CHAPTER 9 – BIOMOLECULES

- All living organisms are made up of similar elements
- In living organisms Carbon and Hydrogen are in abundance with respect to other elements.

How to Analyse Chemical Composition?
- To analyze the chemical composition, we can take any living tissue and grind it in trichloroacetic acid (Cl₃CCOOH) using a mortar and a pestle. We obtain a thick slurry. If we were to strain this through a cheesecloth or cotton we would obtain two fractions
  i. filtrate or the acid-soluble pool,
  ii. retentate or the acid-insoluble fraction.
- Scientists have found thousands of organic compounds in the acid-soluble pool.
- All the carbon compounds that we get from living tissues can be called 'biomolecules'.
- Living organisms have also got inorganic elements and compounds in them.
- Wet weight – weight of living tissue/structure.
- Dry Weight – weight of structure after drying it. (Wet weight – water).
- Ash – if the tissue is fully burnt, all the carbon compounds are oxidised to gaseous form (CO₂, water vapour) and are removed. What is remaining is called 'ash'. This ash contains inorganic elements (like calcium, magnesium etc). (Dry weight – carbon compound)

<table>
<thead>
<tr>
<th>Table: A Comparison of Elements Present in Non-living and Living Matter</th>
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<tbody>
<tr>
<td>Element</td>
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<tr>
<td>Hydrogen (H)</td>
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<tr>
<td>Carbon (C)</td>
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<td>Oxygen (O)</td>
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<td>Nitrogen (N)</td>
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<td>Sulphur (S)</td>
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<td>Sodium (Na)</td>
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<td>Calcium (Ca)</td>
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<td>Magnesium (Mg)</td>
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<td>Silicon (Si)</td>
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<table>
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<tr>
<th>Table: A List of Representative Inorganic Constituents of Living Tissues</th>
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<tbody>
<tr>
<td>Component</td>
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<tr>
<td>Sodium</td>
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<tr>
<td>Potassium</td>
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<tr>
<td>Calcium</td>
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<tr>
<td>Magnesium</td>
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<tr>
<td>Water</td>
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<tr>
<td>Compounds</td>
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Amino acids
- Amino acids are organic compounds containing an amino group and an acidic group as substituents on the same carbon i.e., the α-carbon. Hence, they are called α-amino acids. They are substituted methanes.
- There are four substituent groups occupying the four valency positions. These are hydrogen, carboxyl group, amino group and a variable group designated as R group.
- Based on the nature of R group there are many amino acids. However, those which occur in proteins are only of twentyone types.
- R group = hydrogen  e.g., glycine
- R group = methyl group  e.g., alanine
- R group = hydroxy methyl  e.g., serine.

\[ \text{COOH} \quad \text{H} \quad \text{C} \quad \text{NH}_2 \quad \text{R} \]

Glycine

\[ \text{COOH} \quad \text{H} \quad \text{C} \quad \text{NH}_2 \quad \text{CH}_3 \]

Alanine

\[ \text{COOH} \quad \text{H} \quad \text{C} \quad \text{NH}_2 \quad \text{CH}_2\text{-OH} \]

Serine

- The chemical and physical properties of amino acids are essentially of the amino, carboxyl and the R functional groups.
  - Acidic amino acid – glutamic acid etc.
  - Basic amino acid – lysine
  - Neutral amino acid – valine.
  - Aromatic amino acids – tyrosine, phenylalanine, tryptophan.
- A particular property of amino acids is the ionizable nature of \(-\text{NH}_2\) and \(-\text{COOH}\) groups. Hence in solutions of different pHs, the structure of amino acids changes.

\[
\begin{align*}
\text{(A)} & : \text{H}^+\text{NCH-CONH}_2 \\
\text{(B)} & : \text{H}^+\text{NCH-COO^-} \\
\text{(C)} & : \text{H}^+\text{NCH-COO^-}
\end{align*}
\]

B is called zwitterionic form.

**Lipids**

- Lipids are generally water insoluble. They could be simple fatty acids.
- A fatty acid has a carboxyl group attached to an R group. The R group could be a methyl (-CH\(_3\)), or ethyl (-C\(_2\)H\(_5\)) or higher number of -CH\(_2\) groups (1 carbon to 19 carbons).
  - Palmitic acid has 16 carbons including carboxyl carbon.
  - Arachidonic acid has 20 carbon atoms including the carboxyl carbon.
- Fatty acids could be saturated (without double bond) or unsaturated (with one or more C=C double bonds).
- Another simple lipid is glycerol which is trihydroxy propane.
- Many lipids have both glycerol and fatty acids. Here the fatty acids are found esterified with glycerol. They can be then monoglycerides, diglycerides and triglycerides.
- These are also called fats and oils based on melting point. Oils have lower melting point (e.g., gingely oil) and hence remain as oil in winters.
- Some lipids have phosphorous and a phosphorylated organic compound in them. These are phospholipids. They are found in cell membrane. Lecithin is one example.
- Some tissues especially the neural tissues have lipids with more complex structures.
Nucleotides

- Many carbon compounds have heterocyclic rings like nitrogen bases - adenine, guanine, cytosine, uracil, and thymine.
- When found attached to a sugar, they are called nucleosides. (nucleoside = sugar + nitrogen base). Adenosine, guanosine, thymidine, uridine and cytidine are nucleosides.
- If a phosphate group is also found esterified to the sugar they are called nucleotides. (Nucleotides = nucleosides + phosphate). Adenylic acid, thymidylic acid, guanylic acid, uridylic acid and cytidylic acid are nucleotides.
- Nucleic acids like DNA and RNA consist of nucleotides only. DNA and RNA function as genetic material.

** PRIMARY AND SECONDARY METABOLITES**

- **Primary metabolites** – Biomolecules which are present in all organisms and have identifiable functions and play known roles in normal physiological processes.
- **Secondary metabolites** – In plants, fungus and microbes many compounds other than primary metabolites are present. e.g., alkaloides, flavonoides, rubber, essential oils, antibiotics, coloured pigments, scents, gums, spices. The role or functions of all the secondary metabolites are not known yet. many of them are useful to ‘human welfare’ (e.g., rubber, drugs, spices, scents and pigments). Some secondary metabolites have ecological importance.
BIOMACROMOLECULES

- There is one feature common to all those compounds found in the acid soluble pool. They have molecular weights ranging from 18 to around 800 daltons (Da) approximately. (Micromolecules) \((M_w = <1000 \text{ daltons})\)
- The acid insoluble fraction, has only four types of organic compounds i.e., proteins, nucleic acids, polysaccharides and lipids. These classes of compounds with the exception of lipids, have molecular weights in the range of ten thousand daltons and above. (Macromolecules) \((M_w = >1000 \text{ daltons})\)
- The molecules in the insoluble fraction with the exception of lipids are polymeric substances.
- Lipids are small molecular weight compounds and are present not only as such but also arranged into structures like cell membrane and other membranes. When we grind a tissue, we are disrupting the cell structure. Cell membrane and other membranes are broken into pieces, and form vesicles which are not water soluble. Therefore, these membrane fragments in the form of vesicles get separated along with the acid insoluble pool and hence in the macromolecular fraction. Lipids are not strictly macromolecules.
- The acid soluble pool represents roughly the cytoplasmic composition. The macromolecules from cytoplasm and organelles become the acid insoluble fraction. Together they represent the entire chemical composition of living tissues or organisms.

<table>
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<tr>
<th>TABLE: Average Composition of Cells</th>
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<tbody>
<tr>
<td>Component</td>
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<tr>
<td>Water</td>
</tr>
<tr>
<td>Proteins</td>
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<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Lipids</td>
</tr>
<tr>
<td>Nucleic acids</td>
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<tr>
<td>Ions</td>
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PROTEINS

- Proteins are polypeptides. They are linear chains of amino acids linked by peptide bonds.
- Each protein is a polymer of amino acids. As there are 21 types of amino acids (e.g., alanine, cysteine, proline, tryptophan, lysine, etc.), a protein is a heteropolymer and not a homopolymer.
- A homopolymer has only one type of monomer repeating ‘n’ number of times.
- Amino acids can be essential or non-essential. Essential amino acids are supplied in diet while our body prepares non essential amino acids.
- Proteins carry out many functions in living organisms, some transport nutrients across cell membrane, some fight infectious organisms, some are hormones, some are enzymes, etc.
- Collagen is the most abundant protein in animal world.
- Ribulose bisphosphate Carboxylase-Oxygenase (RUBISCO) is the most abundant protein in the whole of the biosphere.
POLYSACCHARIDES

- Polysaccharides are long chains of sugars. They are threads (literally a cotton thread) containing different monosaccharides as building blocks.

- **Cellulose** is a polymeric polysaccharide consisting of only one type of monosaccharide i.e., glucose. Cellulose is a homopolymer.

- **Starch** is a variant of this but present as a storehouse of energy in plant tissues. Animals have another variant called **glycogen**.

- **Inulin** is a polymer of fructose.

- In a polysaccharide chain (say glycogen), the right end is called the reducing end and the left end is called the non-reducing end. It has branches.

- Starch forms helical secondary structures. In fact, starch can hold I₂ molecules in the helical portion. The starch-I₂ is blue in colour. Cellulose does not contain complex helices and hence cannot hold I₂.

- Plant cell walls are made of cellulose. Paper made from plant pulp is cellulose. Cotton fibre is cellulose.

- There are more complex polysaccharides in nature. They act as building blocks, amino-sugars and chemically modified sugars (e.g., glucosamine, N-acetyl galactosamine, etc.).

- Exoskeletons of arthropods, for example, have a complex polysaccharide called **chitin**. These complex polysaccharides are heteropolymers.
NUCLEIC ACIDS

- Present in acid insoluble fraction of all living tissues.
- These are polynucleotides. For nucleic acids, the building block is a nucleotide. A nucleotide has three chemically distinct components. One is a heterocyclic compound (N2 bases, the second is a monosaccharide and the third a phosphoric acid or phosphate.)
- Adenine, Guanine, Uracil, Cytosine, and Thymine are N2 containing bases. Adenine and Guanine are substituted purines while the rest are substituted pyrimidines.
- The sugar found in polynucleotides is either ribose (a monosaccharide pentose) or 2’ deoxyribose.
- A nucleic acid containing deoxyribose is called deoxyribonucleic acid (DNA) while that which contains ribose is called ribonucleic acid (RNA).

STRUCTURE OF PROTEINS

- Proteins are heteropolymers containing strings of amino acids. \
- Biologists describe the protein structure at four levels.
  - **Primary structure** – 
    It is linear structure of protein.
    the left end represented by the first amino acid and the right end represented by the last amino acid.
    The first amino acid is also called as N-terminal amino acid. The last amino acid is called the C-terminal amino acid.
  - **Secondary structure** –
    The linear protein thread is folded in the form of a helix (similar to a revolving staircase). In proteins, only right handed helices are observed.
  - **Tertiary structure** –
    The long protein chain is also folded upon itself like a hollow wollen ball, giving rise to the tertiary structure. This gives us a 3-dimensional view of a protein. Tertiary structure is absolutely necessary for the many biological activities of proteins.
  - **Quaternary structure** –
    Some proteins are an assembly of more than one polypeptide or subunits. The manner in which these individual folded polypeptides or subunits are arranged with respect to each other (e.g. linear string of spheres, spheres arranged one upon each other in the form of a cube or plate etc.) is the architecture of a protein otherwise called the quaternary structure of a protein.
    e.g., Adult human haemoglobin consists of 4 subunits. Two of these are identical to each other. Hence, two subunits of α type and two subunits of β type together constitute the human haemoglobin (Hb).
NATURE OF BOND LINKING MONOMERS IN A POLYMER

- In a **polypeptide** or a **protein**, amino acids are linked by a **peptide bond** which is formed when the carboxyl (-COOH) group of one amino acid reacts with the amino (-NH₂) group of the next amino acid with the elimination of a water moiety (the process is called dehydration).

- In a **polysaccharide** the individual monosaccharides are linked by a **glycosidic bond**. This bond is also formed by dehydration. This bond is formed between two carbon atoms of two adjacent monosaccharides.

- In a **nucleic acid** a phosphate moiety links the 3'-carbon of one sugar of one nucleotide to the 5'-carbon of the sugar of the succeeding nucleotide. The bond between the phosphate and hydroxyl group of sugar is an **ester bond**. As there is one such ester bond on either side, it is called **phosphodiester bond**.

Nucleic acids exhibit a wide variety of secondary structures. One of the secondary structures exhibited by DNA is the famous Watson-Crick model.

**Watson-Crick Model**

- According to this model DNA exists as a double helix. The two strands of polynucleotides are antiparallel i.e., run in the opposite direction.
- The backbone is formed by the sugar-phosphate-sugar chain.
- The nitrogen bases are projected more or less perpendicular to this backbone but face inside.
- A and G of one strand compulsorily base pairs with T and C, respectively, on the other strand. There are two hydrogen bonds between A and T. There are three hydrogen bonds between G and C.
- Each strand appears like a helical staircase.
- Each step of ascent is represented by a pair of bases. At each step of ascent, the strand turns 36°.
- One full turn of the helical strand would involve ten steps or ten base pairs.
- On drawing a line diagram, the pitch would be 34Å. The rise per base pair would be 3.4Å. This form of DNA with the above mentioned salient features is called **B-DNA**.
- There are more than a dozen forms of DNA named after English alphabets with unique structural features.
living organisms contain thousands of organic compounds. These compounds or biomolecules are present in certain concentrations (expressed as mols/cell or mols/litre etc.).

- all these biomolecules have a turn over. This means that they are constantly being changed into some other biomolecules and also made from some other biomolecules through chemical reactions. Together all these chemical reactions are called metabolism.

- These metabolic reactions result in the transformation of biomolecules like removal of CO\textsubscript{2} from amino acids making an amino acid into an amine, removal of amino group in a nucleotide base; hydrolysis of a glycosidic bond in a disaccharide, etc.

- Majority of these metabolic reactions are always linked to some other reactions or the metabolites are converted into each other in a series of linked reactions called metabolic pathways.

- Flow of metabolites through metabolic pathway has a definite rate and direction. This metabolite flow is called the dynamic state of body constituents.

- Another feature of these metabolic reactions is that every chemical reaction is a catalysed reaction. There is no uncatalysed metabolic conversion in living systems.

- The catalysts which hasten the rate of a given metabolic conversation are also proteins. These proteins with catalytic power are named enzymes.

**METABOLIC BASIS FOR LIVING**

- Metabolic pathways can lead to a more complex structure from a simpler structure (for example, acetic acid becomes cholesterol) = anabolic pathways, or lead to a simpler structure from a complex structure (for example, glucose becomes lactic acid in our skeletal muscle) = catabolic pathways.

- Anabolic pathways, as expected, consume energy. While, catabolic pathways lead to the release of energy, which is stored in the form of chemical bonds in ATP (adenosine triphosphate).

**THE LIVING STATE**

- Many chemical compounds or metabolites, or biomolecules, are present at concentrations characteristic of each of them.
  
  e.g., the blood concentration of glucose in a normal healthy individual is 4.5-5.0 mM, while that of hormones would be nanograms/mL.

- all living organisms exist in a steady-state characterised by concentrations of each of these biomolecules. These biomolecules are in a metabolic flux. Any chemical or physical process moves spontaneously to equilibrium.

- The steady state is a non-equilibrium state. Because systems at equilibrium cannot perform work.
• **the living state is a non-equilibrium steady-state to be able to perform work**; living process is a constant effort to prevent falling into equilibrium. This is achieved by energy input. Metabolism provides a mechanism for the production of energy. Hence the living state and metabolism are synonymous. Without metabolism there cannot be a living state.

**ENZYMES**

• Almost all enzymes are **proteins**. There are some nucleic acids that behave like enzymes. These are called **ribozymes**.

• An enzyme like any protein has a primary structure, secondary and the tertiary structure.

• In tertiary structure, the backbone of the protein chain folds upon itself, the chain criss-crosses itself and hence, many crevices or pockets are made. One such pocket is the 'active site'.

• An active site of an enzyme is a crevice or pocket into which the substrate fits. Thus enzymes, through their active site, catalyse reactions at a high rate.

• Enzyme catalysts differ from inorganic catalysts in many ways. Inorganic catalysts work efficiently at high temperatures and high pressures, while enzymes get damaged at high temperatures (above 40°C).

However, enzymes isolated from organisms who normally live under extremely high temperatures (e.g., hot vents and sulphur springs), are stable and retain their catalytic power even at high temperatures (upto 80°-90°C). Thermal stability is thus an important quality of such enzymes isolated from thermophilic organisms.

**Chemical Reactions**

• Chemical compounds undergo two types of changes.

  A **physical change** simply refers to a change in shape without breaking of bonds. This is a physical process. Another physical process is a change in state of matter: when ice melts into water, or when water becomes a vapour.

  when bonds are broken and new bonds are formed during transformation, this will be called a chemical reaction. For example:

  \[ \text{Ba(OH)}_2 + H_2SO_4 \rightarrow \text{BaSO}_4 + 2H_2O \]

  hydrolysis of starch into glucose is an organic chemical reaction.

• **Rate of a physical or chemical process** refers to the amount of product formed per unit time. It can be expressed as:

  \[ \text{rate} = \frac{\delta P}{\delta t} \]

  Rate can also be called velocity if the direction is specified.

• Rates of physical and chemical processes are influenced by temperature among other factors.

• A general rule is that **rate doubles or decreases by half for every 10°C change** in either direction. Catalysed reactions proceed at rates vastly higher than that of uncatalysed ones. e.g.,

  \[ \text{carbon dioxide} + \text{water} \xrightarrow{\text{carbonic anhydrase}} \text{carbonic acid} \]

  In the absence of any enzyme this reaction is very slow, with about 200 molecules of \( \text{H}_2\text{CO}_3 \) being formed in an hour.

  However, by using the enzyme carbonic anhydrase, the reaction speeds about 600,000 molecules being formed every second.

• A multistep chemical reaction, when each of the steps is catalysed by the same enzyme complex or different enzymes, is called a metabolic pathway. For example,

  \[ \text{Glucose} \rightarrow 2 \text{Pyruvic acid} \]

  \[ C_6H_{12}O_6 + O_2 \rightarrow 2C_3H_4O_3 + 2H_2O \]
This reaction is actually a metabolic pathway in which glucose becomes pyruvic acid through ten different enzyme catalysed metabolic reactions.

- This pathway provides different products in different conditions –
  - In our skeletal muscle, under anaerobic conditions, lactic acid is formed.
  - Under normal aerobic conditions, pyruvic acid is formed.
  - In yeast, during fermentation, the same pathway leads to the production of ethanol (alcohol).

How do Enzymes bring about such High Rates of Chemical Conversions?

- Enzymes, i.e. proteins with three dimensional structures including an 'active site', convert a substrate (S) into a product (P). Symbolically, this can be depicted as:
  \[ S \rightarrow P \]
- Substrate 'S' has to bind the enzyme at its 'active site' within a given cleft or pocket. The substrate has to diffusetowards the 'active site'.
- There is thus, an obligatory formation of an 'ES' complex. E stands for enzyme. This complex formation is a transient phenomenon.
- During the state where substrate is bound to the enzyme active site, a new structure of the substrate called transition state structure is formed.
- Very soon, after the expected bond breaking/making is completed, the product is released from the active site. In other words, the structure of substrate gets transformed into the structure of product(s).
- There could be many more 'altered structural states' between the stable substrate and the product. all otherintermediate structural states are unstable. Stability is something related to energy status of the molecule or the structure.
- If 'P' is at a lower level than'S', the reaction is an exothermic reaction. One need not supply energy (by heating) in order to form the product.
- However, whether it is an exothermic or spontaneous reaction or an endothermic or energy requiring reaction, the 'S' has to go through a much higher energy state or transition state.
- The difference in average energy content of'S' from that of this transition state is called 'activation energy'.
- Enzymes eventually bring down this energy barrier making the transition of'S' to 'P' more easy.

Nature of Enzyme Action

- Each enzyme (E) has a substrate (S) binding site in its molecule so that a highly reactive enzyme-substrate complex (ES) is produced. This complex is short-lived and dissociates into its product(s) P and the unchanged enzyme with an intermediate formation of the enzyme-product complex (EP).
- The formation of the ES complex is essential for catalysis.

\[ E + S \rightarrow ES \rightarrow EP \rightarrow E + P \]

- The catalytic cycle of an enzyme action can be described in the following steps:
  1. First, the substrate binds to the active site of the enzyme, fitting into the active site.
  2. The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.
  3. The active site of the enzyme, now in close proximity of the substrate breaks the chemical bonds of the substrate and the new enzyme-product complex is formed.
  4. The enzyme releases the products of the reaction and the free enzyme is ready to bind to...
another molecule of the substrate and run through the catalytic cycle once again.

**Factors Affecting Enzyme Activity**
Factors affecting Enzyme activity are temperature, pH, change in substrate concentration or binding of specific chemicals that regulate its activity.

1. **Temperature and pH**
   Enzymes generally function in a narrow range of temperature and pH. Each enzyme shows its highest activity at a particular temperature and pH called the optimum temperature and optimum pH. Low temperature preserves the enzyme in a temporarily inactive state whereas high temperature destroys enzymatic activity because proteins are denatured by heat.

2. **Concentration of Substrate**
   With the increase in substrate concentration, the velocity of the enzymatic reaction rises at first. The reaction ultimately reaches a maximum velocity ($V_{max}$) which is not exceeded by any further rise in concentration of the substrate. This is because the enzyme molecules are fewer than the substrate molecules and after saturation of these molecules, there are no free enzyme molecules to bind with the additional substrate molecules.

   The activity of an enzyme is also sensitive to the presence of specific chemicals that bind to the enzyme. When the binding of the chemical shuts off enzyme activity, the process is called inhibition and the chemical is called an inhibitor.

   **Competitive inhibition** –
   When the inhibitor closely resembles the substrate in its molecular structure and inhibits the activity of the enzyme, it is known as competitive inhibitor. Due to its close structural similarity with the substrate, the inhibitor competes with the substrate for the substrate-binding site of the enzyme. Consequently, the substrate cannot bind and as a result, the enzyme action declines, e.g., inhibition of succinic dehydrogenase by malonate which closely resembles the substrate succinate in structure.

   Such competitive inhibitors are often used in the control of bacterial pathogens.

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**Classification and Nomenclature of Enzymes**
Enzymes are divided into 6 classes each with 4-13 subclasses and named accordingly by a four-digit number.

1. **Oxidoreductases/dehydrogenases**: Enzymes which catalyse oxidoreduction between two substrates $S$ and $S'$ e.g.,
   
   $S$ reduced + $S'$ oxidised $\rightarrow$ $S$ oxidised + $S'$ reduced.

2. **Transferases**: Enzymes catalysing a transfer of a group, G (other than hydrogen) between a pair of substrate $S$ and $S'$ e.g.,

   $S$ - G + $S'$ $\rightarrow$ $S$ + $S'$ - G

3. **Hydrolases**: Enzymes catalysing hydrolysis of ester, ether, peptide, glycosidic, C-C, C-halide or P-N
bonds.

4. **Lyases:** Enzymes that catalyse removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds.

\[
\begin{array}{c}
X \quad Y \\
C \quad C
\end{array}
\rightarrow X - Y + C = C
\]

5. **Isomerases:** Includes all enzymes catalysing inter-conversion of optical, geometric or positional isomers.

6. **Ligases:** Enzymes catalysing the linking together of 2 compounds, e.g., enzymes which catalyse joining of C-O, C-S, C-N, P-O etc. bonds.

**Co-factors**

- Enzymes are composed of one or several polypeptide chains. However, there are a number of cases in which **non-protein constituents called co-factors** are bound to the enzyme to make the enzyme catalytically active.
- In these instances, the protein portion of the enzymes is called the **apoenzyme**.
- Three kinds of cofactors may be identified: prosthetic groups, co-enzymes and metal ions.
- **Prosthetic groups** are organic compounds and are distinguished from other cofactors in that they are tightly bound to the apoenzyme.
  For example, in peroxidase and catalase, which catalyze the breakdown of hydrogen peroxide to water and oxygen, haem is the prosthetic group and it is a part of the active site of the enzyme.
- **Co-enzymes** are also organic compounds but their association with the apoenzyme is only transient, usually occurring during the course of catalysis. Furthermore, co-enzymes serve as co-factors in a number of different enzyme catalyzed reactions. The essential chemical components of many coenzymes are vitamins, e.g., coenzyme nicotinamide adenine dinucleotide (NAD) and NADP contain the vitamin niacin.
- **Metal ions** – A number of enzymes require metal ions for their activity which form coordination bonds with side chains at the active site and at the same time form one or more coordination bonds with the substrate, e.g., zinc is a cofactor for the proteolytic enzyme carboxypeptidase. Catalytic activity is lost when the co-factor is removed from the enzyme which testifies that they play a crucial role in the catalytic activity of the enzyme.