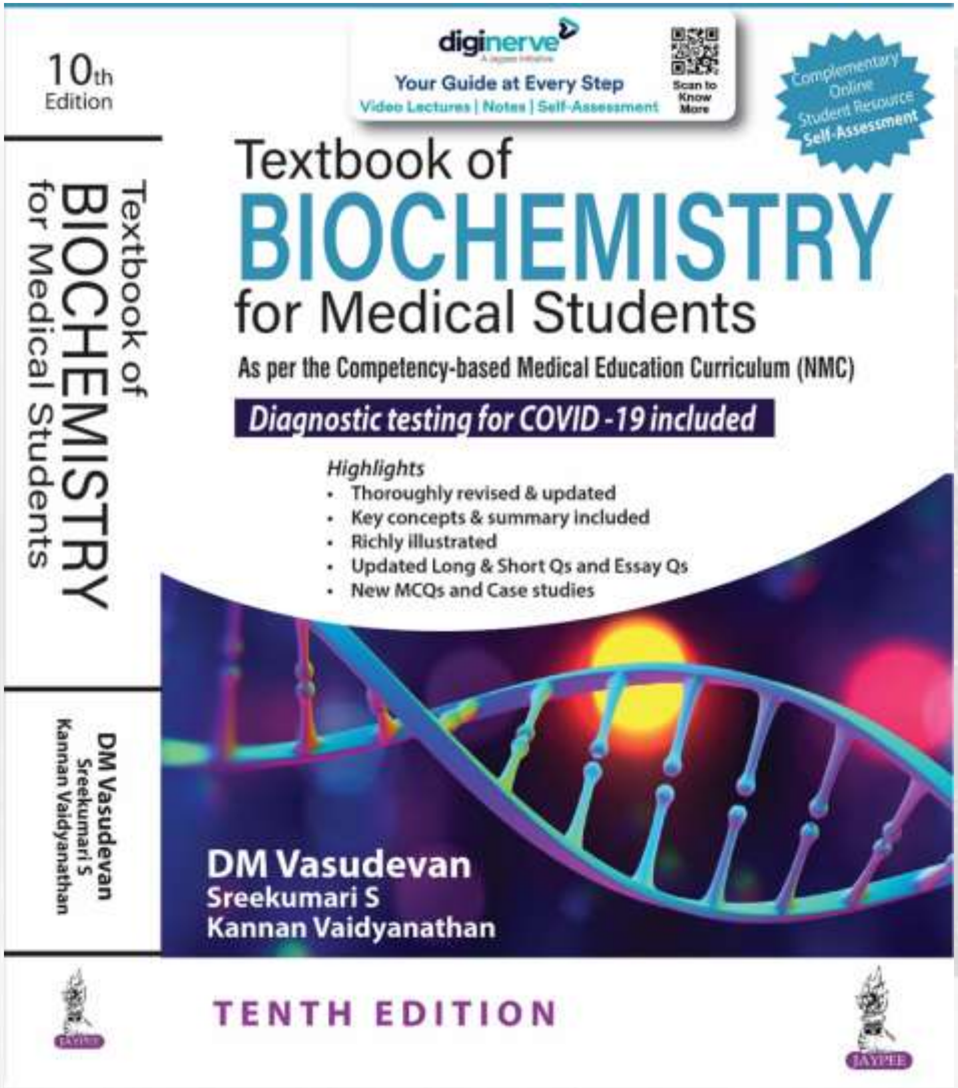
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BIOCHEMISTRY
for Medical Students

As per the Competency-based Medical Education Curriculum (NMC)

Diagnostic testing for COVID-19 included

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- Thoroughly revised & updated
- Key concepts & summary included
- Richly illustrated
- Updated Long & Short Qs and Essay Qs
- New MCQs and Case studies

DM Vasudevan
Sreekumari S
Kannan Vaidyanathan

TENTH EDITION

Textbook of BIOCHEMISTRY for Medical Students
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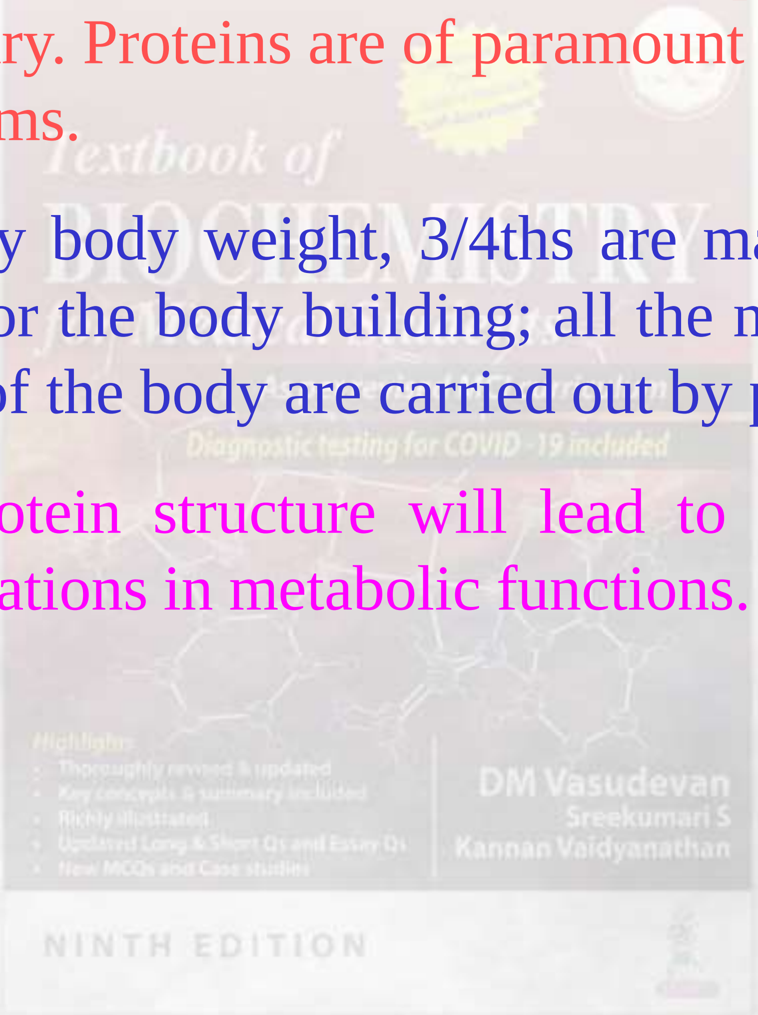
Chapter 3B:

Proteins, Structure and Function

Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION



The word protein is derived from Greek word, “proteios” which means primary. Proteins are of paramount importance for biological systems.

Out of the total dry body weight, 3/4ths are made up of proteins. Proteins are used for the body building; all the major structural and functional aspects of the body are carried out by protein molecules.

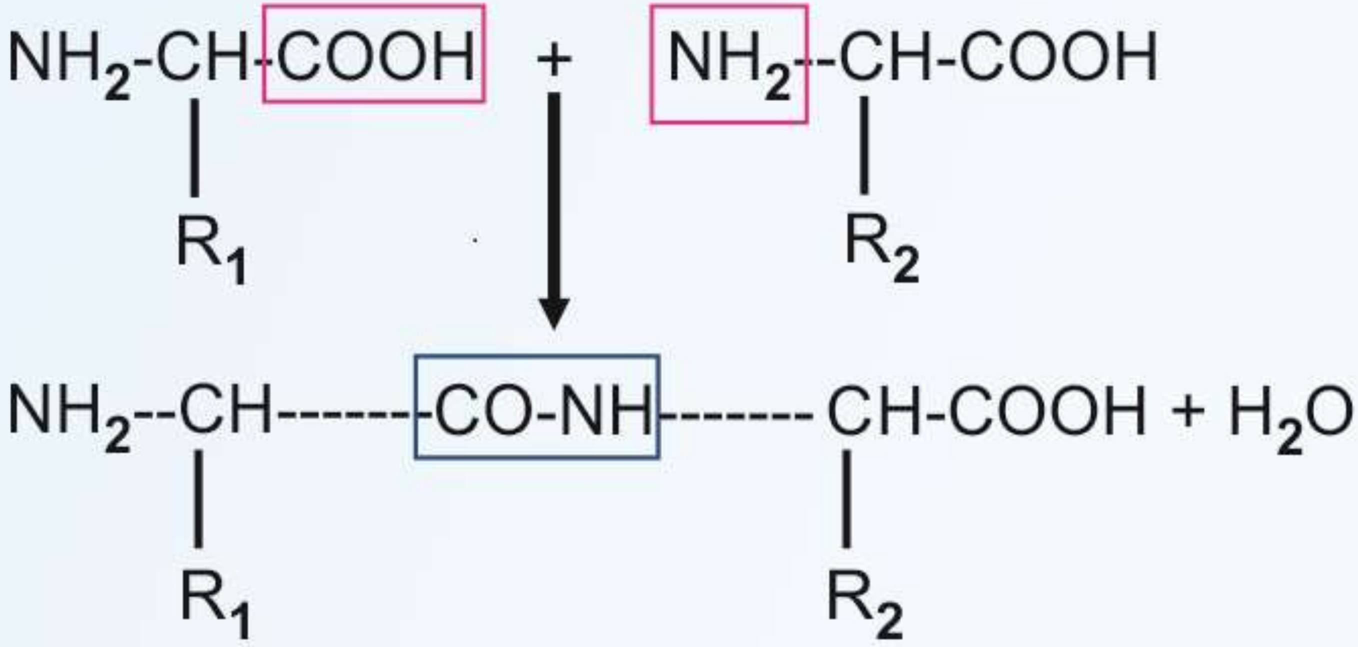
Abnormality in protein structure will lead to molecular diseases with profound alterations in metabolic functions.

Proteins contain Carbon, Hydrogen, Oxygen and Nitrogen as the major components. Sulphur and Phosphorus are minor constituents.

Nitrogen is characteristic of proteins. The nitrogen content of ordinary proteins is 16% by weight.

All proteins are polymers of amino acids.

Peptide bond formation



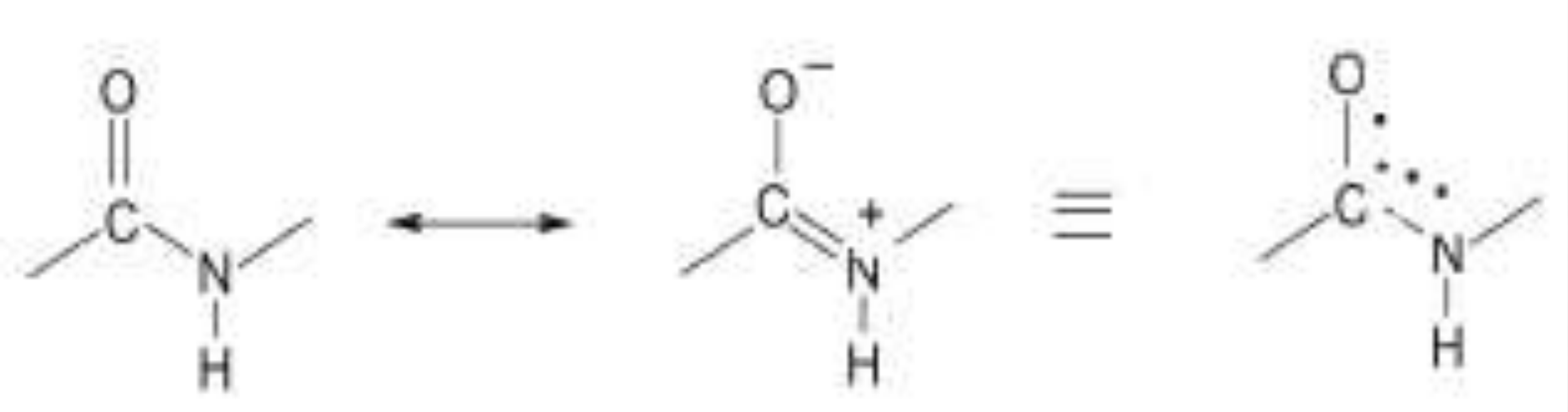
$$\underset{\displaystyle R_1}{NH_2\text{-}CH\text{-}}\boxed{COOH} + \boxed{NH_2}\text{-}\underset{\displaystyle R_2}{CH\text{-}COOH}$$

$$\downarrow$$

$$\underset{\displaystyle R_1}{NH_2\text{--}CH}\text{------}\boxed{CO\text{-}NH}\text{------}\underset{\displaystyle R_2}{CH\text{-}COOH} + H_2O$$

The peptide bond is a partial double bond.

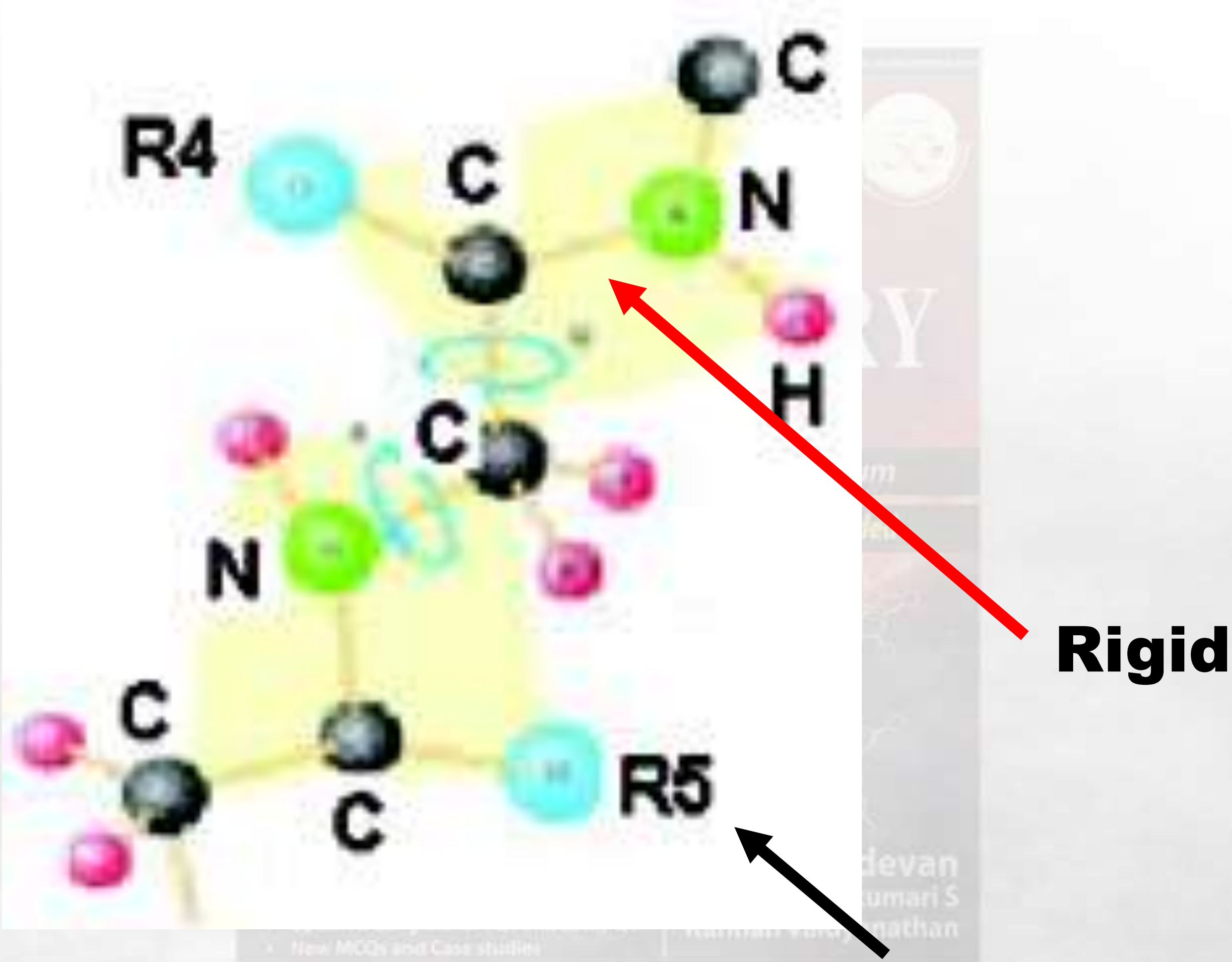
The C–N bond is ‘trans‘ in nature and there is no freedom of rotation



The distance is 1.32 Å which is midway between single bond (1.49 Å) and double bond (1.27Å).

Characteristics of Peptide Bond

- Partial double bond
- C-N bond is “trans” in nature
- No freedom of rotation (due to partial double bond nature)
- Side chains are free to rotate
- The angles of rotation known as **Ramachandran angles, determine the spatial** orientation of the peptide chain



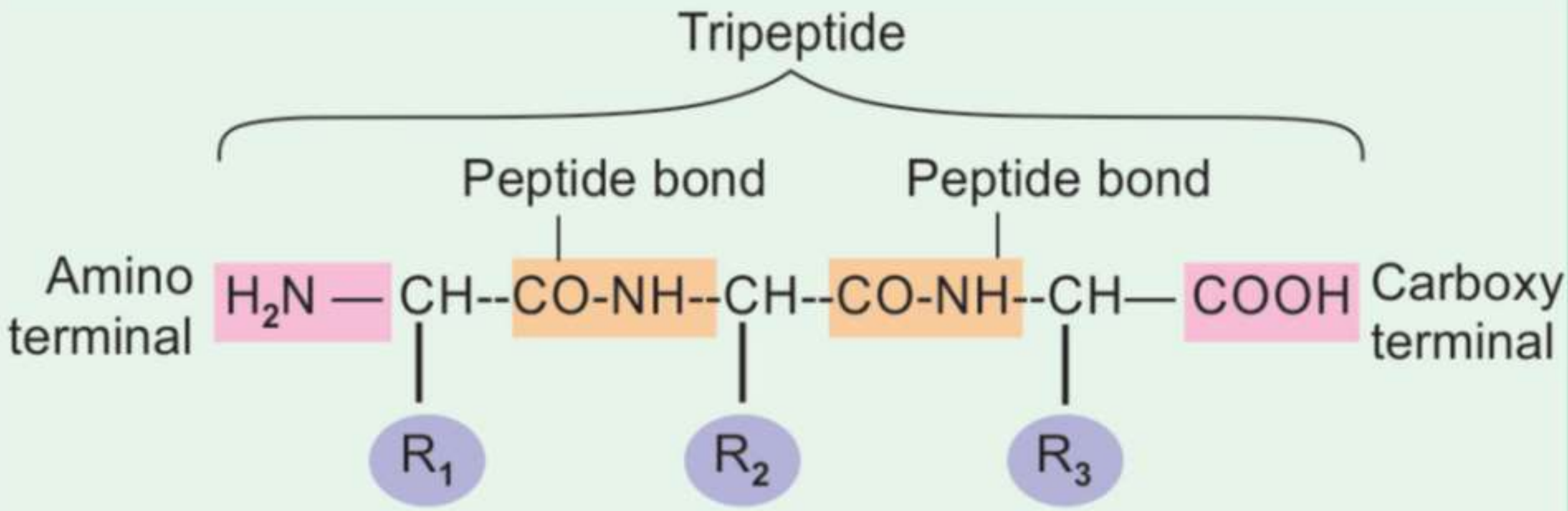
Rigid

The side chains are free to rotate on either side of the peptide bond.

Numbering of Amino Acids in Proteins

- In a polypeptide chain, at one end there will be one free alpha amino group.
- This end is called the **amino terminal (N-terminal) end and the** amino acid contributing the alpha-amino group is named as the **first amino acid.**
- Biosynthesis of the protein starts from the amino terminal end.

- The other end of the polypeptide chain is the **carboxy terminal end (C-terminal), where** there is a free alpha carboxyl group which is contributed by the **last amino acid.**
- All other alpha amino and alpha carboxyl groups are involved in peptide bond formation.



$$H_2N — \underset{\displaystyle R_1}{\underset{|}{CH}}\text{--}CO\text{-}NH\text{--}\underset{\displaystyle R_2}{\underset{|}{CH}}\text{--}CO\text{-}NH\text{--}\underset{\displaystyle R_3}{\underset{|}{CH}} — COOH$$

R_1,R_2 and R_3 are side chains

Dipeptide (2 amino acids)

Tri peptide (3) (ex: Glutathione)

Oligo peptide (5-10)

Poly peptide (10-50)

Protein (>50)

Branched and Circular Proteins

- Generally, the polypeptide chains are linear.
- However, branching points in the chains may be produced by interchain **disulphide bridges.**
- The covalent disulphide bonds between different polypeptide chains in the same protein (interchain) or portions of the same polypeptide chain (intrachain) are also part of the primary structure.

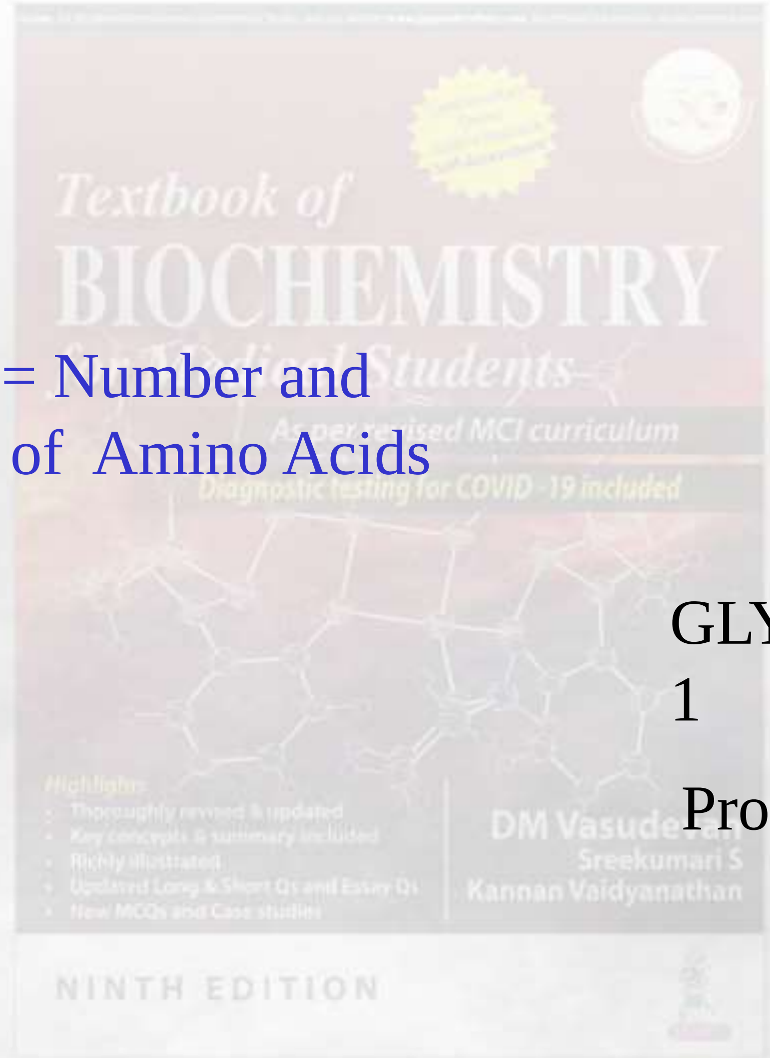
Pseudopeptides

- Rarely, instead of the alpha COOH group the **gamma carboxyl group of glutamic acid may** enter into peptide bond formation, e.g. Glutathione (gamma-glutamyl-cysteinyl-glycine).
- The term **pseudopeptide (or isopeptide) is** used to denote such a peptide bond formed by carboxyl group, other than that present in alpha position.
- Very rarely, protein may be in a circular form, e.g. Gramicidin.

Definitions of Levels of Organization

1. **Primary structure** of protein means the order of amino acids in the polypeptide chain and the location of disulfide bonds, if any.
2. **Secondary structure** is the steric relationship of amino acids, close to each other.
3. **Tertiary structure** denotes the overall arrangement and interrelationship of the various regions, or domains of a single polypeptide chain.
4. **Quaternary structure** results when the proteins consist of two or more polypeptide chains held together by noncovalent forces.

Organisation of Oroteins



Primary
Secondary
Tertiary
Quaternary

Primary Structure = Number and Unique Sequence of Amino Acids

Protein A	Protein B
GLY--ALA--VAL	GLY--VAL--ALA
1 2 3	1 2 3

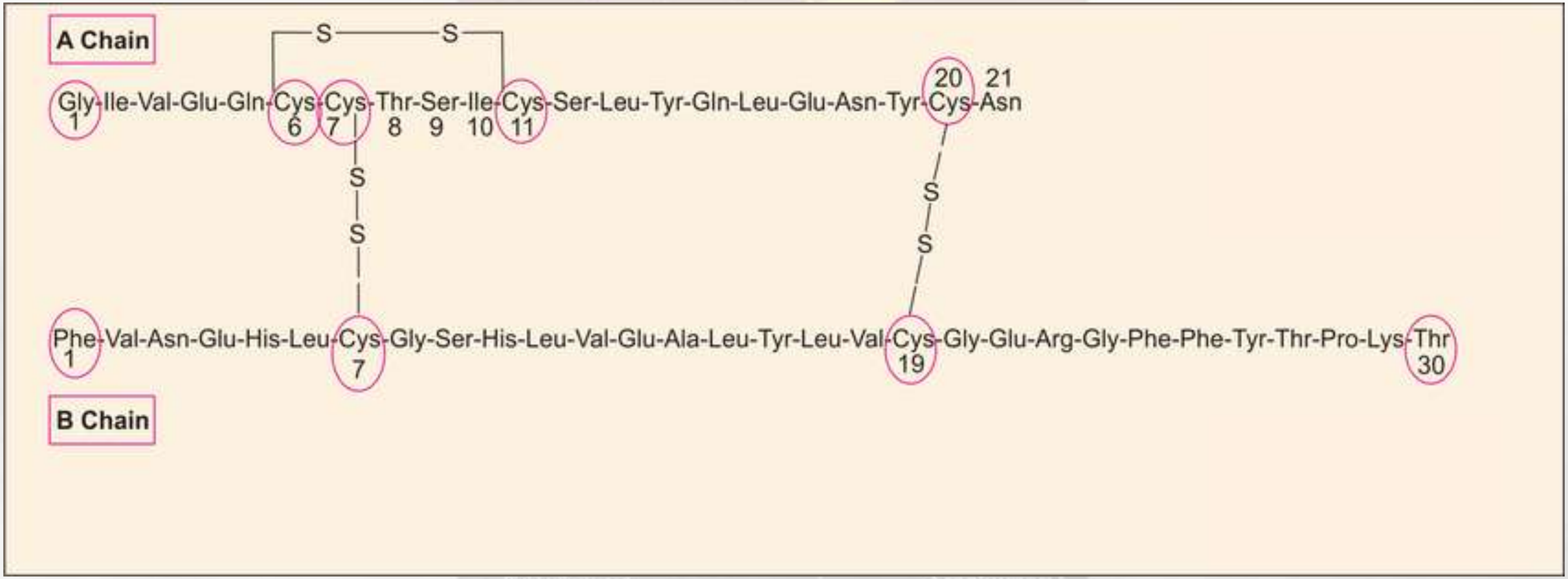
In a tripeptide, there are 3 amino acids, but these 3 can be any of the total 20 amino acids.

Thus $20^3 = 8000$ different permutations and combinations are possible in a tripeptide.

An ordinary protein having about 100 amino acids, will have 20^{100} different possibilities.

By changing the sequence of combination of 20 amino acids, nature produces enormous number of markedly different proteins.

INSULIN Primary Structure



A Chain

S—S

Gly-Ile-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn

1 6 7 8 9 10 11 20 21

S—S

S—S

Phe-Val-Asn-Glu-His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr

1 7 19 30

B Chain

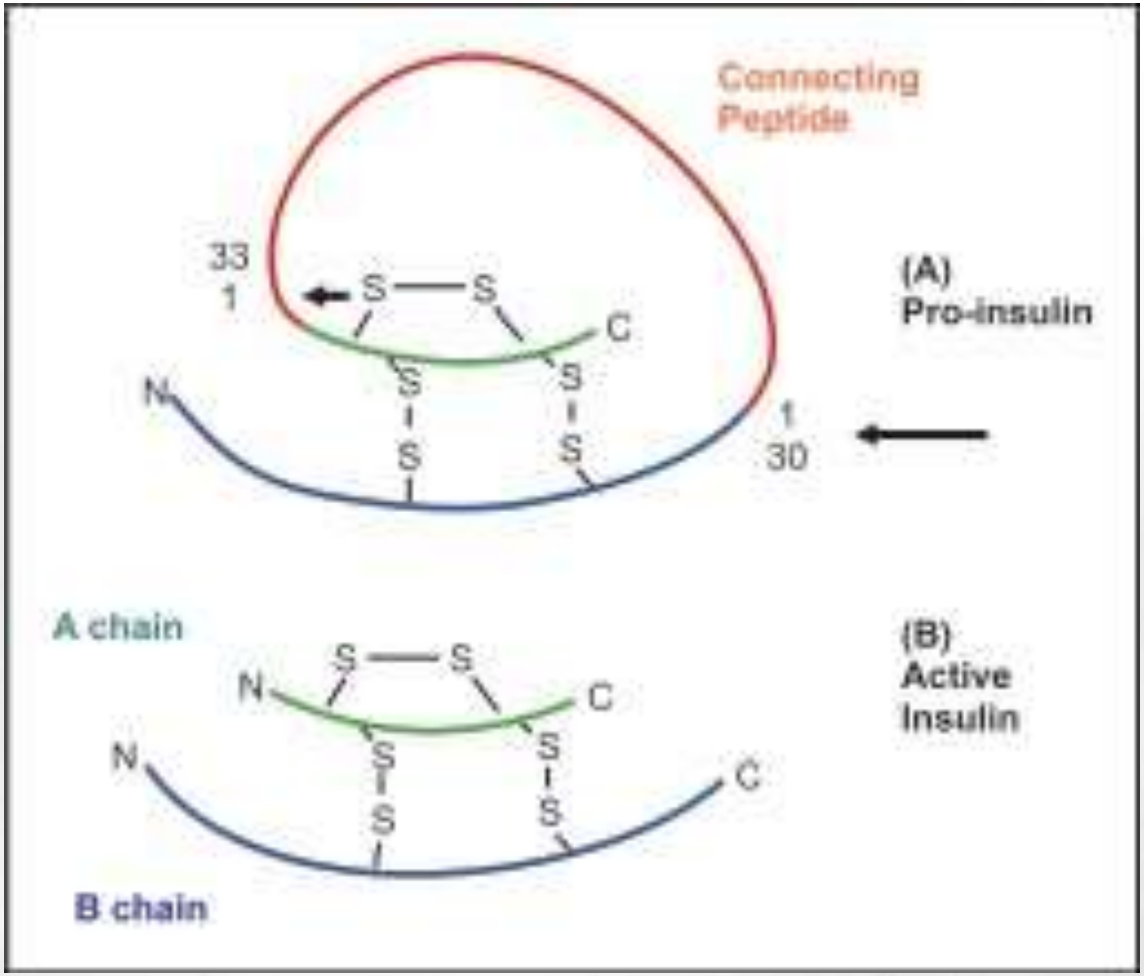
Primary Structure of Insulin

- Insulin has **two polypeptide chains.**
- **A** chain (**Glycine chain) has 21 amino acids**
- B (**Phenyl alanine) chain has 30 amino acids.**
- They are held together by **two interchain disulphide bonds.**
- A chain 7th cysteine and B chain 7th cysteine are connected.

Primary Structure of Insulin

- Similarly, A chain 20th cysteine and B chain 19th cysteine are connected.
- There is another **intrachain disulphide bond between 6th and** 11th cysteine residues of A chain.
- Species variation is restricted to amino acids in position 8, 9 and 10 in A chain and in C-terminal of B chain.
- Amino acid sequence has been conserved to a great extent during evolution.

Conversion of Pro-insulin to active Insulin



Arrows show the site of action of proteolytic enzymes.

A protein with a specific primary structure, when put in solution, will automatically form its natural three dimensional shape.

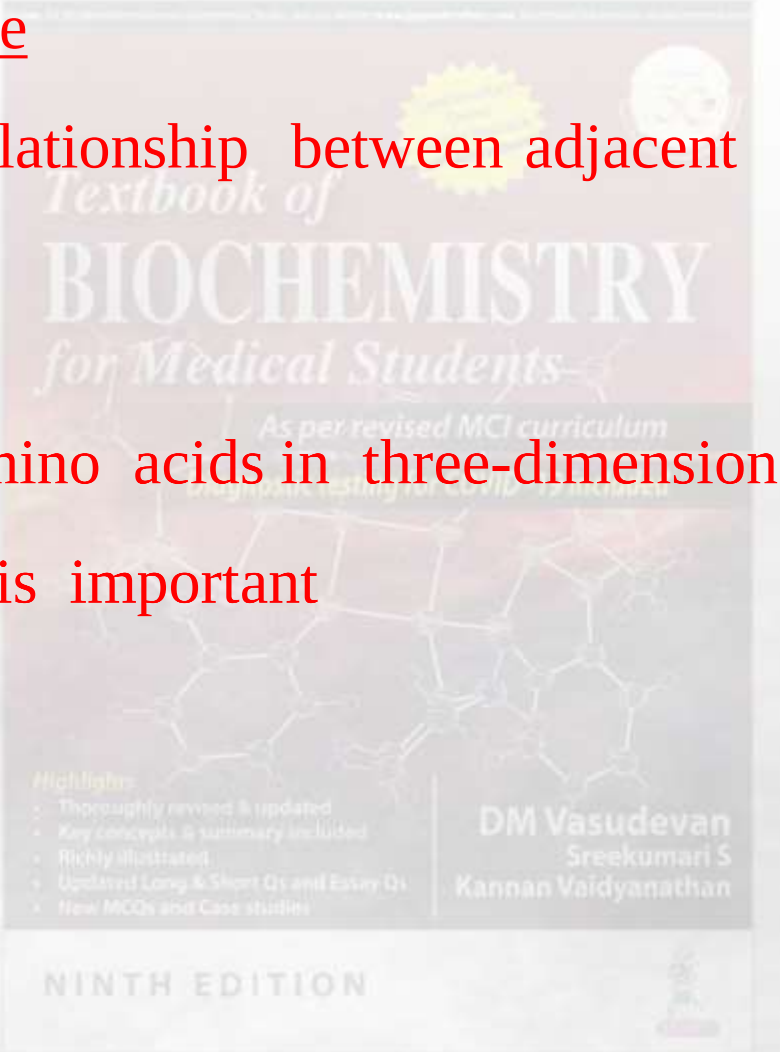
Primary structure determines other levels of organisation

Mutation

B chain 6th Glu --> Val

HbA --> HbS

Structure - function relationship



Secondary structure

Configurational relationship between adjacent 3 - 4 amino acid residues

Tertiary structure

Relationship of amino acids in three-dimensional rganisation

Primary structure is important

Forces Keeping the Structure

Primary -- Peptide linkage
Disulphide linkage

Secondary and Tertiary
Hydrogen bonds
Electrostatic (ionic)
Hydrophobic bonds
van der Waals force

Hydrogen Bond

Hydrogen releasing

- -NH (imidazole, indole, peptide)
- -OH (Serine, Threonine)
- -NH2 (Arginine, Lysine)

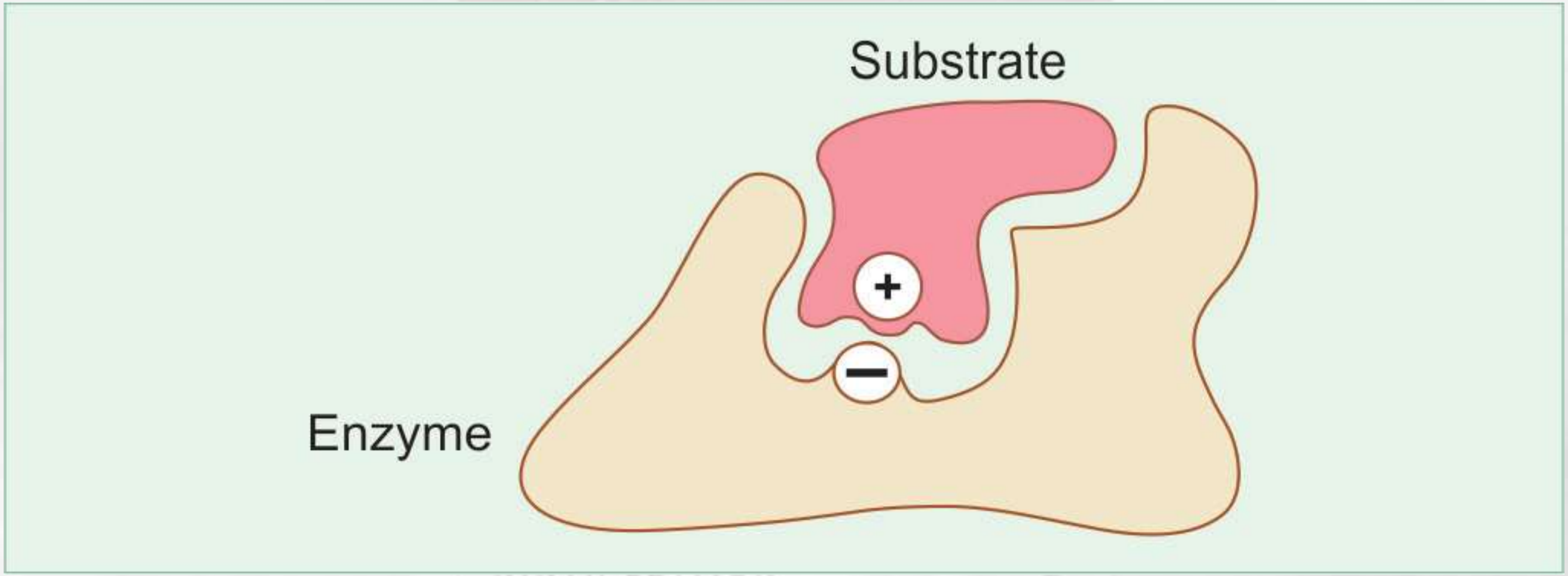
Hydrogen accepting

- COO- (Aspartic, Glutamic)
- C=O (peptide)

Electrostatic or ionic bonds

+ ve : Lysine, Arginine, Histidine

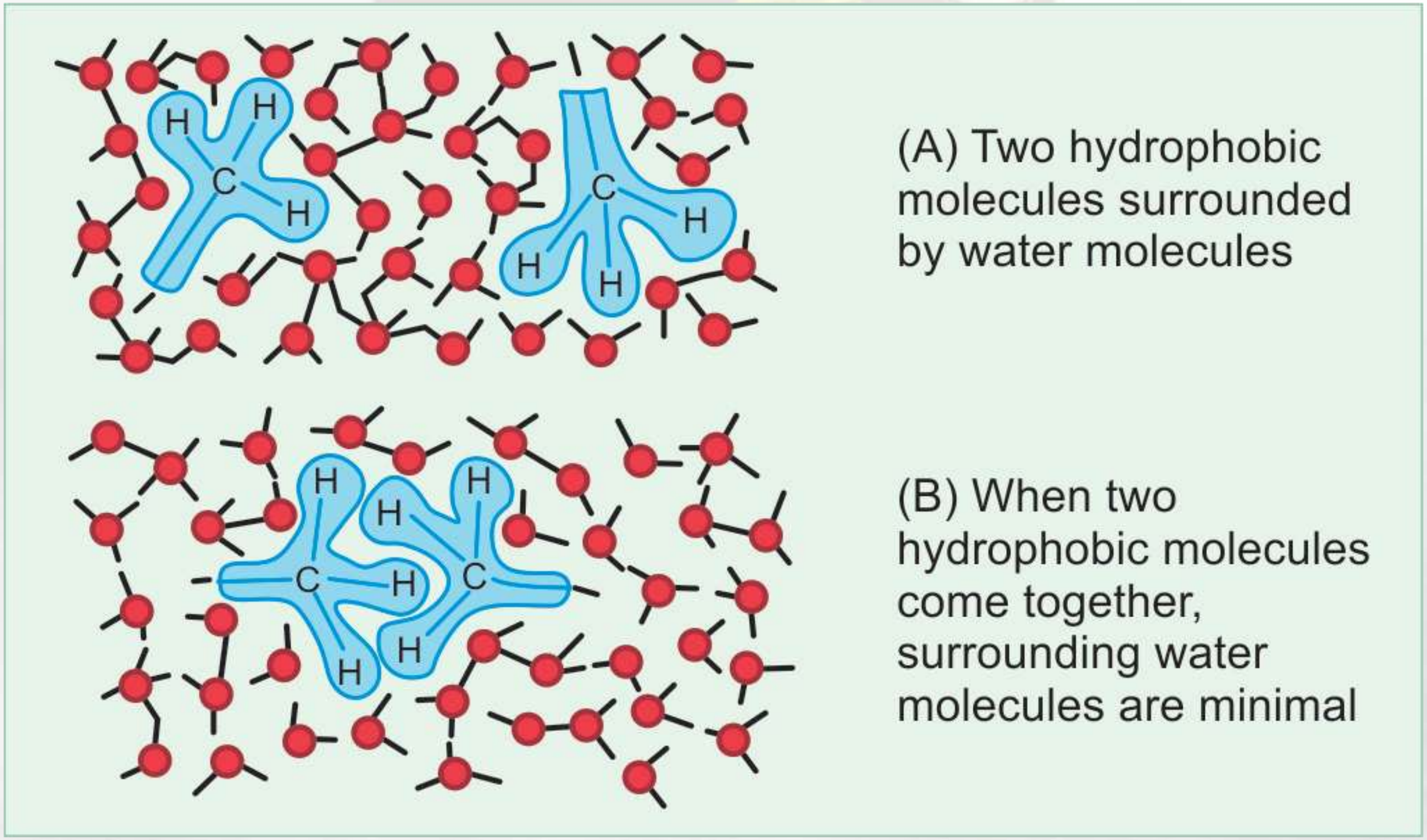
\- ve : Aspartic, Glutamic



Hydrophobic Bonds

Non-polar hydrobic side chains

V L I M P W



(A) Two hydrophobic molecules surrounded by water molecules

(B) When two hydrophobic molecules come together, surrounding water molecules are minimal

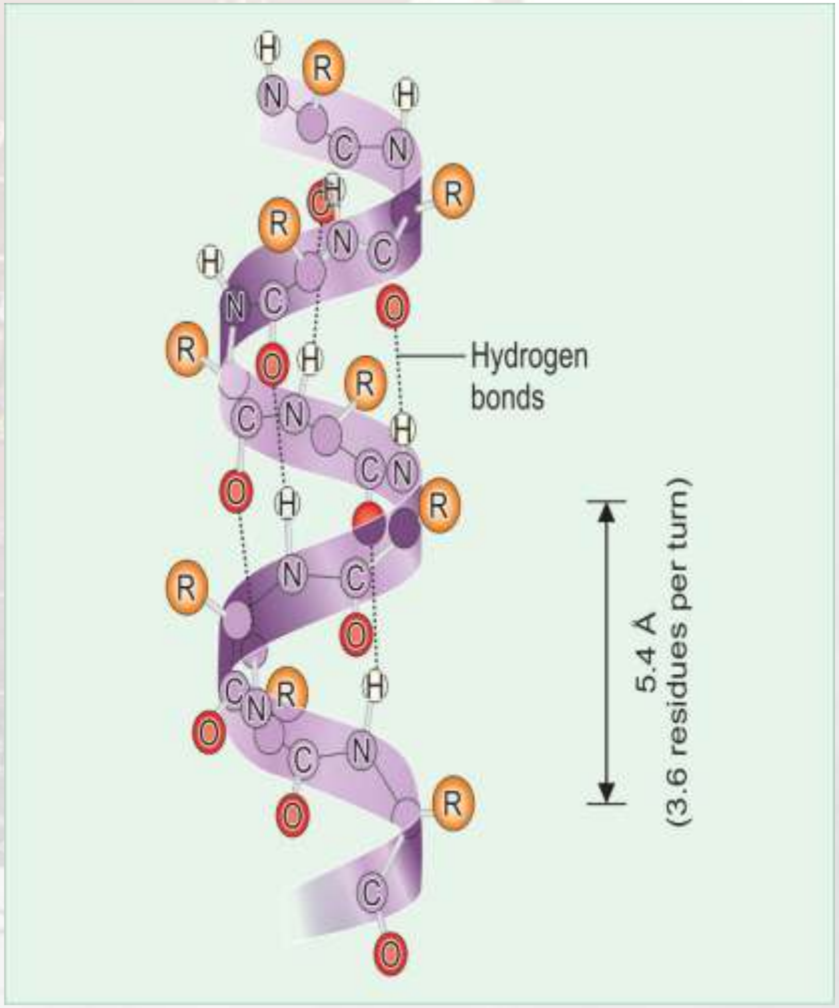
Van der Waals Forces

- Attractive forces operating between all atoms due to oscillating dipoles
- Dependent on distance between atoms
- Weak forces
- Contributes maximum towards stability of protein

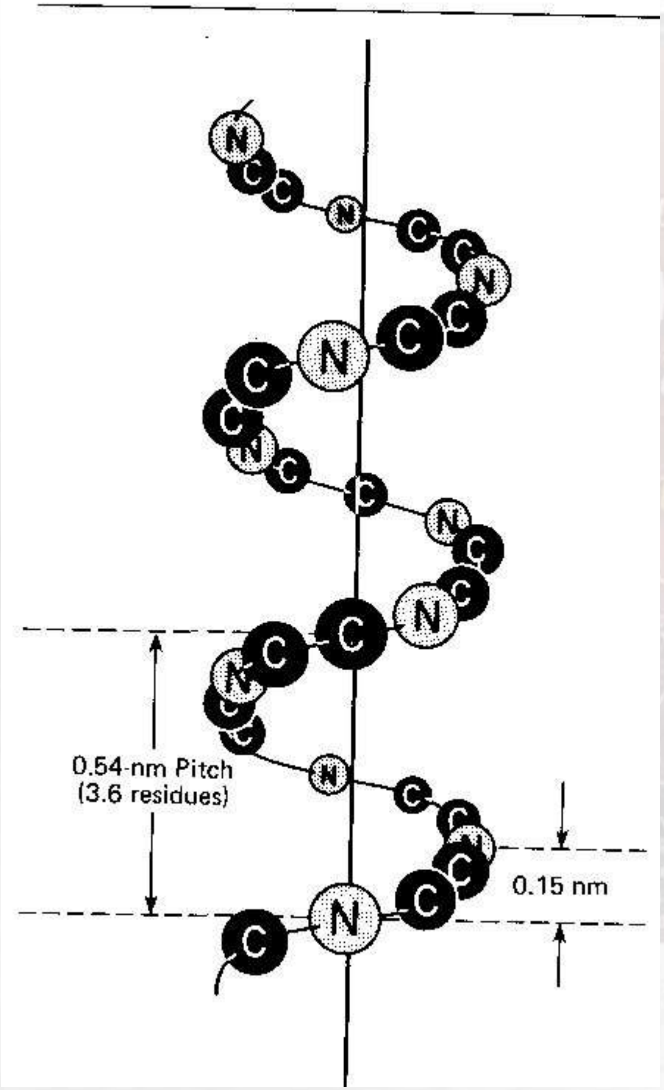
Alpha Helix

- **Most common and stable conformation; e.g., Hemoglobin**

1. Right handed; Spiral structure.
2. Polypeptide bonds backbone



Alpha Helix



3. **Structure is stabilized by hydrogen bonds between NH and C=O groups.**

4. Each turn by 3.6 residues;
5. Distance between each residue is 0.15 nm

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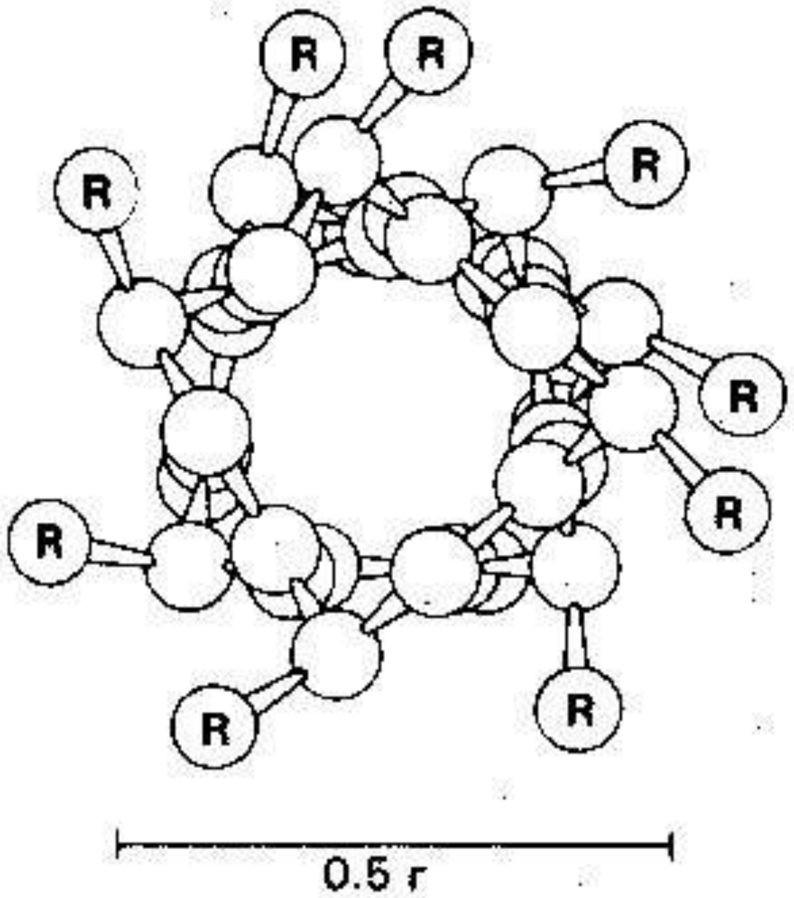
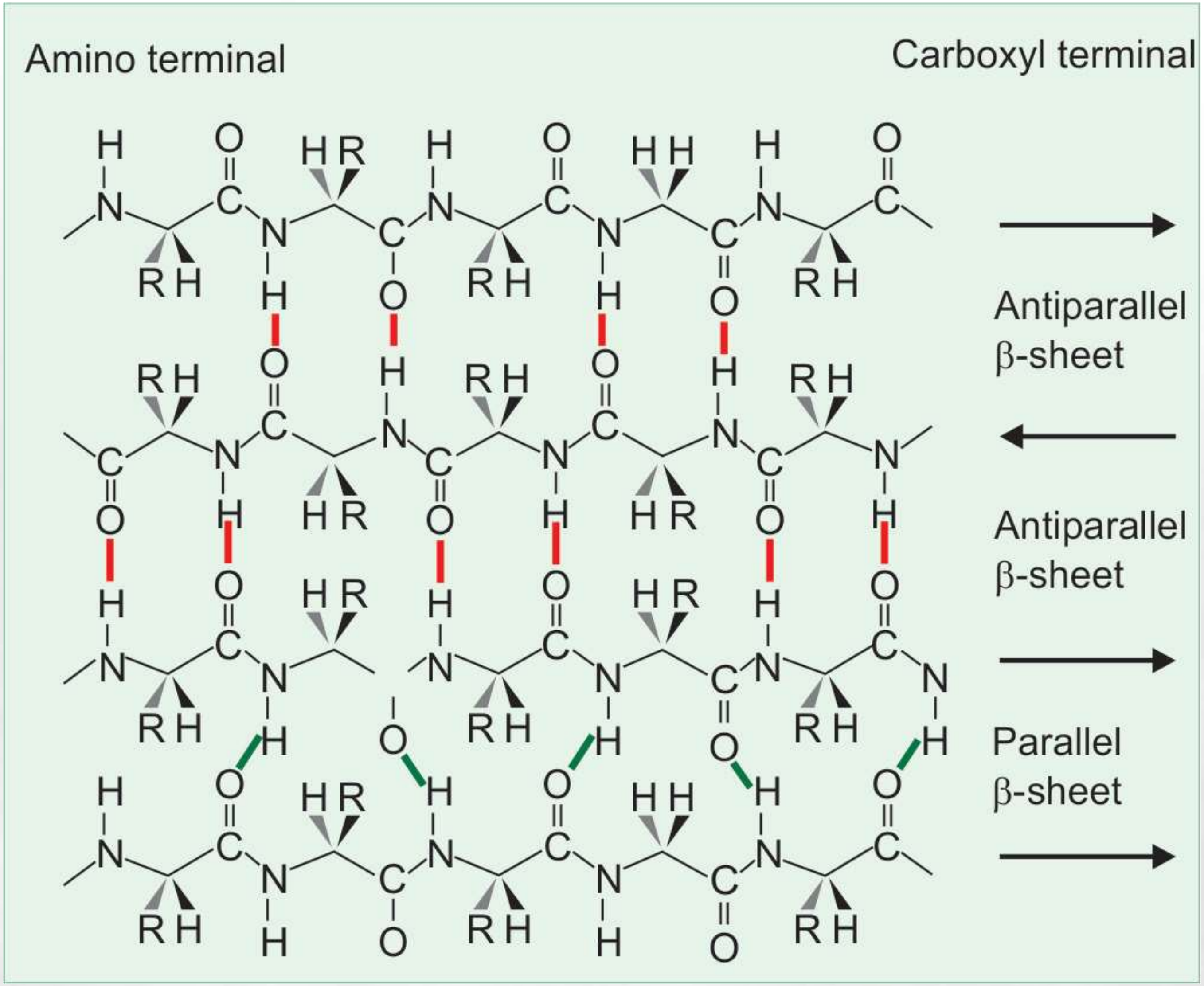


6. Side chains of amino acids extend outward.

Beta-pleated Sheet

- Polypeptide chains fully extended.
- Stabilised by hydrogen bonds between NH and C=O groups of neighbouring polypeptide segments.
- Intrachain disulfide bridges stabilise these bends.
- Adjacent strands in a sheet can run in the same direction (parallel) or in opposite direction (anti parallel sheet)
- Silk Fibroin (anti parallel)
- Flavodoxin (parallel)
- Carbonic anhydrase (both)

Structure of Beta-pleated Sheet



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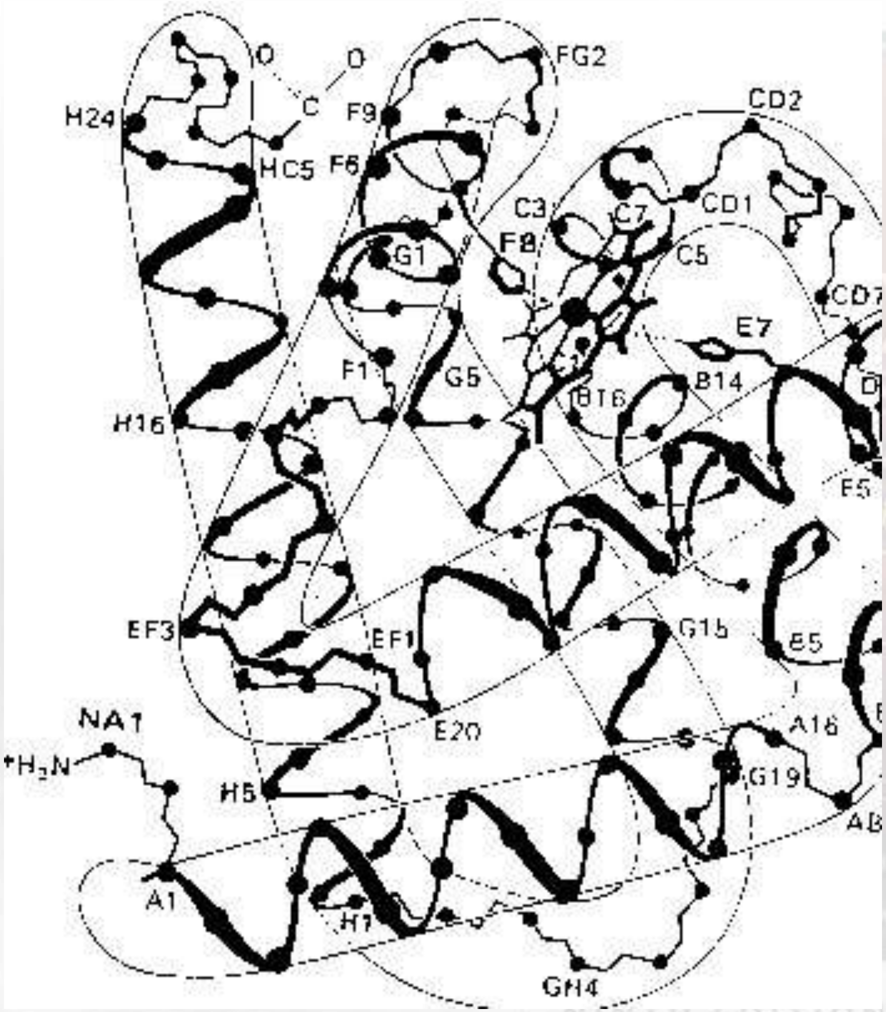
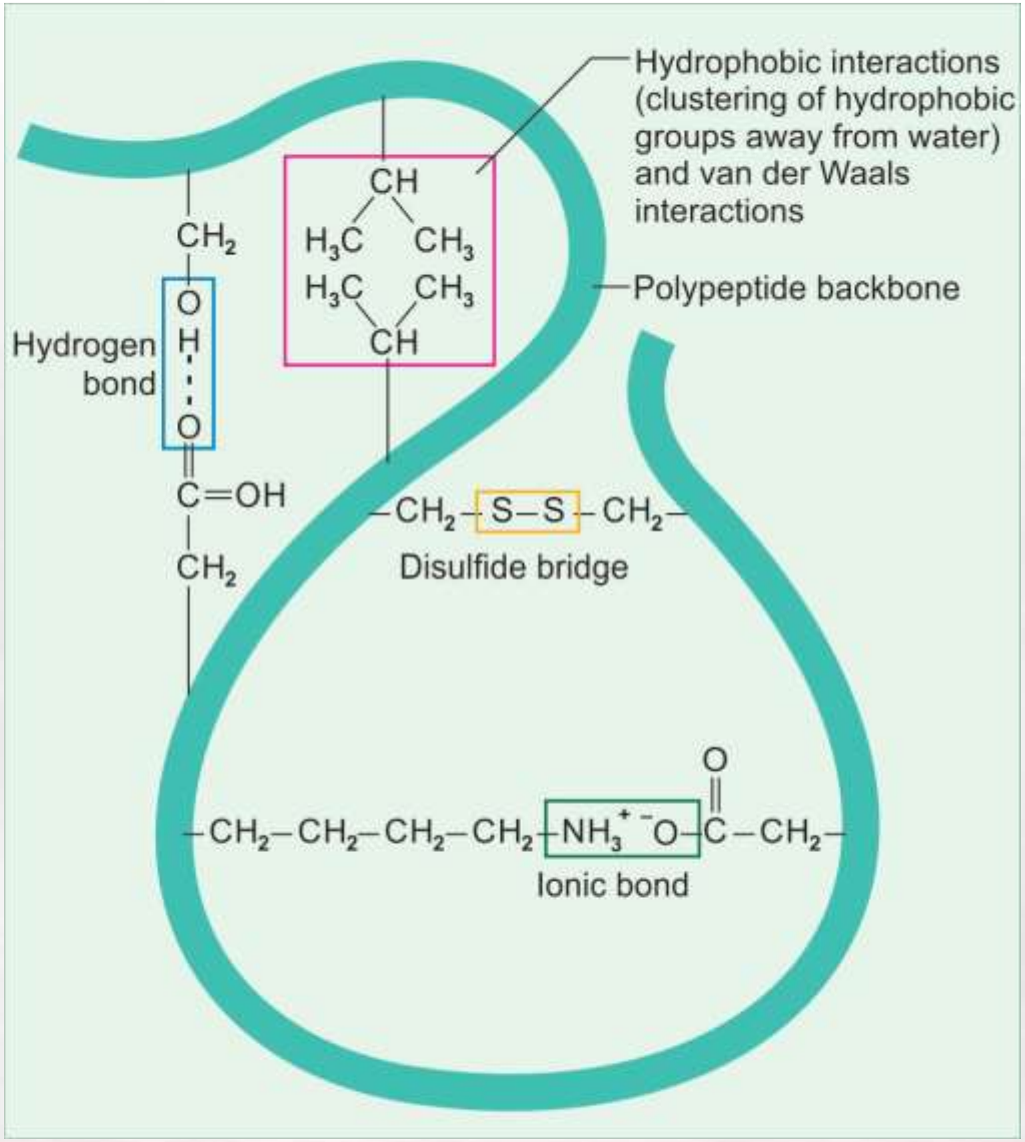


Three dimensional structure of myoglobin

The tertiary structure denotes three dimensional structure of the whole protein

The tertiary structure is maintained by hydrophobic bonds, electrostatic bonds and van der Waals forces. The tertiary structure acquired by native protein is always thermodynamically most stable.

Forces Keeping the Tertiary Structure



Domain

- **Domain is the term used to denote a compact** globular functional unit of a protein.
- A domain is a relatively independent region of the protein, and may represent a functional unit.
- The domains are usually connected with relatively flexible areas of protein.
- Phenyl alanine hydroxylase enzyme contains 3 domains, one regulatory, one catalytic and one protein-protein interaction domains.

Motif

In a protein, a structural motif is a superstructure. Different structural motifs are:

a) **Beta hairpin**: This is very common. Two antiparallel beta-strands are connected by a tight turn of a few amino acids between them.

b) **Helix-loop-helix**: This consists of alpha-helices bound by a looping stretch of amino acids. This motif is seen in transcription factors.

c) **Zinc finger**: Two beta-strands with an alpha-helix end folded over to bind a zinc ion. This is important in DNA binding proteins.

d) **Helix-turn-helix**: Two alpha-helices joined by a short strand of amino acids and found in many proteins that regulate gene expression.

Specific Motifs in proteins

Protein	Structural motif present
Myoglobin	Alpha-helix and beta-pleated sheet
Collagen	Triple helix
Keratin	Coiled coil
Elastin	No specific motif
Superoxide dismutase	Antiparallel, beta-pleated sheet

Quaternary Structure

Polypeptides aggregate to form one functional protein.

Loss of function when the subunits are dissociated.

The forces that keep the quaternary structure are hydrogen bonds, electrostatic bonds, hydrophobic bonds and van der Waals forces.

Quaternary Structure

Subunit or monomer

Combination: Dimer (2) tetramer (4)

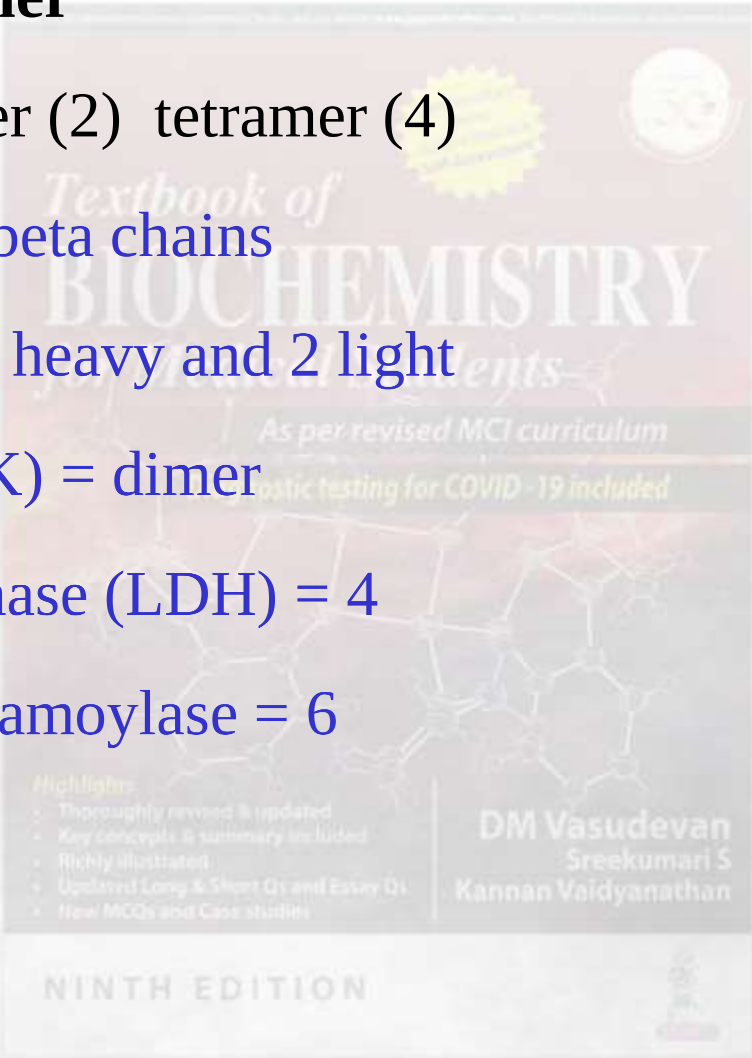
Hb: 2 alpha and 2 beta chains

Immunoglobulin: 2 heavy and 2 light

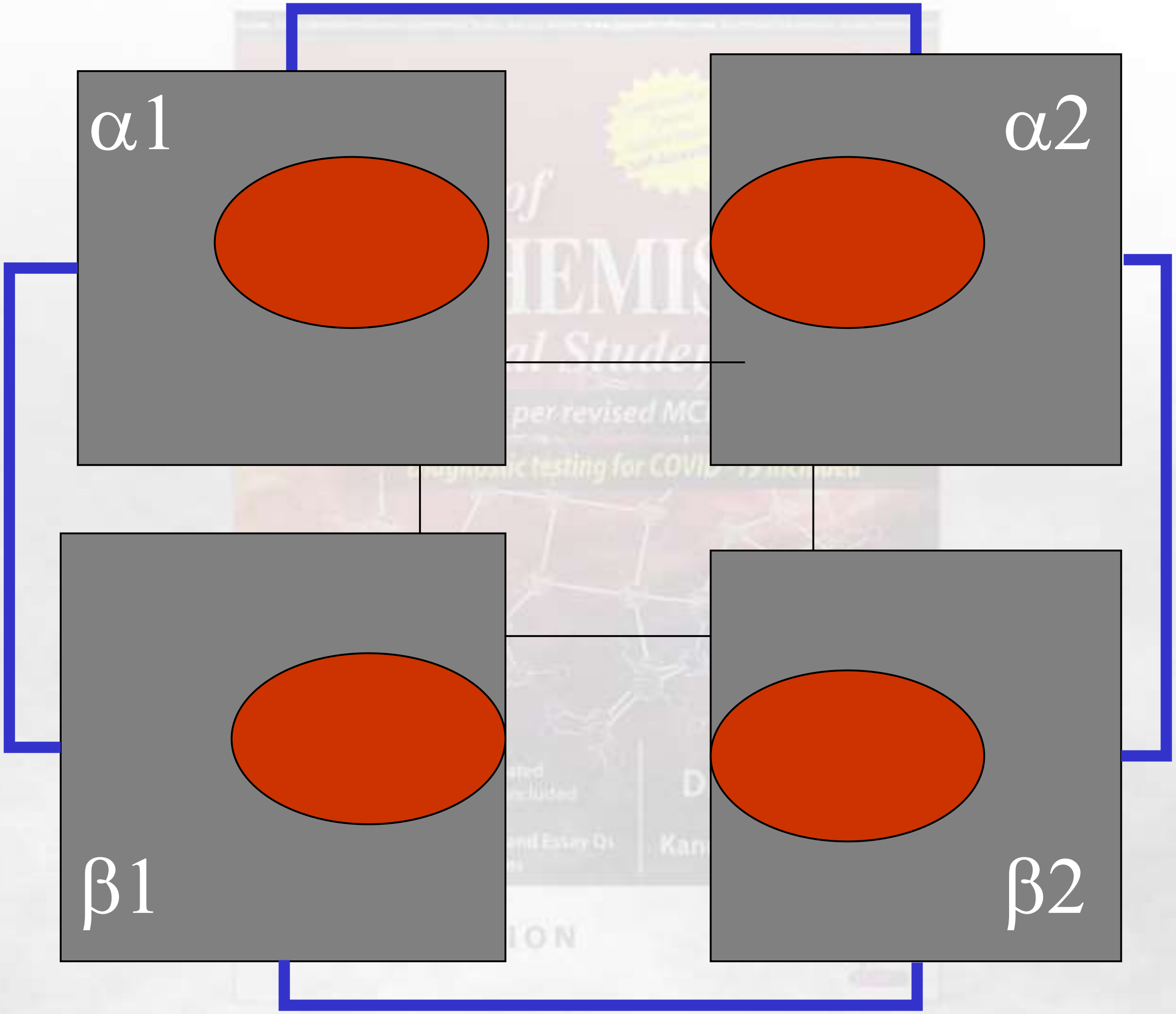
Creatine kinase (CK) = dimer

Lactate dehydrogenase (LDH) = 4

Aspartate transcarbamoylase = 6



Hemoglobin



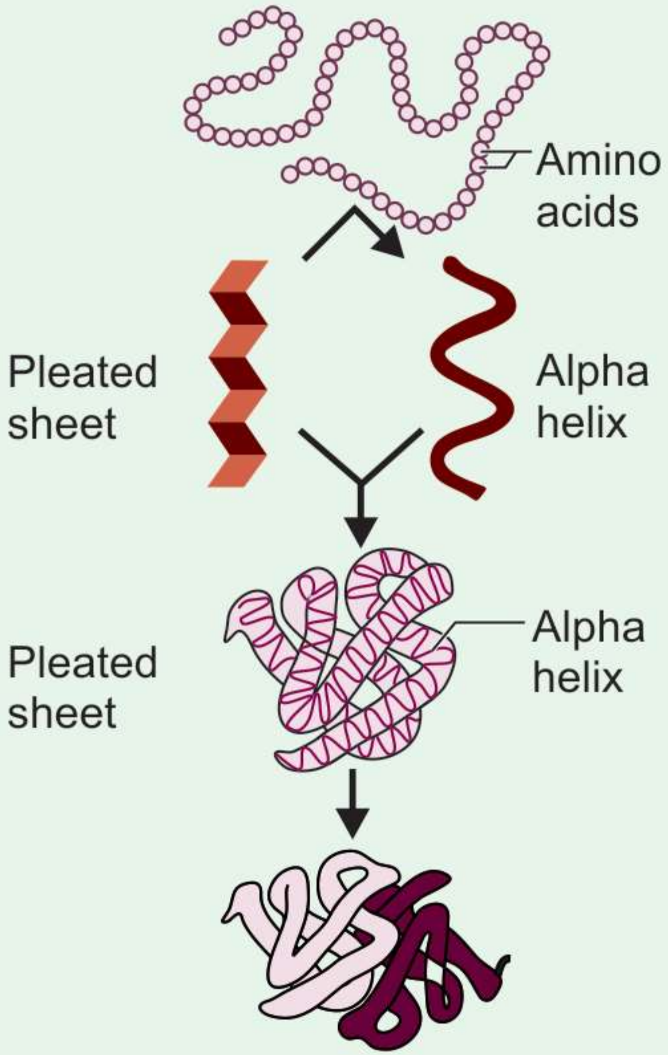
Configuration and Conformation

Configuration of a protein denotes the spatial relationship between particular atoms, e.g. L and D amino acids.

Conformation means the spatial relationship of every atom in a molecule, e.g. rotation of a portion of the molecule.

Levels of Organisation

1. **Primary structure** of protein means the order of amino acids in the polypeptide chain and the location of disulfide bonds, if any.
2. **Secondary structure** is the steric relationship of amino acids, close to each other.
3. **Tertiary structure** denotes the overall arrangement and inter-relationship of the various regions, or domains of a single polypeptide chain.
4. **Quaternary structure** results when the proteins consist of two or more polypeptide chains are held together by non-covalent forces.



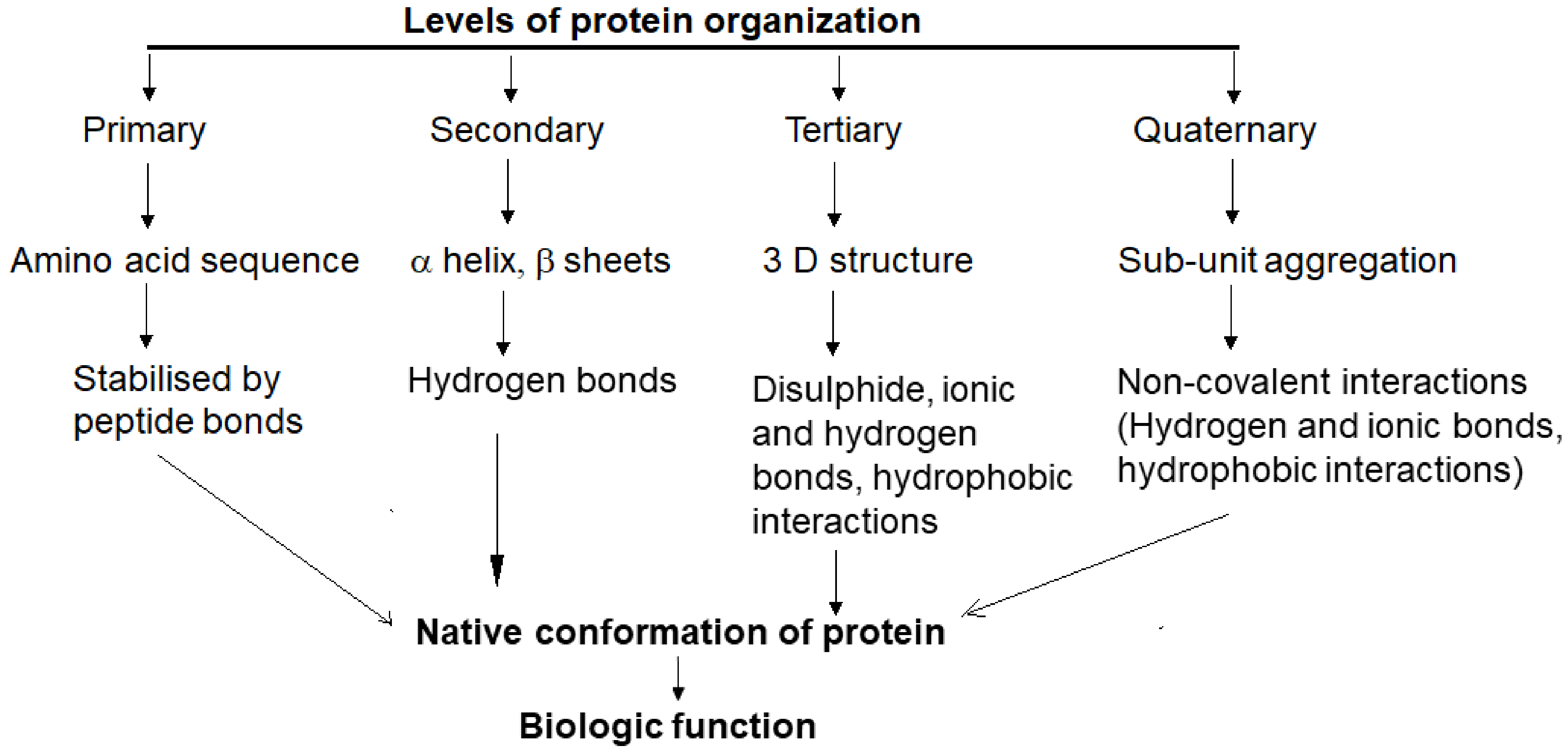
Primary structure
is determined by the sequence of amino acids

Secondary structure
occurs when the amino acids are linked by hydrogen bonds

Tertiary structure
is formed when alpha helices and beta sheets are held together by weak interactions

Quaternary structure
consists of more than one polypeptide chain

Levels of protein organization



Primary → Amino acid sequence → Stabilised by peptide bonds

Secondary → α helix, β sheets → Hydrogen bonds

Tertiary → 3 D structure → Disulphide, ionic and hydrogen bonds, hydrophobic interactions

Quaternary → Sub-unit aggregation → Non-covalent interactions (Hydrogen and ionic bonds, hydrophobic interactions)

Native conformation of protein

Biologic function

Structure-Function Relationship

- The three dimensional structural conformation provides and maintains the functional characteristics.
- The three dimensional structure, in turn, is dependent on the primary structure.
- Any difference in the primary structure may produce a protein which cannot serve its function.

Examples

1. **Enzymes** - The first step in enzymatic catalysis is the binding of the enzyme to the substrate. This, in turn, depends on the structural conformation of the active site of the enzyme, which is precisely oriented for substrate binding.

2. **Hemoglobin** - Binding of oxygen to one heme facilitates oxygen binding by other subunits. Even a single amino acid substitution alters the structure and thereby the function.

3. **Collagen** - Collagen forms a superhelical cable where the 3 polypeptide chains are wound around itself.

- In collagen, every 3rd residue is a glycine.
- The only amino acid that can fit into the triple stranded helix is glycine.
- Triple helix of collagen is stabilized by the steric repulsion of the rings of hydroxyproline and also by the hydrogen bonds between them.

Proteins

Study of Protein Structure

Sequence analysis

Complete Hydrolysis

6 N HCl; 110 C; 18-36 hr ; anaerobic conditions

No. of polypeptide chains
Dansyl Chloride
N-terminal amino acid

Partial Hydrolysis

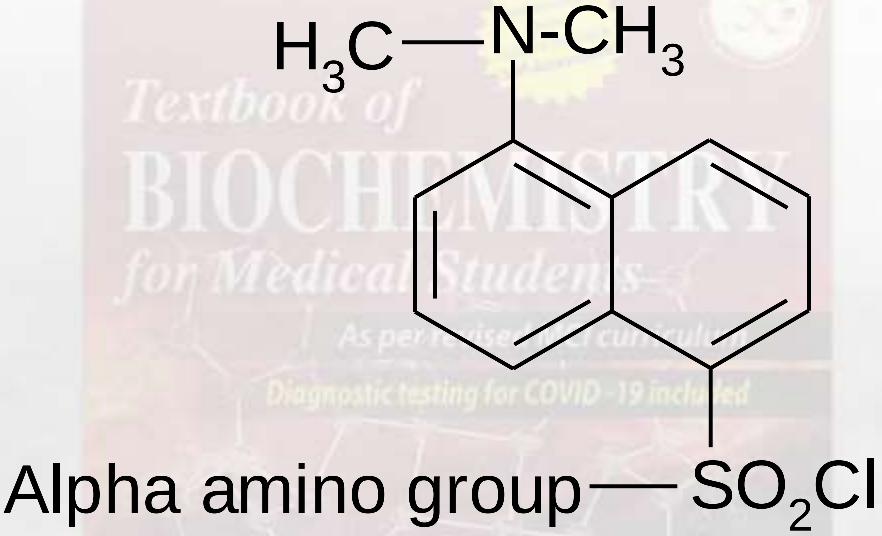
Trypsin

Carboxyl group of Arg / Lys

G--A--K-||-M--V--R-||-G--V—

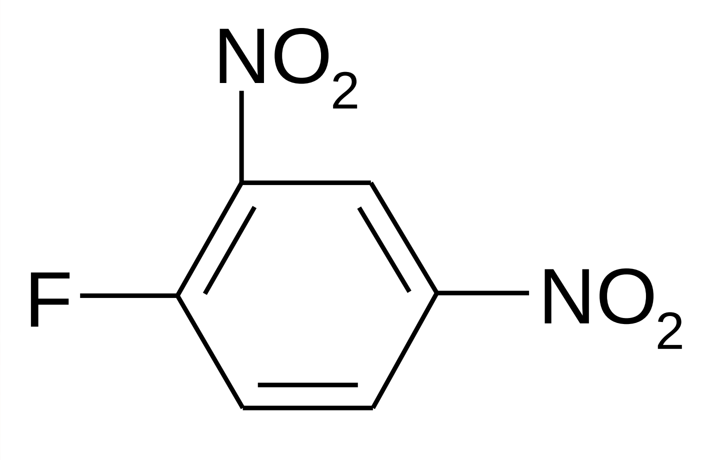
Chymotrypsin

End Group Analysis Dansyl Chloride



N-terminal amino acid Dansyl Derivative

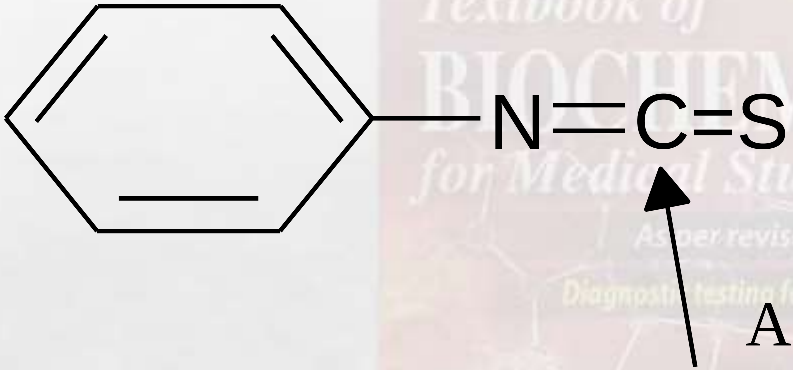
End Group Analysis Sanger’s Reagent



Fluoro dinitro benzene (FDNB) Dinitro phenyl derivative

Edman’s Degradation

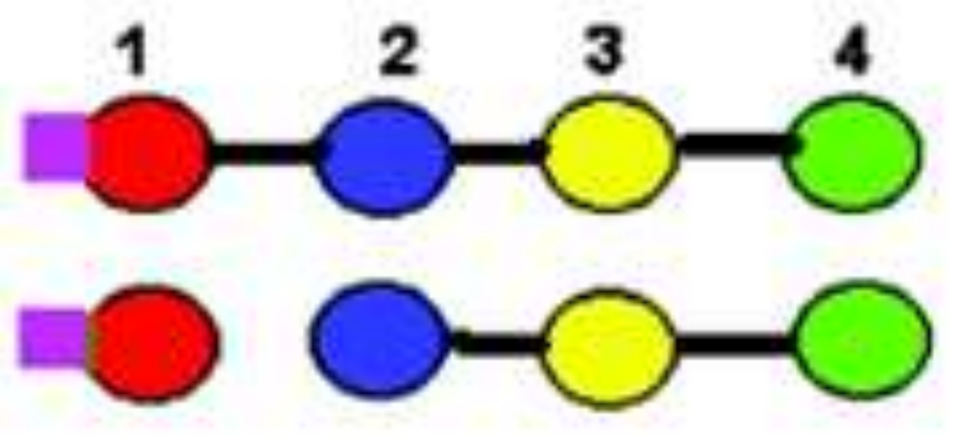
Phenyl iso thio cyanate



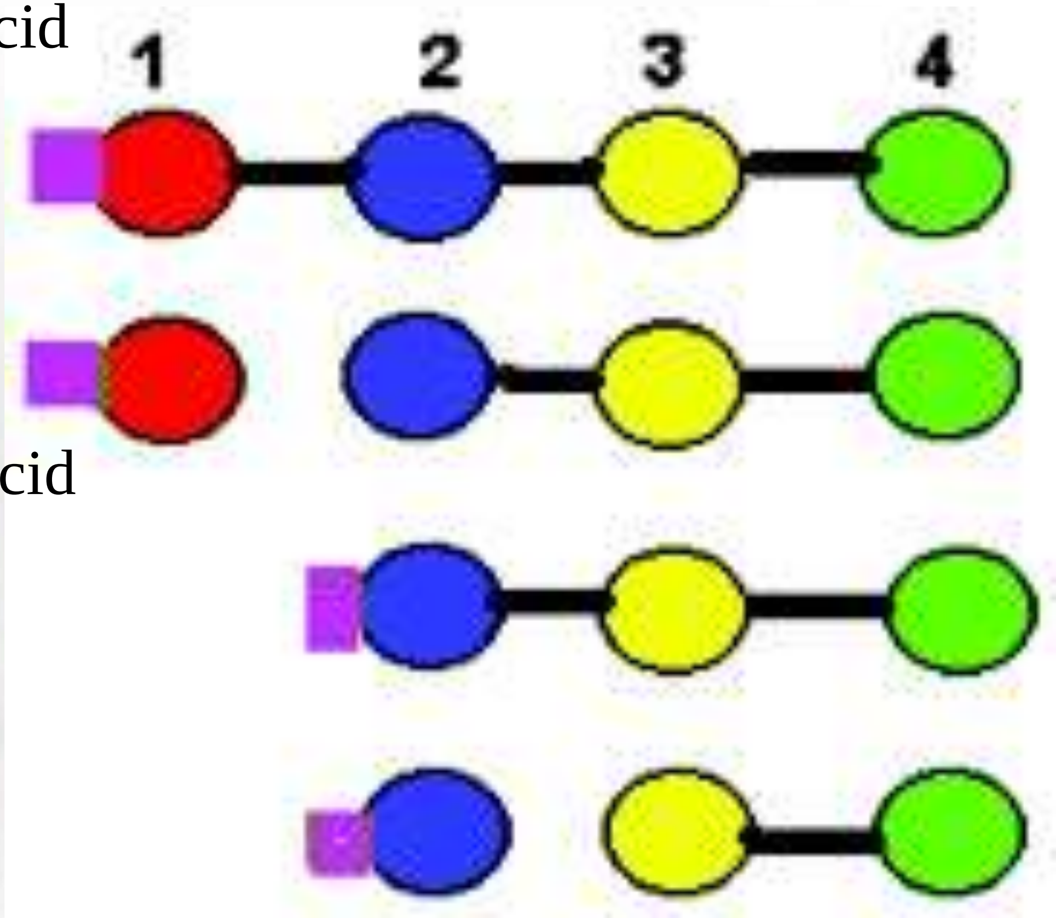
Amino group of First a.a.

Label first amino acid

Release



Steps in Edman's degradation process.



Label first amino acid

Release

Label next amino acid

Release

Steps in Edman's degradation process.

Carboxy Peptidase

Sequential release of amino acids from C-terminus

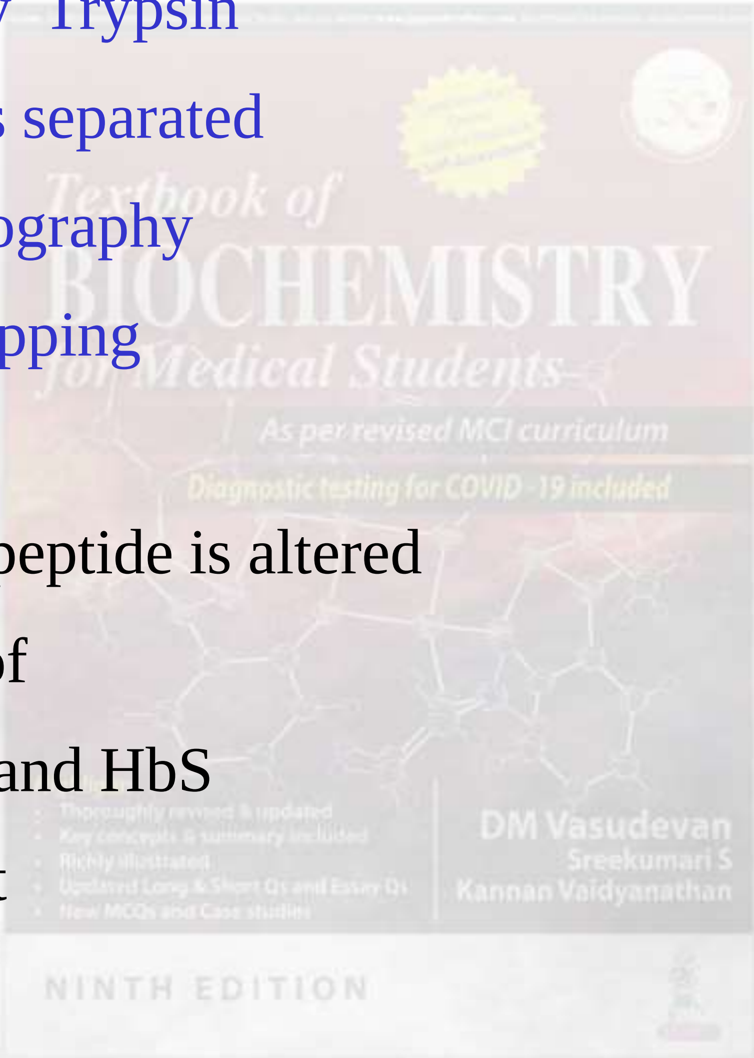
1-----2------3-----4----5

1-----2------3-----4 5

1-----2------3 4 5

1-----2 3 4 5

Ingram’s Technique Protein Finger Printing



Protein digested by Trypsin

Mixture of peptides separated

by chromatography

Peptide mapping

Mutation

position of peptide is altered

Peptide mappings of

Hb in HbA and HbS

are different

NORMAL

ABNORMAL