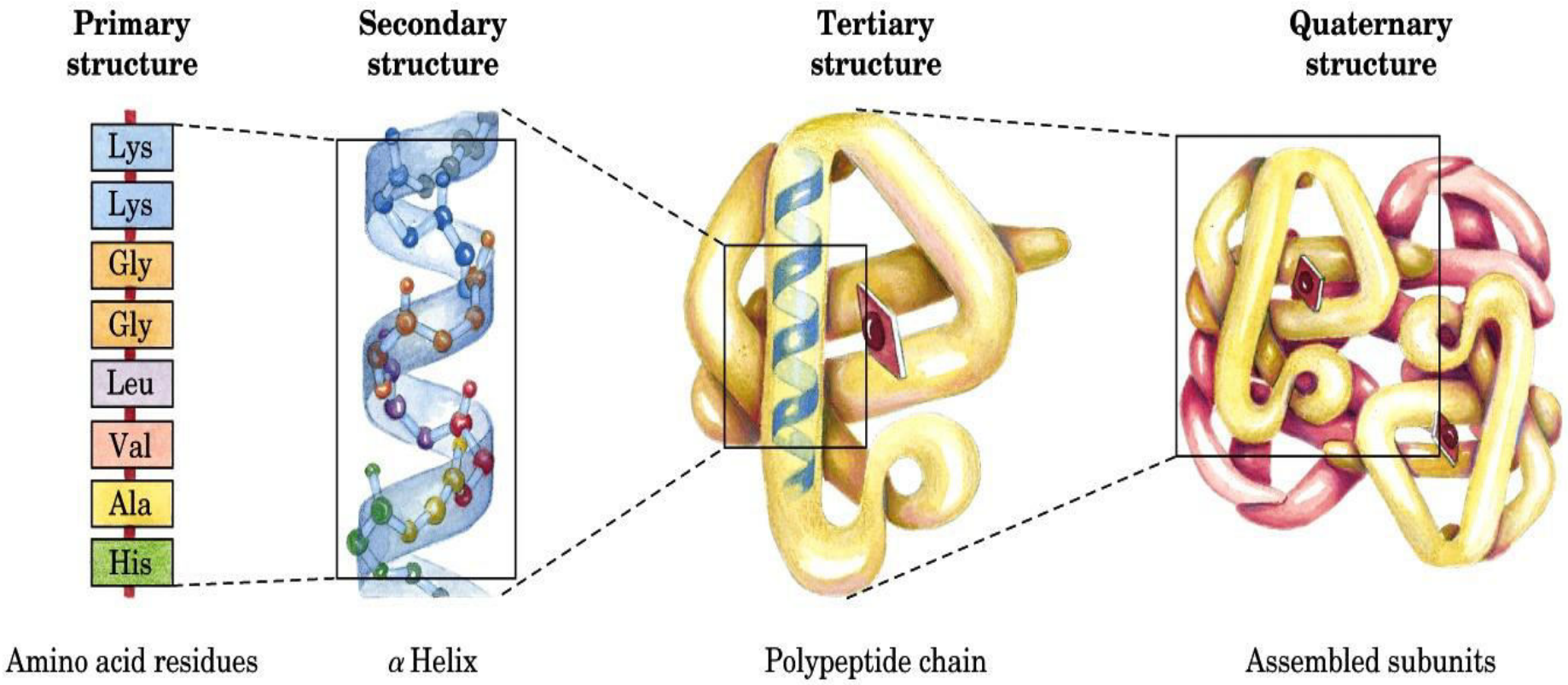
Levels of Protein Structure



Primary structure

- Means order of amino acid residues present in protein and location of disulphide bonds if any

- I f primary structure is known we can know the following things:

1. Total number of amino acid residues
2. N and C terminal amino acids
3. Order or sequence of amino acids
4. Types or composition of amino acids

- The bonds responsible for primary structure are covalent and permanent bonds (peptide bonds and disulphide bonds)

Protein

- In 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.

- N terminal is usually written on the left hand side when the sequence of the protein is denoted

- The other end is the carboxy terminal end which is contributed by last amino acid

- Amino acid residues in polypeptide are named by changing the suffix-ine to –yl e.g Glycine to Glycyl

- NH_2-Gly-Ala-Val-COOH is named as Glycyl-analyl-valine

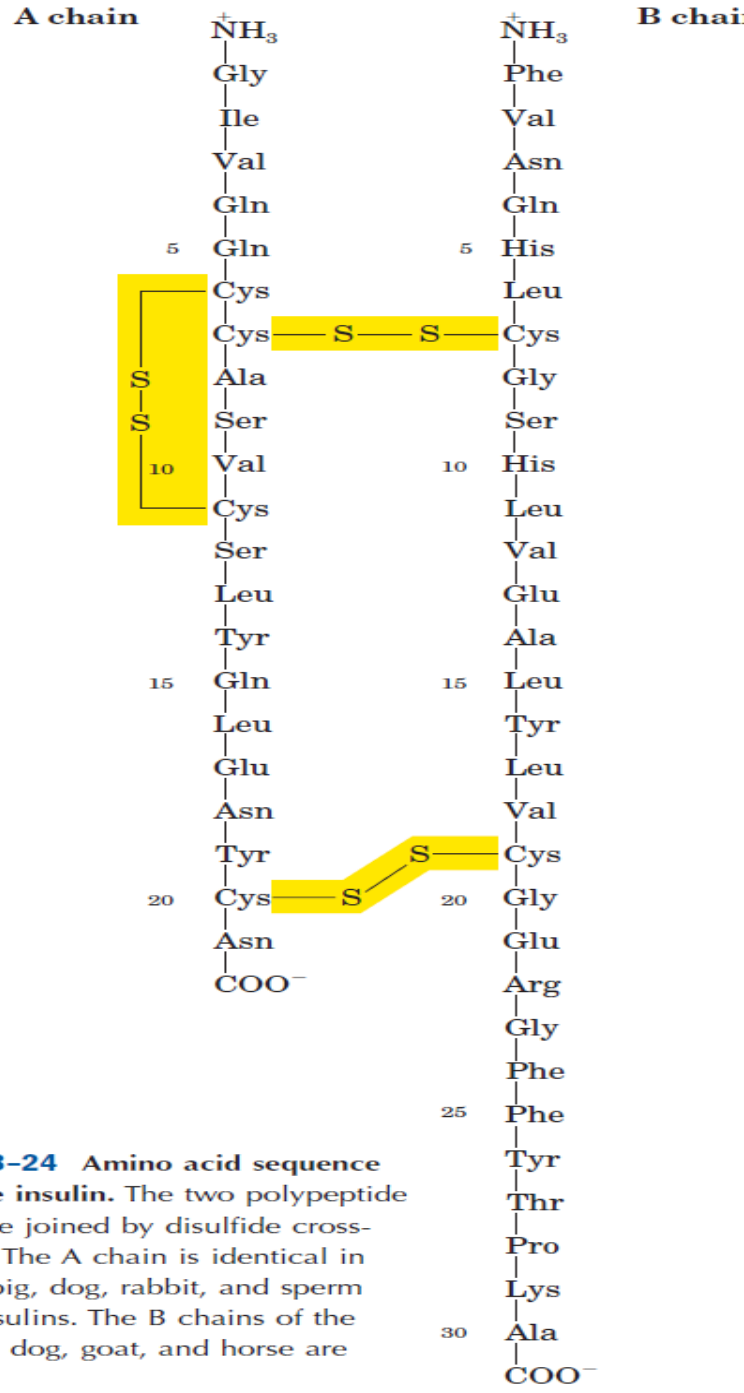
Branched Protein

- Generally the polypeptide chains are linear

- Branching points may be produced by the interchain disulphide bridges

- The covalent disulphide bonds between different polypeptide chains in same protein (interchain) or portions of the same polypeptide chain (intrachain)

- Good example is INSULIN



E 3–24 Amino acid sequence ine insulin. The two polypeptide are joined by disulfide cross- es. The A chain is identical in , pig, dog, rabbit, and sperm insulins. The B chains of the ig, dog, goat, and horse are al.

Circular Protein

- Generally the polypeptide chains are linear
- Rarely may be in circular form
- Example is Gramicidin

Psuedo Protein

- Usually most peptide bonds are formed between α-COOH and α-NH3+ group

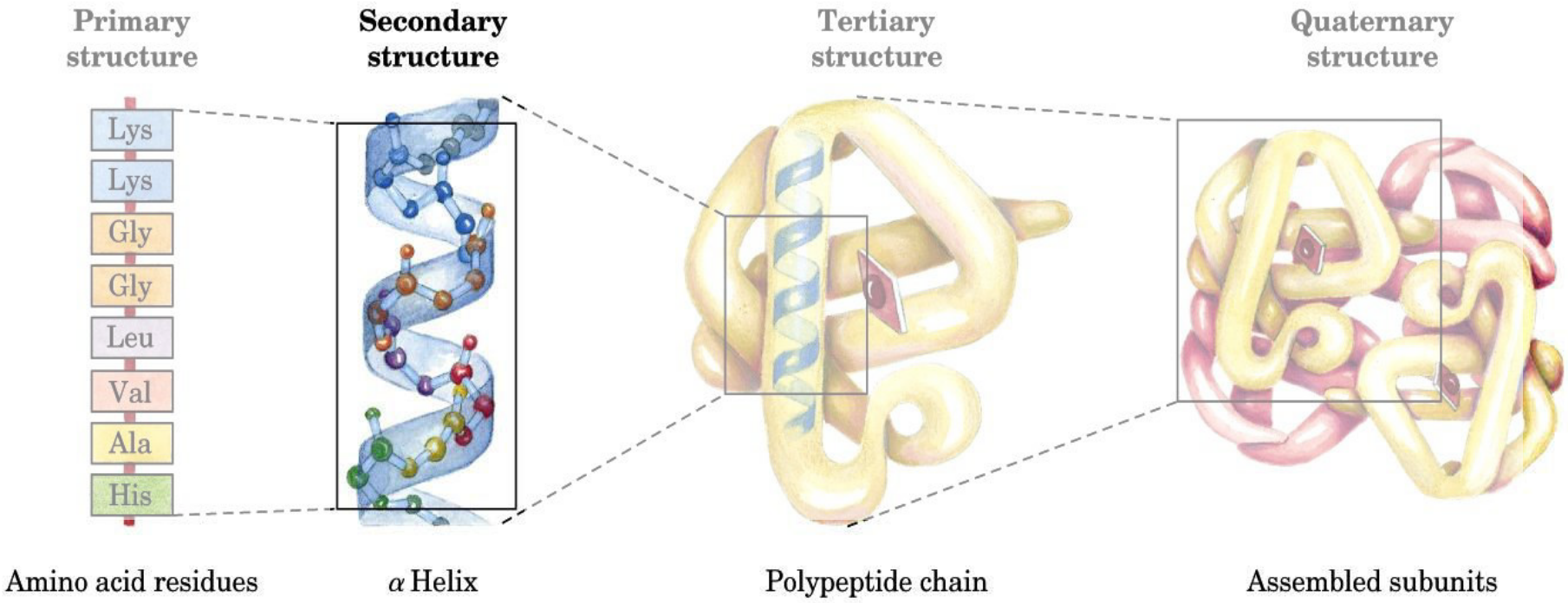
- Sometimes non α-COOH group is involved in peptide bond

- Eg. Glutathione(Glu-Cys-Gly)

- γ-Carboxy group of Glutamate forms peptide bond with the –NH3+ group of Cysteine

- Such a bond is sometimes called psuedopeptide bond

Secondary structure = local folding of residues into regular patterns



Secondary structure

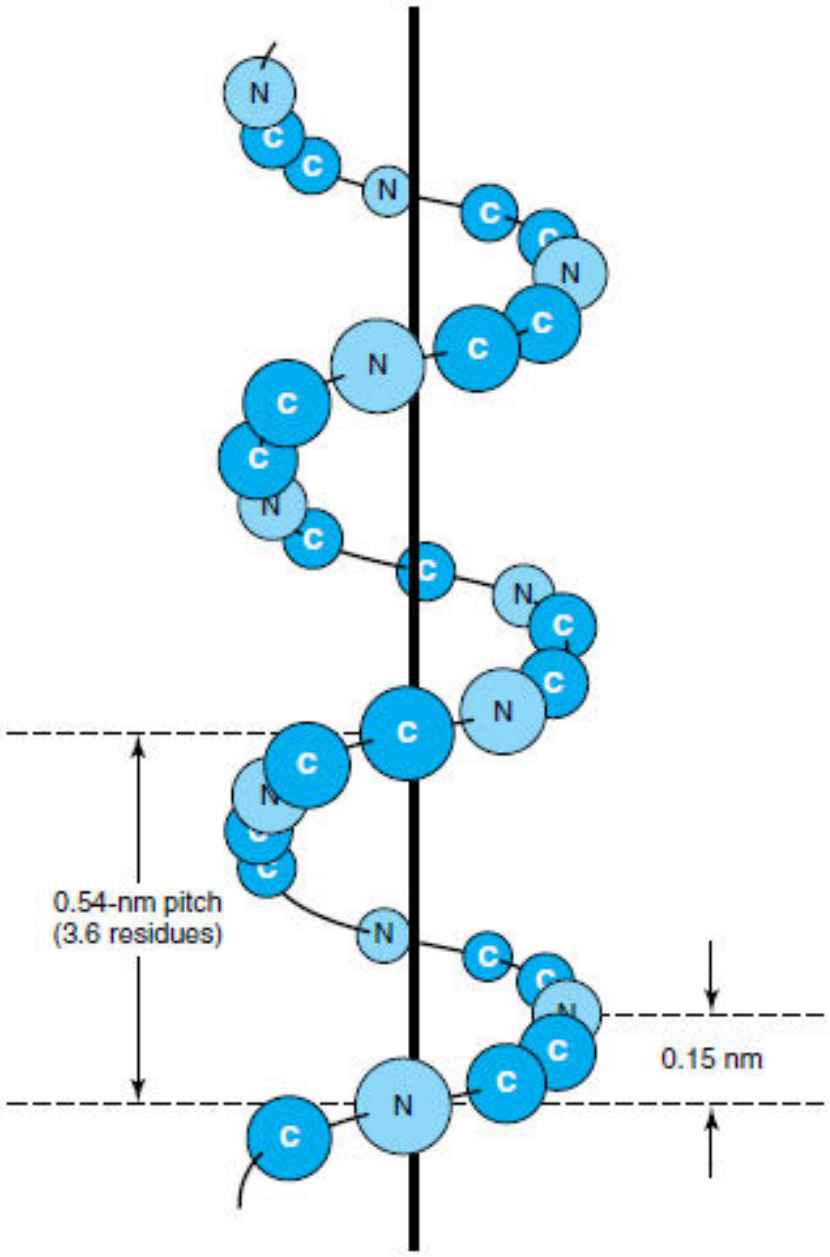
- Folding of polypeptide chain due to non covalent bondings between neighboring or closely placed amino acid residues in primary structure

- Folding pattern can occur periodically i.e regularly (α-Helix and β-Pleated sheets) or occasionally (turns and loops)

- Regularly occuring folding gives rise to two well defined structural patterns called α-Helix and β-Pleated sheets

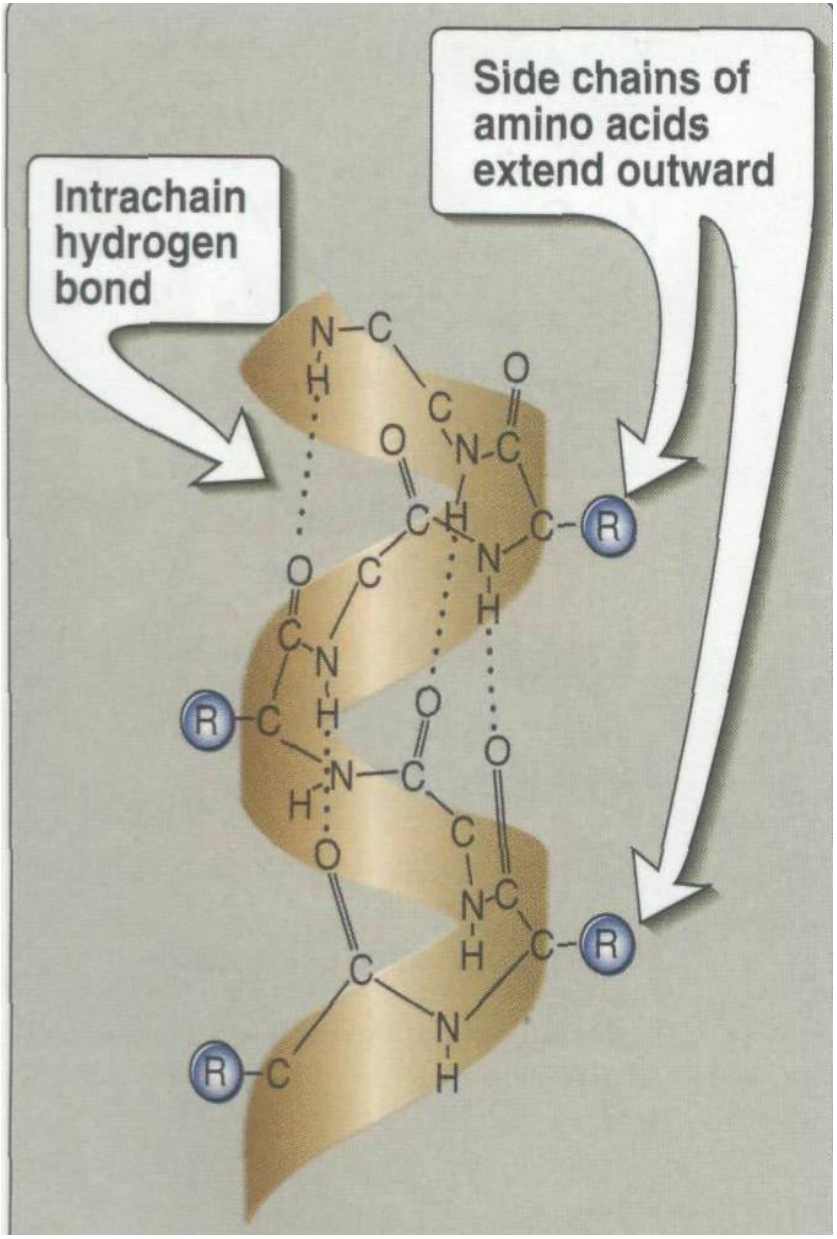
- Secondary structures are stabilized by hydrogen bonding between H and N or O atoms of peptide bonds

The α-helix



- Most **stable conformation** of a protein; commonly found in long **fibrous proteins** like collagen and aplha keratins
- Is a **right handed** helix in which polypeptide chain is folded into a helical pattern around a central axis
- Each turn contains **3.6** amino acids and is **0.54nm** long
- Each residue is **0.15nm** apart in the turn

α-helix



- Stabilized by hydrogen bonding between the peptide-bond carbonyl oxygens and amide hydrogens.

- The hydrogen bonds extend up the spiral from the carbonyl oxygen of one peptide bond to the -NH - group of a peptide linkage four residues ahead in the polypeptide.

- R groups of amino acids project outwards

- Alpha helix usually lie on the surface of a protein molecule but ca also be buried deep into the interior of the protein

Amphipathic α-helix

•Many α helices have predominantly hydrophobic R groups on one side of the axis of the helix and predominantly hydrophilic ones on the other.

•These amphipathic helices are well adapted to the formation of interfaces between polar and nonpolar regions such as the hydrophobic interior of a protein and its aqueous environment.

• Clusters of amphipathic helices can create a channel, or pore, that permits specific polar molecules to pass through hydrophobic cell membranes.

Amino acids disrupting α-helix

Proline:

Imino group is not geometrically compatible with the right-handed spiral of the α-helix.

The nitrogen atom is part of a rigid ring and rotation about the N-Cα bond is not possible so it introduces a destabilizing kink in an *helix*.

The nitrogen atom of a Pro residue in peptide linkage has no substituent hydrogen to participate in hydrogen bonds with other residues.

Charged amino acids (Glu, asp, his, lys, or arg):

disrupt the helix by forming ionic bonds, or by electrostatically repelling each other.

Amino acids disrupting α-helix

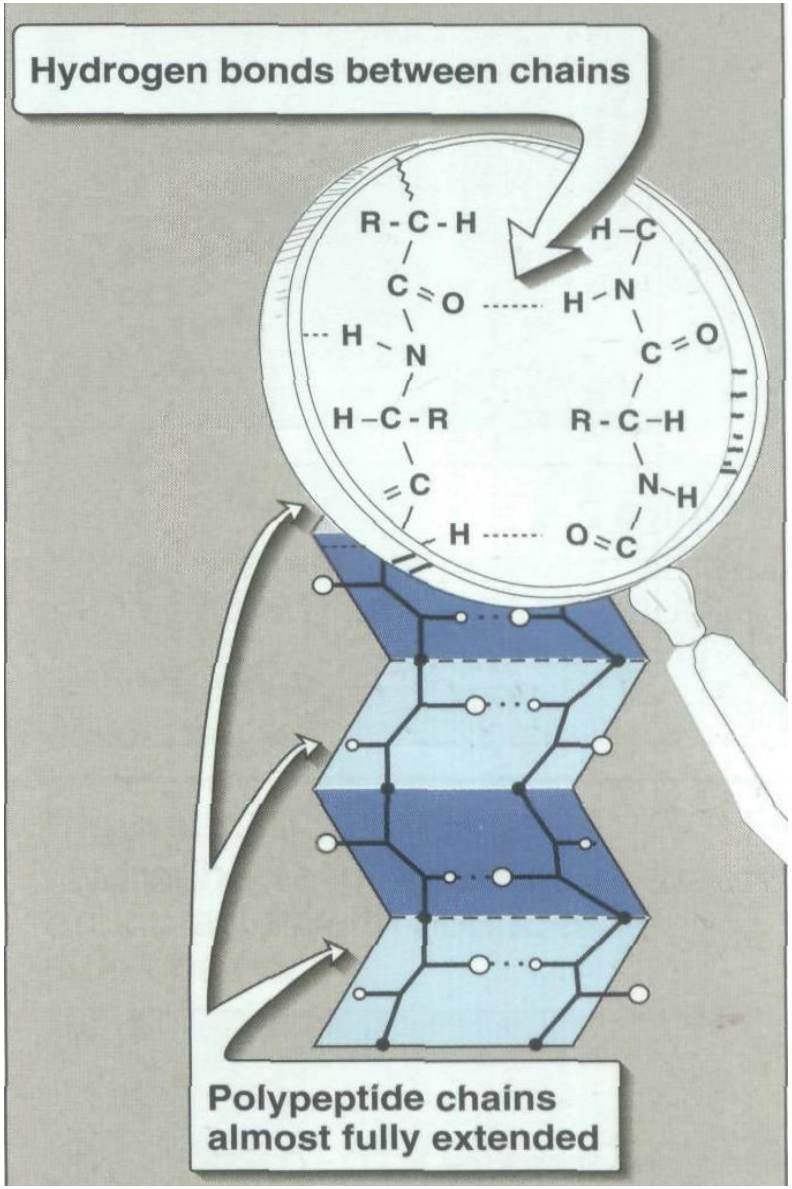
Amino acids with bulky side chains (tryptophan) and aminoacids, such as valine or isoleucine, that branch at the β-carbon:

can interfere with formation of the α-helix if they are present in large numbers.

Glycine:

occurs infrequently in helices as it has more conformational flexibility than the other amino acid residues so polymers of glycine tend to take up coiled structures quite different from an *helix*.

The β-sheet



- Formed when polypeptide chains align together longitudinally; can form between different regions of same polypeptide chain or between different polypeptide chain

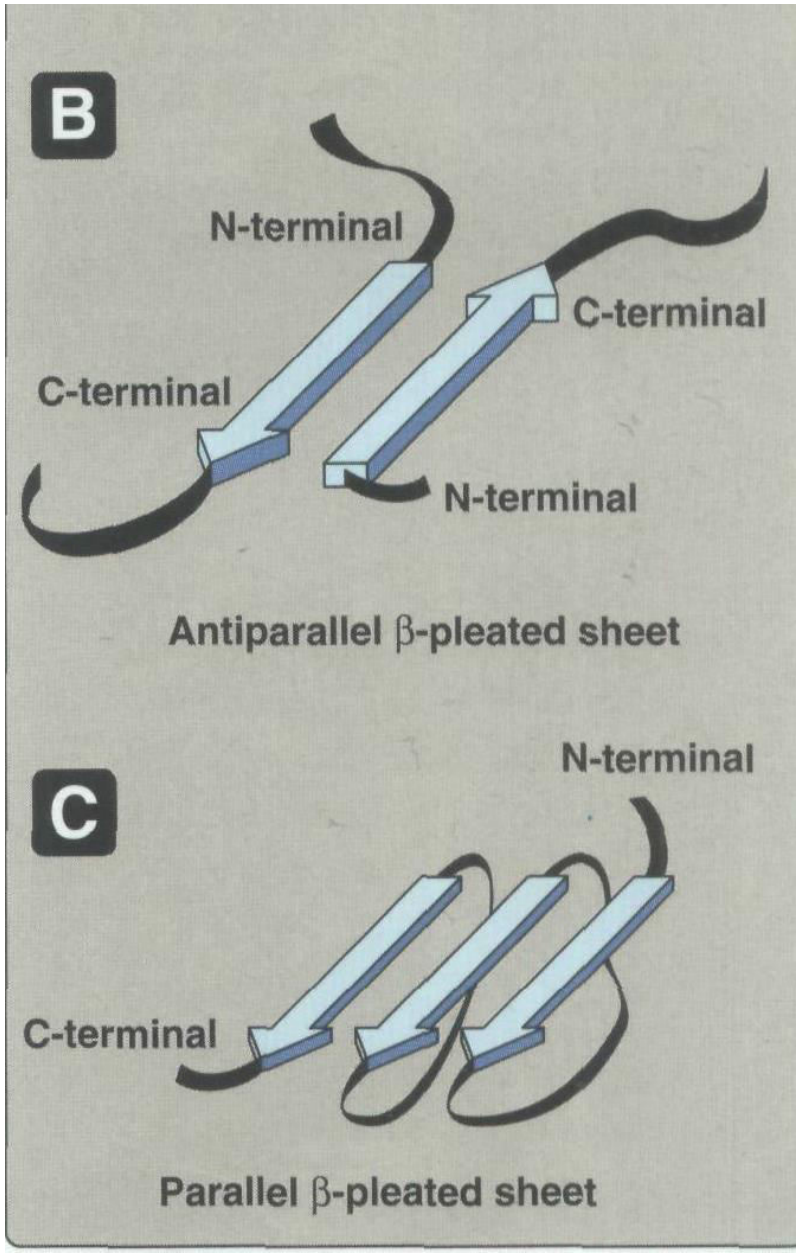
- Polypeptide chain is fully extended with pleated appearance

- Adjacent amino acids are placed 0.35 nm apart and their side chains orient in opposite directions to avoid steric clashes

- Stabilised by hydrogen bonds which can be formed between neighboring polypeptide chain or within a single polypeptide chain folded into segments

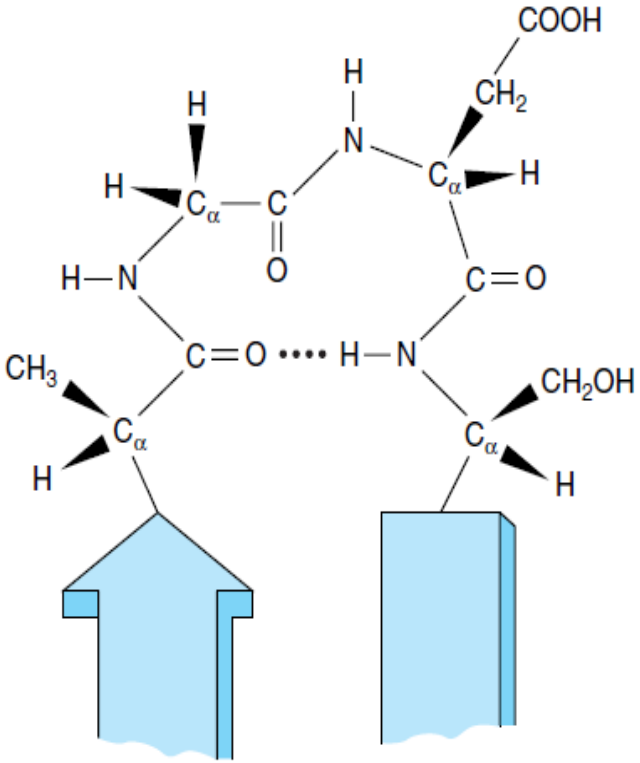
- Two to fifteen strands of polypeptide chain may together form beta-pleated sheet

The β-sheet



- These secondary structures can be either antiparallel or parallel.
- Adjacent chain or regions of the same chain when run in same direction the beta pleated sheet is called parallel
- Adjacent chain or regions of the same chain when run in opposite direction the beta pleated sheet is called antiparallel
- Parallel strand require long loop like structure for cross connections whereas antiparallel strands are

β turns



- A β turn involves four aminoacyl residues, in which the first residue is hydrogen-bonded to the fourth, resulting in a tight 180-degree turn

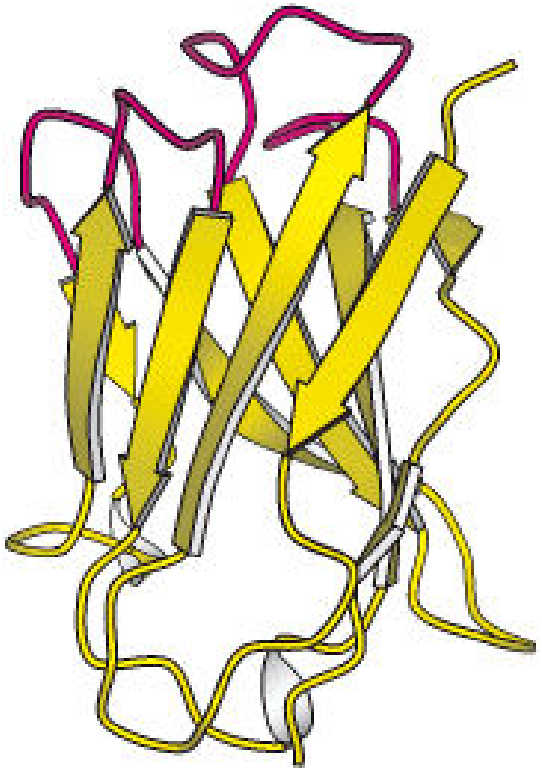
- Generally composed of four amino acids, one of which may be proline that causes a "kink" in the polypeptide chain.

- Glycine, the amino acid with the smallest R-group, is also frequently found in β-turns

- Bends are stabilized by the formation of hydrogen and ionic bonds

- They are usually found on the surface of protein molecules, and often include charged residues (often connect successive strands of antiparallel β-sheets).

Loops



- Loops or Ωloops omega loops are structure responsible for chain reversals
- Do not have regular, periodic structures but their structures are often rigid and well defined.
- Are regions that contain residues beyond the minimum number necessary to connect adjacent regions of secondary structure (anti-parallel beta sheets).
- Lie on the surfaces of proteins and thus often participate in interactions between proteins and other molecules.
- For many enzymes, the loops that bridge domains responsible for binding substrates often contain aminoacyl residues that participate in catalysis.

Super Secondary structural Motifs

- In large globular proteins, alpha helices and beta pleated sheets assemble or organize in different ways to form super secondary structural patterns or motifs.

- These motifs further interact in tertiary and quaternary structure

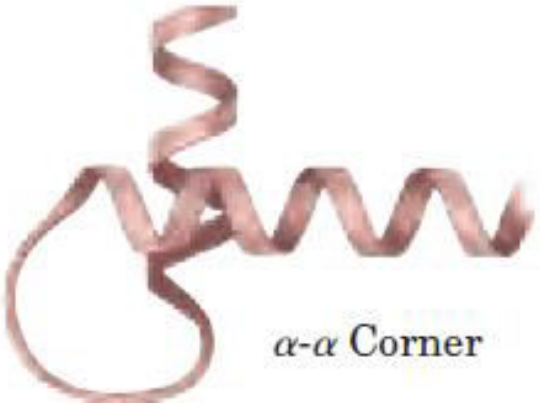
- Some common structure are β-α-β loop; α-α corner, twisted β-sheets,etc.

- These motifs usually occur in different layers of protein

- These simple motifs are further arranged in complex motifs such as β-barrel

Super Secondary structural Motifs

(a) β-α-β Loop

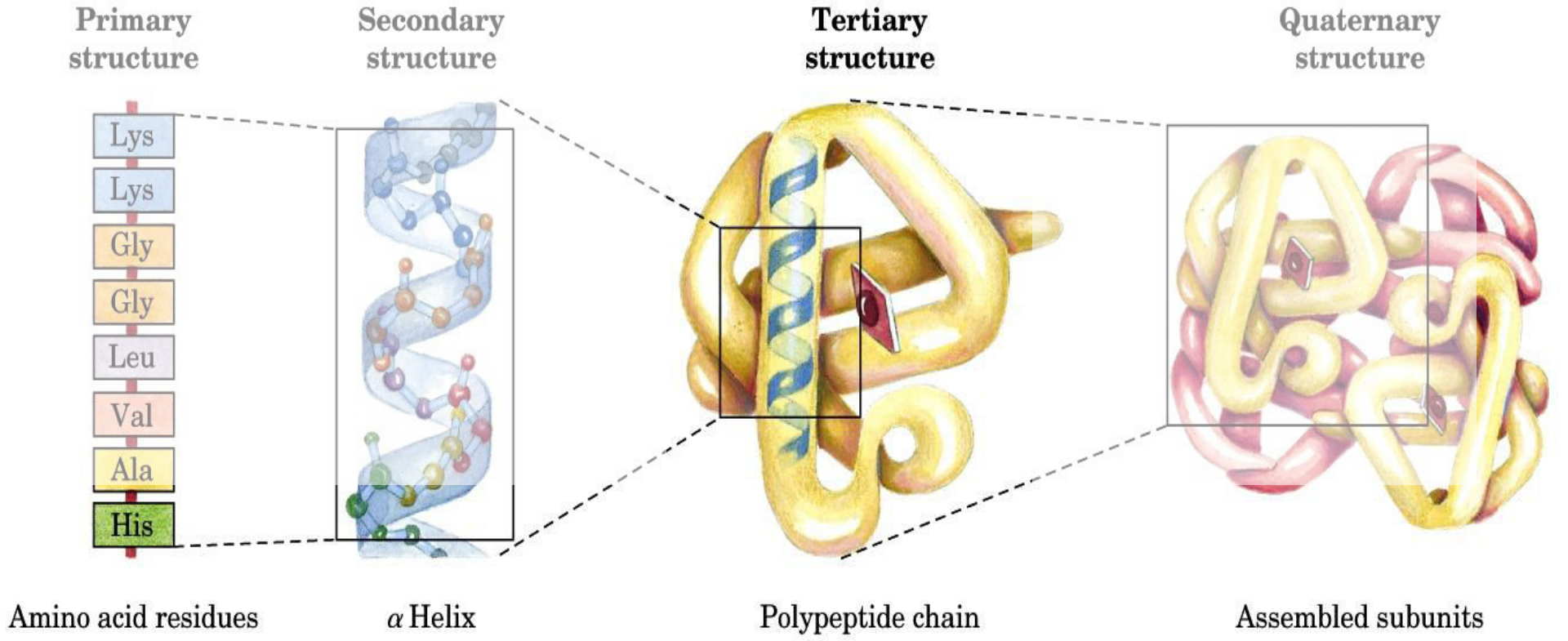


α-α Corner

Twisted β sheet

1) β Barrel

Tertiary structure = global folding of a protein chain



Tertiary structure

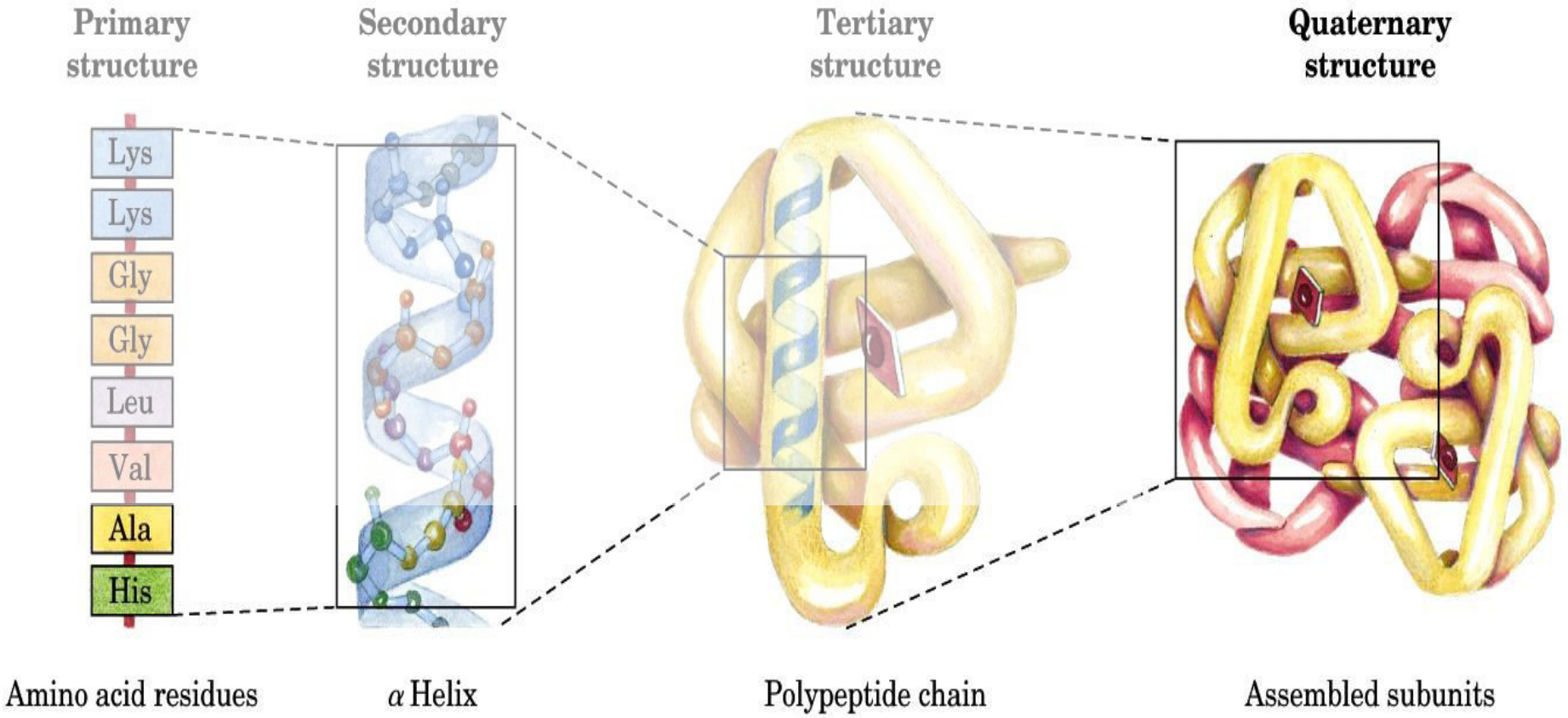
- Denotes three dimensional structure of the whole protein and defines the steric relationship of amino acids which are far apart from each other in the linear sequence but are close in three dimensional aspect

- Tertiary structure are maintained by non covalent interactions such as hydrophobic bonds, electrostatic bonds and vander waals forces

- Tertiary structure is always thermodynamically most stable

- Domain term is used to denote a compact globular functional unit of protein, domains are usually connected by flexible areas of protein for e.g Immunoglobulins

Quaternary structure = Higher-order assembly of proteins



Quaternary structure

- Consist of more than one polypeptide chain (certain polypeptides will aggregate to form one functional protein) is referred as quaternary structure

- The protein will loose its function when the subunits are dissociated

- Quaternary structure are maintained by hydrogen bonds, hydrophobic bonds, electrostatic bonds and vander waals forces

- Depending on the number of monomers, the protein may be termed as dimer(2), tetramer(4),etc. Each polypeptide is termed as subunit or monomer

- Example: Hemoglobin (2 alpha and 2 beta chain); Immmunoglobulin (2 light and 2 heavy chain), Creatine kinase (dimer); Lactate dehydrogenase (tetramer)

Collagen

- Most abundant of the fibrous proteins; constitute more than 25% of the protein mass in the human body.

- Collagen along with other fibrous protein serve as a primary source of structural strength for cells (i.e, the cytoskeleton) and tissues and also provide support to organ

- Skin derives its strength and flexibility from a crisscrossed mesh of collagen and keratin fibers

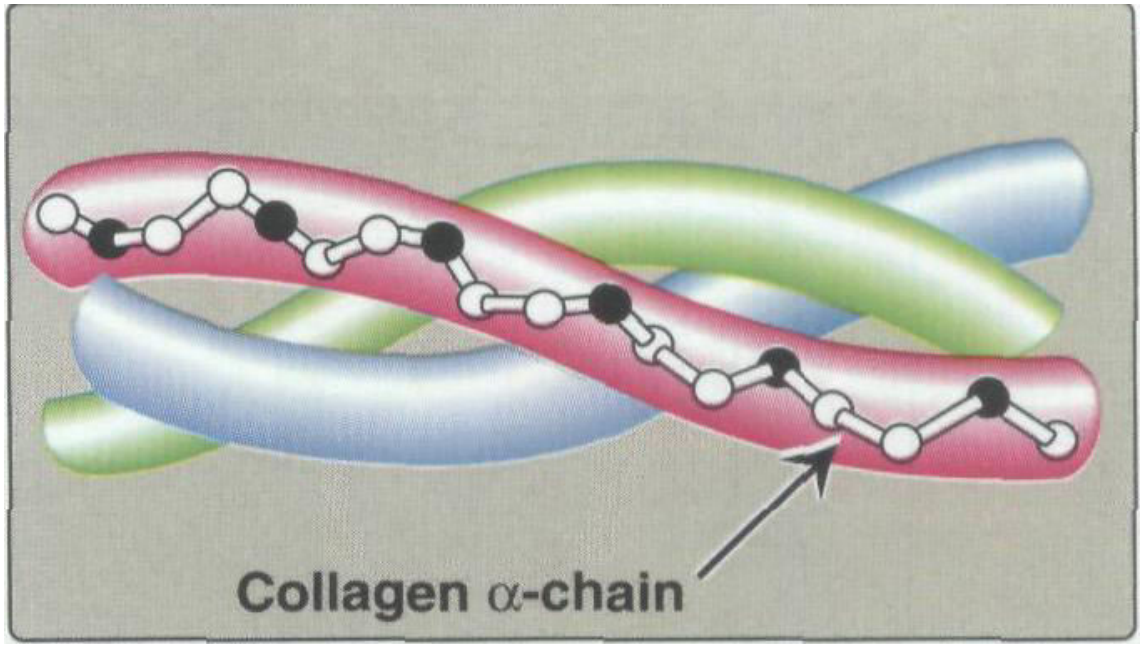
- Bones and teeth are buttressed by an underlying network of collagen fibers analogous to the steel strands in reinforced concrete.

- Also present in connective tissues such as ligaments and tendons.

Collagen

- Collagen provide alignment of cells, so that cell anchorage is possible which in turn help in proliferation and differentiation of cells.
- In blood vessels, if collagen is exposed, platelets adheres and thrombus formation is initiated.
- Various types of collagen found in the tissues.
- The most common collagen, type I contains two chains called α1 and one chain called α2
- Type II collagen contains three α1 chains

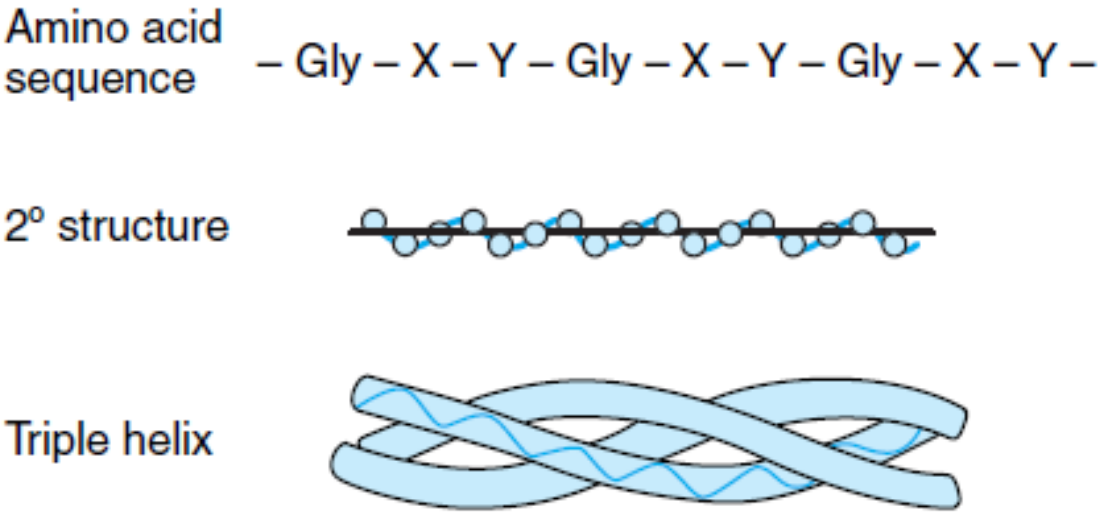
Collagen Triple Helix



- The three polypeptide α-chains(approximately 1000 amino acids long) are held together by hydrogen bonds between the chains.

- A collagen triple helix has 3.3 residues per turn and a rise per residue nearly twice that of an α helix. Each turn is separated by 2.9 Angstrom

Collagen Triple Helix



- **Collagen is rich in proline and glycine,**

- **Proline facilitates the formation of the helical conformation of each α-chain because its ring structure causes "kinks" in the peptide chain.**

- **Glycine, is found in every third position of the polypeptide chain. as it can fit into crowded interior of the collagen triple helix because of its small size**

- **The glycine residues are part of a repeating sequence GLY-X-Y where X is frequently proline and Y is often hydroxyproline or hydroxylysine**

Collagen Triple Helix

- Collagen triple helices are stabilized by hydrogen bonds between residues in different polypeptide chains.

- The hydroxyl groups of hydroxyprolyl residues also participate in interchain hydrogen bonding.

- Additional stability is provided by covalent cross-links formed between modified lysyl residues both within and between polypeptide chains.

- Three intertwined polypeptide strands which twist to the left, wrap around one another in a right-handed fashion to form the collagen triple helix.

- The opposing handedness of this superhelix and its component polypeptides makes the collagen triple helix highly resistant to unwinding ; the same principle used in the steel cables of suspension

Collagen Triple Helix Synthesis

- **Collagen is synthesized by fibroblast intracellularly as large precursor called procollagen (360 KD)**

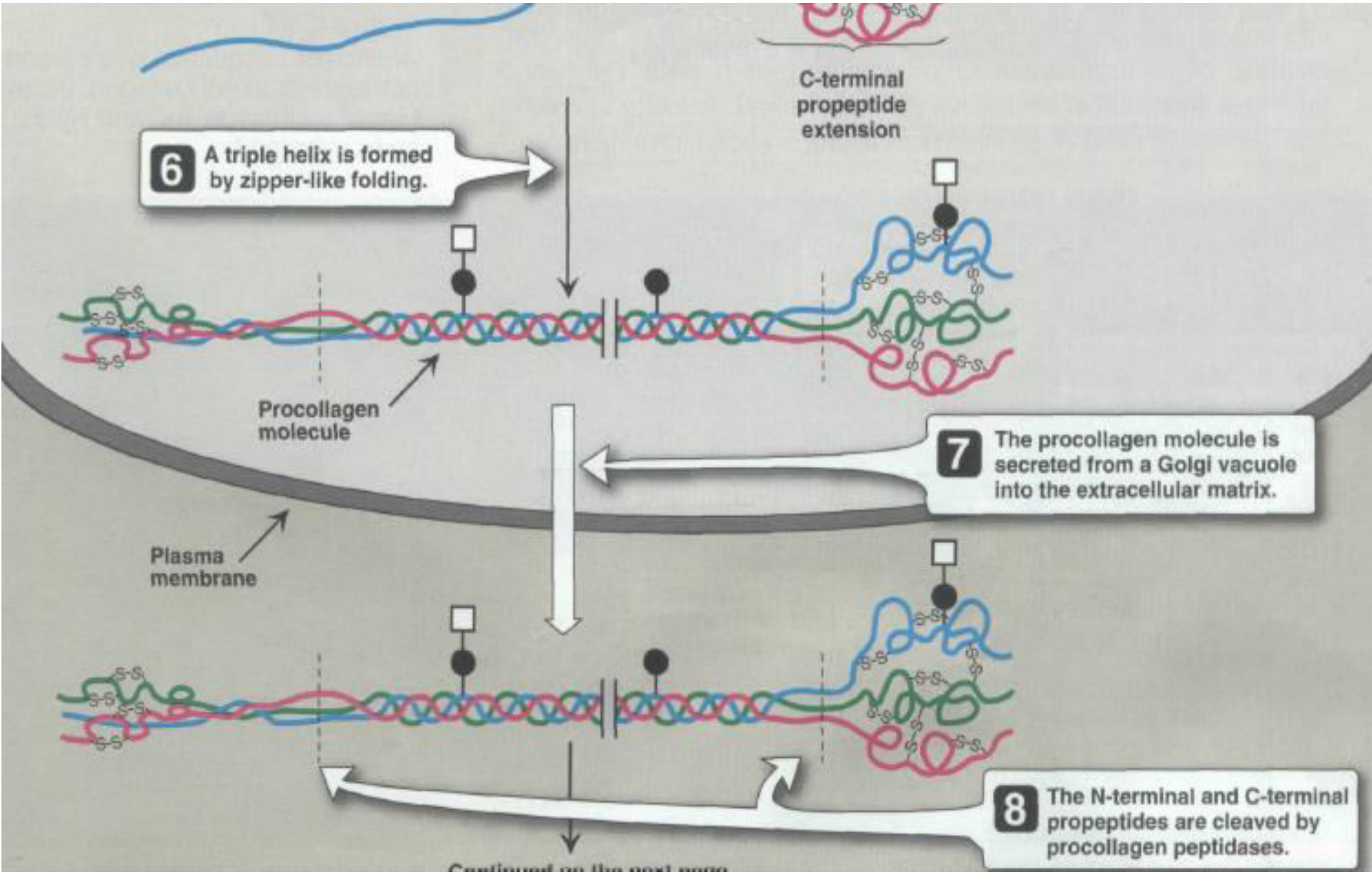
- **Inside the fibroblast; polypeptides are synthesised, proline and lysine residues are hydroxylated and glycosylation of lysine takes place.**

- **Procollagen is then secreted out of cell**

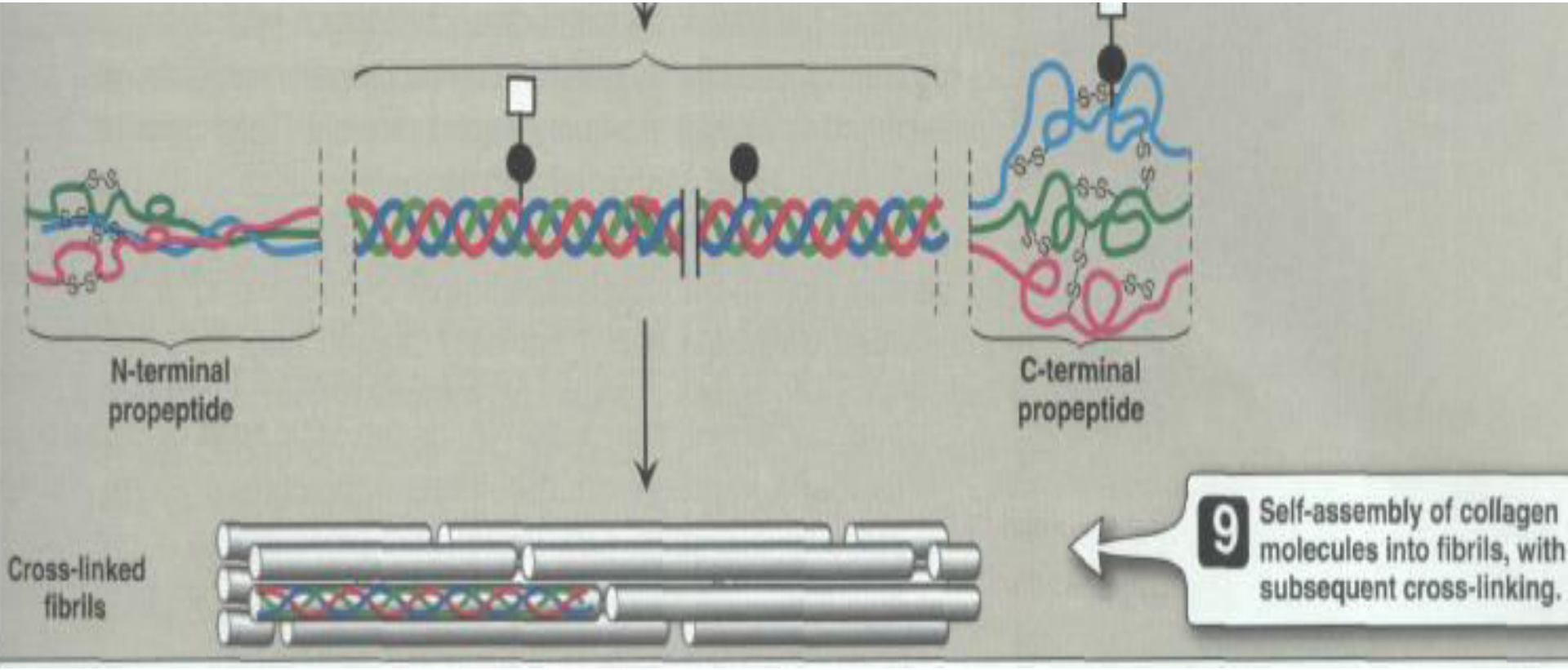
- **The extracellular procollagen is cleaved by specific peptidases to form tropocollagen (150 amino acid in N terminal and 300 amino acids in C terminal are cleaved off)**

- **Tropocollagen molecules are assembled into collagen**

Collagen Triple Helix Synthesis



Collagen Triple Helix synthesis



Hydroxylation of Proline and Lysine

- Proline and lysine residues found in the Y-position of the sequence Gly-X-Y can be hydroxylated to form hydroxyproline and hydroxylysine residues.

- These hydroxylation reactions require molecular oxygen and the reducing agent vitamin C (ascorbic acid) without which the hydroxylating enzymes prolyl hydroxylase and lysyl hydroxylase, are unable to function

- In the case of ascorbic acid deficiency collagen fibers cannot be cross-linked ; greatly decreasing the tensile strength of the assembled fiber resulting deficiency disease is known as scurvy.

- Patients with ascorbic acid deficiency also often show bruises on the limbs as a result of subcutaneous extravasations of blood (capillary fragility)

Cross Links formation in Collagen

- Collagen are strengthened by cross-links between lysine and hydroxy lysine residues.; the crosslinks are formed by lysyl oxidase which converts these amino acids into aldehydes

- Lysyl oxidase is a copper containing enzyme so in copper deficiency collagen synthesis is abnormal

- The aldehyde derivatives of lysine residues can form an aldol condensation such aldol crosslinks are formed near the amino terminal of the chains

- The older the collagen the more the extent of cross linkage.

- In old age the skin, blood vessels and other tissues become less elastic and more stiff contributing a great extent to the medical problems of old people

Collagen diseases

Ehlers-Danlos syndrome (EDS):

- Is a heterogeneous group of generalized connective tissue disorders that result from inheritable defects in the metabolism of fibrillar collagen molecules.

- Can result from—

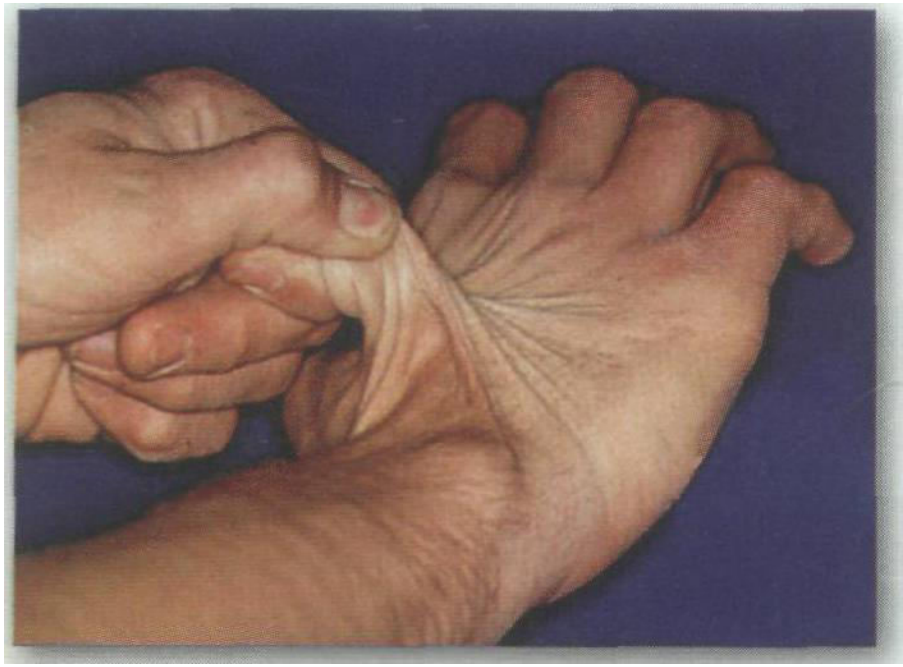
1. Deficiency of collagen-processing enzymes (for example, lysyl hydroxylase deficiency or pro-collagen peptidase deficiency)

2. Mutations in the amino acid sequences of collagen types I, III or V.

- The most clinically important mutations are found in the gene for type III collagen.

Ehlers-Danlos syndrome (EDS)

- Collagen containing mutant chains is not secreted, and is either degraded or accumulated to high levels in intracellular compartments.



- Because collagen type III is an important component of the arteries, potentially lethal vascular problems occur.

- EDS patients also show defects in collagen type I fibrils. This results in stretchy skin, hypermobile and loose joints

Osteogenesis imperfecta

- Also known as brittle bone syndrome, is also a heterogeneous group of inherited disorders

- Caused due to replacement of Glycine by Cysteine. This change disrupts the triple helix near carboxy terminus,hence the polypeptide become excessively glycosylated and hydroxylated

- Unfolding of helix takes place and fibrillar array cannot be formed resulting in brittle bones leading to multiple fractures and skeletal abnormalities

- Retarded wound healing and a rotated and twisted spine leading to a "humped-back" appearance are common features of the disease.

Osteogenesis imperfecta

- **Type I is called osteogenesis imperfect tarda.**

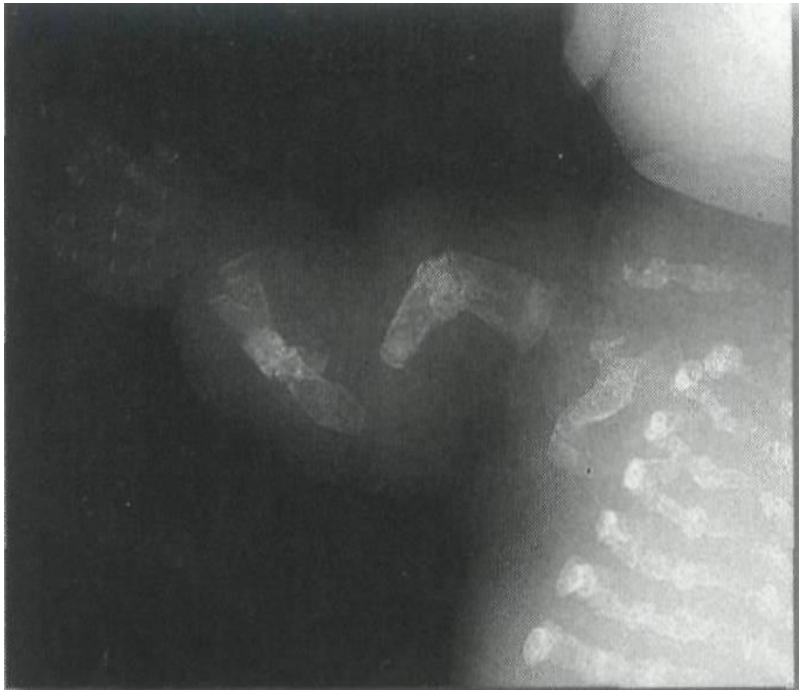
- **This disease present in early infancy with fractures secondary to minor trauma**

- **may be suspected if prenatal ultrasound detects bowing or fractures of long bones.**

Osteogenesis imperfecta

- **Type II osteogenesis imperfecta congenita,**

More severe, and patients die in utero or in the neonatal period of pulmonary hypoplasia.



Most patients with severe OI have mutations in the gene for either the pro-I or pro-II-α of type I collagen.

- **The most common mutations cause the substitution of single amino acids with bulky side chains for the glycine residues**

- **The structurally abnormal Pro-α chain can prevent folding of the protein**

HOMOCYSTINURIA

- Accumulated HOMOCYSTEINE reacts with Lysyl aldehydes to block cross linking

- The skeletal abnormalities, vascular and occular defects are thus produced

Deficiency of Ascorbic acid(Scurvy)

- Defective hydroxylation of Collagen leading to weak collagen formation

- Fragile blood vessels, poor wound healing results due to this abnormality

Lathyrism

- Due to ingestion of Lathyrus sativa or sweet pea
- Toxic agent beta oxalyl amino alanine (BOAA) is present in Lathyrus sativus
- BOAA inhibits lysyl oxidase which interfere with formation of lysyl cross linking