Denaturation of protein:

Protein structures have evolved to function in particular cellular environments. Conditions different from those in the cell can result in protein structural changes, large and small. A loss of three-dimensional structure sufficient to cause loss of function is called denaturation. The denatured state does not necessarily equate with complete unfolding of the protein and randomization of conformation.

Most proteins can be denatured by heat, which affects the weak interactions in a protein (primarily hydrogen bonds) in a complex manner. If the temperature is increased slowly, a protein's conformation generally remains intact until an abrupt loss of structure (and function) occurs over a narrow temperature range. The abruptness of the change suggests that unfolding is a cooperative process: loss of structure in one part of the protein destabilizes other parts. The effects of heat on proteins are not readily predictable.

Proteins can be denatured not only by heat but by extremes of pH, by certain miscible organic solvents such as alcohol or acetone, by certain solutes such as urea and guanidine hydrochloride, or by detergents. Each of these denaturing agents represents a relatively mild treatment in the sense that no covalent bonds in the polypeptide chain are broken. Organic solvents, urea, and detergents act primarily by disrupting the hydrophobic interactions that make up the stable core of globular proteins; extremes of pH alter the net charge on the protein, causing electrostatic repulsion and the disruption of some hydrogen bonding. The denatured states obtained with these various treatments need not be equivalent.

Plasma proteins:

Plasma is present in the blood and constitutes the liquid part of the blood. It is made up of 90% water which is required for hydration of body tissues. 7% of plasma is composed of proteins and looks like a pale yellow liquid. The total concentration of plasma protein in blood is 7-7.5 g/dl.

There are three main groups of plasma protein:

1. **Albumin:** It comprises of 60% of overall plasma protein and hence albumins are the highly abundant plasma proteins. These are produced by the liver and helps in transportation of different components in blood, along with drugs. Albumins also help to maintain water balance in the body and contribute to osmotic pressure.

2. **Fibrinogen:** These comprise of merely 4% of overall plasma proteins. This plasma protein is created by liver and its only function is to make clots and stop bleeding. It is very sticky and is a fibrous coagulant present in blood which produces thrombin. This thrombin then gets transformed into fibrin which is the major protein in blood clot.
3. **Globulin:** These comprise of 36% of overall plasma protein and include protein carriers, enzymes, gamma globulin and antibodies. Globulins are fractionized into alpha, beta and gamma and although all the globulins are manufactured in liver, gamma globulins are especially produced by lymphocytes also termed as plasma cells. Plasma globulins are categorized into four types based on its properties:
   i. **α1-Globulin:** This fraction includes several complex proteins containing carbohydrates and lipids. These are α1-glycoprotein and α-lipoproteins. The normal serum level of α1-globulin is 0.42 g/dl.
   ii. **α2-Globulins:** This fraction also contains complex proteins such as α2-glycoproteins, plasminogen, prothrombin, haptoglobulin, ceruloplasmin (transports Cu) and α2-macroglobulin. The normal serum value of this fraction is 0.67 g/dl.
   iii. **β-Globulins:** This fraction of plasma proteins contain different β-lipoproteins which are very rich in lipid content. It also contains transferrin which transports non-heme iron in plasma. The normal serum value of β-globulins is 0.91 g/dl.
   iv. **γ-Globulins:** These are also called as Immunoglobulins and possess antibody activity. On the basis of their electrophoretic mobility they are classified as IgG, IgA and IgM.

Plasma protein functions:

The functions of plasma proteins include:

1. Osmotic effect of plasma protein maintains fluid as well as electrolyte balance.
2. The plasma protein maintains viscosity of blood.
3. Performs the important function of clotting.
4. Responds with inflammation in case of wound or injury.
5. The gamma globulins act as antibodies and protect our body from infection.
6. Plasma protein also maintains acid base balance.

Amino acids sequences of proteins:

Protein sequencing denotes the process of finding the amino acid sequence, or primary structure of a protein. Sequencing plays a very vital role in Proteomics as the information obtained can be used to deduce function, structure, and location which in turn aids in identifying new or novel proteins as well as understanding of cellular processes. Better understanding of these processes allows for creation of drugs that target specific metabolic pathways.

The enzymes that achieve amino acids sequencing and break down proteins can be divided into exopeptidases and endopeptidases. Endopeptidases break peptide links (the bonds which hold amino acids together) in the middle of polypeptide chains. Exopeptidases break the peptide links of the amino acids at the ends of the chains.

It is often desirable to know the unordered amino acid composition of a protein prior to attempting to find the ordered sequence, as this knowledge can be used to facilitate the discovery of errors in the sequencing process or to distinguish between ambiguous results. Knowledge of the frequency of certain amino acids may also be used to choose which protease to use for digestion of the protein. A generalized method often referred to as amino acid analysis for determining amino acid frequency is as follows:

1. Hydrolyse a known quantity of protein into its constituent amino acids.
2. Separate and quantify the amino acids in some way.

Hydrolysis: Hydrolysis is done by heating a sample of the protein in 6 M hydrochloric acid to 100–110°C for 24 hours or longer.

Separation: The amino acids can be separated by ion-exchange chromatography or hydrophobic interaction chromatography.

Quantitative analysis: Once the amino acids have been separated, their respective quantities are determined by adding a reagent that will form a coloured derivative. If the amounts of amino acids are in excess of 10 nmol, ninhydrin can be used for this; it gives a yellow colour when reacted with proline, and a vivid purple with other amino acids. The concentration of amino acid is proportional to the absorbance of the resulting solution.

***N*-terminal Residue Identification:** *N*-terminal residue identification encompasses a technique which chemically determines which amino acid forms the *N*-terminus of a peptide chain. This information can be used to aid in ordering of individual peptide sequences that were generated using other sequencing techniques that fragment the peptide chain. The *N*-terminal residue identification can be achieved using the following reagents:

1- **Sanger reagent:** 1-fluoro-2,4-dinitrobenzene, or Sanger's reagent (commonly called dinitrofluorobenzene or DNFB) reacts with the amine group in amino acids to produce dinitrophenyl-amino acids. These DNP-amino acids are moderately stable under acid hydrolysis conditions that break peptide bonds. The DNP-amino acids can then be recovered, and the identity of those amino acids can be discovered through chromatography.

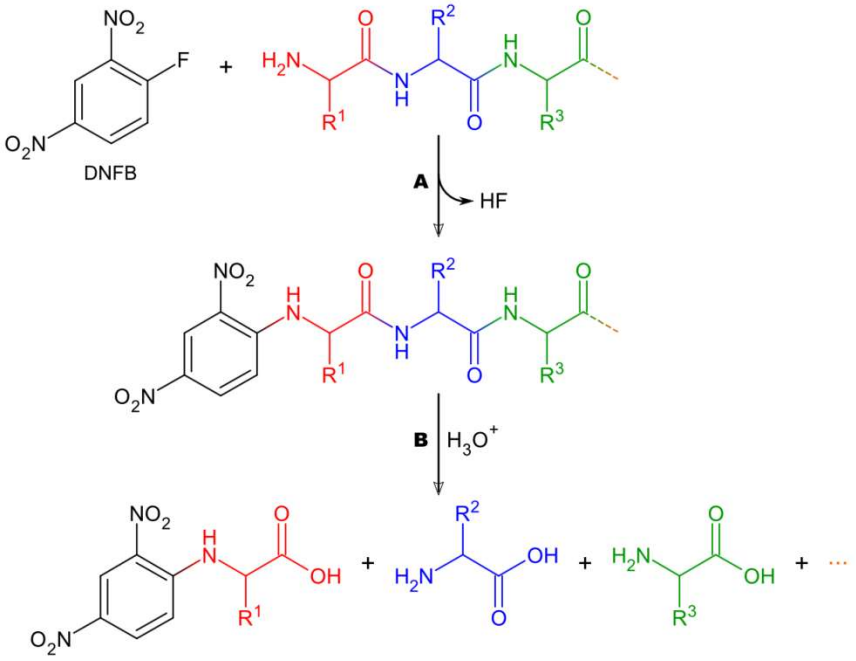

Figure 2: Sanger's method of peptide end-group analysis: A) complex of *N*-terminal end with Sanger's reagent (DNFB), B) total acid hydrolysis of the dinitrophenyl peptide.

2- **Dansyl chloride reagent:** Dansyl chloride or 5-(Dimethylamino) Naphthalene-1-Sulfonyl chloride is a reagent that reacts with the free amino groups of peptides and proteins to produce stable blue- or blue-green–fluorescent sulfonamide adducts.

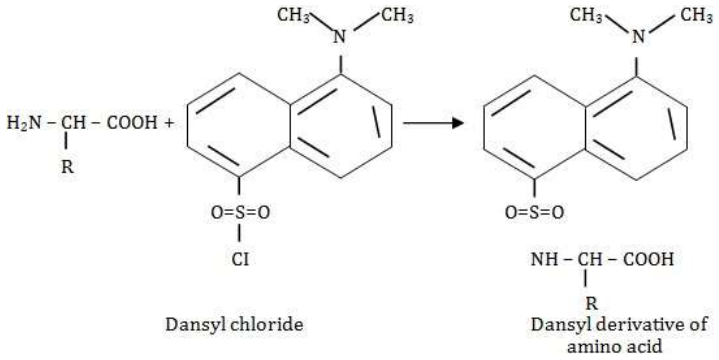

Figure 3: Dansyl chloride complex with amino acid.

3- **Edman reagent:** Edman degradation, developed by Pehr Edman, is a method of sequencing amino acids in a peptide. In this method, Phenyl isothiocyanate is reacted with an uncharged *N*-terminal amino group, under mildly alkaline conditions, to form a cyclical phenyl thiocarbamoyl derivative. Then, under acidic conditions, this derivative of the terminal amino acid is cleaved as a thiazolinone derivative. The thiazolinone amino acid is then selectively extracted into an organic solvent and treated with acid to form the more stable phenyl thiohydantoin (PTH)-amino acid derivative that can be identified using chromatography or electrophoresis.

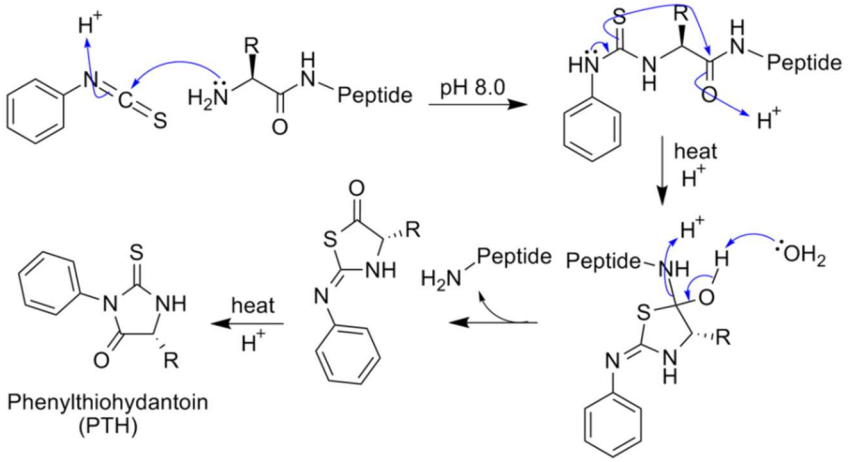

Figure 4: Formation of PTH from the reaction of Phenyl isothiocyanate with peptide.

***C*-terminal residue identification:** The number of methods available for *C*-terminal amino acid analysis is much smaller than the number of available methods of *N*-terminal analysis. The most common method is to add carboxypeptidases to a solution of the protein, take samples at regular intervals, and determine the terminal amino acid by analysing a plot of amino acid concentrations against time. *C*-terminal sequencing would greatly help in verifying the primary structures of proteins predicted from DNA.

Digestion into peptide fragments using endopeptidases: Peptides longer than about 50-70 amino acids long cannot be sequenced reliably by the Edman degradation. Because of this, long protein chains need to be broken up into small fragments that can then be sequenced individually. Digestion is done either by endopeptidases such as trypsin or pepsin or by chemical reagents such as cyanogen bromide. Different enzymes give different cleavage patterns, and the overlap between fragments can be used to construct an overall sequence. The most common endopeptidases are:

1- **Trypsin**: cuts after Arg or Lys, unless followed by Proline.
2- **Chymotrypsin**: cuts after Phe, Trp, or Tyr, unless followed by Pro.
3- **Pepsin:** cuts before Leu, Phe, Trp or Tyr, unless preceded by Pro.
4- **Cyanogen bromide (CNBr):** cuts after methionine residues.

Example: Unknown peptide was treated with trypsin first and again with CNBr. Giving the following sequences:

A) By trypsin (1) Asn-Thr-Trp-Met-Ile-Lys (2) Val-Leu-Gly-Met-Ser-Arg (3) Gly-Tyr-Met-Gln-Phe.
B) By CNBr (4) Gln-Phe (5) Val-Leu-Gly-Met (6) Ile-Lys-Gly-Tyr-Met (7) Ser-Arg-Asn-Thr-Trp-Met.

1- Deduce the sequence of the original peptide.
2- Give the sequence of the reaction of Phenyl isothiocyanate with peptide (5).
3- Give the products of peptide (1) if treated with chymotrypsin.