B.Sc. (Hons.) Biotechnology
Core Course 14: Genomics and Proteomics (BIOT 3014)

Unit 3:

PART-I:

Introduction to protein structure, Chemical properties of proteins.

PART-II

Physical interactions that determine the property of proteins.

PART-I
Structure, function and chemical property of proteins

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Protein function

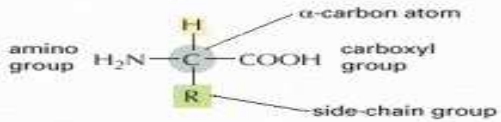
- Proteins are macromolecules found in every biological systems.
- They are the most abundant class of biomolecules representing over 50% of the dry weight of cells.
- They are involved in virtually all biological processes.
- They can transport and store a wide array of ions and small molecules as well as electrons.
- They participate in the reception & transmission of signals as well as stimuli at both intra- and inter-cellular levels.
- They are necessary for providing the mechanical strength and filamentous architecture within and between cells, and consequently essential to cellular contraction and coordinated motion.

Protein composition

- Despite their different structural and/ or functional role, all the proteins are polymers of the 20 amino acids, which are covalently joined together by peptide bonds.
- It is possible to determine the protein3D structure and biological properties from their corresponding protein sequence.
- Proteins differ only in number, nature, and sequential order of their constituent amino acids.
- Each amino acid consists of a central carbon atom (α-carbon), an amino group(-NH2), a carboxyl group(-COOH) and a side chain (R). Differences in side chains distinguish different amino acids.

THE AMINO ACID

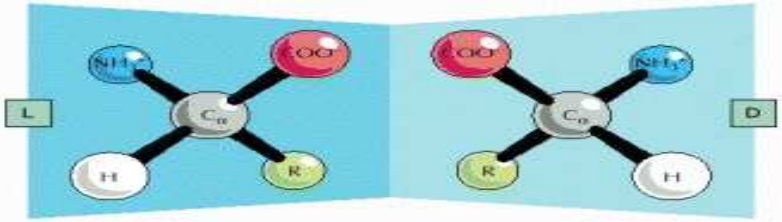
The general formula of an amino acid is



R is commonly one of 20 different side chains. At pH 7 both the amino and carboxyl groups are ionized.

OPTICAL ISOMERS

The α-carbon atom is asymmetric, which allows for two mirror image (or stereo-) isomers, L and D.



Proteins consist exclusively of L-amino acids.

FAMILIES OF AMINO ACIDS

The common amino acids are grouped according to whether their side chains are

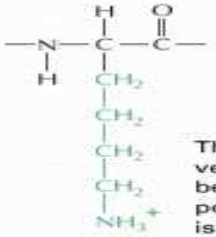
- acidic
- basic
- uncharged polar
- nonpolar

These 20 amino acids are given both three-letter and one-letter abbreviations.

Thus: alanine = Ala = A

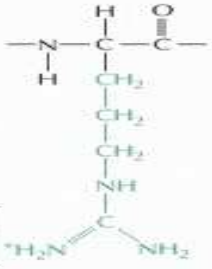
BASIC SIDE CHAINS

lysine (Lys, or K)

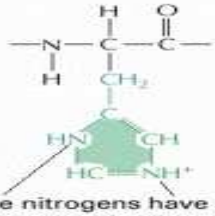


This group is very basic because its positive charge is stabilized by resonance.

arginine (Arg, or R)



histidine (His, or H)



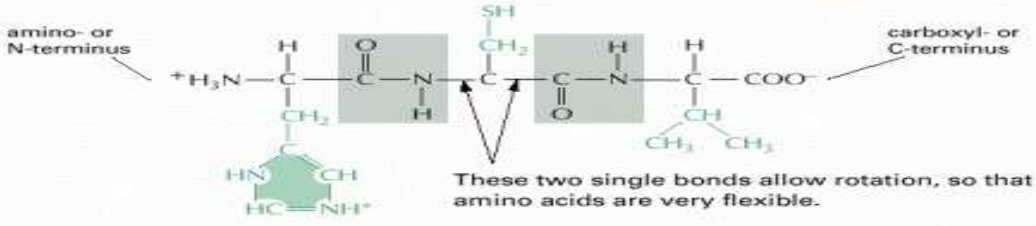
These nitrogens have a relatively weak affinity for an H^+ and are only partly positive at neutral pH.

PEPTIDE BONDS

Amino acids are commonly joined together by an amide linkage, called a peptide bond.

Peptide bond: The four atoms in each *gray box* form a rigid planar unit. There is no rotation around the C–N bond.

Proteins are long polymers of amino acids linked by peptide bonds, and they are always written with the N-terminus toward the left. The sequence of this tripeptide is histidine-cysteine-valine.



These two single bonds allow rotation, so that long chains of amino acids are very flexible.

Classification of 20 amino acids

1. Aliphatic (5 amino acids): glycine, alanine, valine, leucine and isoleucine are poorly soluble in water.

2. Hydroxylic (2 amino acids): serine and threonine are polar and very soluble in water.

3. Acidic or dicarboxylic, and corresponding amides (4 amino acids): aspartic acid and glutamic acid, asparagine and glutamine

4. Basic (3 amino acids): lysine and arginine, histidine.

5. Cyclic (4 amino acids): phenylalanine, tyrosine, tryptophan, proline and imino acid.

6. Sulfur-containing (2 amino acids): methionine and cysteine.

Polar and non polar amino acids found in proteins

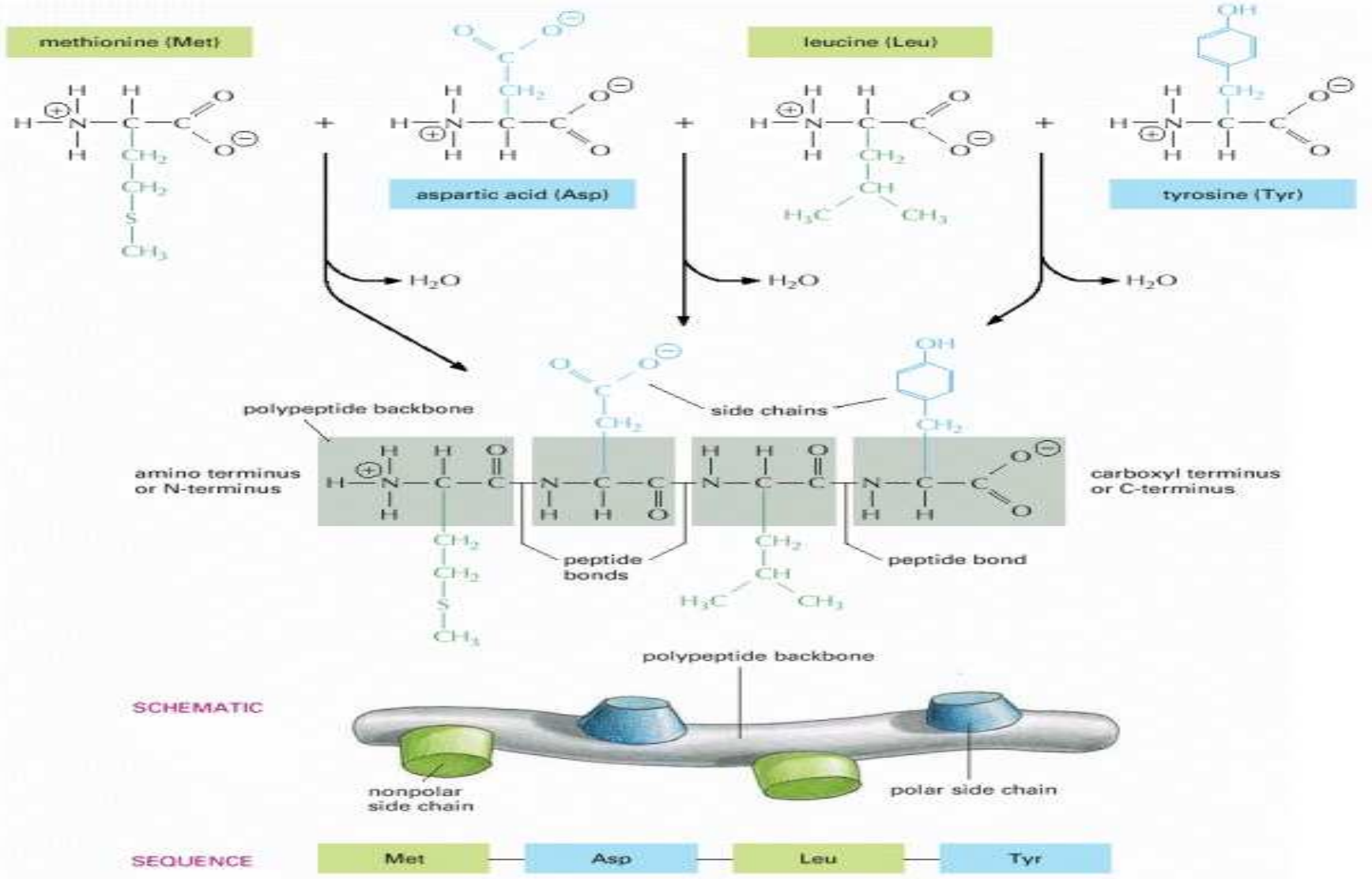
AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS

Structural components of a protein



Primary structure of protein

- The primary structure refers to the linear sequence of amino acids connected through peptide bond along a protein chain and the location of disulfide bonds, between chains or within a chain.
- The dihedral angles (ϕ and ψ) are the angles around the alpha carbon-amide nitrogen bond and alpha carbon-carbonyl carbon bond, respectively.

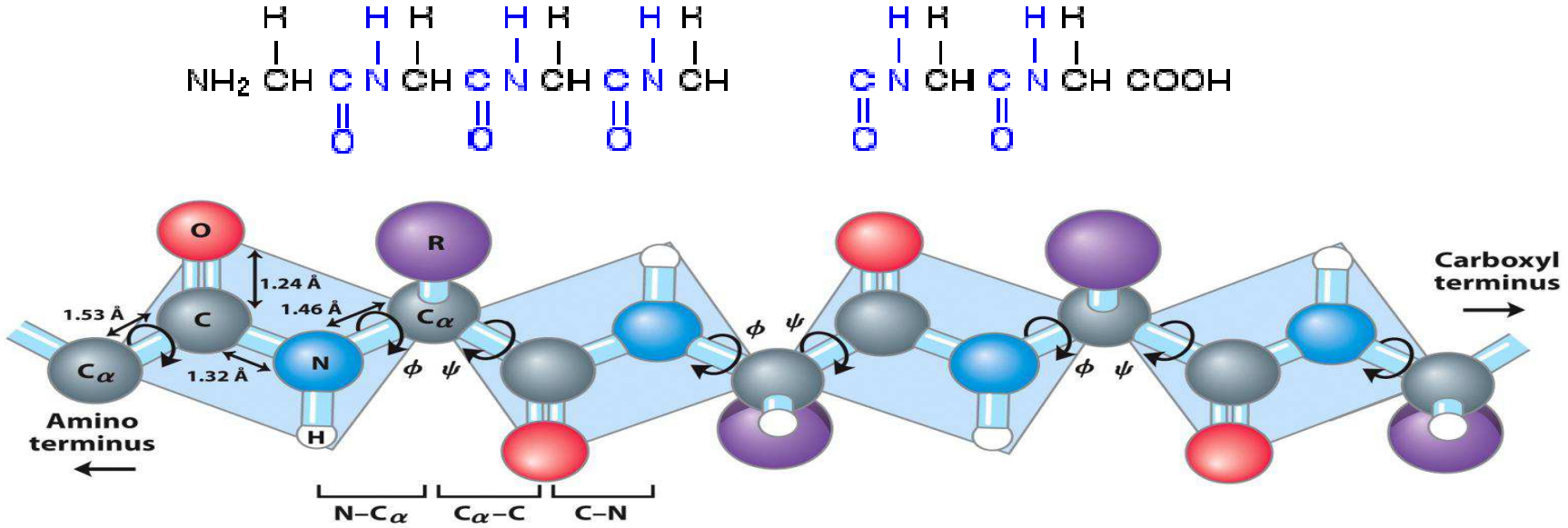
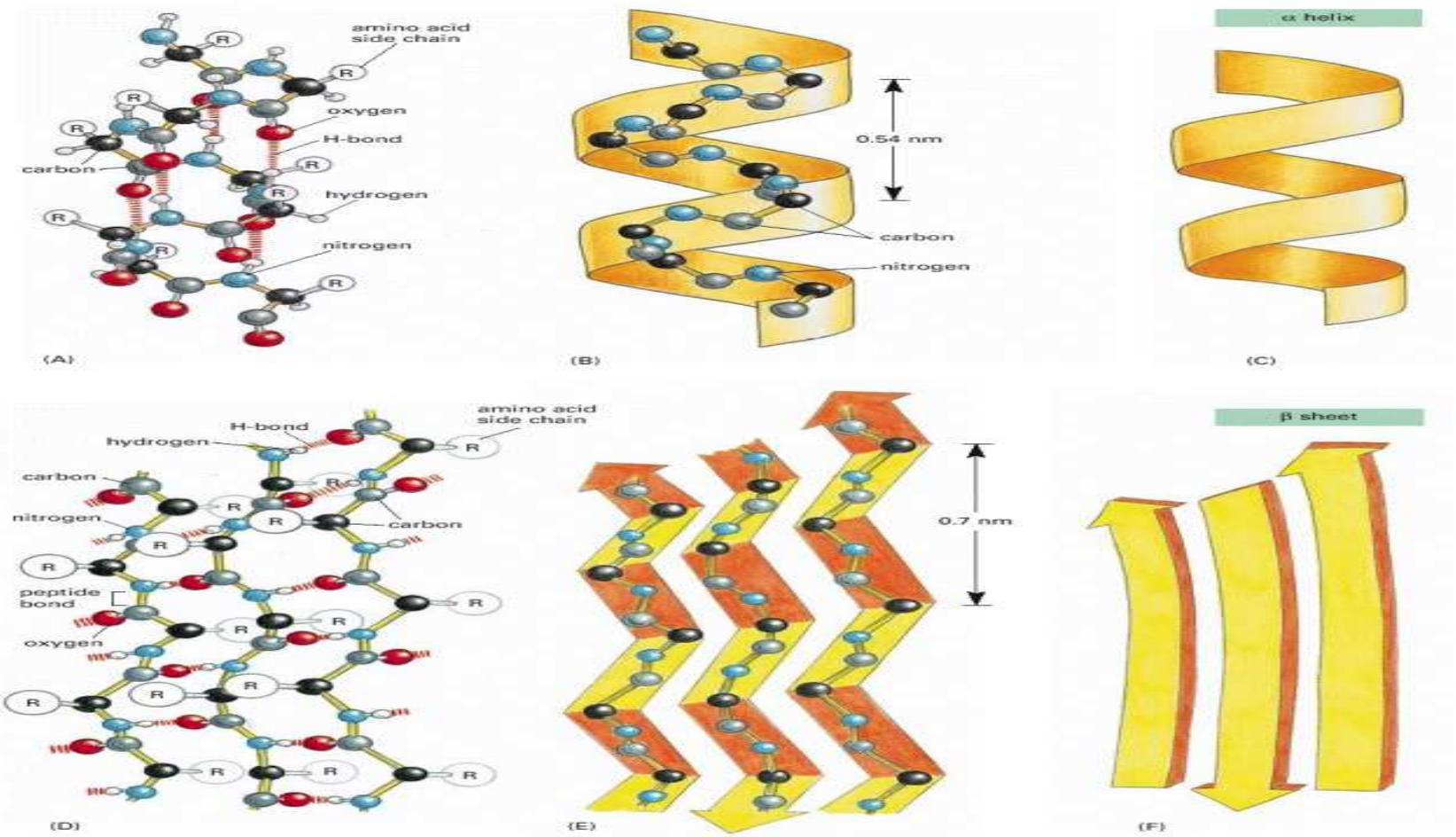


Figure 4-2b
Lehninger Principles of Biochemistry, Sixth Edition

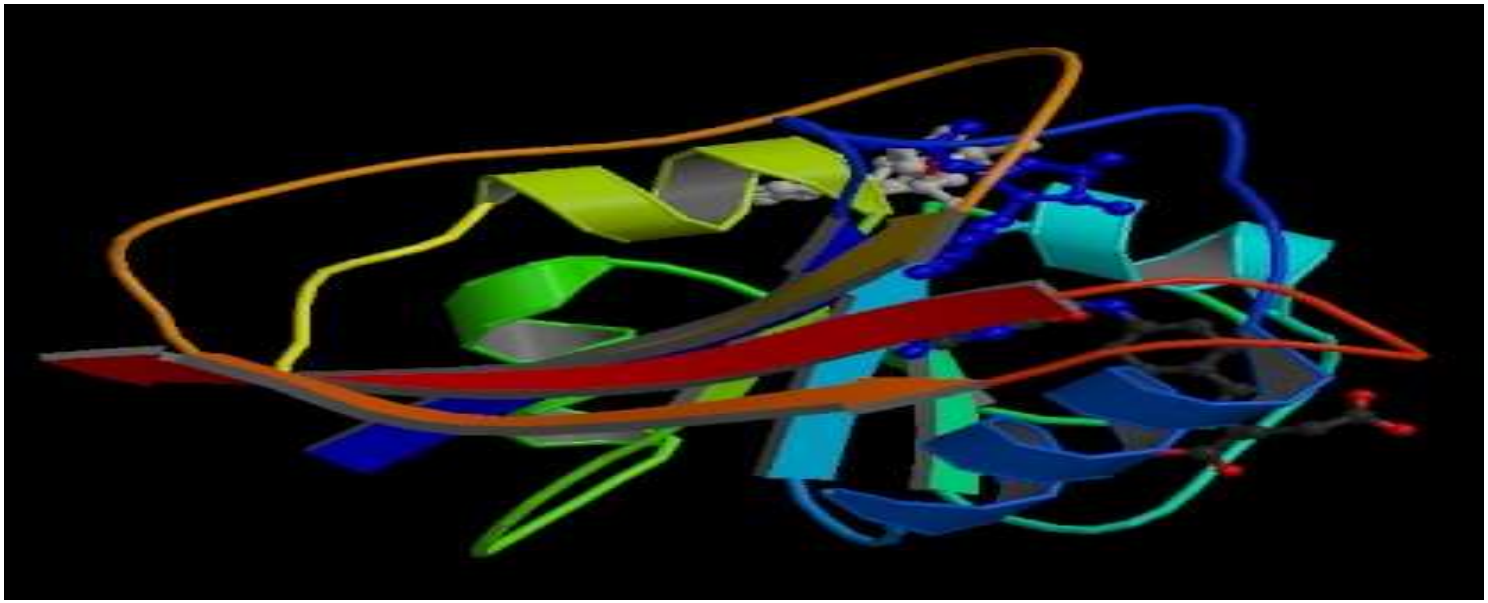
Secondary structure of proteins

Within a long protein chains, there are local regions organized into regular structures known as alpha-helices and beta-pleated sheets (formed by repeating amino acids with the same (ϕ, ψ) angles are called as secondary structural elements) and held together by hydrogen bonds. Irregular arrangement of the polypeptide chain is called the random coil or extended chain.



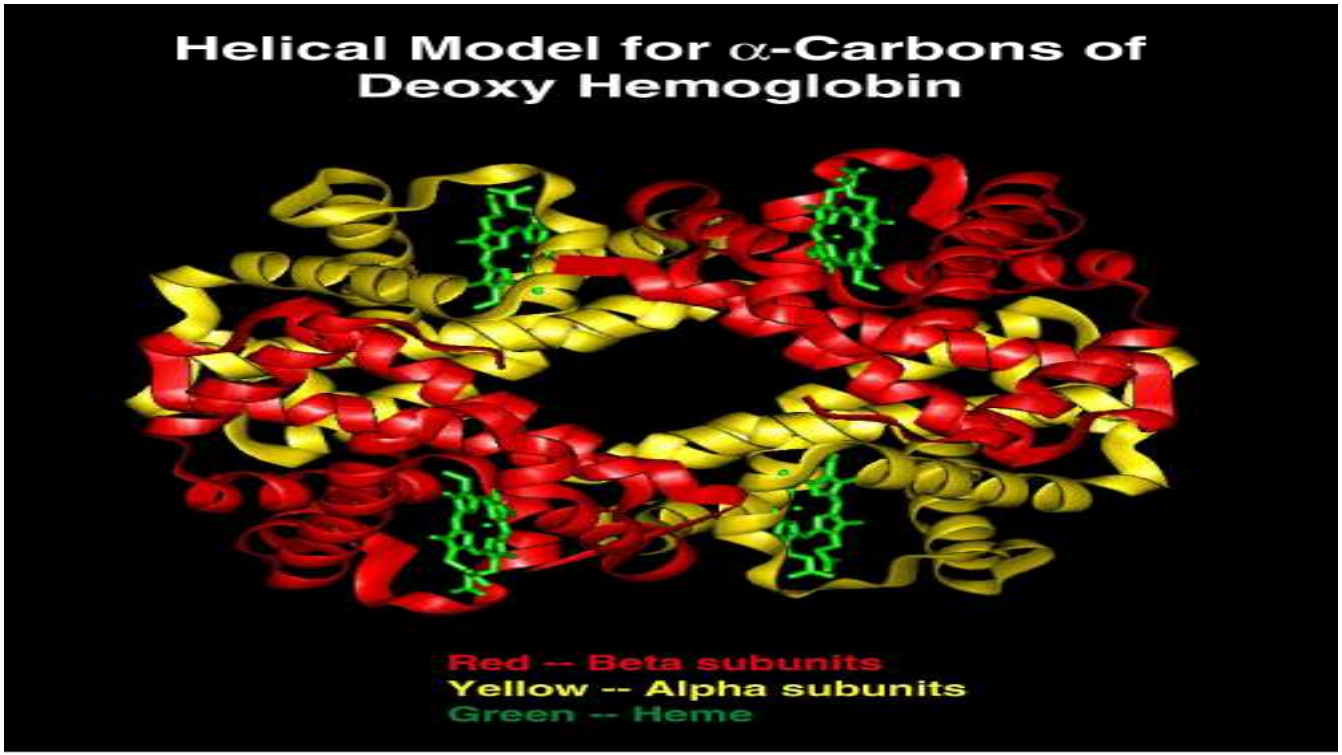
Tertiary structure

Tertiary structure (3D) refers to the overall spatial arrangement of atoms in a protein, held together by several non-covalent interactions such as ionic interactions, hydrogen bonds, van der Waals dispersion and hydrophobic forces between the side chains.



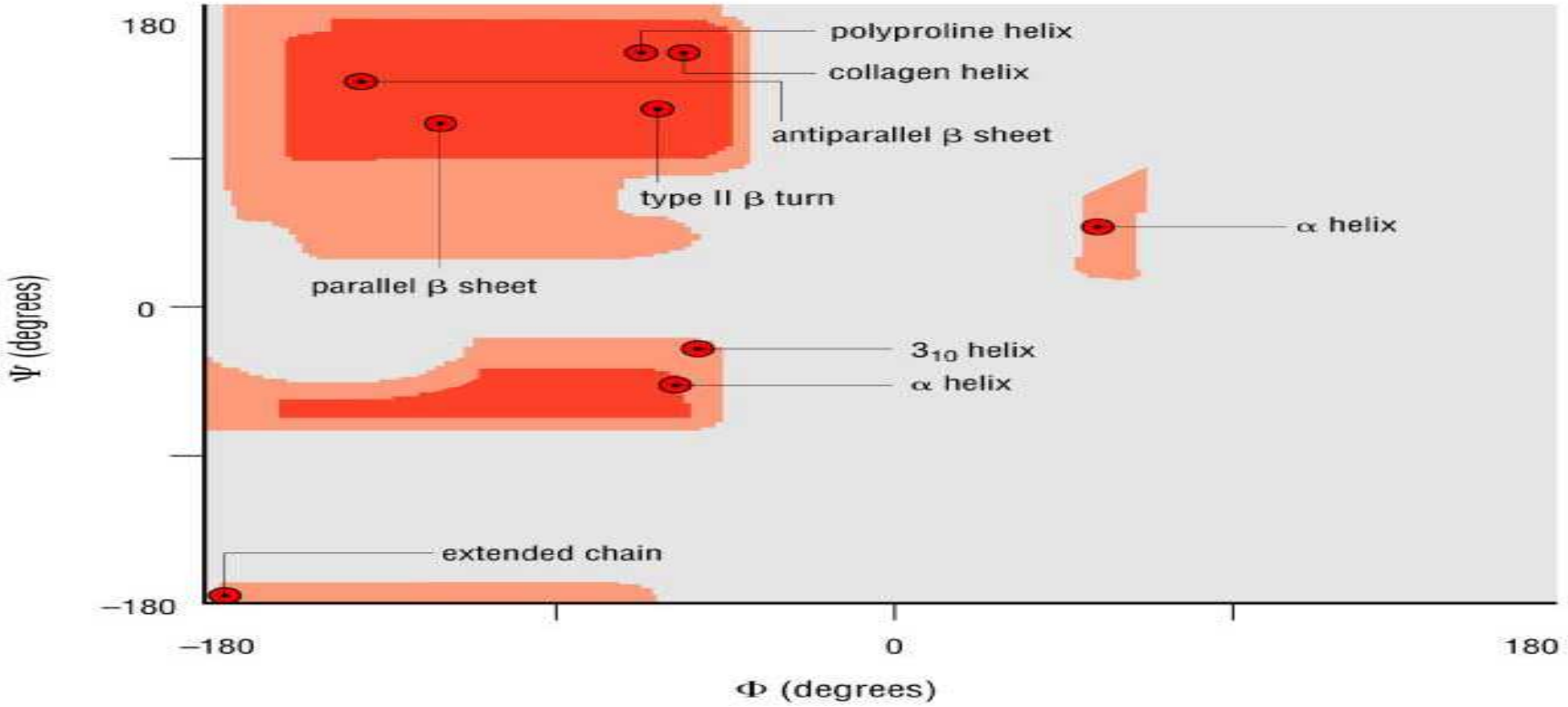
Quaternary Structure

Quaternary structure is formed by the assembly of individual polypeptides into a larger functional clusters i.e., dimer, trimers, or higher order structures. The functional form of hemoglobin is a tetramer.



The Ramachandran Plot

A Ramachandran plot shows the distribution of dihedral angles φ and Ψ that are found in a protein. The ϕ and ψ angles of amino acids in a polypeptide chain or protein are restricted, largely because of steric interactions.



Methods for determining protein structure

- Primary structure: Edman degradation, Mass spectrometry.
- Secondary structure: Circular Dichroism, FTIR.
- Tertiary & quaternary structure: NMR, X-ray crystallography.

Solubility of the proteins

- Proteins exist essentially in either aqueous or membrane environments.
- The presence of polar amino acids located at the surface of proteins favors the solubility of proteins in water and affected mainly by ionic strength and pH.
- At low ionic strength, the solubility of most proteins is relatively high (salting-in effect), but it is reduced when the ionic strength increases (salting-out effect).
- Solubility is minimal at pH values close to their isoelectric point (pI) where the net charge is equal to zero.
- In addition, the protein solubility also depends on temperature and dielectric constant of the solvent.
- When any protein is unfolded or partially denatured, it is generally less soluble than in its native form because of the nonpolar groups are more exposed.
- In general, proteins are insoluble in nonpolar solvents and the extent of their solubility is determined by the interactions of their polar groups with water.

Confirmation and estimation of proteins

- The presence protein can be revealed by the absorbance in the ultraviolet (UV) region around 280 nm due to indole ring (Trp) or an aromatic ring (Phe, Tyr).
- Since, protein contain around 16% nitrogen by mass, it can be analysed by the Kjeldahl method (based on sulfuric acid mineralization and measurement of the released ammonia).
- Biuret reaction also specifically measures peptide bonds, where $CuSO_4$ in alkaline tartrate reacts with a peptide bond to produce a purple compound with maximum absorption at 540 nm.
- Lowry assay (Folin phenol method), in which, copper ions are added to the protein solution in the presence of a phosphomolybdate–tungstate mixture. Amino groups of proteins react with ninhydrin to give a purple product with maximum absorbance at 570 nm.
- A common reagents Coomassie brilliant blue and silver nitrate (for staining proteins) can be used to quantify proteins in solution or included in gels.
- Biological assays (based on their immunological/enzymatic properties of proteins) can be performed with their affinity for specific ligands or their enzymatic activity.

References

- Alberts B, Johnson A, Lewis J, et al. The Shape and Structure of Proteins. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. (https://www.ncbi.nlm.nih.gov/books/NBK26830/)
- Cozzone, A.J. (2002). Proteins: Fundamental Chemical Properties. In eLS, (Ed.). doi:10.1038/npg.els.0001330.

Thank you.

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