What is an Amino acid?

> **"Amino Acids are the organic compounds that combine to form proteins, hence they are referred to as the building components of proteins. These biomolecules are involved in several biological and chemical functions in the human body and are the necessary ingredients for the growth and development of human beings. There are about 300 amino acids that occur in nature."**

> Amino acids are organic compounds containing the basic amino groups (-NH2) and carboxyl groups (-COOH). The ingredients present in proteins are amino acids. Both peptides and proteins are long chains of amino acids. Altogether, there are twenty amino acids, which are involved in the construction of proteins

The general structure of Amino acids is H2NCH RCOOH, and it can be written as:

```
       COOH
        |
H2N – – C – – H
        |
        R
```

There are 20 naturally occurring amino acids and all have common structural features – an amino group (-NH3+), a carboxylate (-COO-) group and a hydrogen-bonded to the same carbon atom. They differ from each other in their side-chain called the R group. Each amino acid has 4 different groups attached to α- carbon.

These 4 groups are:

- Amino group,
- COOH,
- Hydrogen atom,
- Sidechain (R).

General properties of Amino acids

- They have a very high melting and boiling point.
- Amino acids are white crystalline solid substances.
- In taste, few Amino acids are sweet, tasteless, and bitter.
- Most of the amino acids are soluble in water and are insoluble in organic solvents

Classification of Amino Acids

Amino Acid can be classified **based on their structure** and the structure of their side chains i.e. the R chains. Now two basic subcategories are

1] Non-Polar Amino Acids

These are also known as Hydrophobic. The R group can be either of Alkyl groups (with an alkyl chain) or Aromatic groups. The acids falling in this group are stated below. Numbers one to seven are Alkyl and the last two are aromatic

1. Glycine (H)
2. Alanine (CH3)
3. Valine (CH (CH3)2)
4. Methionine (CH2CH2SCH3)
5. Leucine (CH2CH(CH3)2)
6. Isoleucine (-CH(CH3)CH2CH3)
7. Proline (special structure)
8. Phenylalanine
9. Tryptophan

2] Polar Amino Acids

If the side chains of amino acid contain different polar groups like amines, alcohols or acids they are polar in nature. These are also known as Hydrophilic Acids. These are further divided into three further categories.

a) Acidic: If the side chain contains an extra element of carboxylic acid component these are acid-polar amino acids. They tend to donate their hydrogen atom. These are:

- Aspartic Acid (CH2COOH)
- Glutamic Acid (CH2CH2COOH)

b) Basic: These have an extra nitrogen group that tend to attract a hydrogen atom. The three basic polar amino acids are

- Histidine
- Lysine (CH2(CH2)2NH2)
- Arginine

c) Neutral: These are neither acidic nor basic. They have an equal number of amino and carboxyl groups. Also, they have at least one hydrogen component connected to electronegative atoms. Some of these neutral acids are

- Serine (CH2OH)
- Threonine (CH(OH)CH3)
- Asparagine (CH2OHNH2)
- Glutamine (CH2CH2CONH2)
- Cysteine (CH2SH)
- Tyrosine

Amino acid can also be classified on the basis of their need to the human body and their **availability in the human body**

1] Essential Amino Acids

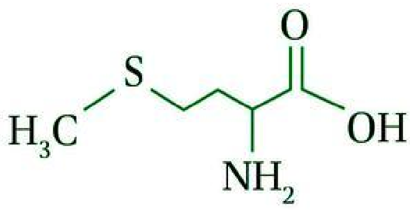
These are the acids that cannot be synthesized in our bodies. We must rely on food sources to obtain these amino acids. They are

- Leucine
- Isoleucine
- Lysine
- Theorine
- Methionine
- Phenylalanine
- Valine
- Tryptophan
- Histidine (conditionally essential)

2] Non-Essential

These acids are synthesized in our bodies itself and we need not rely on outside sources for them. They are either produced in our bodies or obtained from protein breakdowns.

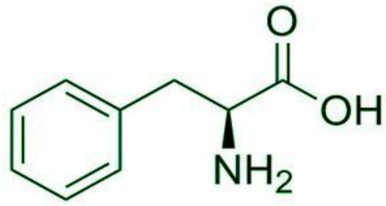
- **Sulphur Containing Amino Acid:** Its structure contains sulphydryl (–SH) groups. **Example:** Cysteine and Methionine



Methionine

- **Aromatic Amino Acid:**

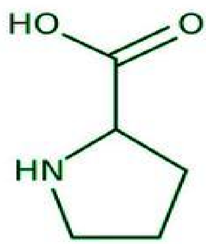
They contain a benzene ring in their structure. **Example:** Phenylalanine and Tyrosine



Phenylalanine

- **Heterocyclic Amino Acids:**

They have a heterocyclic ring on their side chain with at least one element other than carbon. **Example:** Tryptophan, Proline, and Hydroxyproline



Proline

What is Zwitterion?

The term 'Zwitterion' is originally derived from the **German word '<u>zwitter'</u>, which can be roughly translated as 'hybrid' or 'hermaphrodite'.** A zwitterion is an ion that contains two functional groups. In simple terms, it is an ion possessing both positive and negative electrical charges.
Therefore, **zwitterions are mostly electrically neutral (the net formal charge is usually zero).**

Zwitterions are sometimes referred to as "**inner salts**". Usually, dipolar compounds are not classified as zwitterions. The distinction lies in the fact that the plus and minus signs on the amine oxide signify formal charges. Zwitterions may be worthy of medicinal chemistry design considerations when working with acid, basic or neutral leads.

Zwitterion Definition

"A zwitterion is a molecule that has both positive and negative regions of charge." In the solid state, amino acids exist as dipolar ions called zwitterions. While discussing whether a substance is zwitterionic or not, the pH range in which the information is required must be specified (because a sufficiently alkaline solution will change the zwitterion to an anion, and a sufficiently acid solution will change it to a cation).

Some key **Characteristics of Zwitterion** are;

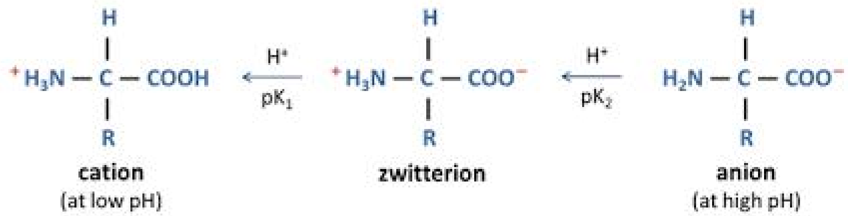
- They can be formed from compounds like ampholytes which contain both acid and base groups in their molecules.
- In this type of ion, the charged atoms are usually held together by one or more covalent bonds.
- Zwitterionic compounds have stable, separated unit electrical charges on atoms.
- These compounds contain quaternary ammonium cations.

Let us further understand the topic by looking at an example of Zwitterion.

Zwitterion Structure

Amino Acids

Amino acids are the most common example of zwitterions. They are made up of an ammonium or amino group which contains a positive charge as well as a carboxyl group which contains a negative charge. The zwitterion form of an amino acid is given below.



Apart from amino acids, any compound that contains acid and base centres can obtain a Zwitterion form. Some more examples include tricine, bicine, solid sulfamic acid, alkaloids like psilocybin amongst others.

Isoelectric Point

- Another main property of a **Zwitterion is that it has an isoelectric point** (represented as pI, pH(I), IEP).
- This point is the pH value at which the charge in molecules is neutral.
- Usually, the net charge on a molecule is greatly affected by the pH of its surrounding environment.
- In this case, molecules can become more charged (positively or negatively) as a result of gain or loss in the number of protons.
- If we look at amino acid, the amino group is a very effective proton acceptor and the carboxyl group is an effective proton donor.
- In addition, **the solubility of a molecule at a given pH is also affected by the pI value.**

Calculation of pH Value

The **pH value at the isoelectric point can be calculated from the equilibrium constants** (acid and base) of the Zwitterion. It is represented by the formula;

$pI=pKa1+pKa22$
Where,

- pI = isoelectric point,
- $Ka1$ = the equilibrium constant of the acid.
- $Ka2$ = the equilibrium constant of the base.

Applications of Zwitterions

1. Zwitterions are widely applied in the process of separating protein molecules via SDS PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) method which is one of the most popular techniques used in molecular biology.
2. They also have great potential to be applied in a wide range of medical and biological related fields.
3. Some popular uses include medical implants, drug delivery, blood contact sensor, separation membrane, as well as antifouling coatings of biomedical implants that help prevent the build-up of microbial adhesion and biofilm formation.
4. In the marine industry, Zwitterionic polymers are used to prevent subaquatic organisms from building up on boats and piers.

Peptide Bond

A peptide bond also sometimes called eupeptide bond is a chemical bond that is formed by joining the carboxyl group of one amino acid to the amino group of another. A peptide bond is basically an amide-type of covalent chemical bond. This bond links two consecutive alpha-amino acids from C1 (carbon number one) of one alpha-amino acid and N2 (nitrogen number two) of another. This linkage is found along a peptide or protein chain.

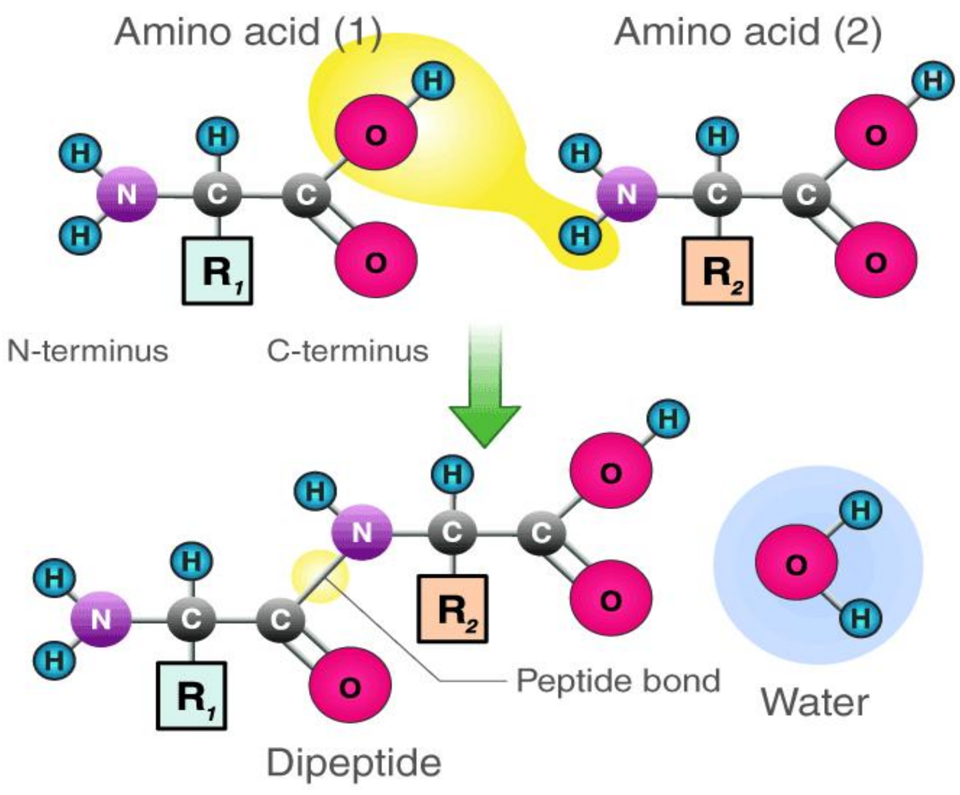
During the formation of this bond, there is a release of water (H2O) molecules. A peptide bond is usually a covalent bond (CO-NH bond) and since the water molecule is eliminated it is considered as a dehydration process. Generally, this process occurs mostly between amino groups.

Meanwhile, a peptide is a Greek word that means "digested". A peptide is a short polymer of amino acid monomers linked by an amide bond.

Peptide Bond Formation or Synthesis

A peptide bond is formed by a dehydration synthesis or reaction at a molecular level. This reaction is also known as a condensation reaction which usually occurs between amino acids.

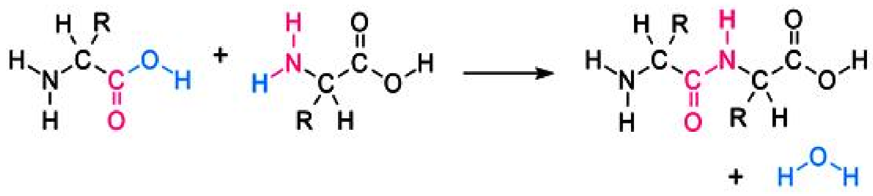
As depicted in the figure given below, two amino acids bond together to form a peptide bond by the dehydration synthesis. During the reaction, one of the amino acids gives a carboxyl group to the reaction and loses a hydroxyl group (hydrogen and oxygen).



The other amino acid loses hydrogen from the NH2 group. The hydroxyl group is substituted by nitrogen thus forming a peptide bond. This is one of the primary reasons for peptide bonds being referred to as substituted amide linkages. Both the amino acids are covalently bonded to each other.

The newly formed amino acids are also called a dipeptide.

Let's have a look at a simpler diagram depicting the formation of the peptide bond.



During the reactions that occur, the resulting CO-NH bond is the peptide bond, and the resulting molecule is an amide. The four-atom functional group -C(=O)NH- is called an amide group or a peptide group.

Characteristics of Peptide Bonds

1. Peptide bonds are strong with partial double bond character:

- They are not broken by heating or high salt concentration.
- They can be broken by exposing them to strong acid or base for a long time at elevated temperature. Also by some specific enzymes (digestive enzymes).

2. Peptide bonds are rigid and planar bonds therefore they stabilize protein structure.

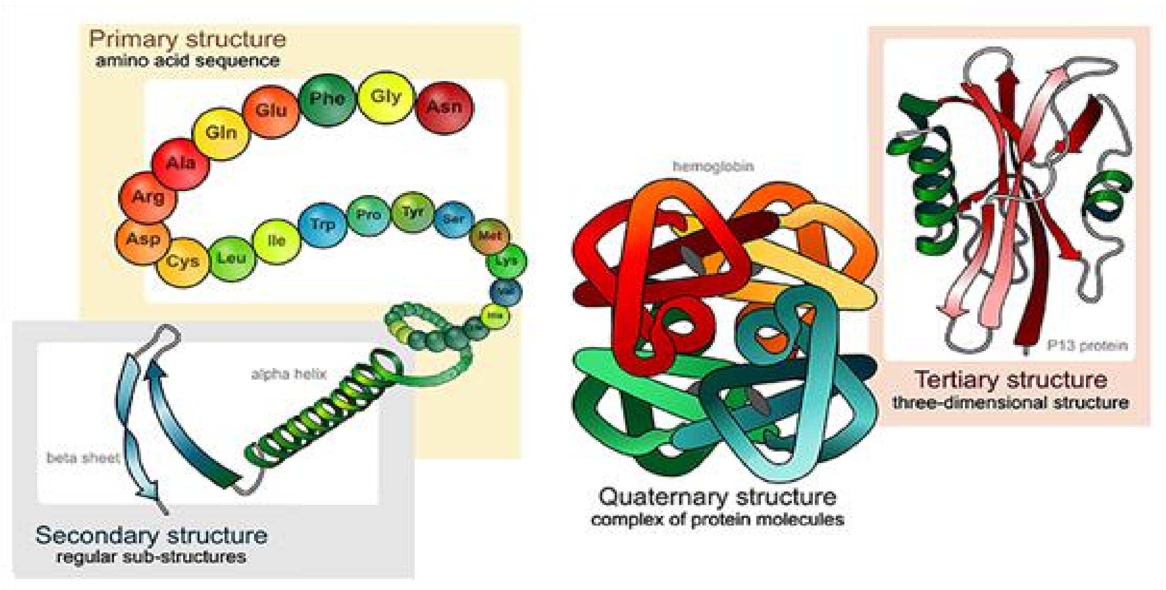
3. Peptide bond contains partial positive charge groups (polar hydrogen atoms of amino groups) and partial negative charge groups (polar oxygen atoms of carboxyl groups).

Different Forms of Peptide Bond

- **Dipeptide =** contains 2 amino acid units.
- **Tripeptide =** contains 3 amino acid units.
- **Tetrapeptide =** contains 4 amino acid units.
- **Oligopeptide =** contains not more than 10 amino acid units.
- **Polypeptide =** contains more than 10 amino acid units, up to 100 residues.
- **Macropeptides =** made up of more than 100 amino acids

Protein Structure

Protein structures are made by condensation of amino acids forming peptide bonds. The sequence of amino acids in a protein is called its primary structure. The secondary structure is determined by the dihedral angles of the peptide bonds, the tertiary structure by the folding of protein chains in space. Association of folded polypeptide molecules to complex functional proteins results in quaternary structure.

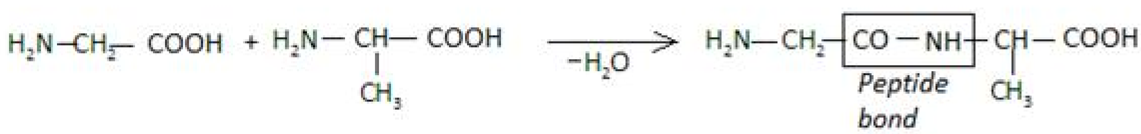


Primary, Secondary, Tertiary and Quaternary Structure of Proteins

Define Protein Structure

Protein structure is defined as a polymer of amino acids joined by peptide bonds.

Let us see how a peptide bond is established from the following reaction:



Formation of Peptide Bond

We can thus see that the peptide bond (-CO-NH) is formed between the amine group of one molecule and the carboxyl group of the adjacent molecule followed by the elimination of a water molecule. This bond is otherwise an amide linkage. When peptide bonds are established among more than ten amino acids, they together form a polypeptide chain. Very often, when a polypeptide chain has a mass exceeding 10000u and the number of amino acids in the chain exceeding 100, we get a protein.

Classification of Proteins

Based on the molecular shape, proteins can be classified into two types.

1. Fibrous Proteins:

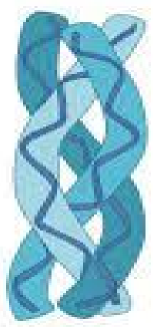
When the polypeptide chains run **parallel** and are held together by hydrogen and disulfide bonds, then the fiber-like structure is formed. Such proteins are generally insoluble in water. These are water-insoluble proteins.

Example – keratin (present in hair, wool, and silk) and myosin (present in muscles), etc.

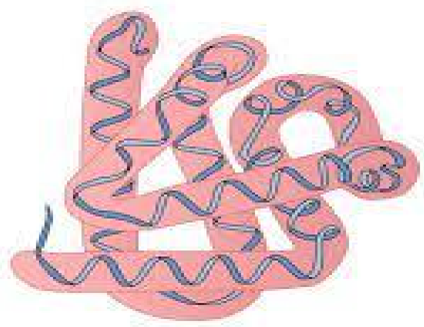
2. Globular Proteins:

This structure results when the chains of polypeptides **coil around** to give a spherical shape. These are usually soluble in water.

Example – Insulin and albumins are common examples of globular proteins.



Fibrous Protein



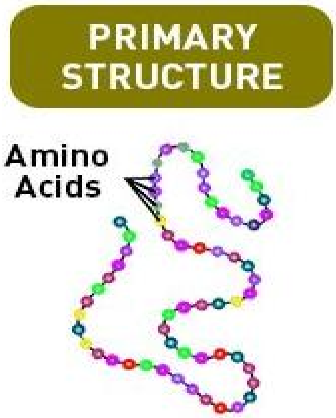
Globular Protein

Levels of Protein Structure

1. Primary Structure of Protein

- The Primary structure of proteins is the exact ordering of amino acids forming their chains.
- The exact sequence of the proteins is very important as it determines the final fold and therefore the function of the protein.
- The number of polypeptide chains together form proteins. These chains have amino acids arranged in a particular sequence which is characteristic of the specific protein. Any change in the sequence changes the entire protein.

The following picture represents the primary protein structure (an amino acid chain). As you might expect, the amino acid sequence within the polypeptide chain is crucial for the protein's proper functioning. This sequence is encrypted in the DNA genetic code. If mutation is present in the DNA and the amino acid sequence is changed, the protein function may be affected.



Primary Structure of Protein

The protein 's primary structure is the amino acid sequence in its polypeptide chain. If proteins were popcorn stringers designed to decorate a Christmas tree, a protein 's primary structure is the sequence in which various shapes and varieties of popped maize are strung together.

Covalent, peptide bonds which connect the amino acids together maintain the primary structure of a protein.

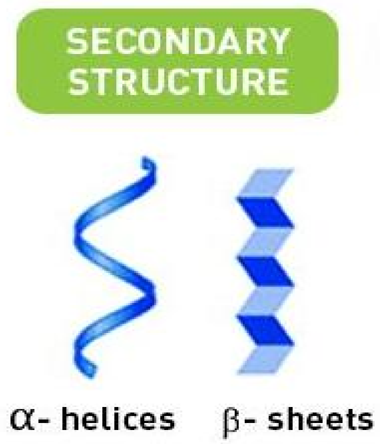
All documented genetic disorders, such as cystic fibrosis, sickle cell anemia, albinism, etc., are caused by mutations resulting in alterations in the primary protein structures, which in turn lead to alterations in the secondary , tertiary and probably quarterly structure.

Amino acids are small organic molecules consisting of a chiral carbon with four substituents. Of those only the fourth the side chain is different among amino acids.

2. Secondary Structure of Protein

Secondary structure of protein refers to local folded structures that form within a polypeptide due to interactions between atoms of the backbone.

- The proteins do not exist in just simple chains of polypeptides.
- These polypeptide chains usually fold due to the interaction between the amine and carboxyl group of the peptide link.
- The structure refers to the shape in which a long polypeptide chain can exist.
- They are found to exist in two different types of structures α – helix and β – pleated sheet structures.
- This structure arises due to the regular folding of the backbone of the polypeptide chain due to hydrogen bonding between -CO group and -NH groups of the peptide bond.
- However, segments of the protein chain may acquire their own local fold, which is much simpler and usually takes the shape of a spiral an extended shape or a loop. These local folds are termed secondary elements and form the proteins secondary structure.



Secondary Structure of Protein

(a) α – Helix:

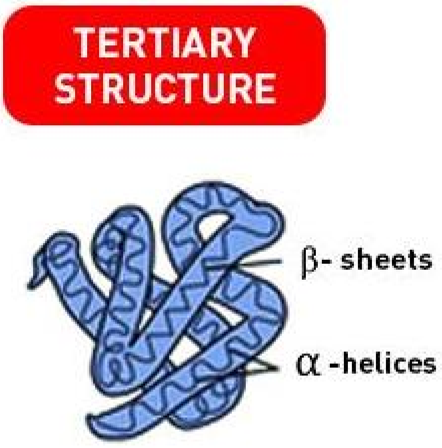
α – Helix is one of the most common ways in which a polypeptide chain forms all possible hydrogen bonds by twisting into a right-handed screw with the -NH group of each amino acid residue hydrogen-bonded to the -CO of the adjacent turn of the helix. The polypeptide chains twisted into a right-handed screw.

(b) β – pleated sheet:

In this arrangement, the polypeptide chains are stretched out beside one another and then bonded by intermolecular H-bonds. In this structure, all peptide chains are stretched out to nearly maximum extension and then laid side by side which is held together by intermolecular hydrogen bonds. The structure resembles the pleated folds of drapery and therefore is known as β – pleated sheet

3. Tertiary Structure of Protein

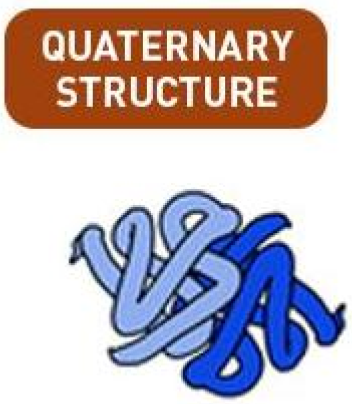
- This structure arises from further folding of the secondary structure of the protein.
- H-bonds, electrostatic forces, disulphide linkages, and Vander Waals forces stabilize this structure.
- The tertiary structure of proteins represents overall folding of the polypeptide chains, further folding of the secondary structure.
- It gives rise to two major molecular shapes called fibrous and globular.
- The main forces which stabilize the secondary and tertiary structures of proteins are hydrogen bonds, disulphide linkages, van der Waals and electrostatic forces of attraction.



Tertiary Structure of Protein

4. Quaternary Structure of Protein

The spatial arrangement of various tertiary structures gives rise to the quaternary structure. Some of the proteins are composed of two or more polypeptide chains referred to as sub-units. The spatial arrangement of these subunits with respect to each other is known as quaternary structure.



Quaternary Structure of Protein

The exact amino acid sequence of each protein drives it to fold into its own unique and biologically active three-dimensional fold also known as the tertiary structure. Proteins consist of different combinations of secondary elements some of which are simple whereas others are more complex. Parts of the protein chain, which have their own three-dimensional fold and can be attributed to some function are called **"domains"**. These are considered today as the evolutionary and functional building blocks of proteins.

Many proteins, most of which are enzymes contain organic or elemental components needed for their activity and stability. Thus the study of protein evolution not only gives structural insight but also connects proteins of quite different parts of the metabolism.

DENATURATION AND RENATURATION OF PROTEIN

What is Denaturation of Protein?

Denaturation is a process by which a protein loses its quaternary structure, tertiary structure or secondary structure which makes it biologically active. During denaturation, the forces which hold the 3D structure of the protein molecule is disrupted. As a result, the protein molecule loses its natural properties and its biological activity. Proteins become biologically active due to protein folding. Denaturation causes unfolding of the polypeptide chain, leading to disorganization of the 3D structure of the protein. Once they lose their 3D structure, they become functionally inactive or nonfunctional.

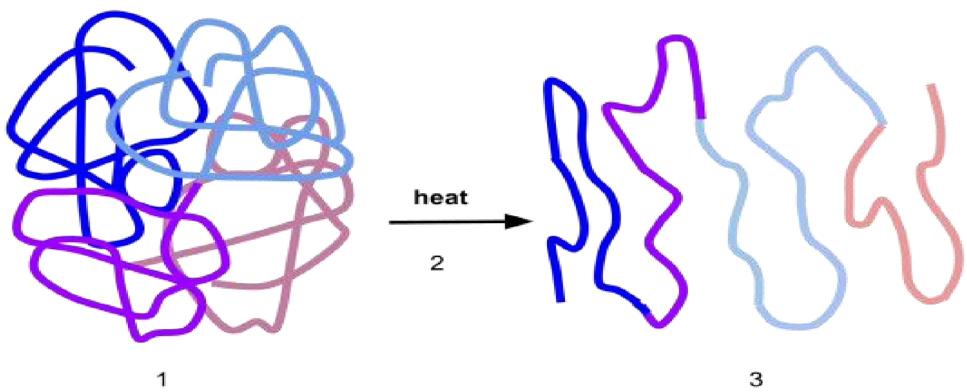


Figure 01: Denaturation of Protein

Denaturation of proteins can be achieved by applying some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent, radiation or heat, etc. Cells die when the proteins of a cell are denatured. Most importantly, when a protein is denatured, it cannot fulfil its function. For example, when enzymes are denatured, they cannot catalyze biochemical reactions. They also show a loss of solubility to protein aggregation.

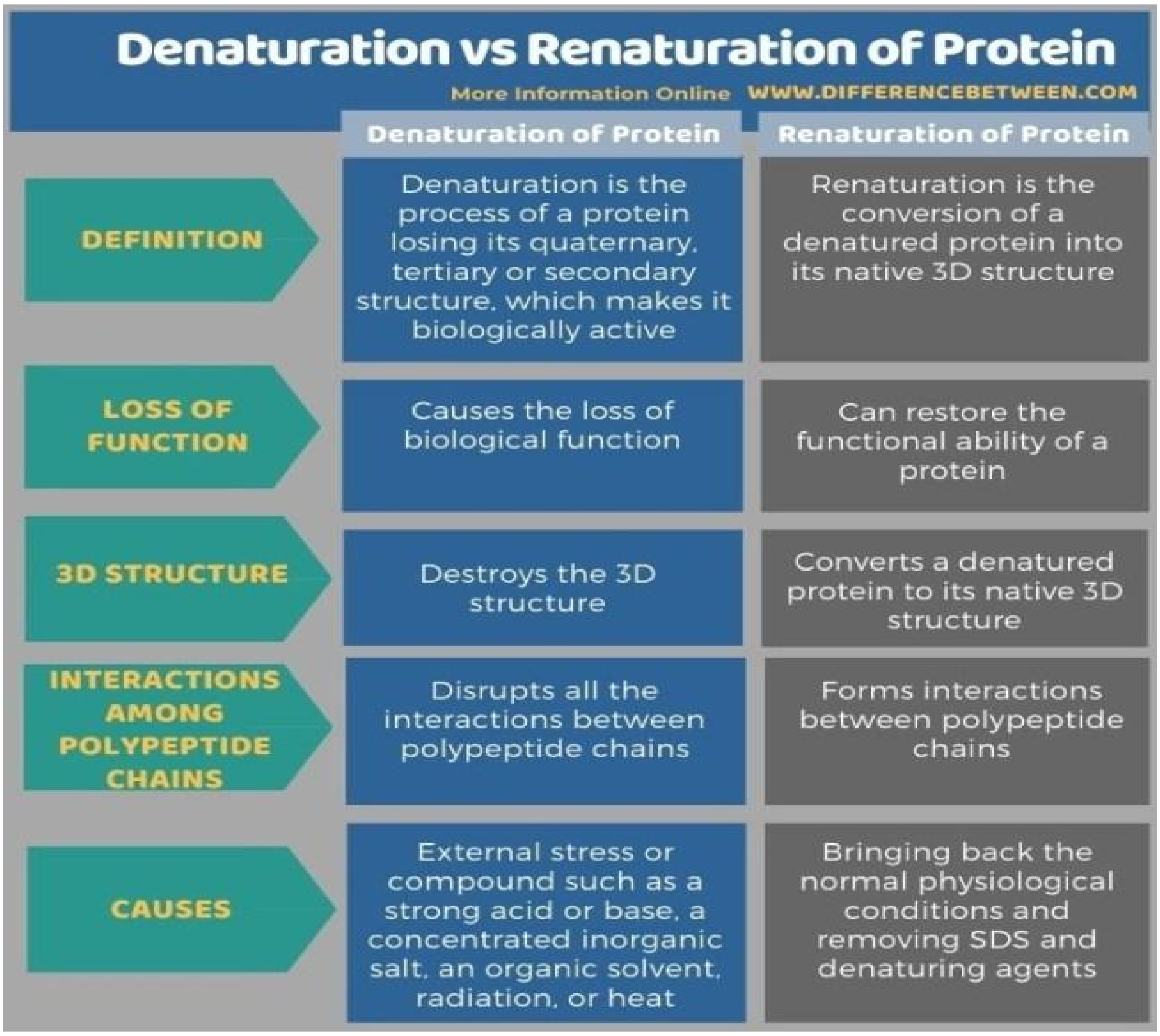
What is Renaturation of Protein?

Renaturation of a protein is the conversion of a denatured protein back into its native 3D structure. Therefore, it involves the reconstruction of a protein molecule after losing its original structure. Renaturation is the inverse process of denaturation. Renaturation is sometimes reversible. However, renaturation is not common and easy as denaturation. One way of renaturing a protein is removing the SDS and denaturing agents following denaturation during PAGE or IEF protein identification. When the physiological conditions are placed back, the protein folding may occur and restore its original 3D conformation.

What is the Difference Between Denaturation and Renaturation of Protein?

Denaturation is the process of a protein losing its quaternary structure, tertiary structure or secondary structure, which makes it biologically active. On the other hand, renaturation is the conversion of a denatured protein into its native 3D structure. So, this is the key difference between denaturation and renaturation of protein.

Moreover, denaturation causes the loss of the biological function of a protein, while renaturation can restore the functional ability of a protein.



Denaturation vs Renaturation of Protein

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	Denaturation of Protein	Renaturation of Protein
DEFINITION	Denaturation is the process of a protein losing its quaternary, tertiary or secondary structure, which makes it biologically active	Renaturation is the conversion of a denatured protein into its native 3D structure
LOSS OF FUNCTION	Causes the loss of biological function	Can restore the functional ability of a protein
3D STRUCTURE	Destroys the 3D structure	Converts a denatured protein to its native 3D structure
INTERACTIONS AMONG POLYPEPTIDE CHAINS	Disrupts all the interactions between polypeptide chains	Forms interactions between polypeptide chains
CAUSES	External stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent, radiation, or heat	Bringing back the normal physiological conditions and removing SDS and denaturing agents