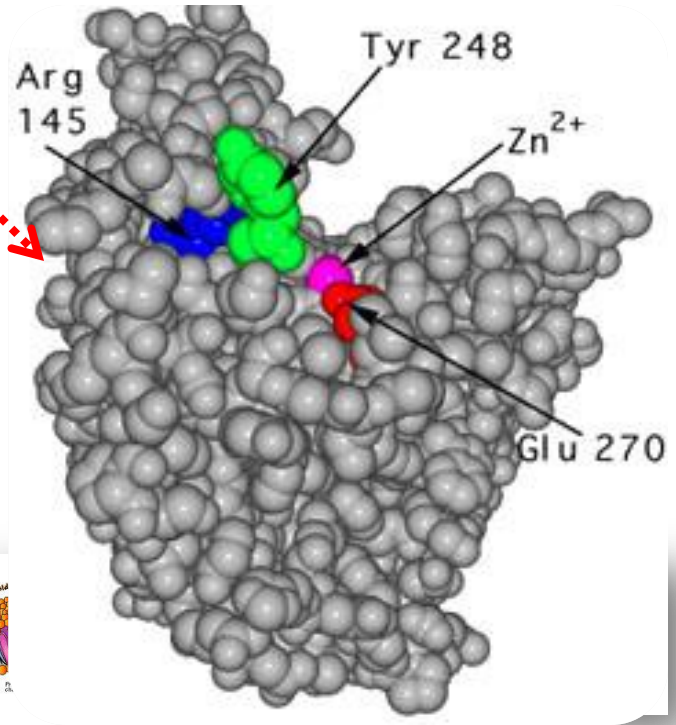
3D structure of active site- contd..

- These amino acid residues are very important to the activity of the enzymes that is why they are called **BINDING or CATALYTIC residues**.
- Other amino acid residues are not directly involved in the active site events but they are crucial for the stabilization of the 3D conformation of the entire enzyme and active site so they are called **structural amino acid residues** (most of the AA).





Cleft or pocket of active site

- In all enzymes, substrate molecules are bound to a part called CLEFT or POCKET
- Water (H2O) is excluded unless it is a reactant
- NON-POLAR character of most of the CLEFT enhances binding of substrate by HYDROPHOBIC interactions
- Cleft may also contains POLAR residues
- It creates a microenvironment in which certain of these residues acquire special properties essential for catalysis

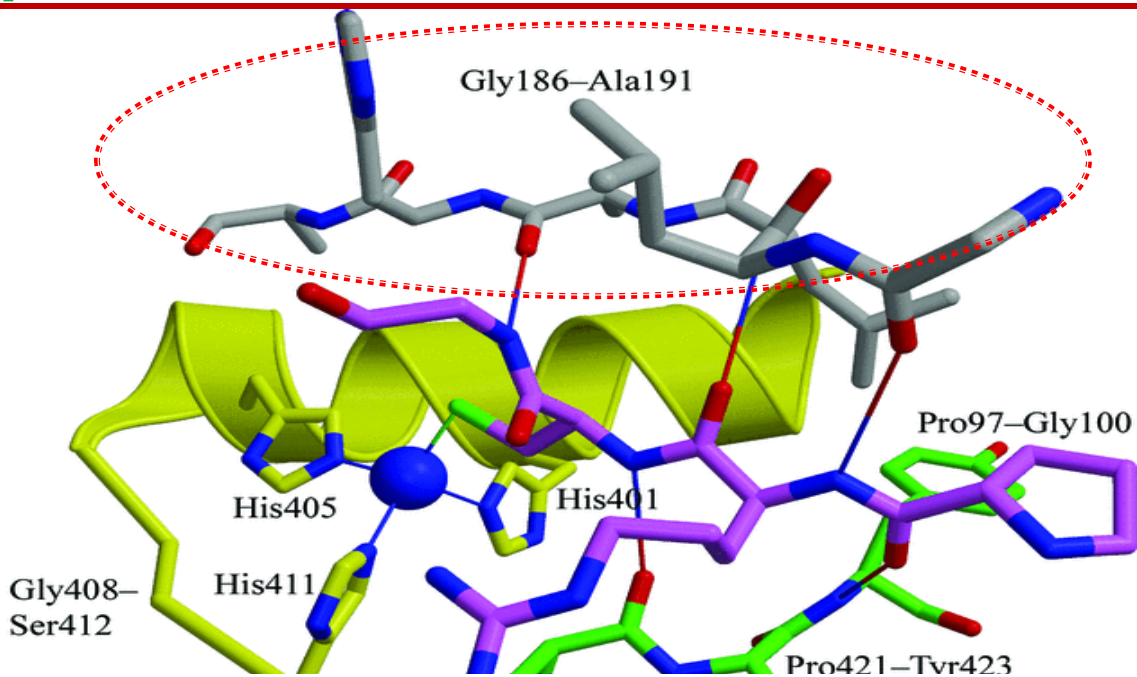


Fig. Ball-and-stick drawing of the active-site cleft. The cleft is delineated by the bulge-edge strand, residues 186-191, shown in grey, which runs horizontally across the figure.

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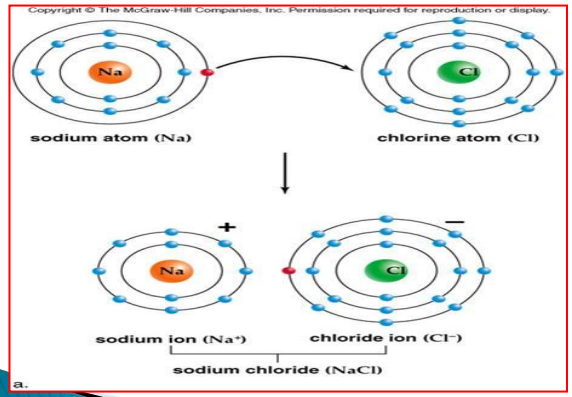
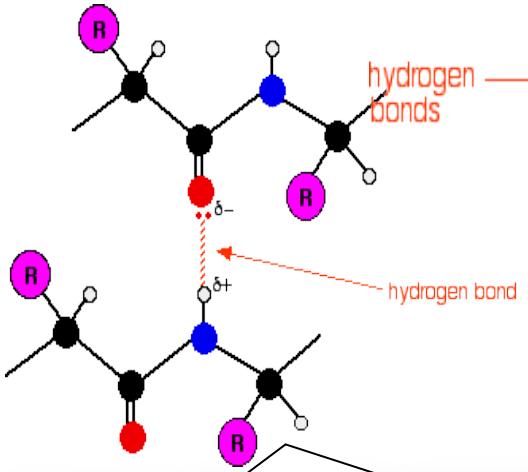
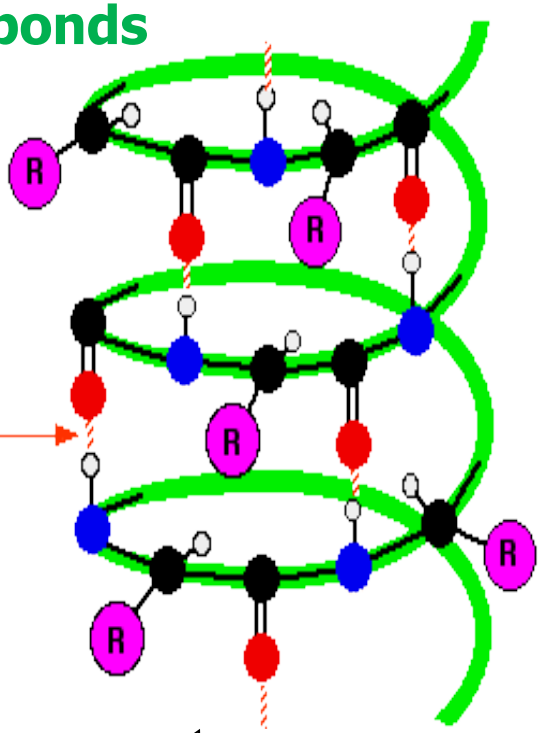
How substrates bind to enzymes?

- **Substrates are bound to enzymes by multiple weak interactions**
- **The non-covalent interactions in Enzyme-Substrate complexes are much weaker than covalent bonds**
- **Interactions are attributed to:**
  - **Ionic bonds**
  - **Hydrogen bonds**
  - **Hydrophobic interactions and**
  - **van der Waals forces**







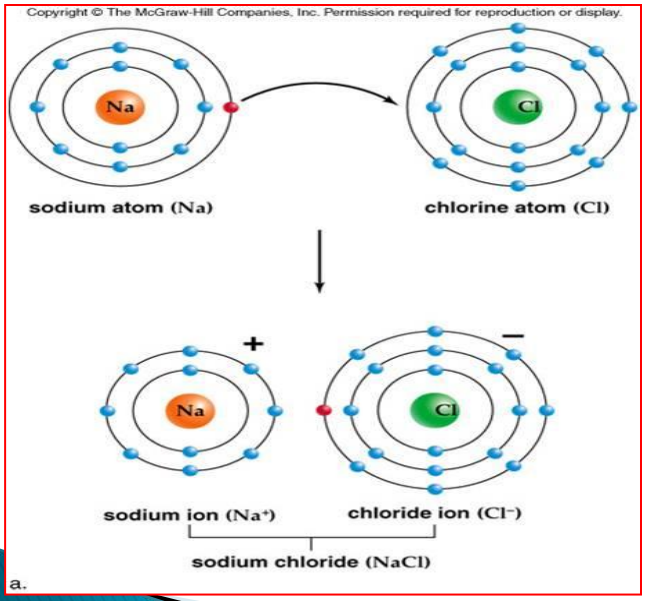
Hydrogen bonding in β-pleated sheet structure of protein/ enzyme

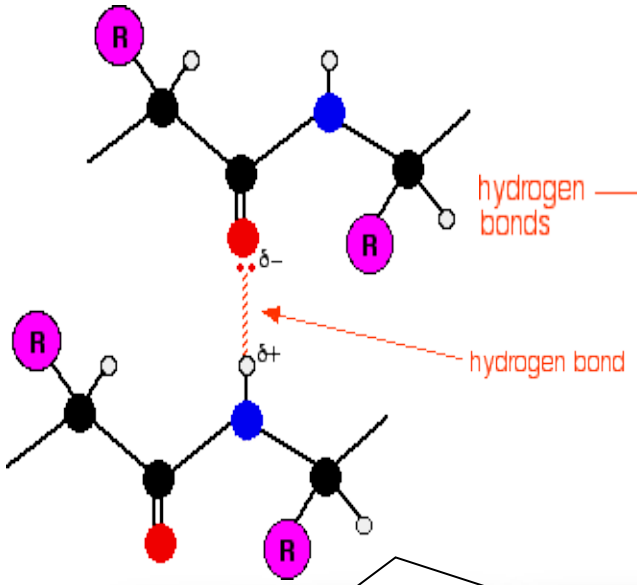
Hydrogen bonding in α-helix structure of protein/enzyme

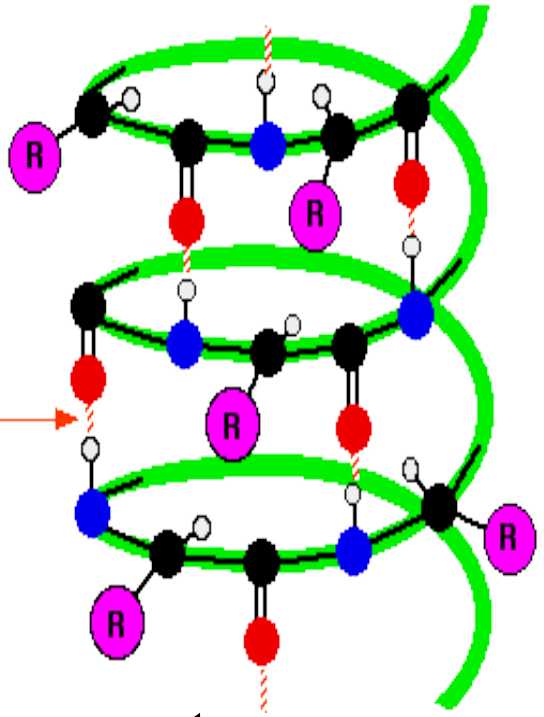
Page 6 in Handouts

How substrates bind to enzymes?

- van der Waals forces only become significant when numerous substrate atoms can simultaneously come close to many enzyme atoms
- HYDRONGEN BONDING between enzyme and substrate often enforces a high degree of specificity to the active site







Hydrogen bonding in β-pleated sheet structure of protein/ enzyme

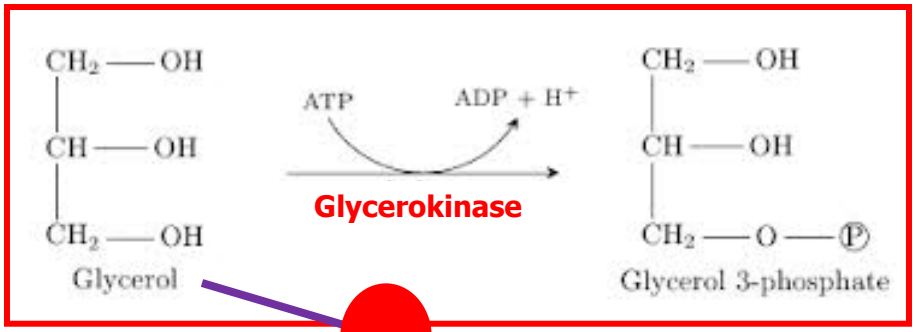
Hydrogen bonding in α-helix structure of protein/enzyme

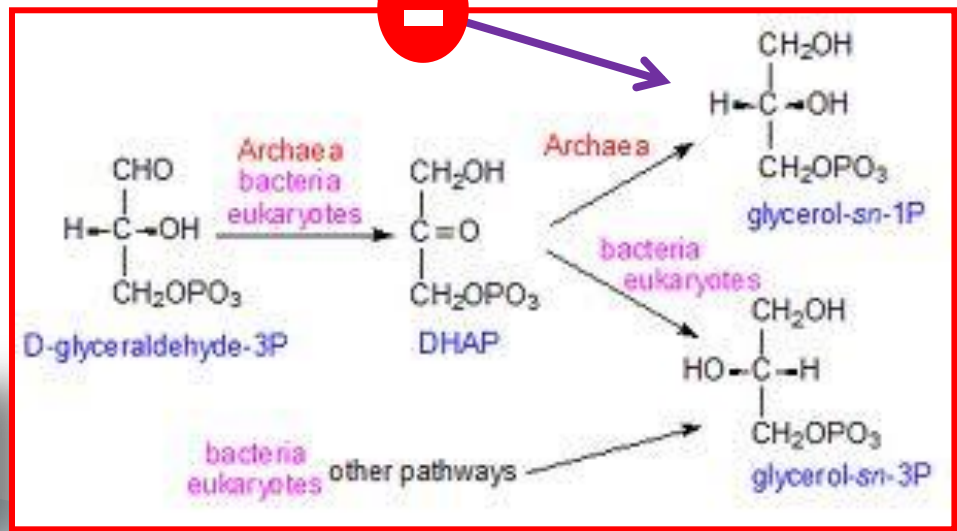
Page 6 in Handouts

Enzyme-substrate interactions

Roles of the atoms of active site on E-S interactions:

- **The specificity of binding depends on the precisely defined arrangement of atoms in an active site.**
- **Example- Glycerol is a symmetric molecule, consequently it is assumed that both the – CH_2OH groups would react IDENTICALLY.**
- **This was proven not to be the case by OGSTON (1948) rather his experiment showed that an asymmetric enzyme, which attacks a symmetrical molecule, could distinguish between its identical groups.**

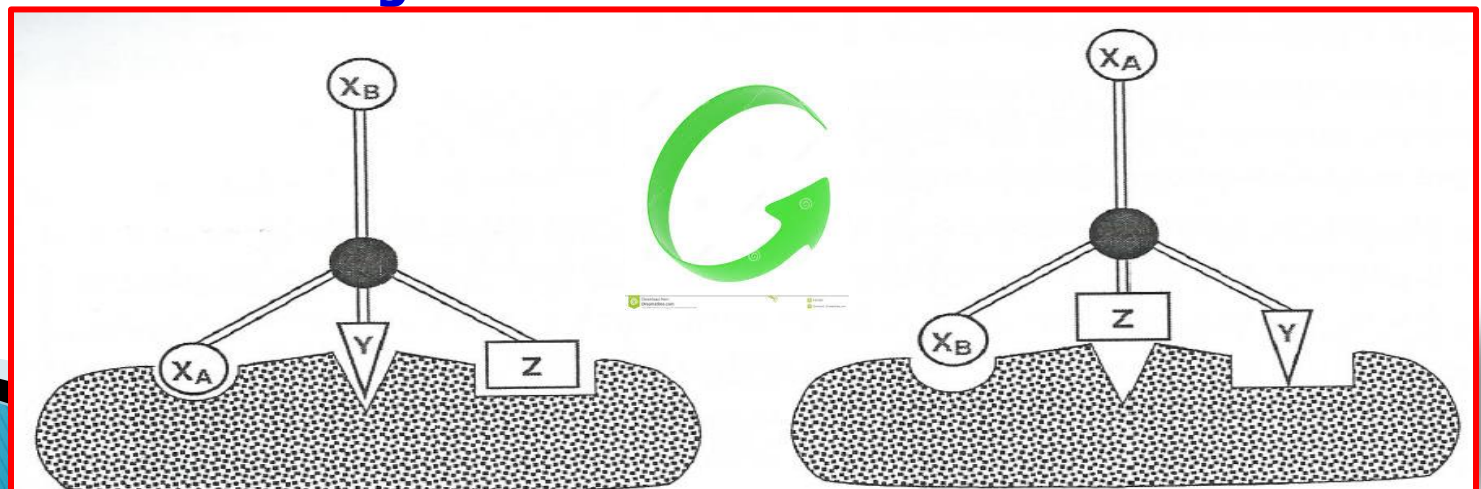




Enzyme-substrate interactions

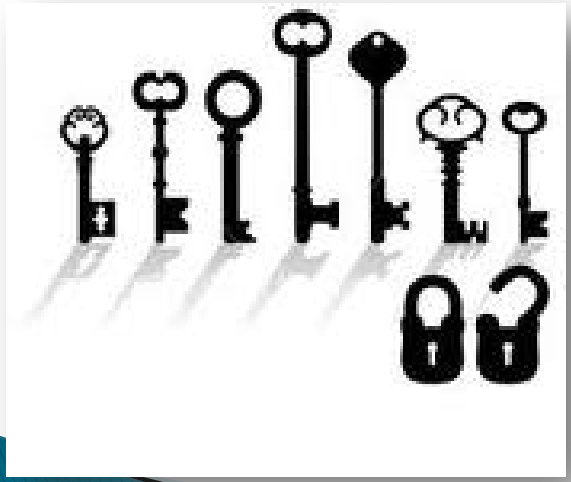
Roles of the atoms of active site on E-S interactions:

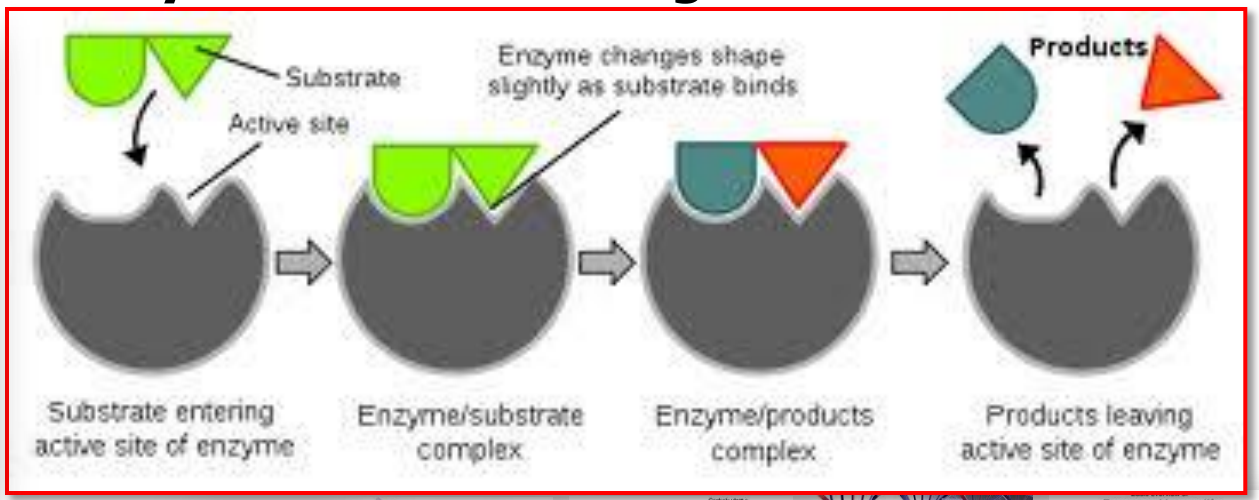
- **Consider, a molecule has 2 hydrogen atoms Xa and Xb, a group Y and another group Z bonded tetrahedrally to a carbon atom.**
- **Now suppose the enzyme binds 3 groups of this substrate i.e. Xa, Y and Z at 3 complimentary sites.**
- **In contrast the other combination Xb, Y and Z cannot be bonded to this active site, because the 2 hydrogen atoms Xa and Xb are not sterically equivalent in terms of Y and Z.**
- **Therefore the bonding of the substrate depends on the precisely defined arrangement of atoms in the active site.**

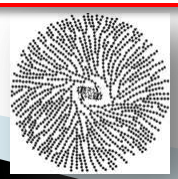


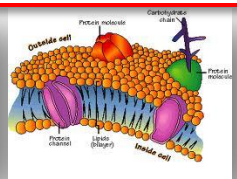
Lock and key model?

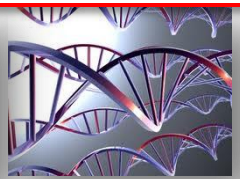
- Lock and Key Model of enzyme was first described by **E. Fishcer in 1890**
- **The enzyme and substrate recognize each other by Lock and Key recognition process is also called STATIC RECOGNITION**
- **To fit into an ACTIVE SITE the substrate must have a matching or complementary shape which is called "Lock and Key Model" of enzyme substrate recognition**

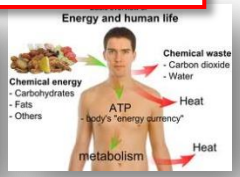




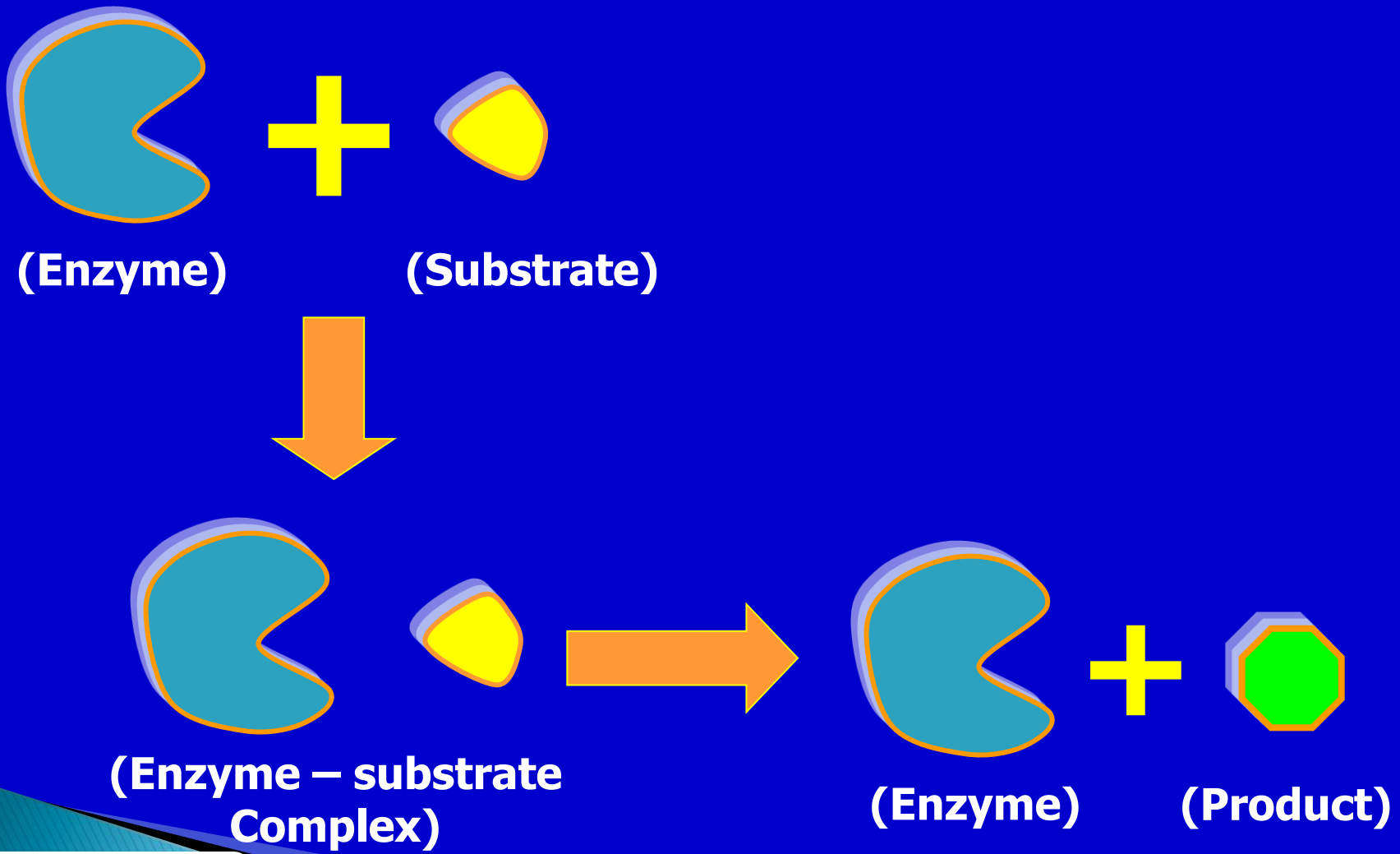






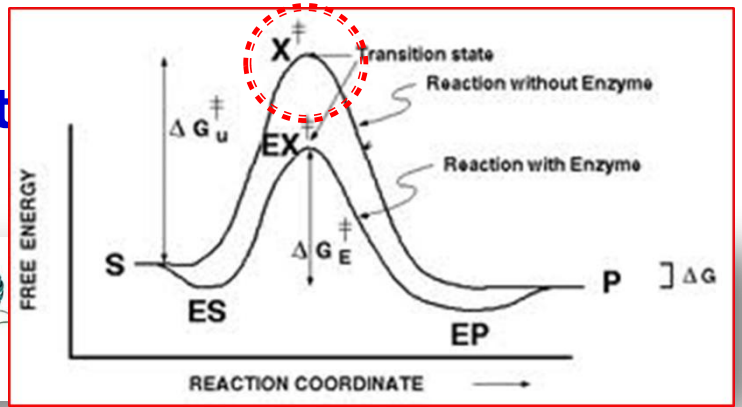


Lock and Key Model?

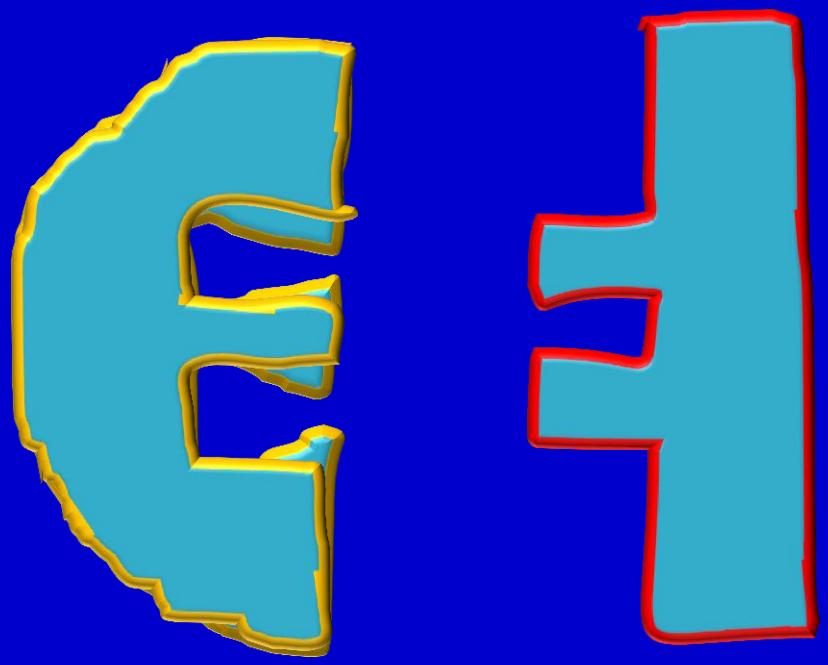


Induced-fit model?

- Active sites of some enzymes are not rigid
- In 1958, D.E. Koshland predicted that the binding of substrate modifies the shapes of some enzymes
- Active sites of some enzymes are become complementary to the shape of the substrate as soon as they bind to the enzyme
- As it is a substrate induced active site/ binding so it is called INDUCED-FIT model
- Induced-fit implies DISTORTION on the enzyme and substrate which helps to change the shape of the active site at a friendly state
- This distortion occurs during the transition state of the reaction
- This currently exists as the dominant model for enzymatic catalysis.



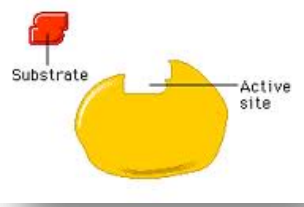
Induced-fit Model?



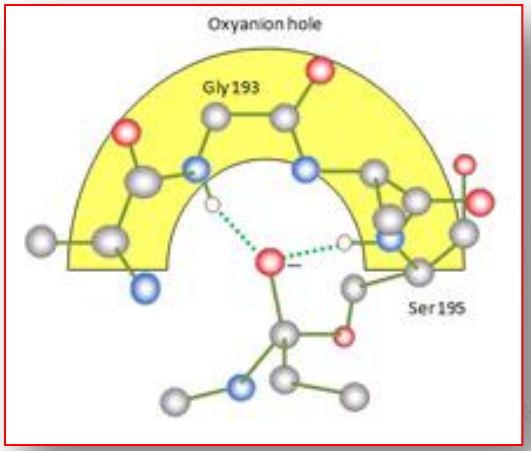
(Enzyme) (Substrate)

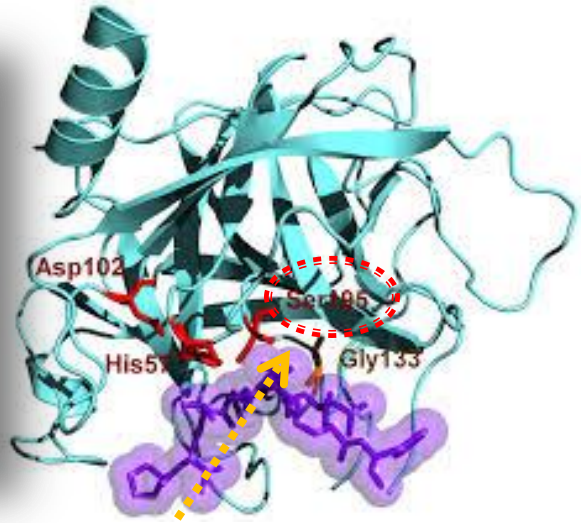
(Enzyme-Substrate Complex)

Structures & mode of actions



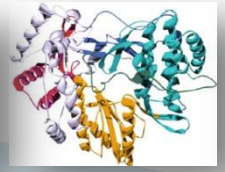
- To illustrate these general principles of enzymes in more detailed manner; two specific enzymes will be discussed:
  - Chymotrypsin
  - Carboxypeptidase A

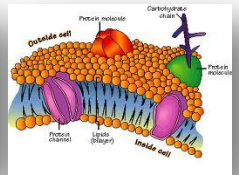


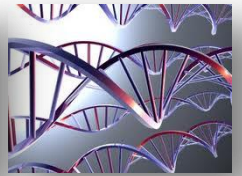


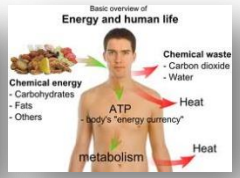
Chymotrypsin:

- Chymotrypsin together with ELASTASE and trypsin belong to a diverse group of enzymes that is commonly referred to as the Serine Protease.
- These enzymes are so named because a serine residue belonging to the enzyme's active site plays a critical role in the catalytic process.



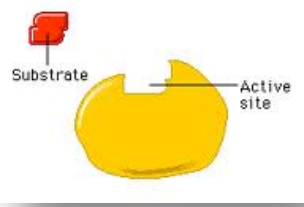






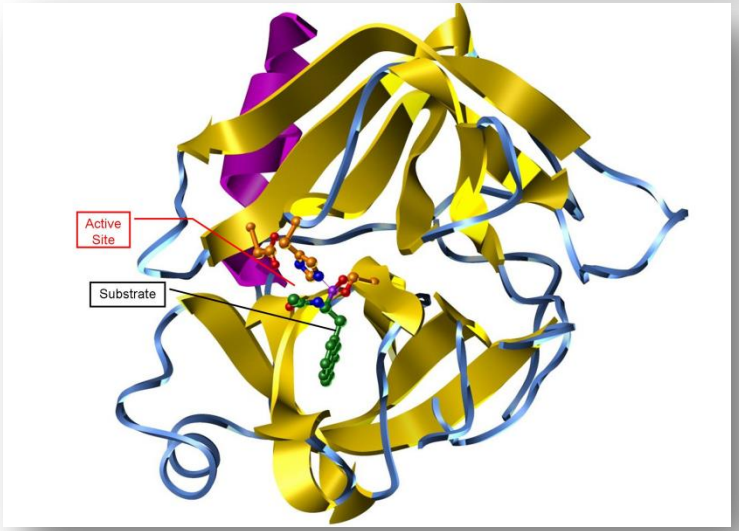
Pg 9-10 in Handouts

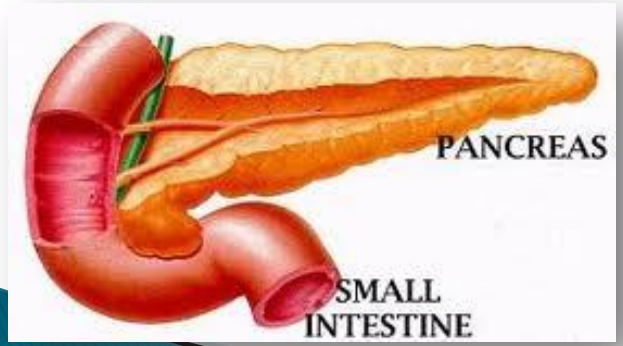
Structures & mode of actions

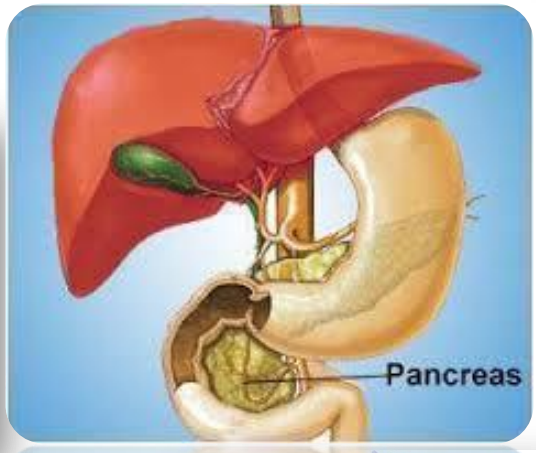


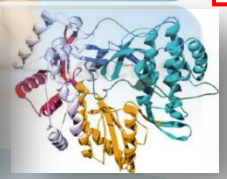
Chymotrypsin: contd…

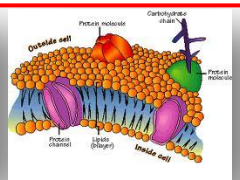
- Chymotrypsin is a 25kD enzyme, consisting of 245 amino acids, 3 polypeptide chains connected by 2 inter-chain disulfide bonds.
- Chymotrypsin is synthesized in the pancreas and is responsible for hydrolysis of proteins in the small intestine.

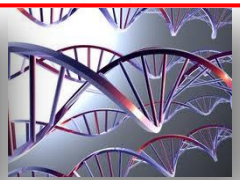


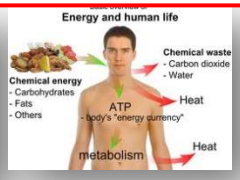






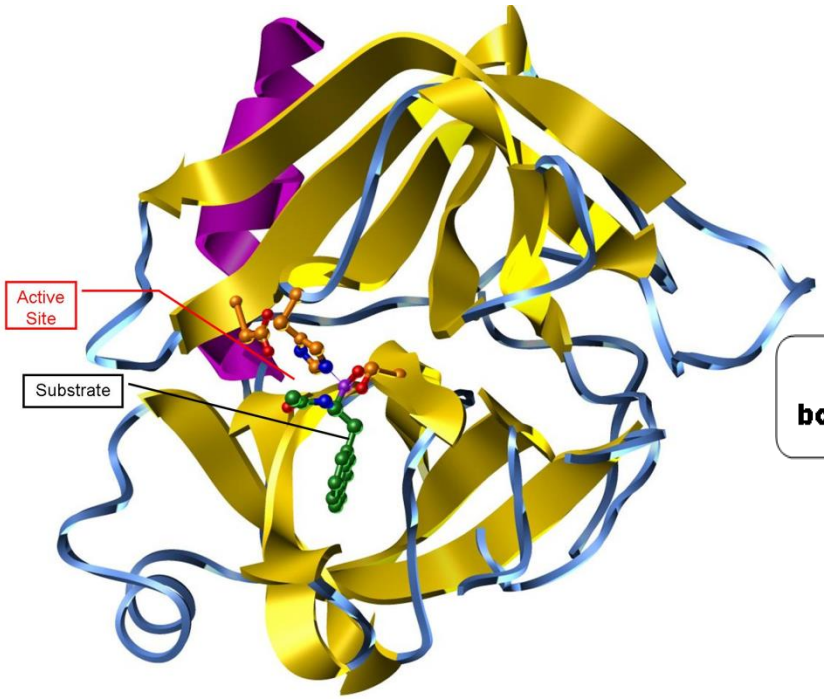




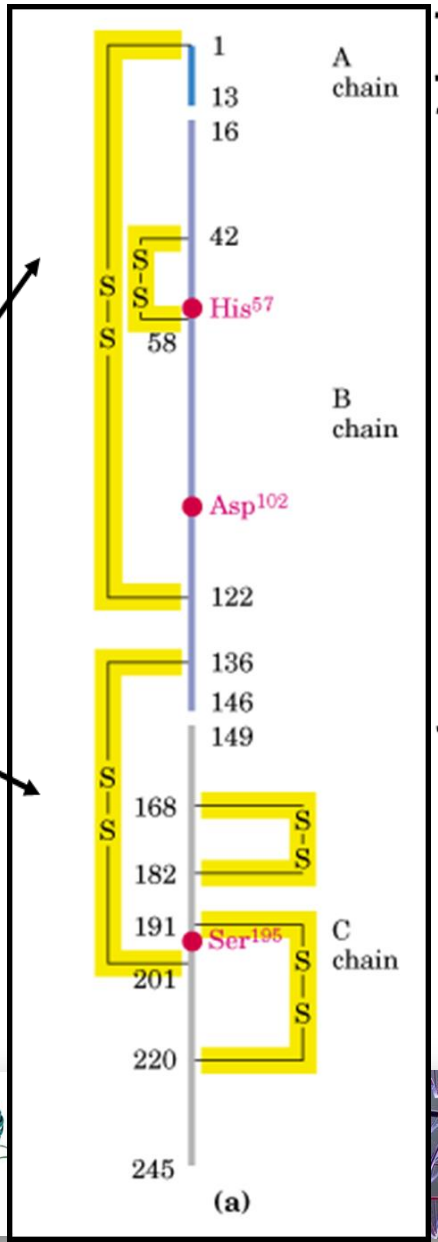


Structures & mode of actions

Chymotrypsin: contd…



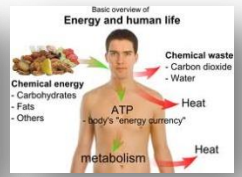
Disulfide bonds/ bridges



A Chain

B Chain

C Chain

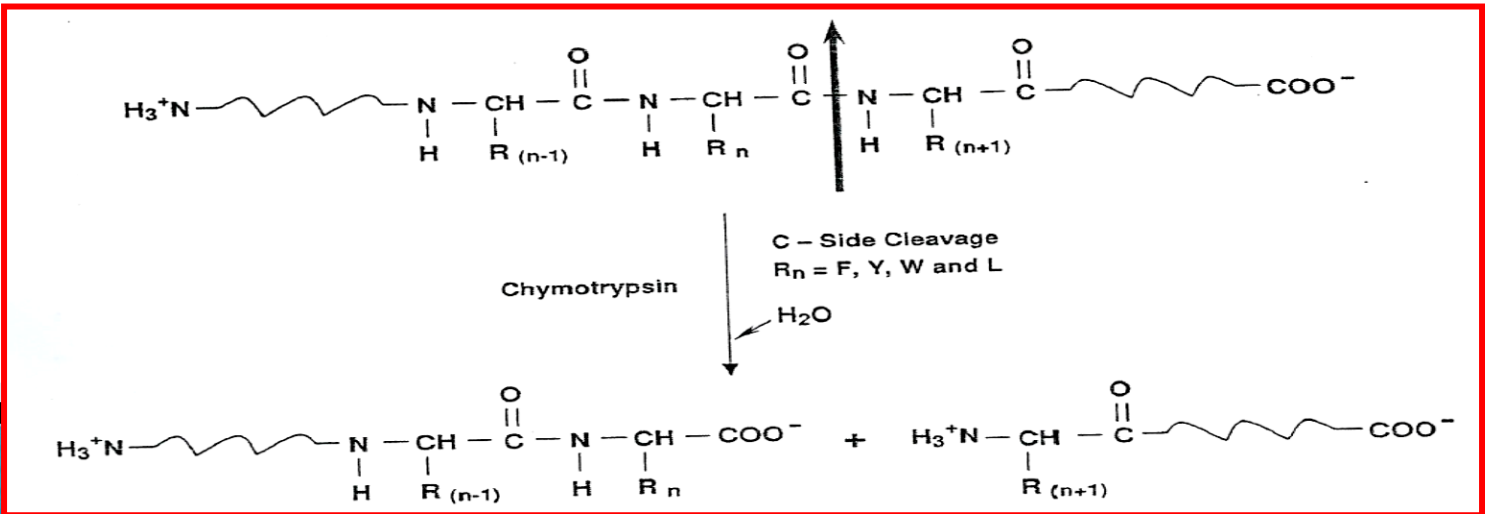


Structures & mode of actions

Chymotrypsin: contd…

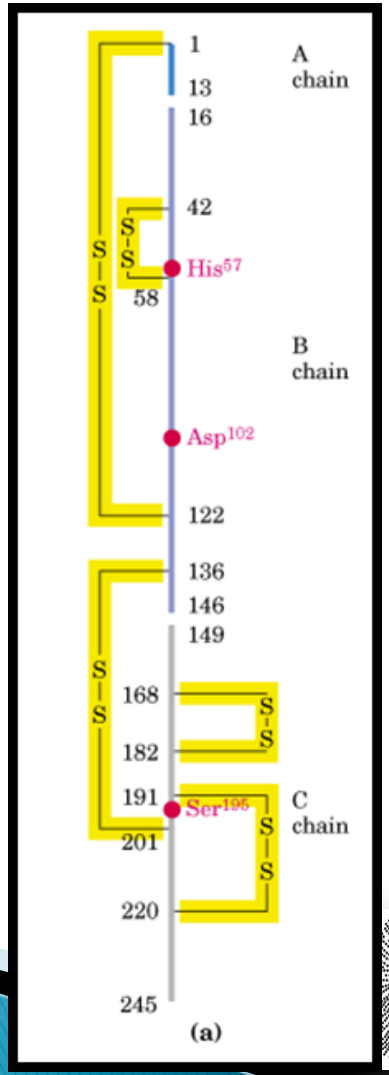
- It catalyzes the hydrolysis of peptide bonds.
- It does not cleave all peptide bonds, instead it is selective for peptide bonds on the –COOH side of the aromatic amino acid residues such as-
  - Tyrosine
  - Tryptophan and
  - Phenylalanine

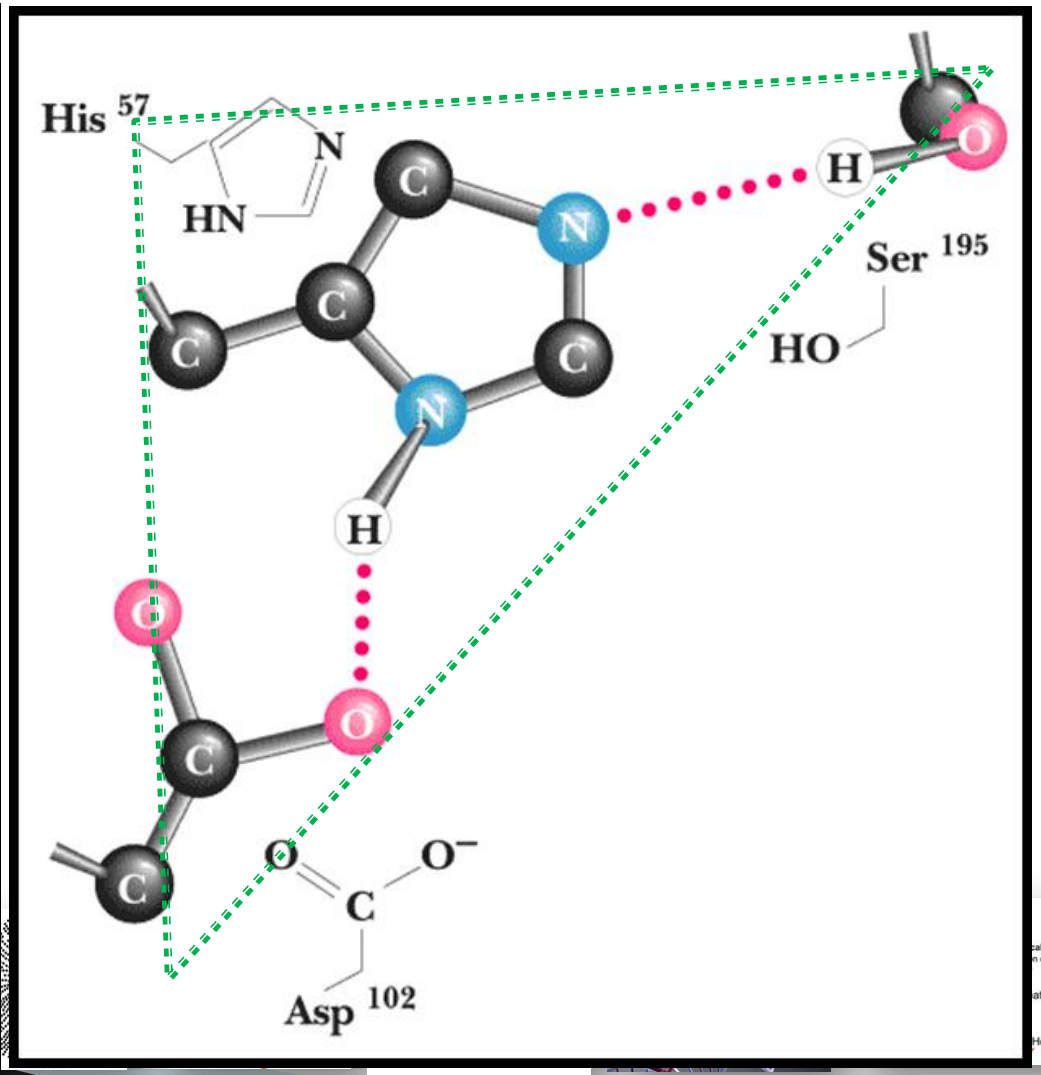
  and of large hydrophobic residues e.g. Leucine
- In addition, it can also act as an esterase for esterification reaction



Hydrolysis by chymotrypsin

- **$Ser_{195,}$ His_{57} and Asp_{102} form a catalytic TRIAD. This functions as an alternating donor and acceptor of protons.**

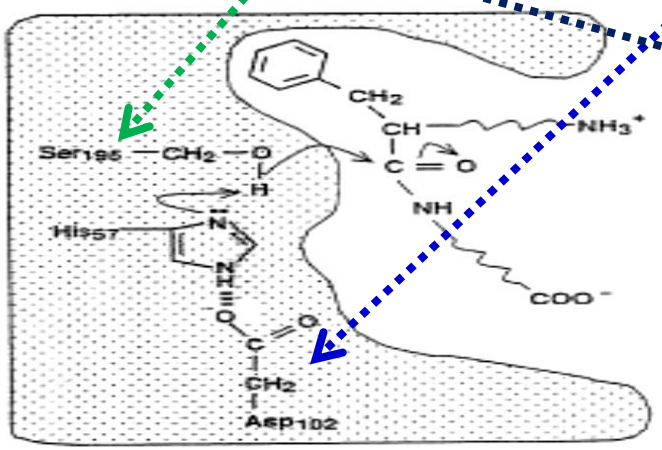




Hydrolysis by chymotrypsin

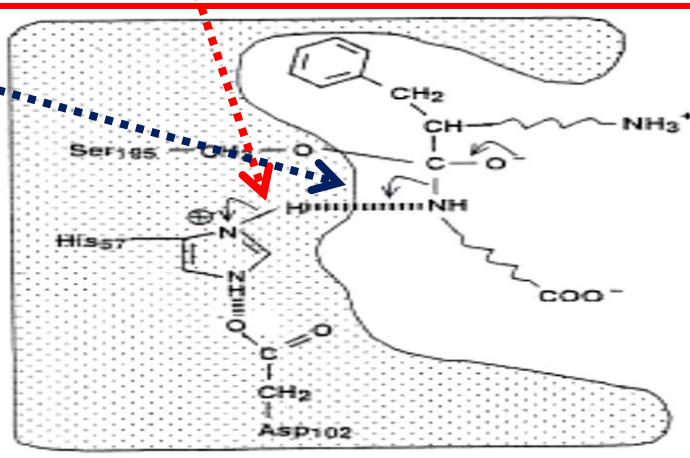
ACYLATION: Step 1-3

- Acylation starts with a nucleophilic attack by the oxygen atom of the hydroxyl group of Ser195 on the carbonyl carbon atom of the susceptible peptide bond.
- A proton is then transferred from Ser195 to His57. The catalytic triad facilities this transfer, i.e. ASP102 precisely orientates the imidazolium ring of His57 and it also neutralizes the newly acquired positive charge that develops on it.
- A tetrahedral transition state is thus formed.



STEP 1

Polypeptide substrate binds noncovalently with side chains of hydrophobic pocket to form the enzyme-substrate complex



STEP 2

H^+ is transferred from Ser to His to yield a tetrahedral transition state

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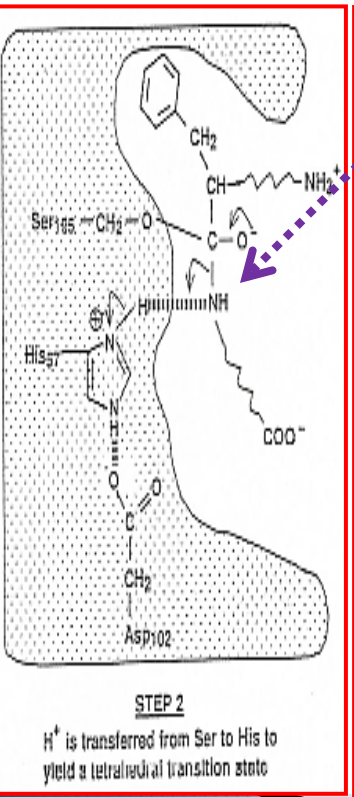
Hydrolysis by chymotrypsin

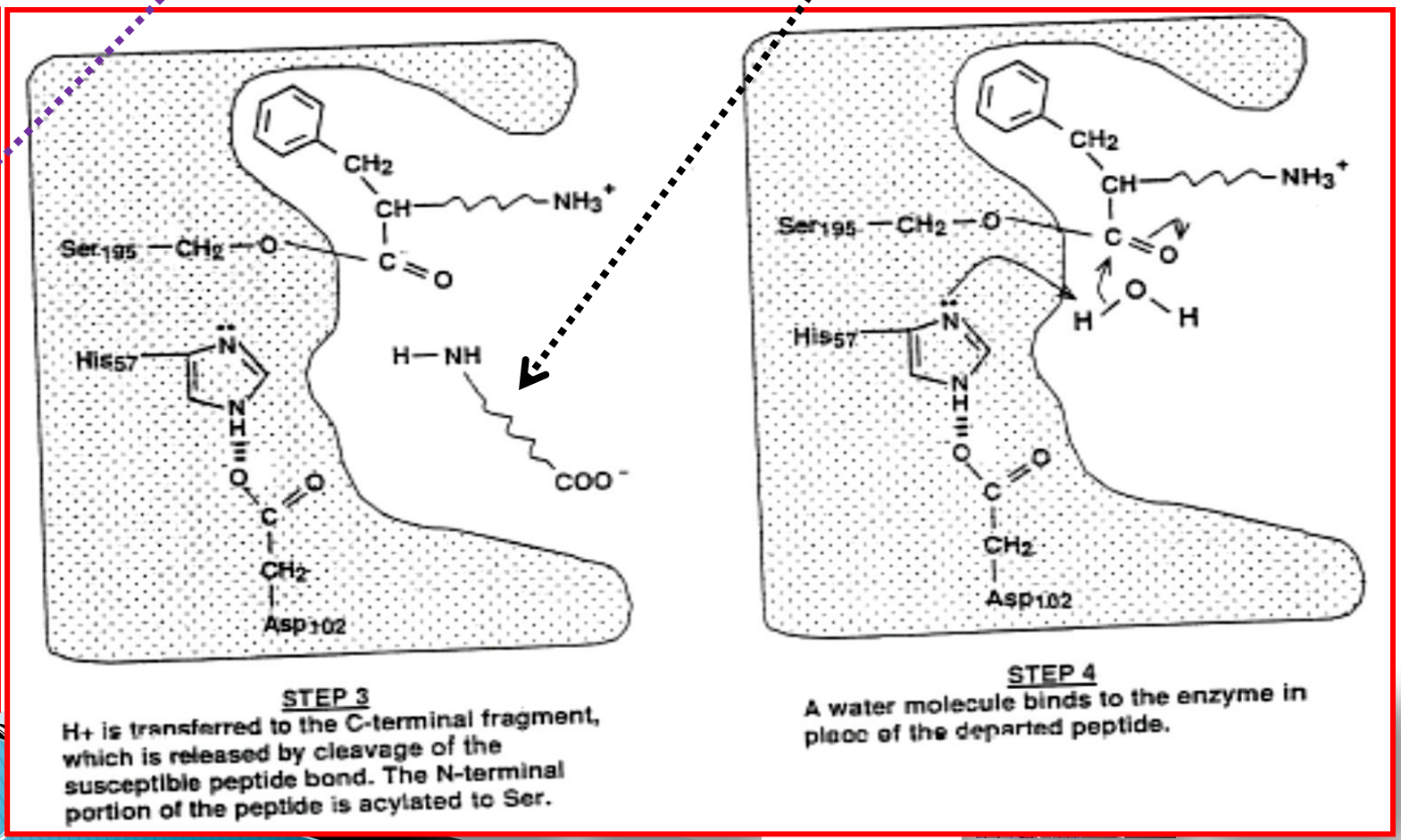
ACYLATION: Step 1-3 contd..

- The proton from His57 is the donated to the N atom of the susceptible peptide bond. The bond is cleaved and the amine component of the substrate diffuses away.



STEP 2

H^+ is transferred from Ser to His to yield a tetrahedral transition state



STEP 3

H+ is transferred to the C-terminal fragment, which is released by cleavage of the susceptible peptide bond. The N-terminal portion of the peptide is acylated to Ser.

STEP 4

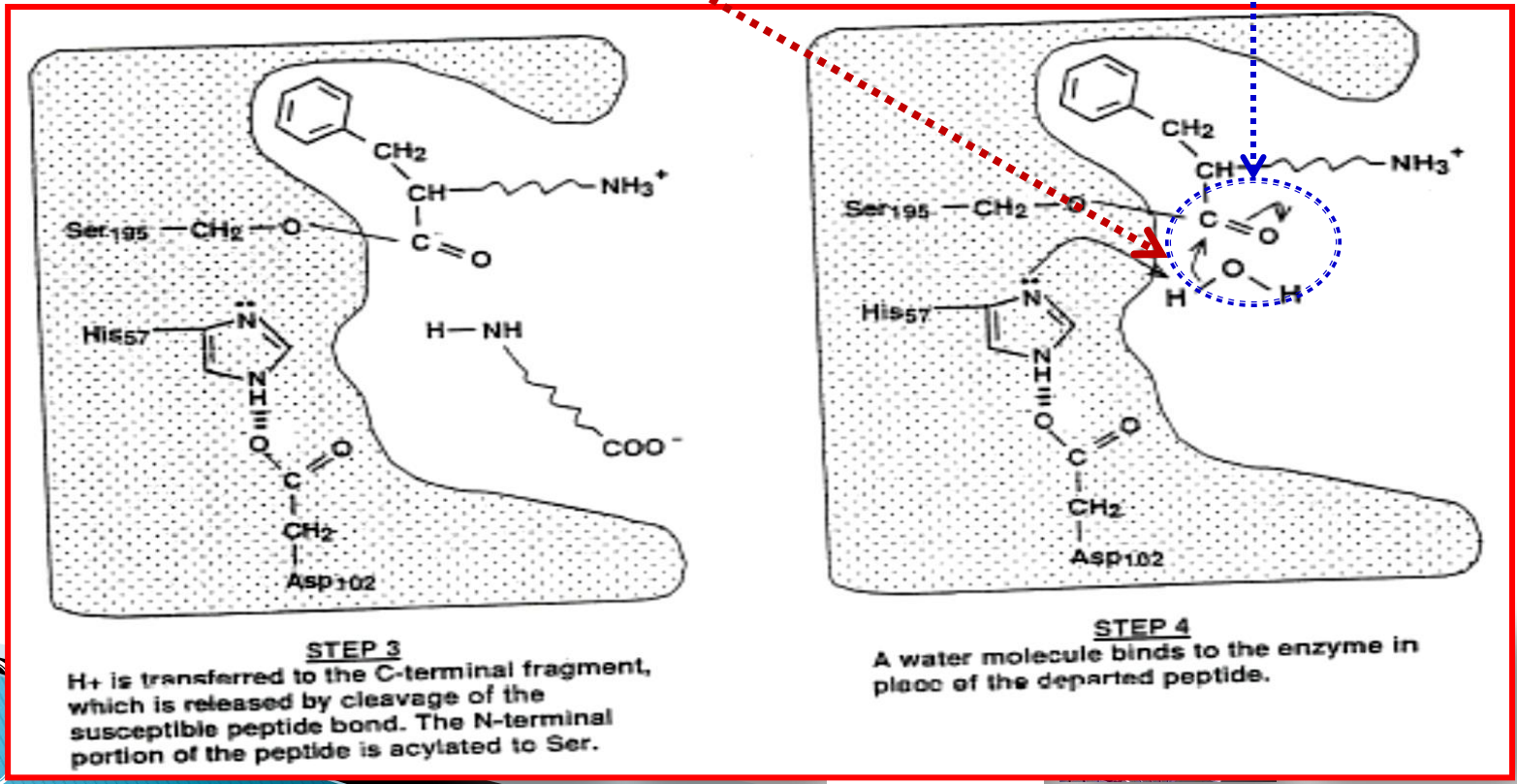
A water molecule binds to the enzyme in place of the departed peptide.

Page 11-12 in Handouts

Hydrolysis by chymotrypsin

DEACYLATION: Step 4-6

- **Deacylation is the reverse of the acylation process with water replacing the amine component.**
- **The catalytic triad, i.e. His_{57} draws a proton from H_2O and the OH- ion attacks carbonyl carbon atom of the acyl group that is attached to Ser_{195}.**



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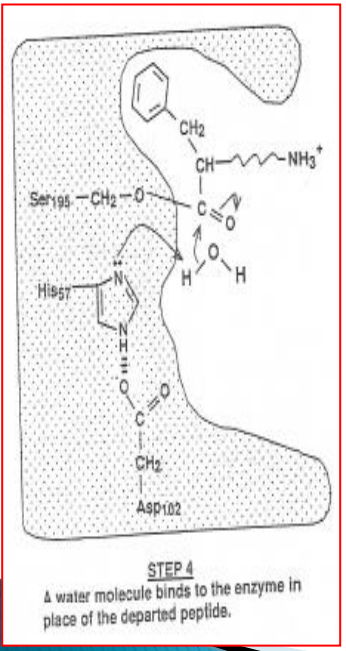
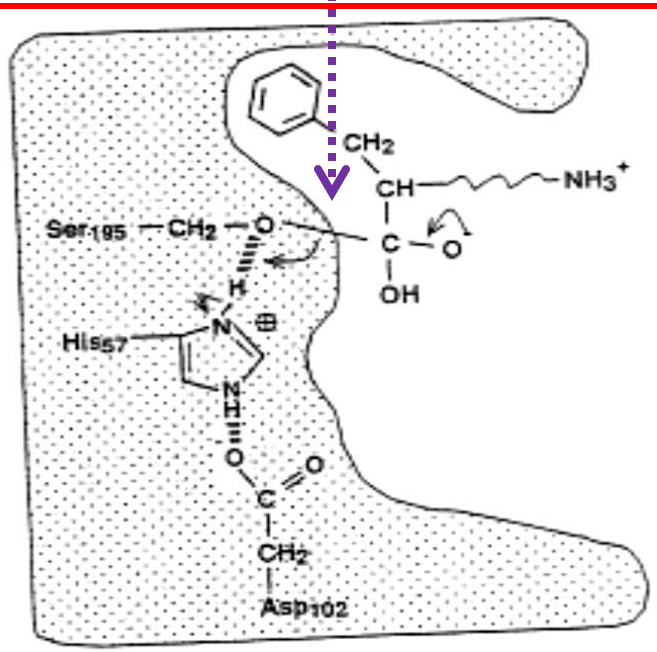
Hydrolysis by chymotrypsin

DEACYLATION: Step 4-6 contd…

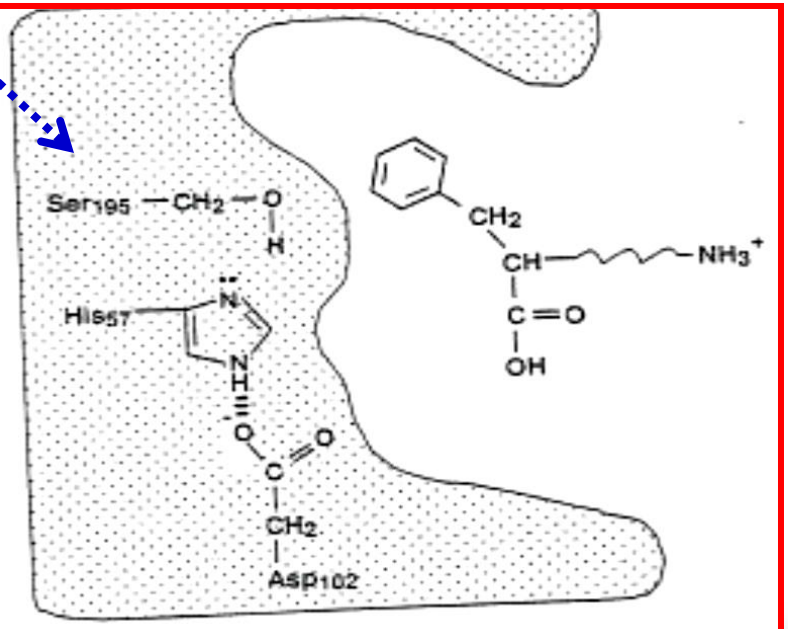
- **A tetrahedral transition state is formed.**
- **His_{57} then donates a proton to Ser_{195}, which releases the acid component of the substrate.**
- **The free enzyme is generated for another round of catalysis.**



STEP 4
A water molecule binds to the enzyme in place of the departed peptide.



STEP 5
The water molecule transfers its proton to His and its OH^- to the remaining portion of the substrate.



STEP 6
The second peptide fragment is released, the acyl bond is cleaved, the proton is transferred back to Ser, and the enzyme returns to its initial state ready fort another round of catalysis.

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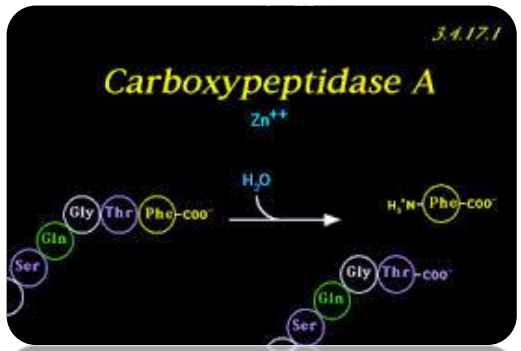
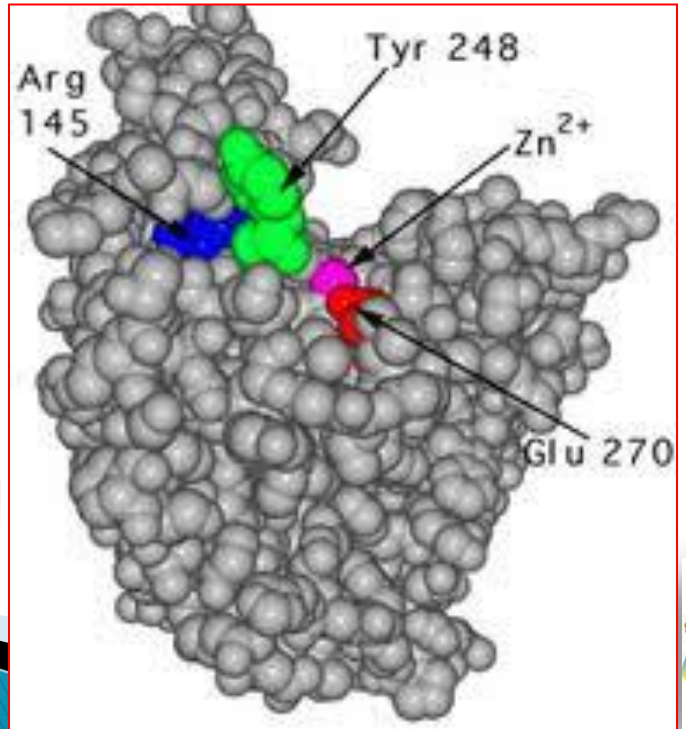
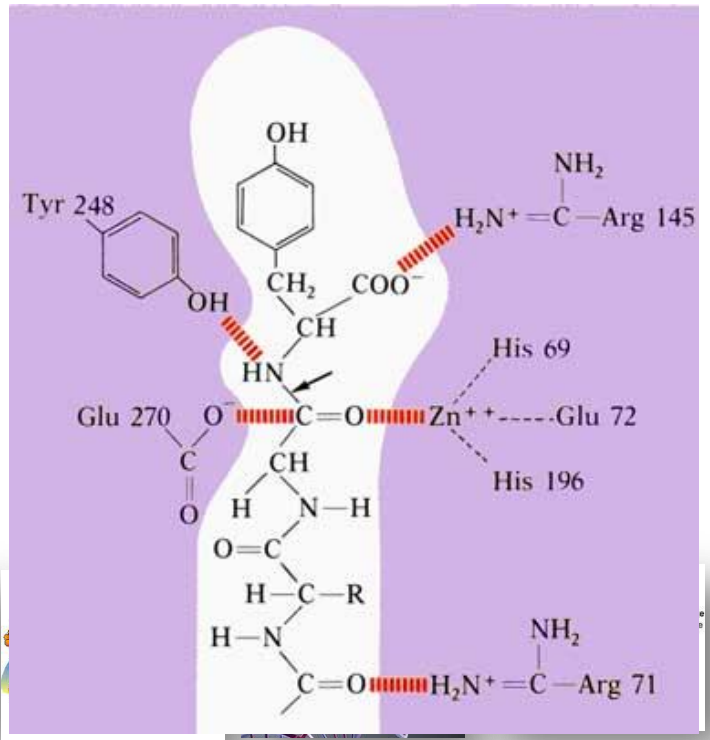
Carboxypeptidase

- This enzyme belongs to a group of enzymes that are commonly referred to as the Zinc Proteases.
- Carboxypeptidase hydrolyzes the CARBOXYL TERMINAL AMINO ACID residues of proteins and peptides hence it is called carboxypeptidase.
- Hydrolysis occurs most readily when the terminal residue is an aromatic or bulky aliphatic side chain.

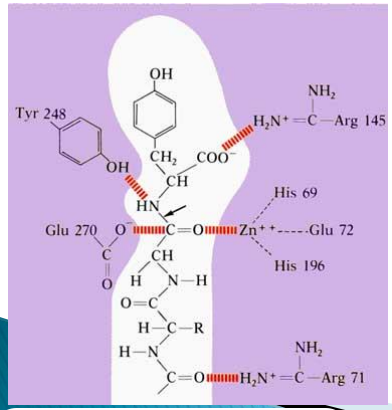


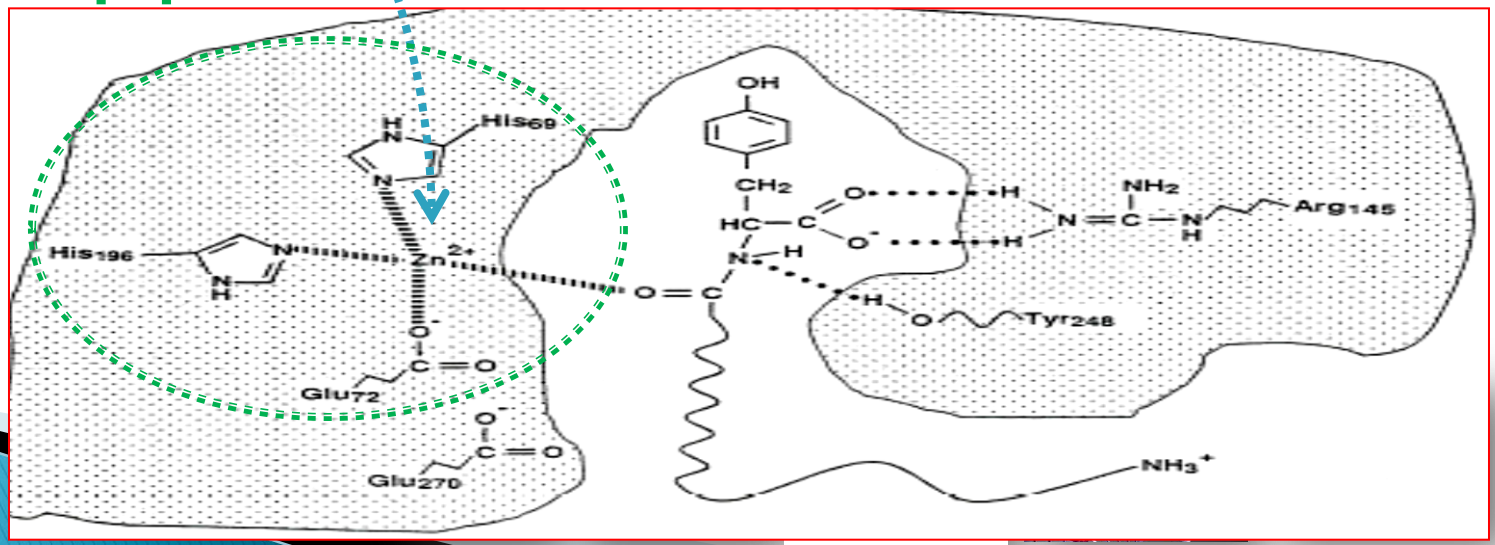




Carboxypeptidase - Mecahanism

- **The following amino acid residues are critical to the catalytic mechanisms of Carboxypeptidase A:**
  - **Zn^{2+} Ion:**
    - **This is an essential cofactor for catalytic activity.**
    - **It's electron-withdrawing character, increases the polarity of the susceptible carbonyl carbon atom.**
  - **His_{69}, His_{196} and Glu_{72}:**
- **His_{69}, His_{196} and Glu_{72} are responsible for holding the Zn^{2+} ion in position so as to interact optimally with the susceptible peptide bond of the substrate.**





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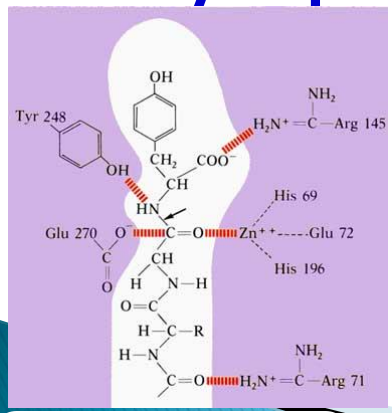
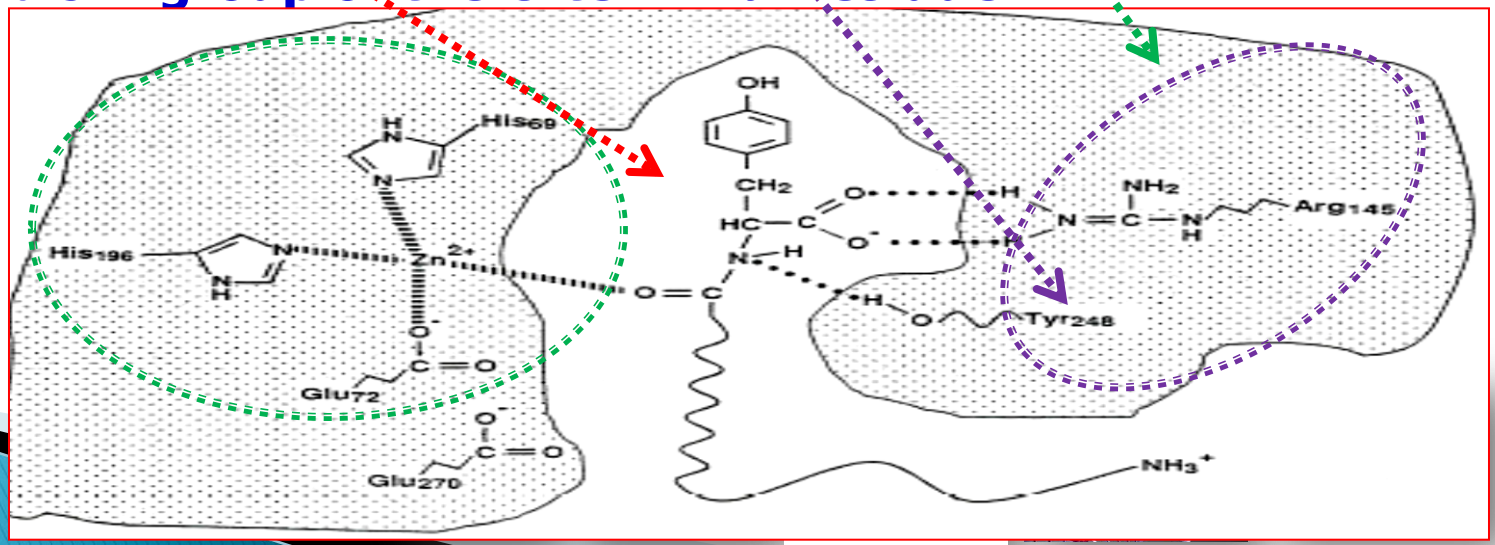
Carboxypeptidase - Mecahanism

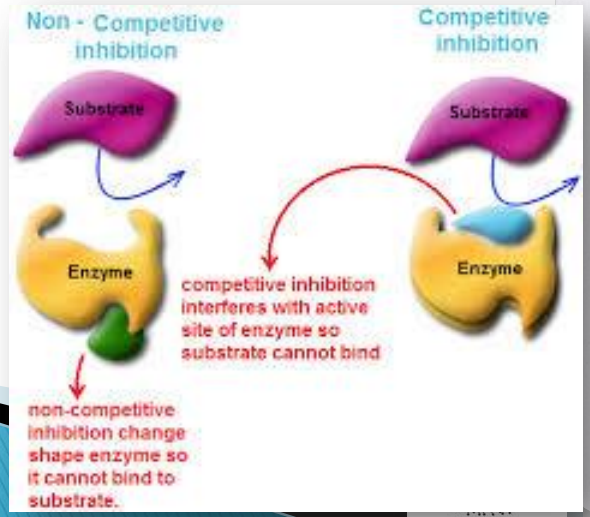
- **Tyr_{248}:**
- Tyr_{248} in conjunction with Zn^{2+} interacts with the substrate to sensitize the susceptible peptide bond in the substrate, which is subsequently cleaved by H_2O.
- **Tyr_{248} and Arg_{145}:**
- Tyr_{248} and Arg_{145} have important hydrogen bonding interactions with the substrate and these interactions account for the carboxyl-terminal specificity of this protease.
- **Deep pocket:**
- Deep pocket helps anchor the substrate by binding the hydrophobic R-group of the C-terminal residue.





Enzyme inhibition

- The inhibition of enzymatic activity by specific small molecules and ions is important because it serves as a major control mechanisms in biological system.
- Many drugs and toxic agents act by inhibiting enzymes.
- Inhibition can also be used to study the mechanism of enzyme action.
- Enzyme inhibition can be classified into TWO major classes such as-
  - Irreversible inhibition and
  - Reversible inhibition



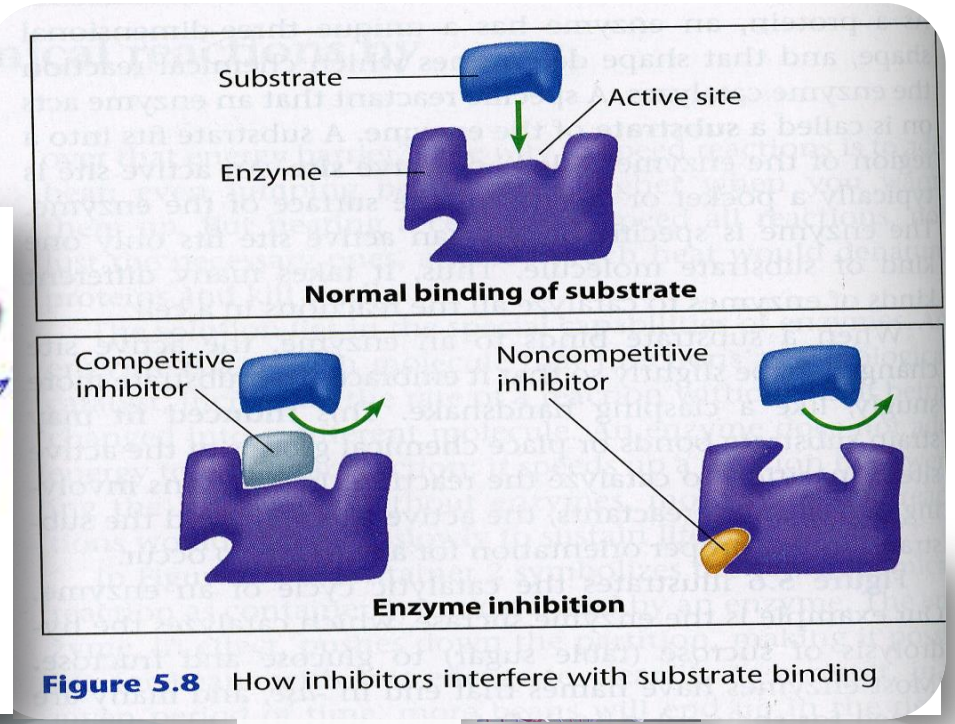


Figure 5.8 How inhibitors interfere with substrate binding

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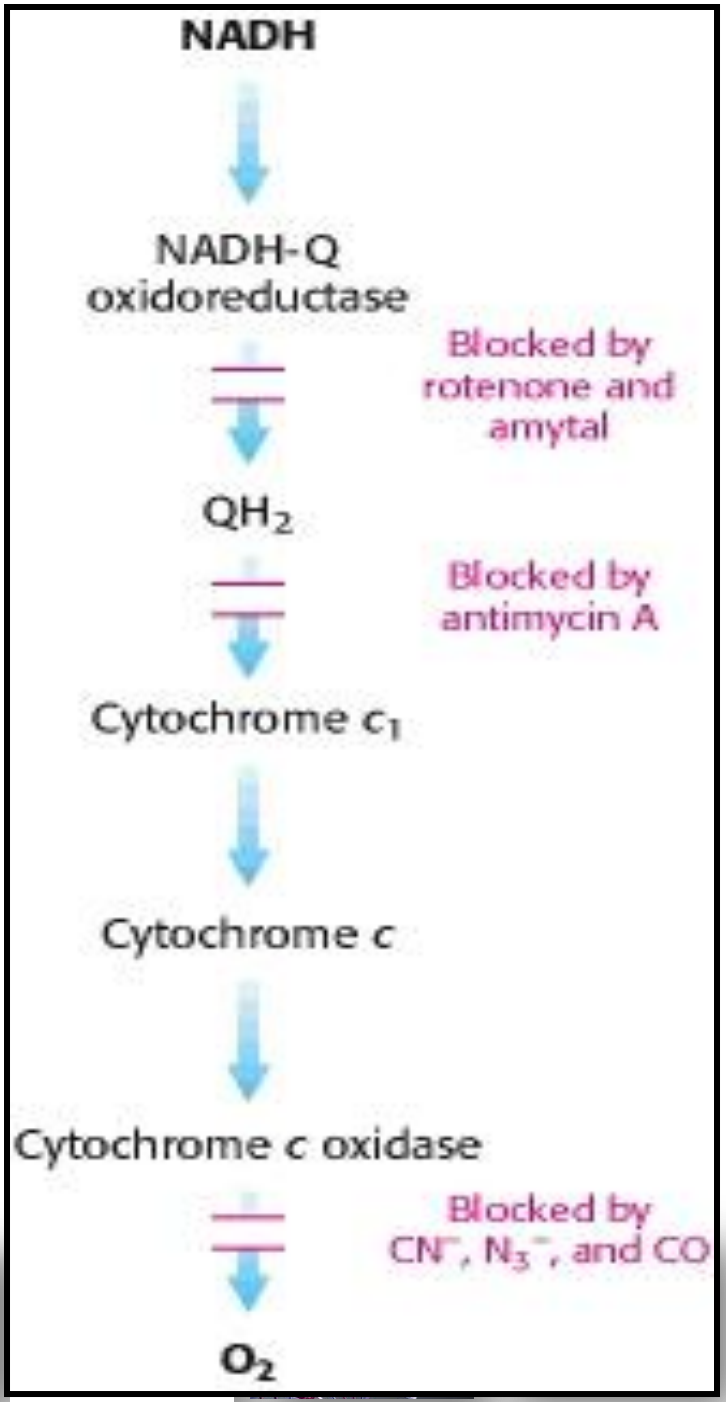
Enzyme inhibition

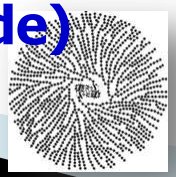
Irreversible inhibition:

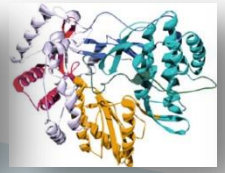
- **Some substances bind COVALENTLY with enzymes so as to inactivate them irreversibly.**
- **All irreversible enzyme inhibitors are toxic substances either neutral or man-made e.g.**
  - **Cyanide**
    - **Reacts with enzyme metal ions, Fe2+, Zn2+**

Irreversible inhibitors react with some functional group in the active site, to block the substrate from binding to the active site and inactive the enzyme.

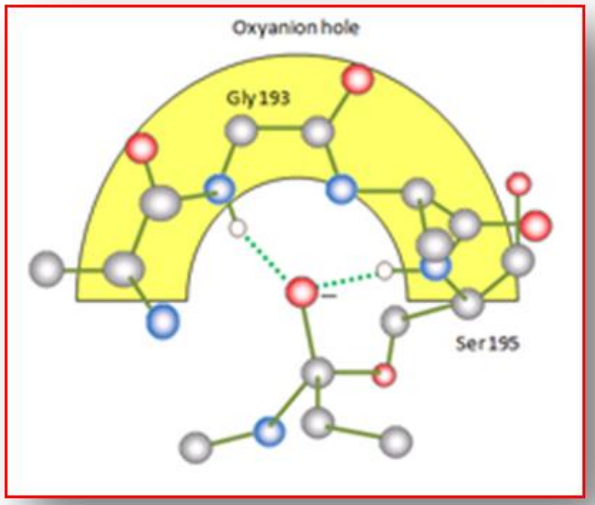
Example – DFP (next slide)





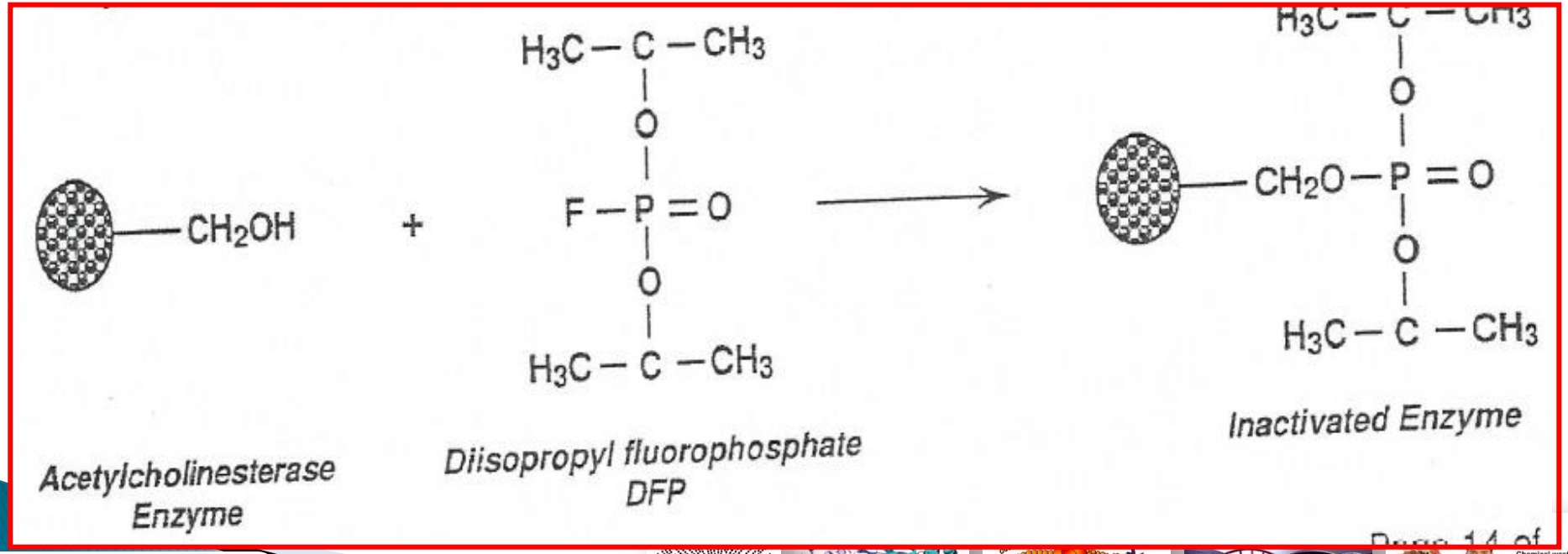


Enzyme inhibition



Irreversible inhibition: contd…

- **Diisopropyl fluorophosphate (DFP)**
  - DFP acts as an irreversible inhibitor of enzymes that contain an essential SERINE residue in their active site e.g. Chymptrypsin or acetylcholinesterase (ACE). **Binds with serine covalently.**

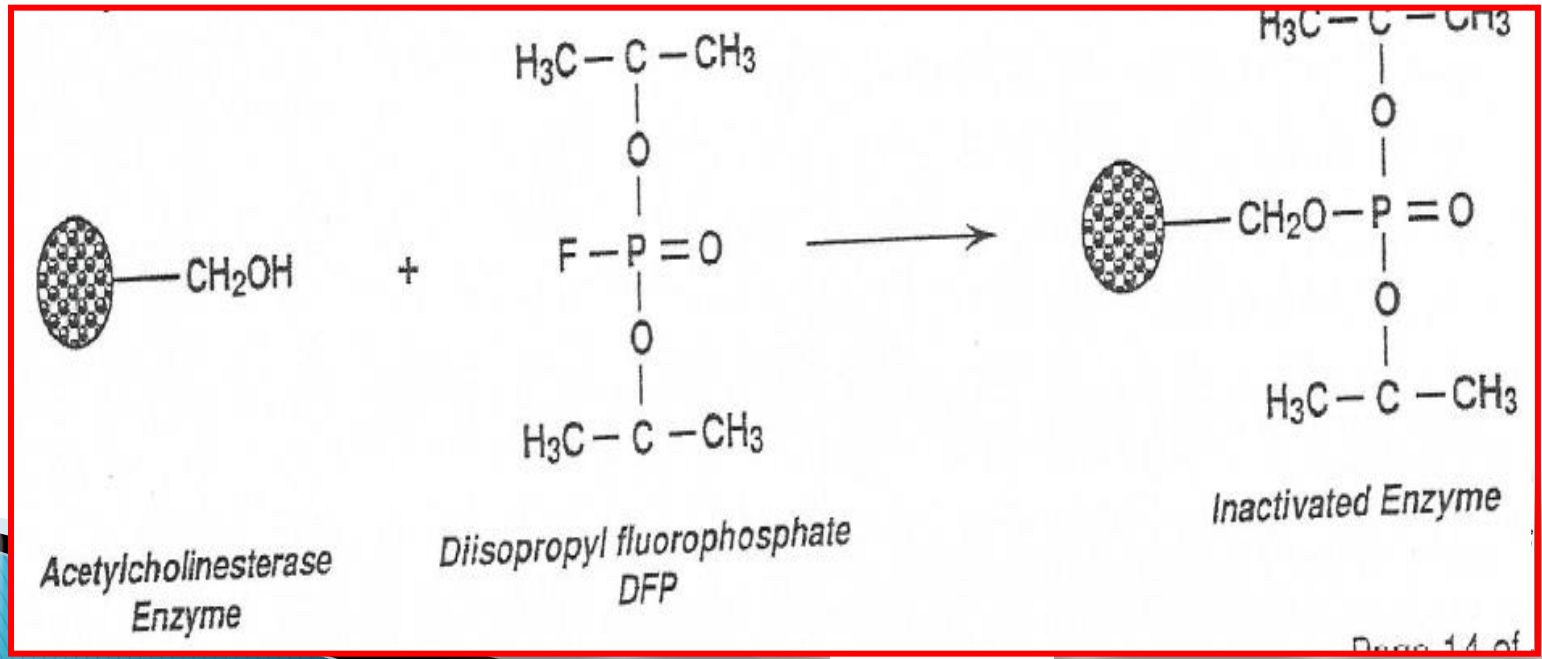


Enzyme inhibition

Irreversible inhibition: contd…

- Diisopropyl fluorophosphate (DFP)

- Acetylecholinesterase (ACE) is essential for nerve conduction and its inhibiton by DFP causes rapid paralysis of vital functions.
- Numerous insecticides and nerve gases are potent acetylcholinesterase (ACE) inhibitors which are usually used as biological weapons during war.



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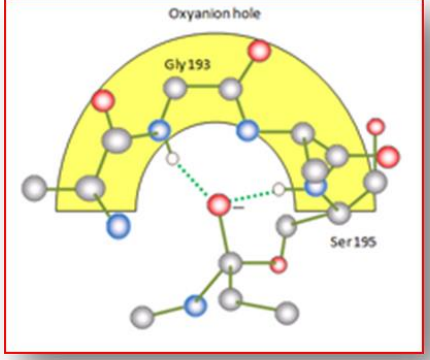
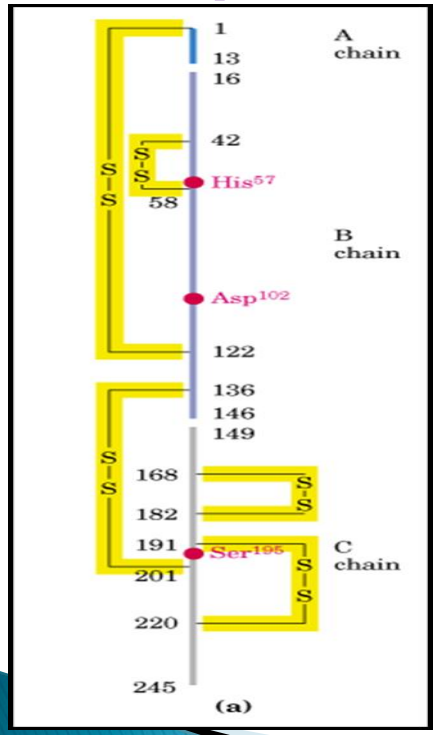
Enzyme inhibition

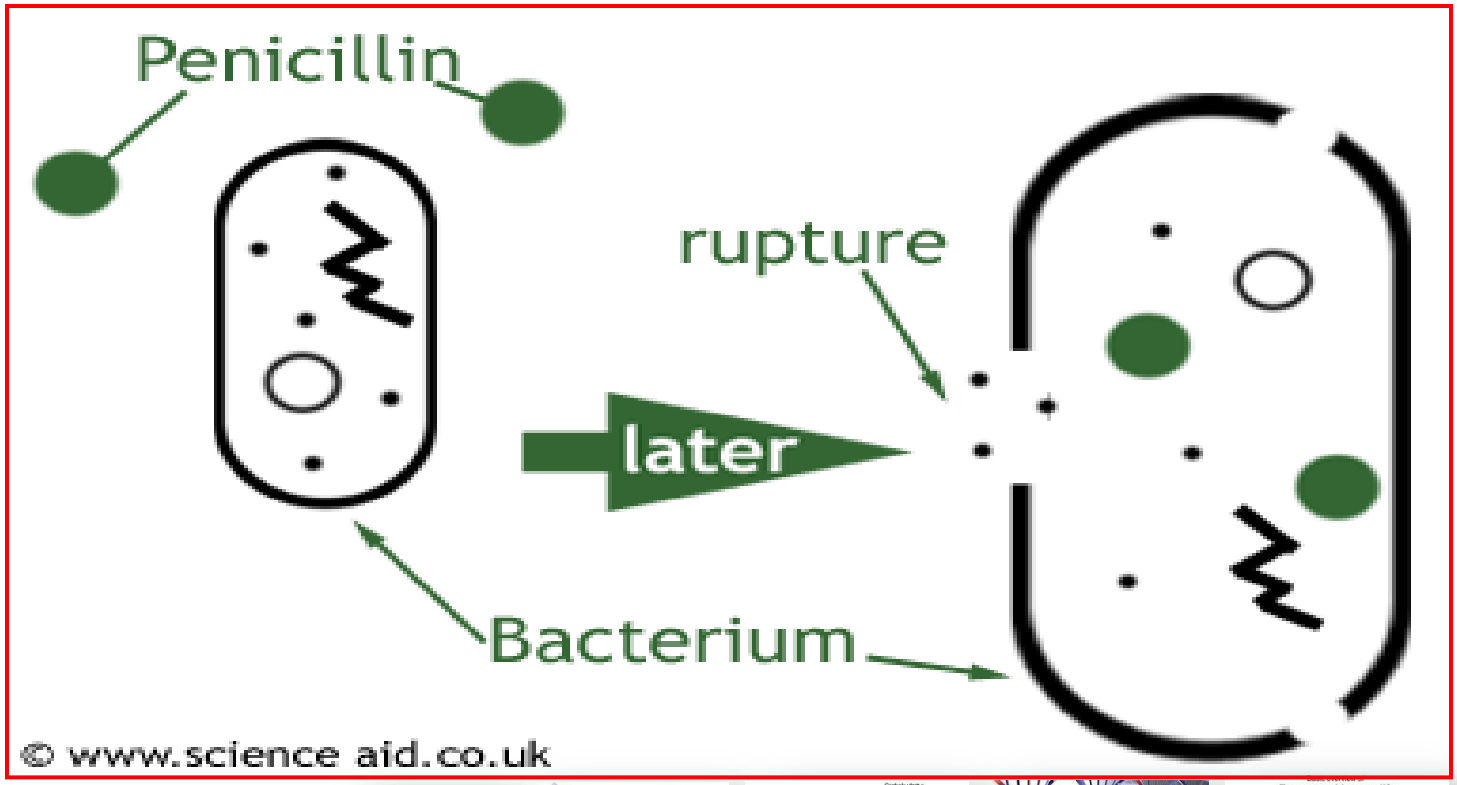


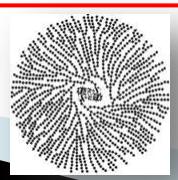
Irreversible inhibition: contd…

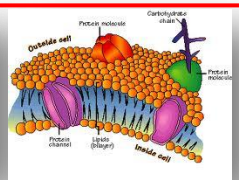
- **Penicillin:**

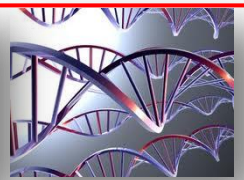
- Penicillin reacts covalently with active site serine residue and inhibits enzymes that are involved in bacterial cell wall synthesis

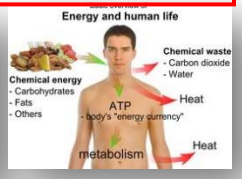




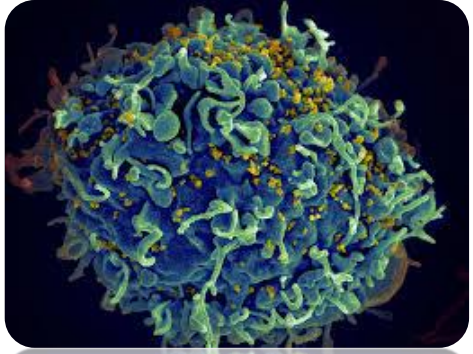


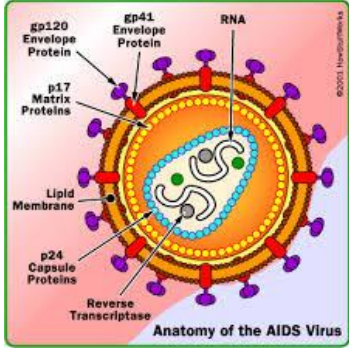






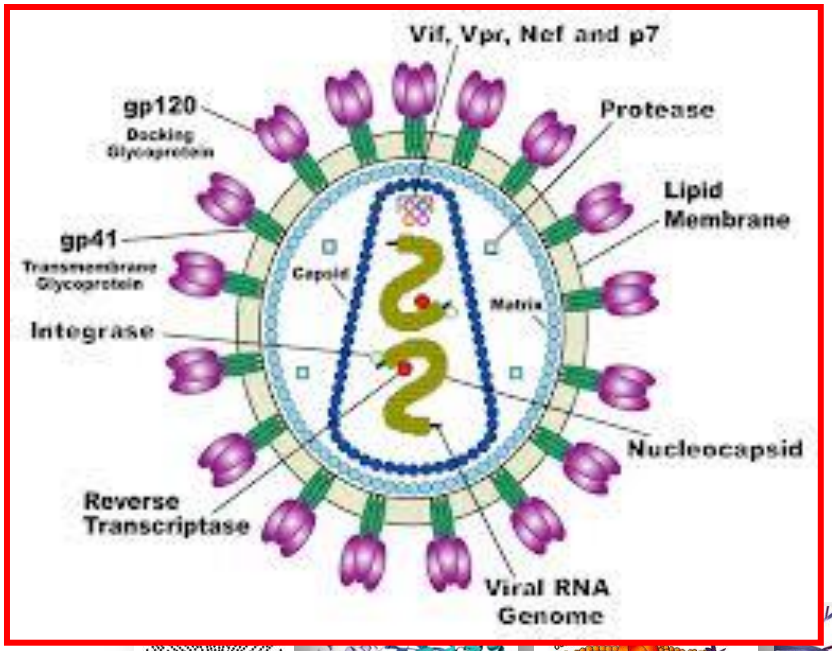
Enzyme inhibition

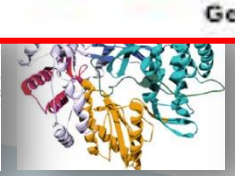


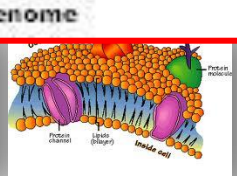


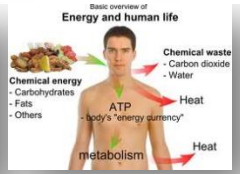
Irreversible inhibition: contd…

- **Multi-drug therapy**
  - Currently the most effective HIV (AIDS) treatment is a multi-drug therapy that includes protease inhibitors.
  - This inhibitor inactivate a viral enzyme that is crucial in viral replication.





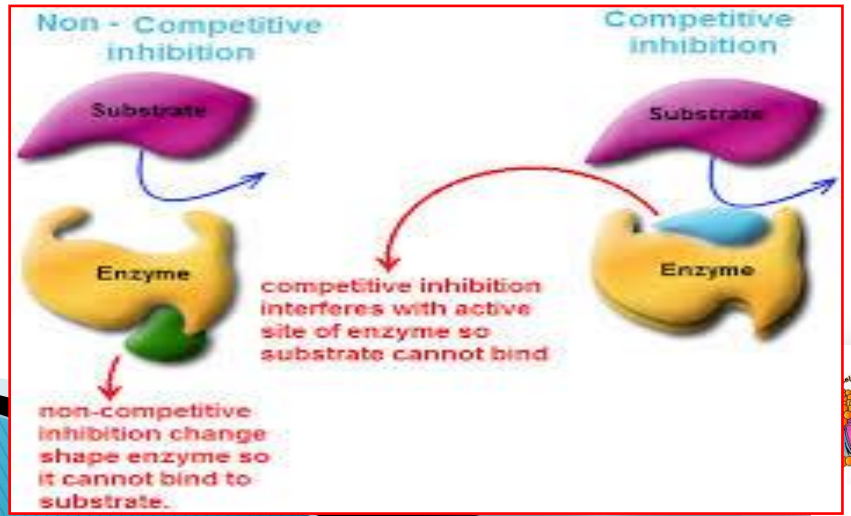




Enzyme inhibition

Reversible inhibition:

- **Reversible inhibition is classified into three sub-classes:**
  - **Competitive inhibition**
  - **Non-competitive inhibition**
  - **Uncompetitive inhibition**
- **The various modes of reversible inhibition all involve the NON-COVALENT binding of an inhibitor to the enzymes, but they differ in their mechanisms of binding.**
- **By this way, they decrease the enzymes activity and this is how they affect the kinetics of the reaction.**



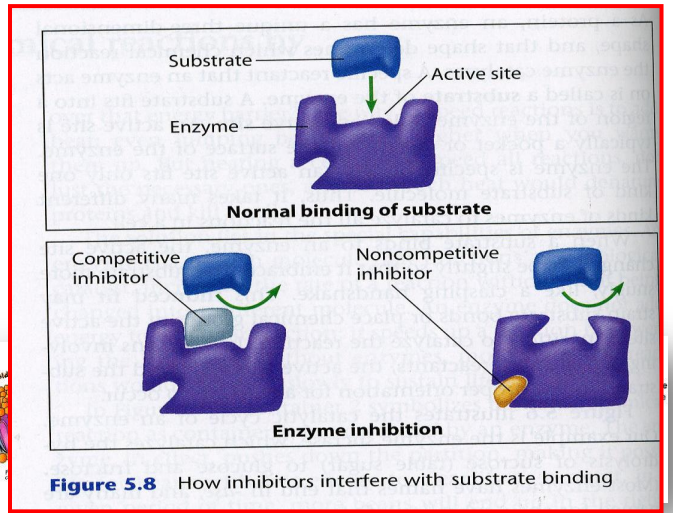


Figure 5.8 How inhibitors interfere with substrate binding

Enzyme inhibition

(1) Competitive inhibition:

- A competitive inhibitor (I) is a substance that reversibly binds with the free form of an enzyme (E) to produce a binary EI complex that is incapable of binding a substrate (S).
- Therefore when E, S, and I are present, E can bind with S to yield ES, or E can bind with I to yield EI.
- However, E cannot bind simultaneously with S and I to yield a ternary complex EIS.

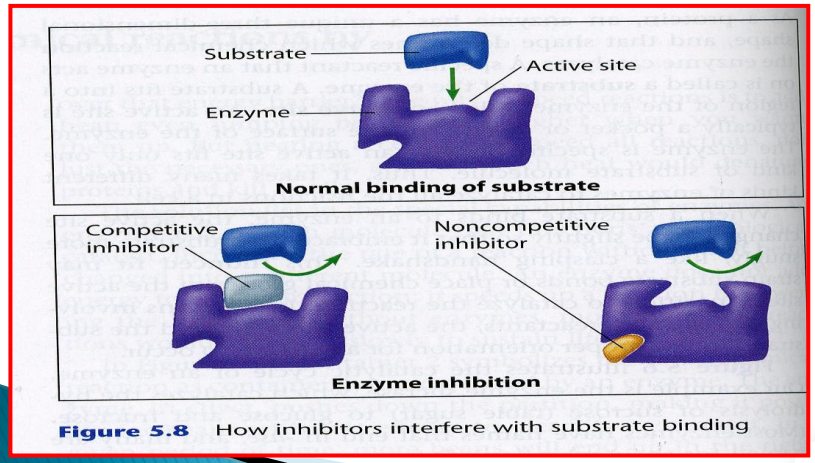
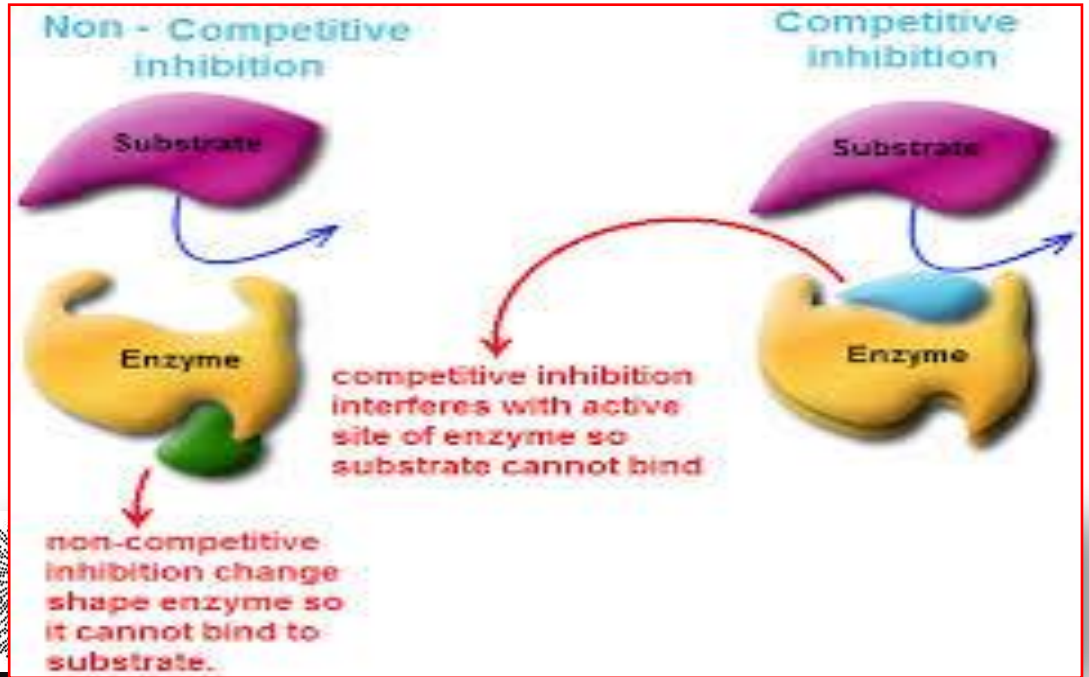


Figure 5.8 How inhibitors interfere with substrate binding



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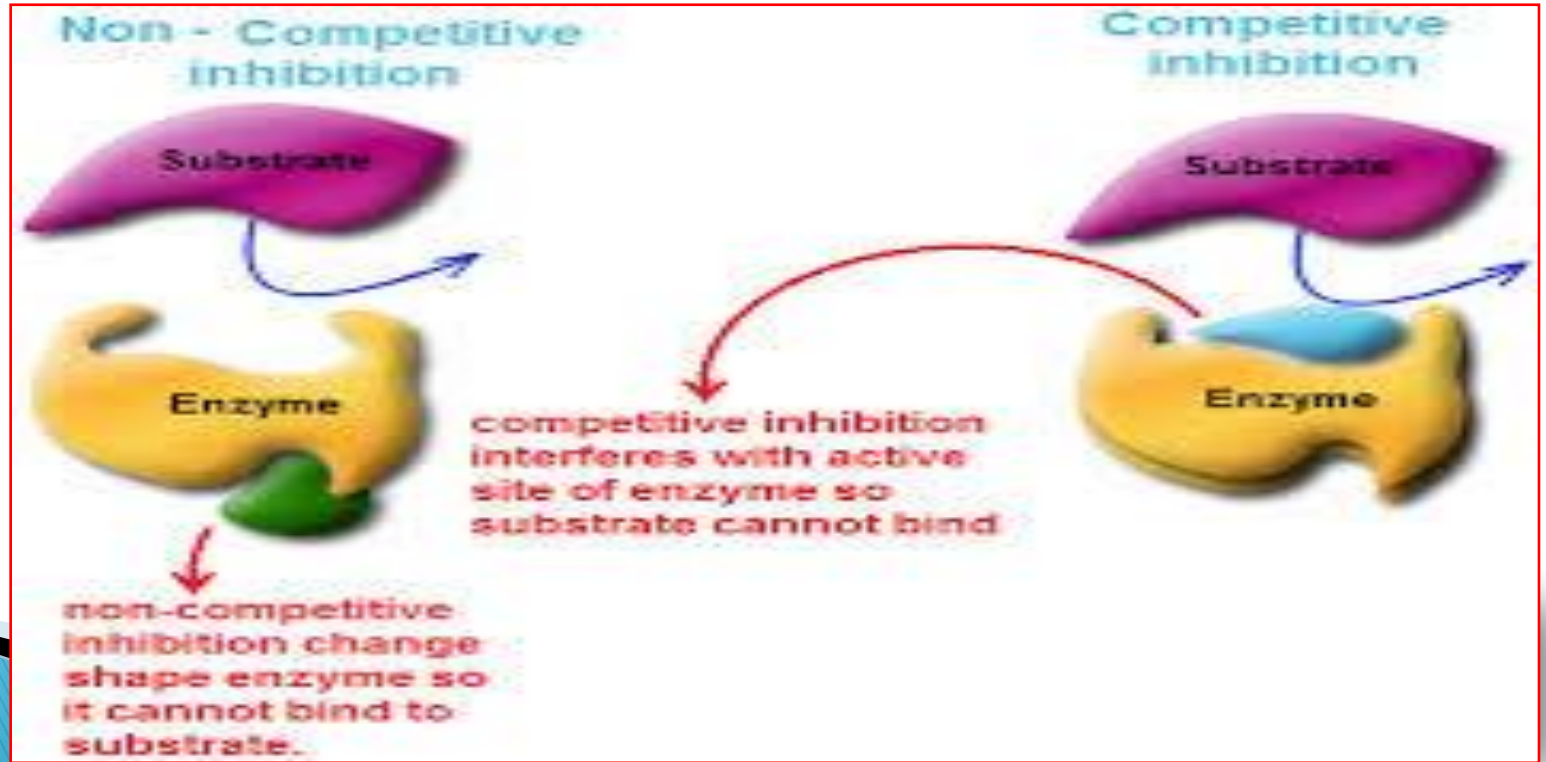
Enzyme inhibition

(2) Non-competitive inhibition:

- This form of inhibition occurs when an inhibitor (I) molecule or ion can bind to a second site on an enzyme surface (NOT on the active site) but an inhibitor site.
- Non-competitive inhibitor do not resemble to substrate.
- Inhibitor binding results in distortion or modification of the enzyme's conformation, which prevents product formation.



Enzyme inhibition

(2) Non-competitive inhibition: contd…

- The EIS ternary complex may either be totally or partially inactive in terms of catalyzing the substrate to product reaction.
- In pure non-competitive inhibition, with total inactivation, inhibitor binding does not affect substrate binding.

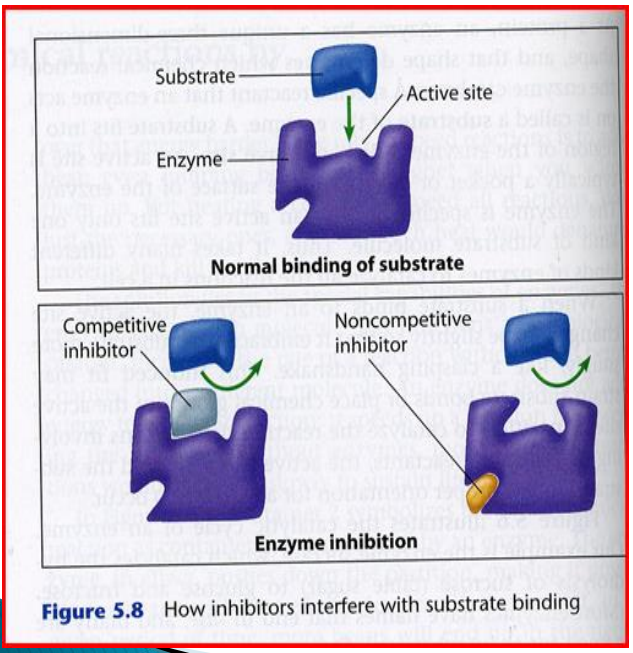
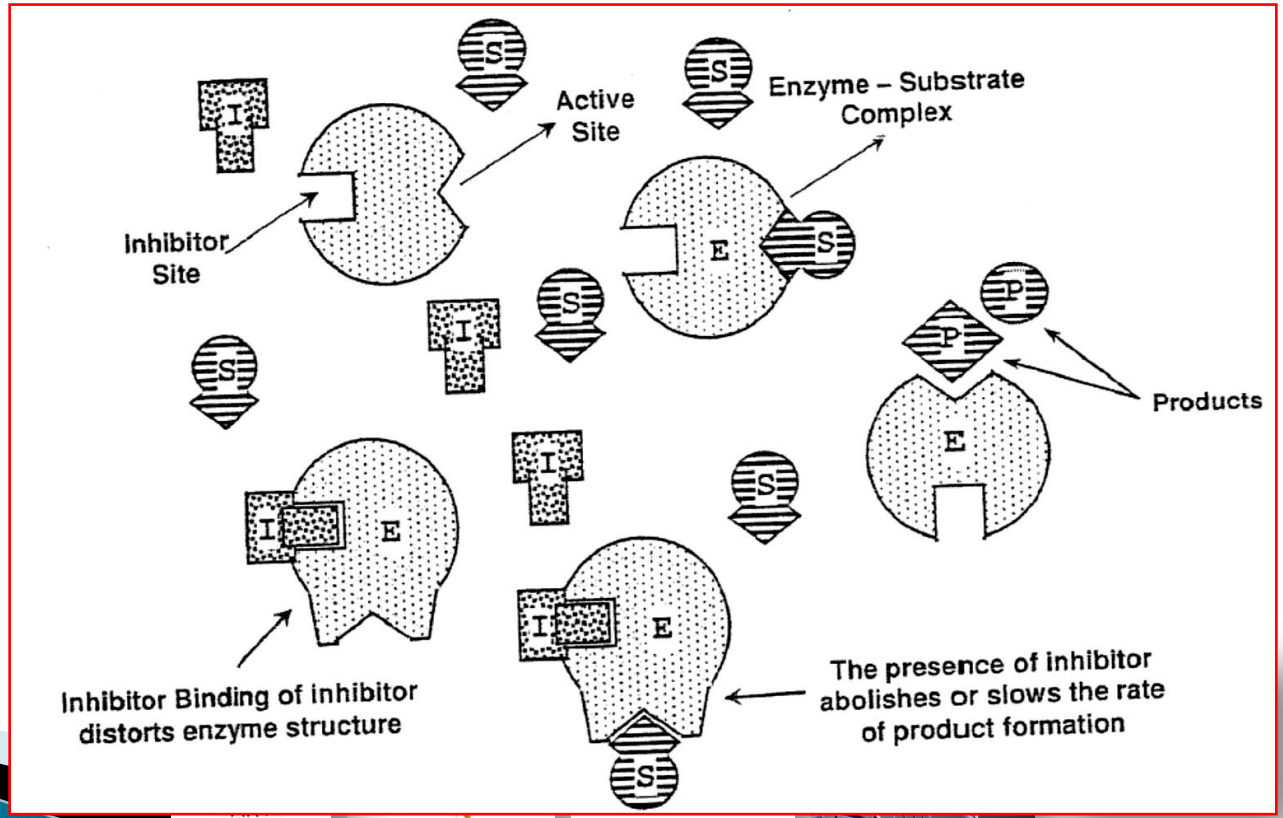


Figure 5.8 How inhibitors interfere with substrate binding



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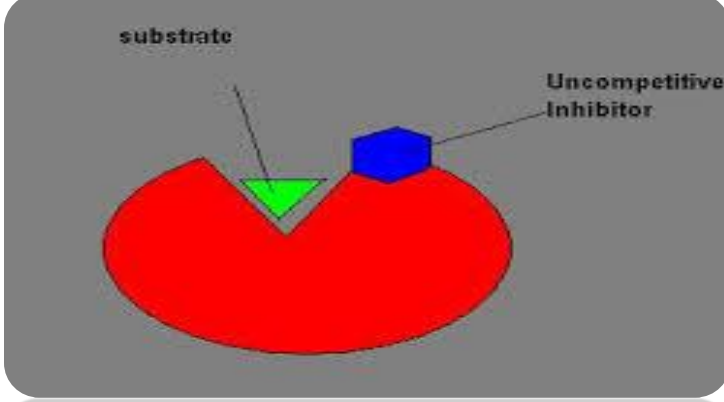
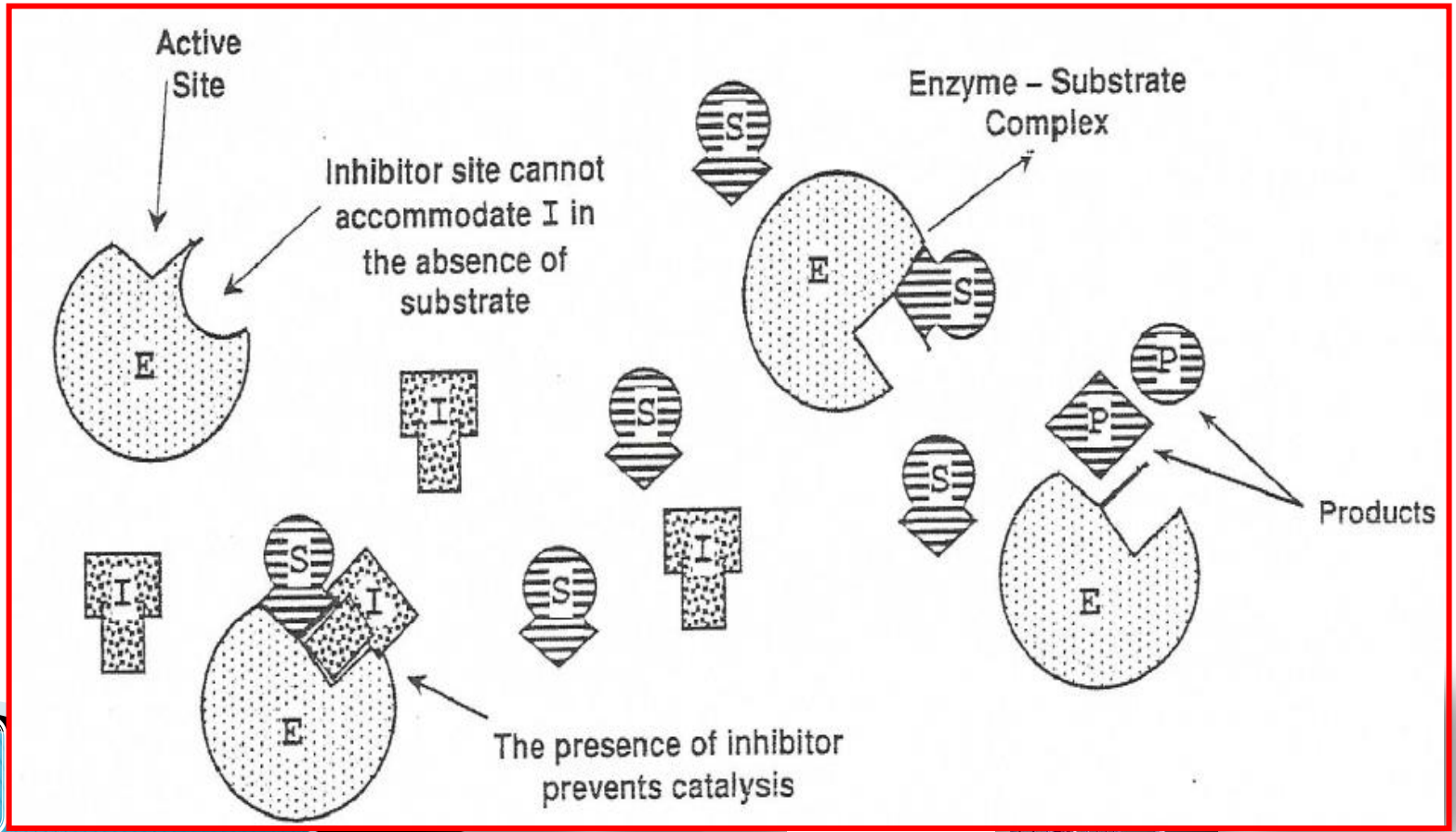
Enzyme inhibition



(3) Un-competitive inhibition:

- The inhibitor binds exclusively to the enzyme-substrate complex (ES).
- Substrate binding results in a modification of the enzyme's conformation, which promotes the binding of the inhibitor.



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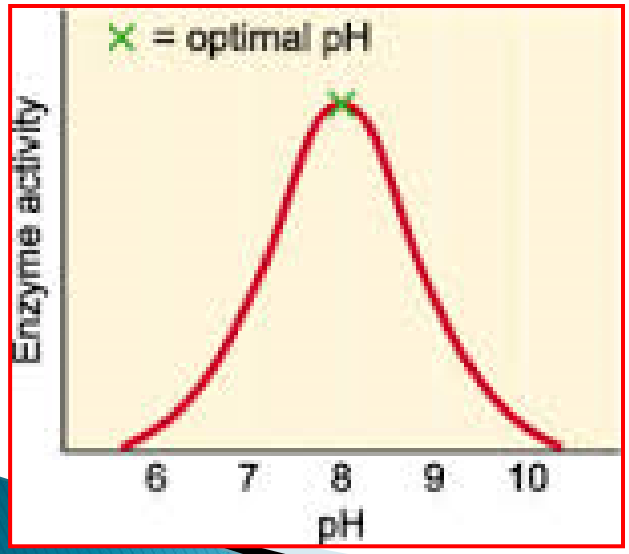
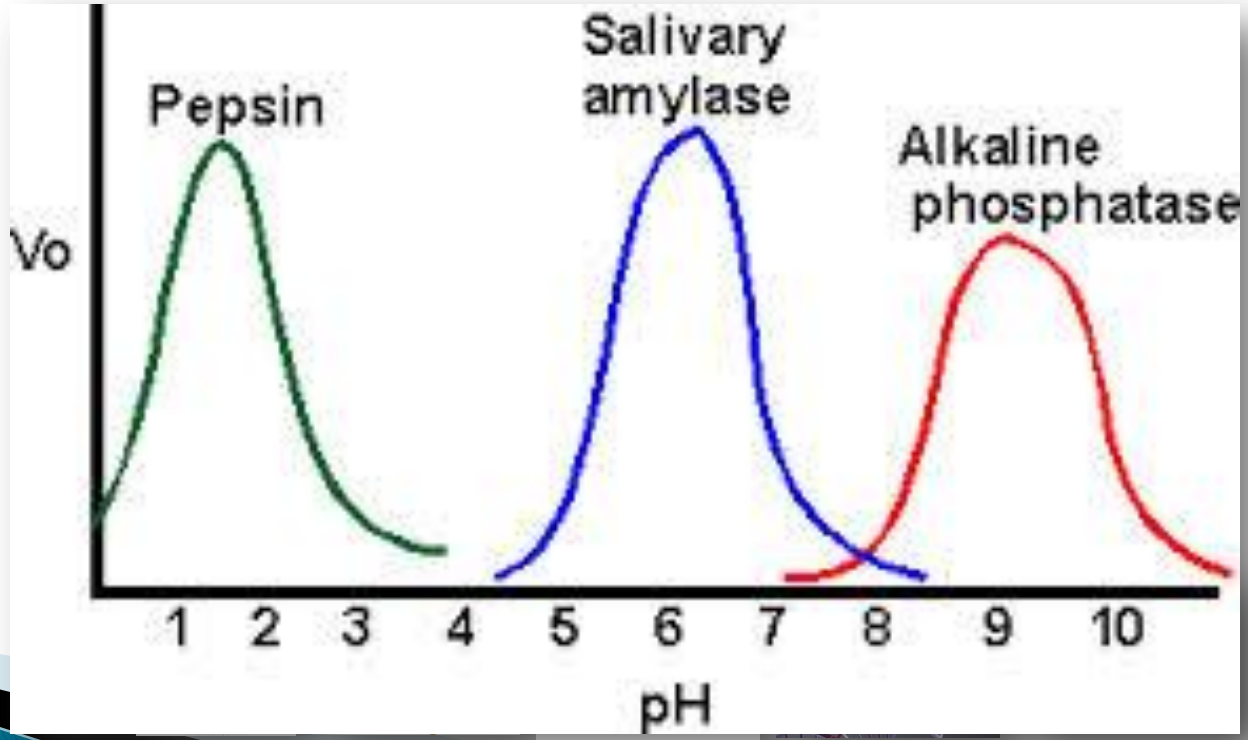
Factors affect enzyme activity

Effects of pH on enzyme activity:

- Most enzymes have an optimal pH at which their activity is maximal.
- If an enzyme is kept saturated with substrate at all pH values tested, many enzymes would have a pH activity profile that is characterized by a "bell-shaped" curve.
- However, pH activity profiles of certain enzymes may vary considerably.

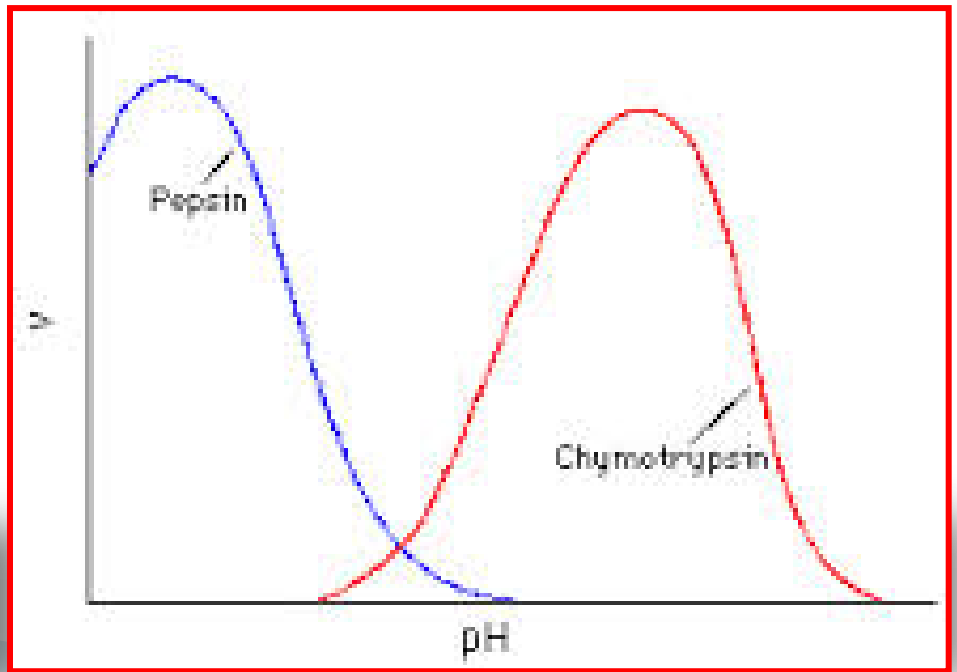




Factors affect enzyme activity

Effects of pH on enzyme activity: contd…

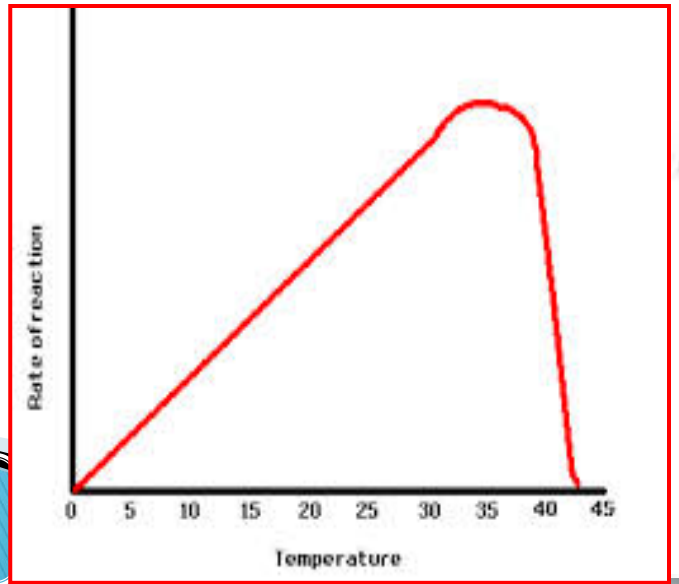
- The pH activity profile of an enzyme is dependent on the acid-base behavior of the enzyme and the substrate, i.e.
  - Any ionizable R-groups belong to the active site (catalytic AA residue)
  - Ionizable R-groups of the structural AA residues and
  - Ionizable groups belonging to the substrate molecule that is involved in binding interactions with the enzyme.
- It is notable that the optimal pH of an enzyme is not necessary the pH of the cellular environment.
- Thus it may be that the intracellular pH may exert some control of the activity of an enzyme.

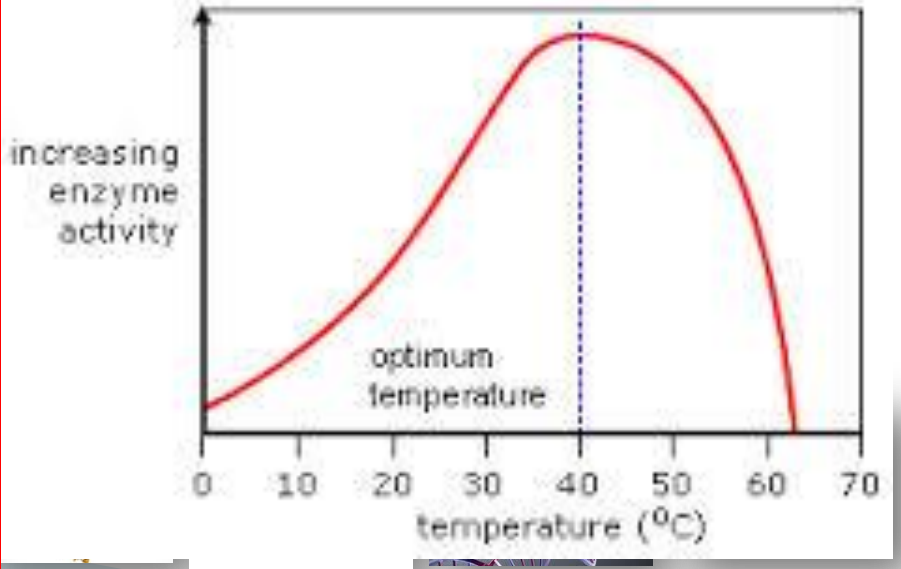


Factors affect enzyme activity

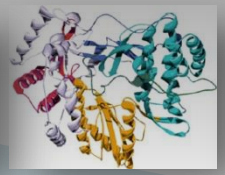
Effects of temperature on enzyme activity:

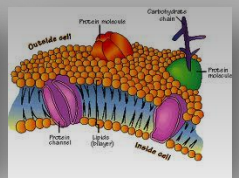
- The rates of enzyme catalyzed reaction generally increase with temperature, within the T° range in which the enzyme is stable and retains full activity.
- Although enzyme catalyzed reactions have an optimum temperature, the peak in such a plot of catalytic activity versus temperature results because enzymes being proteins are denature by heat and become inactive.
- Most enzymes are denatured or inactivated at temperatures above 55°C to 60°C.

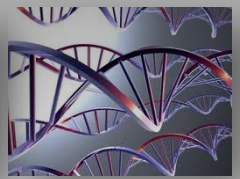


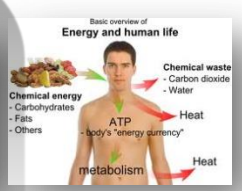


The End




Protein molecule
Carbohydrate chain
Outside cell
Protein molecule
Protein channel
Lipids (bilayer)
Inside cell




Basic overview of
Energy and human life
Chemical waste
- Carbon dioxide
- Water
Chemical energy
- Carbohydrates
- Fats
- Others
ATP
- body's "energy currency"
Heat
metabolism
Heat