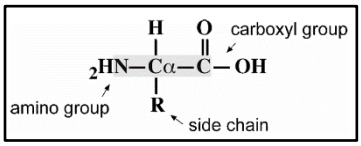
UNIT 4 –

Amino acids –



- **Def –** small molecules that contain a free amino group (NH_2) and a free carboxyl group (COOH) linked to a central carbon (Cα), which is attached to a hydrogen and a side chain group (R).
- Building blocks of proteins
- 20 naturally occurring amino acids → differ only by the side chain group (R).
- Chemical reactivities of the R groups → determine the specific properties of the amino acids

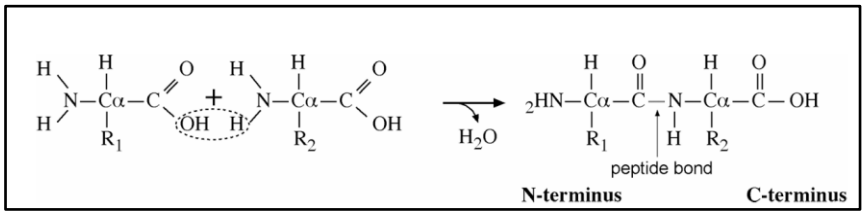
- **Classification of amino acids –**
  REMAINING

- **Glycine –**
  - ✓ Smallest amino acid
  - ✓ R group = hydrogen (H) atom → ∴ more flexible confirmations

- **Proline –**
  - ✓ Cyclic structure since side chain forms bond with its own backbone → ∴ very rigid

Peptide formation –

- Formed when two amino acids are covalently joined together between the carboxyl group of one amino acid and amino group of another.



- Condensation reaction → involves removal of elements of water from the two molecules
- Resulting product = dipeptide
- Polypeptide / Protein = a linear polymer of >50 amino acid residues + has a well – defined 3 – dimensional arrangement
- Peptide = a polymer with < 50 residues + without a well – defined 3 – dimensional structure
- Numbered beginning with the residue containing the amino group (N – terminus) and ending with the residue containing the carboxyl group (C – terminus)
- Backbone atoms involved in the formation of peptide bond –
  1. Nitrogen of amino group
  2. Alpha carbon to which side chain is attached
  3. Carbon of the carbonyl group

Ramachandran Plot (RC Plot) –

1. **Planar Nature of Peptide bonds –**
   - Polypeptides or proteins → most commonly, linear and unbranched polymers – composed of amino acids linked together by peptide bonds (amide linkages between formed between alpha amino group of one amino acid and the alpha carboxyl group of adjacent amino acid).
   - **Condensation reaction** → water molecule is released → linked amino acids are referred to as **'amino acid residues'**
   - **Thermodynamics** → endergonic process → $\Delta G° \sim 21$ kJ / mol
   - **Peptide C – N Bond chemistry** → Partial double bond character
     - ✓ Peptide bond length = 1.33 A° (intermediate between C – N single bond = 1.49 A° and C = N double bond = 1.27 A°)
     - ✓ 40% double bonded character due to resonance
     - ✓ Oxygen (partial negative charge) + Nitrogen (partial positive charge) → a small electric dipole
     - ✓ Keep the peptide bond in a rigid planar configuration
     - ✓ 6 atoms – Cα, C, and O of 1st amino acid and N, H and Cα of 2nd amino acid lie in the same plane.

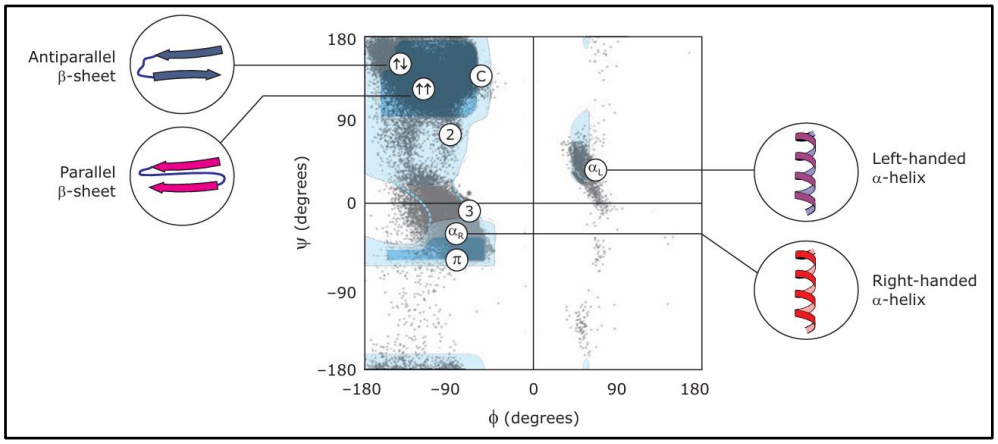
2. **Tortional angles / Dihedral angles –**
   - **Def –** Angle of rotation above the peptide bond
   - Rotation is not completely free due to planar nature of the peptide bonds + steric hindrance from side chain R group
   - **2 types –**
     (a) phi (ϕ) – along the N – Cα bond
     (b) psi (Ψ) – along the Cα – C bond
     ] can vary over a range of 0 to 360°

3. **Description of RC plot –**
   - Ramachandran plot = Ramachandran diagram = [ϕ, Ψ] plot
   - Developed in 1963 – by G. N. Ramachandran, C. Ramakrishnan and V. Sasisekharan
   - Provides a simple 2 – dimensional graphic representation of all possible protein structures when ϕ (on x – axis) and Ψ (on y – axis) angles of particular protein are plotted against each other
   - Developed using theoretical methods, mathematical calculations and model building
   - Plots entire conformational space + shows sterically allowed and disallowed regions

   - White areas = Sterically disallowed confirmations
     - ✓ Any non – bonding inter atomic distance < its corresponding Van der Waals radii
     - ✓ spherically disallowed for all amino acids except Glycine → unique as it lacks a side chain

   - Dark blue regions = allowed regions
     - ✓ Conformations where there are no steric interferences
     - ✓ Most ϕ and Ψ angles fall within these allowed regions of the RC plot
     - ✓ Glycine → less sterically restricted → allowed range of torsional angles covers a larger area of the RC plot
     - ✓ Proline → cyclic side chain limits its range of ϕ values to around -60° → most conformationally restricted amino acid residue
   - Light blue regions = conformation having outer limit van der Waals distances i.e., the atoms are allowed to come a little closer together
   - D and L form → have their side chain oriented differently with respect to the CO group
     ∴ different allowed ϕ and Ψ angles
     eg – β - sheet made up of L-amino acid occupies upper left quadrant
     β - sheet made up of same amino acids of D - form would occupy lower right quadrant

4. **Applications of RC plot –**
   (a) Predicting secondary structure of a protein
   (b) Predicting quality of protein structure determined using experimental methods (X – ray crystallography, NMR and Cryo – EM) + homology modelling and *ab – initio* methods
      ✓ Good quality → all sets of torsional angles in allowed area
      ✓ Bad quality (low resolution) → multiple torsional angles in forbidden region



Hierarchy of proteins –

Level	Definition
Primary structure (1°)	• A linear amino acid sequence of a protein • Simplest level with amino acids linked together through peptide bonds.
Secondary structure (2°)	• Local conformation of peptide chain • Highly regular and repeated arrangement of amino acid residues stabilised by hydrogen bonds between main chain atoms of the C=O Group and the NH group of different residues.
Supersecondary structures	• Intermediate between secondary and tertiary structures • Two or three secondary structural elements forming a unique functional domain, a recurring structural pattern conserved in evolution.
Tertiary structure (3°)	• 3-dimensional arrangement of various secondary structural elements and connecting regions.
Quaternary structure (4°)	• Association of several polypeptide chains into a protein complex • Maintained by non – covalent interactions • **'Monomers' or 'subunits'** = individual polypeptide chains

Stabilizing forces → Non-covalent interactions –

1. Electrostatic Interaction (EI)
- Occur when excessive (-ve) charge in 1 region is neutralized by (+ve) charge in another region.
- Formation of salt bridges b/w oppositely charged residues.
- Long range (>15 A°)

2. Hydrogen Bonds (H – bonds)
- Similar to dipole – dipole interactions
- Type of EI involving H from one residue and O from another
- H → from H – bond → slightly donor grp (NH) (+vely) charged

  PARTIAL BOND (< 3 A°)

  O → from H – bond → slightly acceptor grp (C=O) (- vely) charged
- Dominant factor in determining different protein 2° structures
- Can occur between both main chain and side chain items atoms

3. Disulphide bridges
- Covalent bonds between S atoms of Cysteine residue

4. Van der Waals forces
- Instantaneous interactions between atoms when they become transient dipoles (can induce another transient dipole nearby)
- Oscillating dipoles → result in attractive force
- VDW <<< weaker than EI & H – bond → ∴ secondary effect on protein structure

REMAINING – REFER HIGHLIGHTED XIN XIONG

The Anfinsen Experiment

- conducted by Christian B. Anfinsen in the 1950s
- Involved the denaturation and renaturation of ribonuclease A (RNase). Anfinsen chose ribonuclease A (RNase A), an enzyme found in the pancreas, as his model protein. RNase A has a well-defined structure and enzymatic activity, making it ideal for the experiment.
- Aimed to understand how proteins fold into their native conformations + to determine whether the native structure of a protein is uniquely determined by its primary structure (the sequence of amino acids) or if other factors, such as the presence of disulfide bonds, play a crucial role in the folding process.

Steps of Anfinsen Experiment –

Step 1 – Isolation of Native RNase A

Anfinsen first isolated the active form of RNase A from the pancreas. This "native" protein had a specific folded structure and could efficiently break down RNA molecules.

↓

Step 2 – Denaturation

RNase was denatured by treating it with 8 M urea to break down hydrogen bonds responsible for the protein's secondary structure (local folding patterns).

↓

Step 3 – Reduction

The denatured RNase was then reduced by adding beta-mercaptoethanol (β-ME) to break the disulfide bonds, a specific type of linkage between amino acid side chains, crucial for the protein's tertiary structure (overall 3D shape), and create a fully reduced enzyme.

↓

Step 4 – Incubation

The reduced enzyme was incubated in the presence of air, the denaturing agents (urea and beta-mercaptoethanol) removed through dialysis, allowing the disulfide bonds to reform spontaneously.

↓

Step 5 – Activity Assay

The enzyme activity was measured after each step to assess the extent of renaturation. The refolded protein regained its enzymatic activity, demonstrating that the information necessary for proper folding resided within the amino acid sequence itself.

Results –

The experiment showed that the reduced RNase recovered its native activity and structure when incubated in the presence of air. This was interpreted as evidence that the native structure of a protein is uniquely determined by its primary structure, as the enzyme was able to fold back into its native conformation without any external guidance – **'The Anfinsen Dogma'**

Significance –

Anfinsen's experiment provided compelling evidence for the "thermodynamic hypothesis" of protein folding. This hypothesis states that the amino acid sequence of a protein encodes the information needed for it to fold into its most stable and functional conformation under physiological conditions. The specific interactions between the amino acid side chains (attractions, repulsions) guide the folding process, leading to the unique three-dimensional structure.

This knowledge is crucial for understanding protein function in health and disease, and for the development of new drugs that target specific protein structures.

Limitations and Controversies –

It has been shown that the spontaneous recovery of activity may not be as complete as initially reported, and that the presence of a reshuffling mixture is necessary to achieve full native activity.

Additionally, the role of disulfide bonds in the folding process has been reevaluated, with some studies suggesting that they may not be as crucial as previously thought.

Secondary Structure Prediciton

- **Def –** prediction of the conformational state of each amino acid residue sequence as one of the 3 possible states – namely Helices (H), Strands (E) or Coils (C).
- Structural regularity serves the foundation for prediction algorithms
- **Applications of PSSP –**
    1. Classification of proteins
    2. Separation of protein domains and functional motifs
    3. intermediate step in tertiary structure prediction as in threading analysis
- 2° structures → much more conserved than sequences during evolution

  ∴ correctly identifying secondary structure elements (SSE) → helps to guide sequence alignment or improve existing sequence alignment of distinctly related sequences

REFER CLASS NOTES AND HIGHLIGHTED XIN XIONG