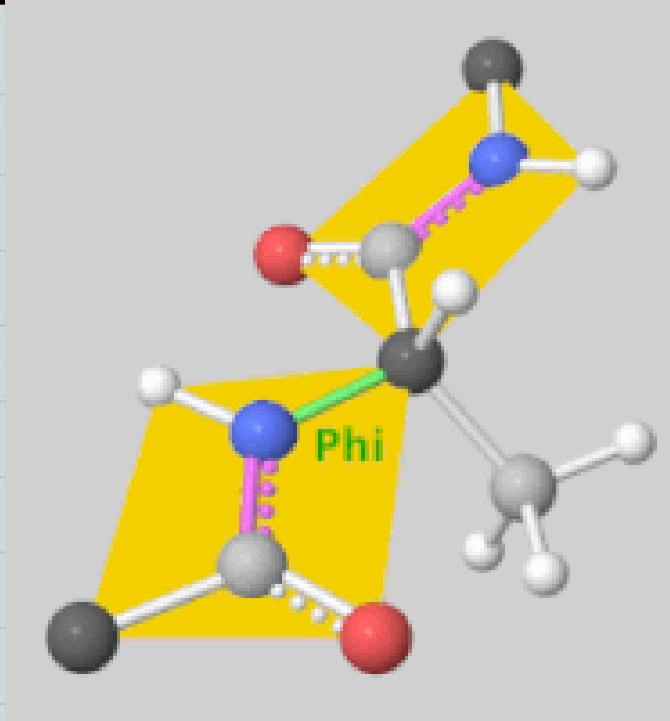
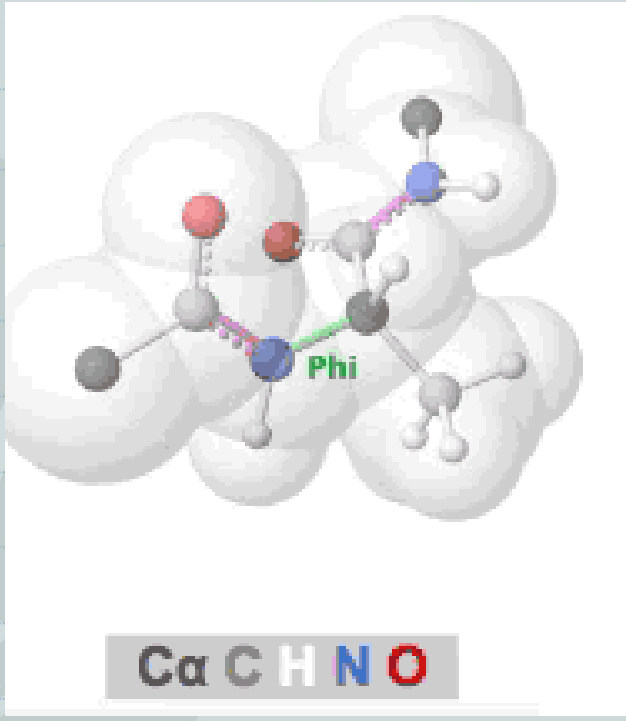
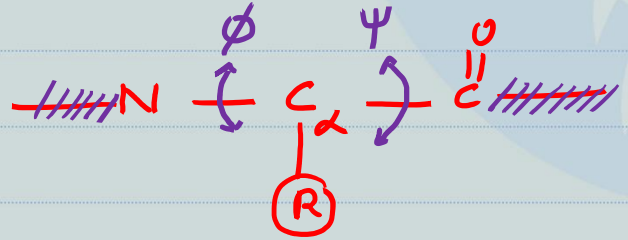
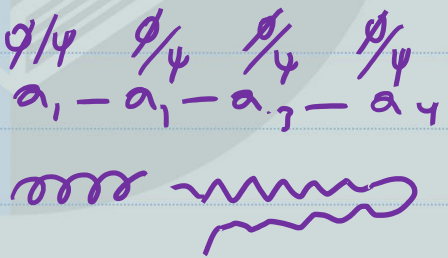
LECTURE 12

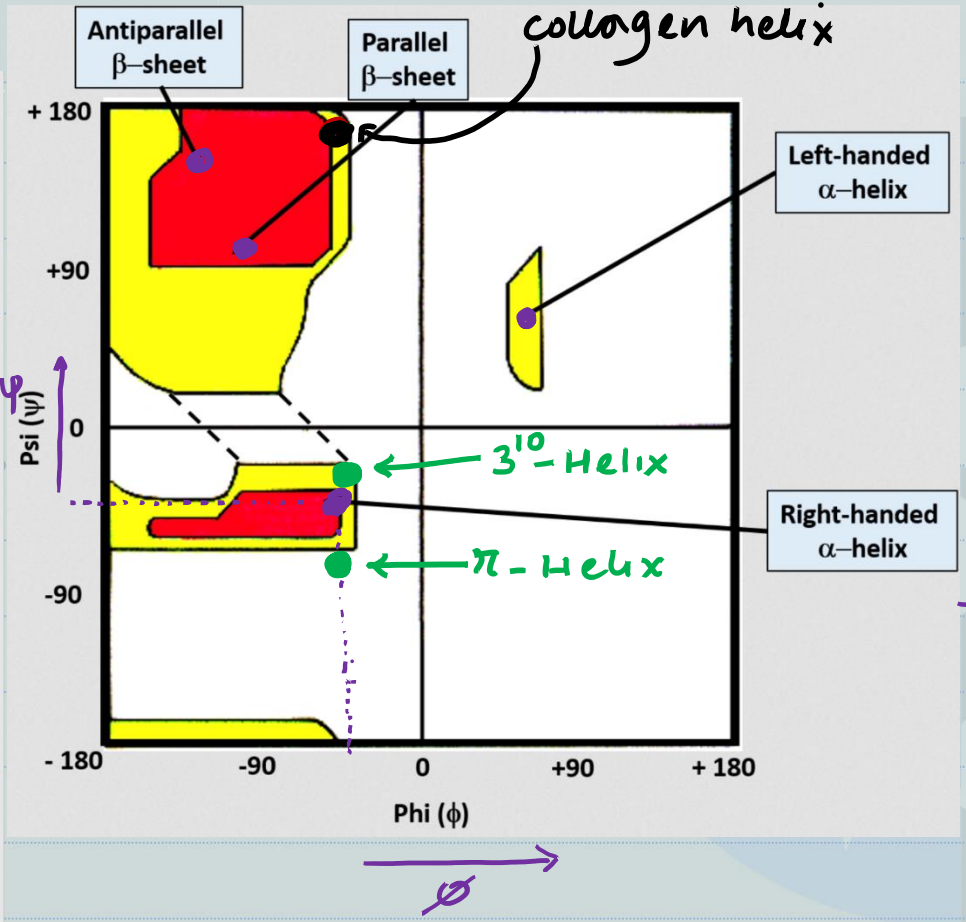
Protein- Secondary & tertiary Structures


Phi


Phi
Cα C H N O


ϕ
ψ
O
N
Cα
R


ϕ/ψ ϕ/ψ ϕ/ψ ϕ/ψ
a1 – a1 – a3 – a4



Secondary structure	ϕ	ψ
RH α-helix (L-aa)	- 57	- 47
LH α-helix (D-aa)	+ 57	+ 47
Parallel β-sheets	- 119	+ 113
Anti Parallel β-sheets	- 139	+ 135
3_{10} helix	-49	-26
Π-helix	-55	-70
Collagen Tripple helix	-51	+153

$a_1 - a_2 - a_3 - a_4 - a_5 - a_6$

ϕ = ?

ψ = ?



RH Alpha-Helix:

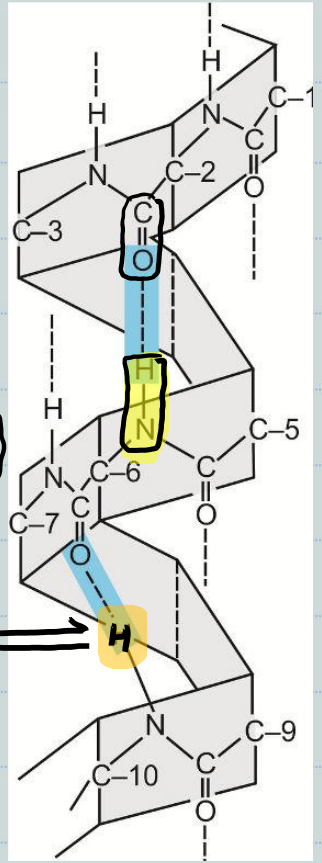
most common 2° str

eg α-Keratin

Trans-membrane region of protein

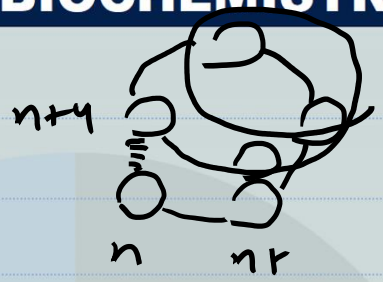
- Right handed (L-amino acids)
- One helix has 3.6 amino acids
- Helix height 5.4 A° (0.54 nm)
- Distance between two amino acids = 1.5 A°
- ϕ, ψ dihedral angles near −57°, −47° → [−60 −30 (α-helix)]
- Intra Chain hydrogen bonding: n→n+4
- First formed secondary structure
- Slow exchange amide H/D in NMR

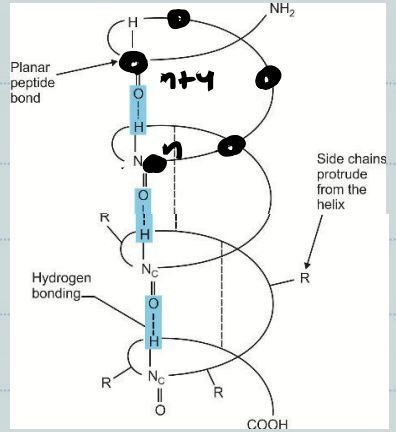




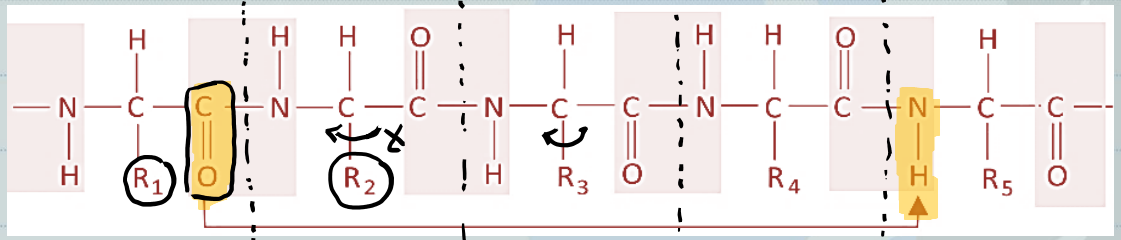
Hydrogen Bonds:

- Main Chain C=O of (n) → N-H of (n+4)





- Number of hydrogen bond: number of amino acids - 4



- R- chain donot participate in H- bonding & If participate (Ser, Thr, Asn, Gln) than destabilize α-Helix

What is the length and number of hydrogen bonds in a polypeptide with 20 amino acid residues in a single contiguous α- helix spanning hydrophobic core of plasma membrane?

(1) 3 nm and 16

(2) 2 nm and 16

(3) 10.8 nm and 32

(4) 30 nm and 32

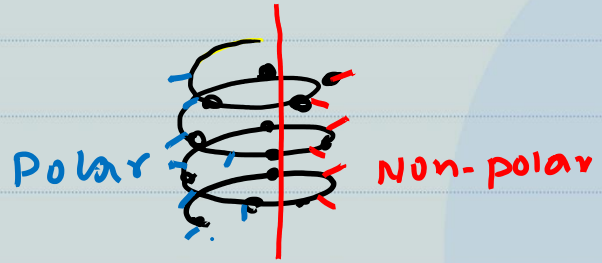
α - Helix : distance bet^{n} 2 aa = 1.5 A$^{\circ}$

Number of H- bond = No of aa - 4

- 20 x 1.5 A$^{\circ}$ = 30 A$^{\circ}$ = 3 nm
- 20 - 4 = 16

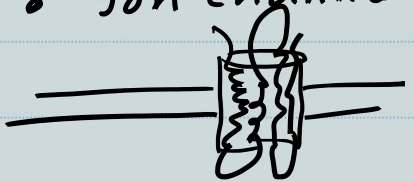
Ampipathic and hydrophobic alpha helix

–val – asp – asp – val – phe – ser – gln –val – cys – thr – his - leu - asp- thr – leu - lys



Amipathic α-Helix

- Transporters
- Ion channel



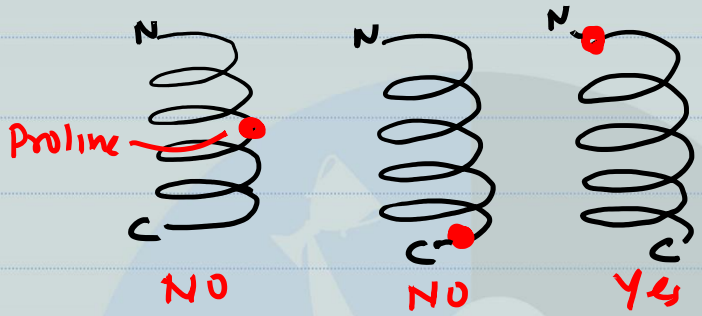
– val- leu- leu -Ala-phe- val- leu –

↓

Hydrophobic α-Helix

- Transmembrane protein – 20 aa
- Core of protein

- Disrupt α helix
- Proline
- Hydroxy-proline



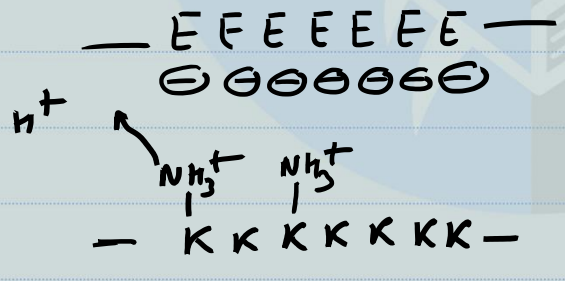
- Hydrophobic aa are preferred — Alanine
- N-terminal side of proline for cis-peptide bond

—EEDEED—

- continous acidic acid aa
- continous basic acid aa

⊖⊖⊖ ⊕⊕⊕ — At pH = 7 → Strong Repulsion → NO → α-Helix

— KKKKKKK —



at low pH → α-Helix

COO^- $COOH$

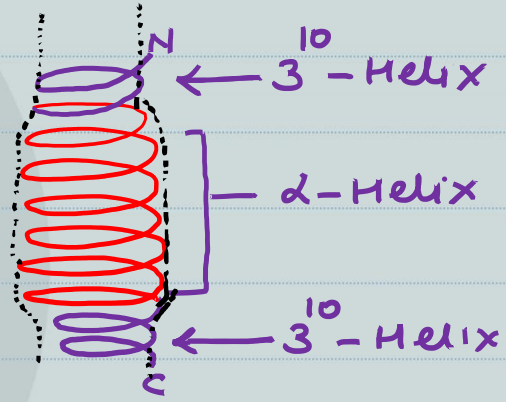
At high pH → α-Helix

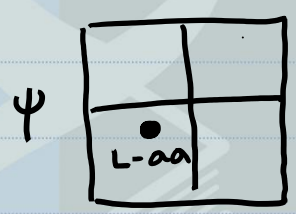
3_{10}-helix

3 = Number of aa in Helix

10 = Numbe of atoms in Helix

- Observed as extensions of α-helices found at either their N- or C- termini.
- ϕ, ψ dihedral angles near −49°, −26° = (−75)
- Right-handed helical structure
- n → n+3 (n) → C=O ////// H — N — (n+3)
- Distance between two amino acids 2.0 Å or 0.2 nm.



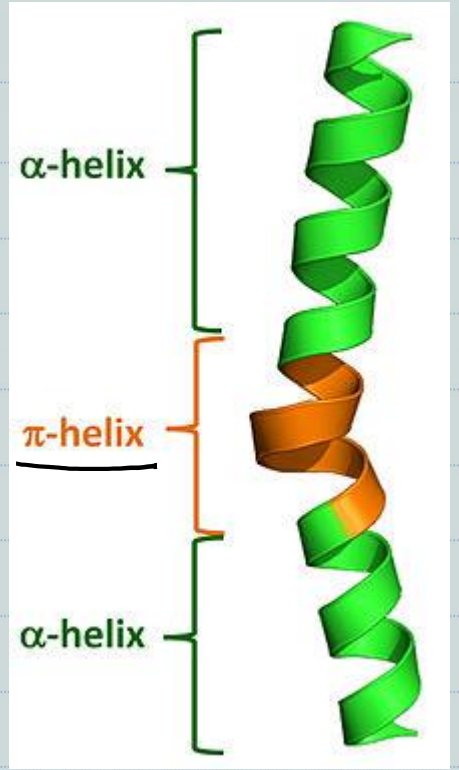


α - Helix = 3.6_{13} - Helix

π-helix = 4.1_{16} Helix

2.2_7 Helix → $n \rightarrow n+2$

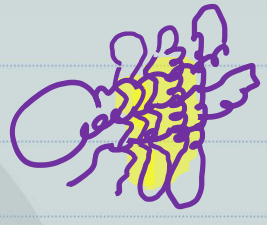
- ✓ Right-handed helical structure
- ✓ Observed at center of long α-helices
- ✓ 4.1 residues per turn & 16 atoms
- ✓ ϕ, ψ dihedral angles near −55°, −70°
- n→n+5 (n) C=O ////// H — N — n+5
- Distance between two amino acids 1.15 Å



3_{10}	α-Helix (3.6_{13})	π-Helix (4.1_{16})
• 3 aa	3.6 aa	• 4.1 aa
• ϕ = −49, ψ = −26	ϕ = −57, ψ = −47	ϕ = −55, ψ = −70
• n→n+3	n→n+4	n→n+5
• 2 A°	1.5 A°	• 1.15 A°

Beta Sheets:

- Silk fibroin protein — Antiparallel β-sheet
- Porin membrane protein



- Common in protein after alpha helix
- Generally seen in interior of globular protein
- Distance between two amino acids 3.5 A°

Parallel



- Inter/intra chain hydrogen bonding

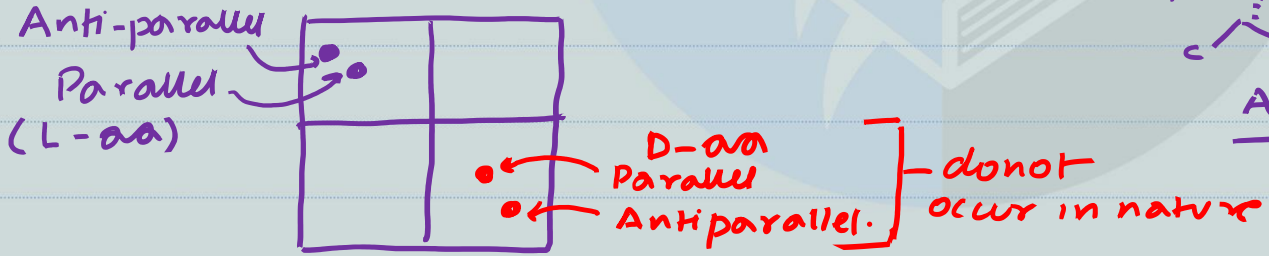


- ϕ, ψ are about –140°, +135° in Antiparallel sheets



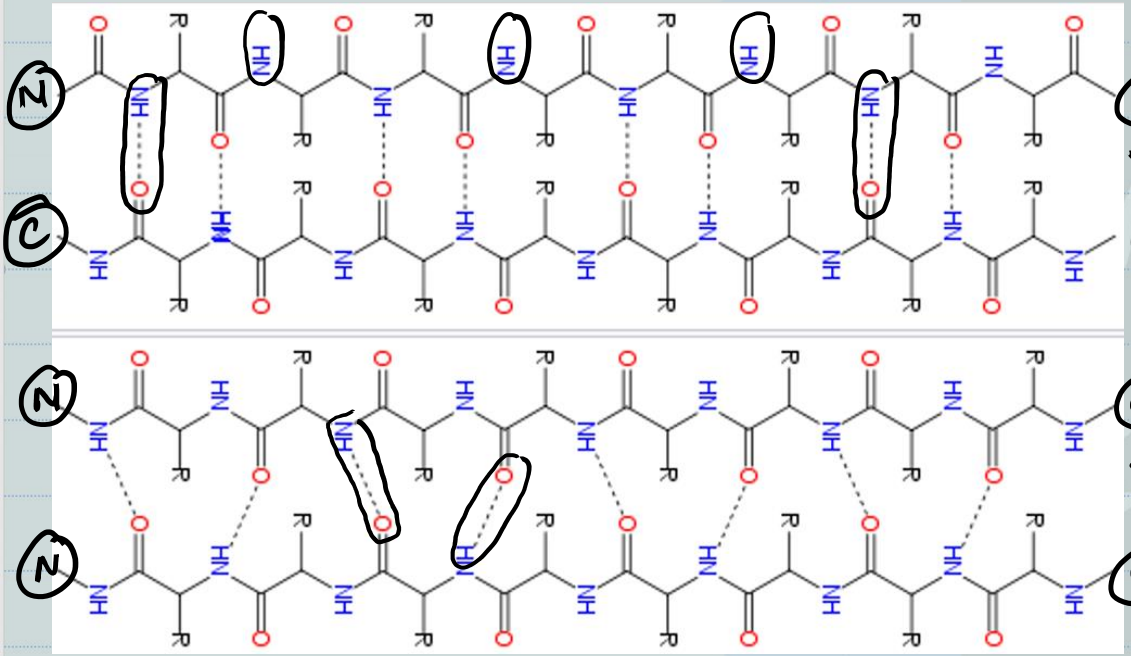
Antiparallel

- ϕ, ψ are about –120°, +115° in Parallel sheets



50%. H/D – Exchange of amide hydrogen in NMR

Hydrogen bonding occurs between neighboring polypeptide chains



← Anti-parallel

Hydrogen bond = 180°

→ Stable, more common

← Parallel

H- bond = 150°

→ Unstable

→ lesser seen

Rare Amino Acids

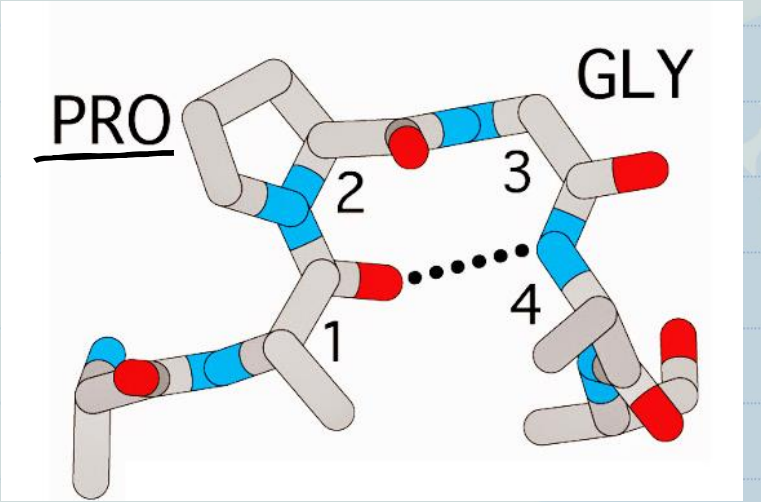
- Proline — cis peptide bm
- Acidic
- Basic

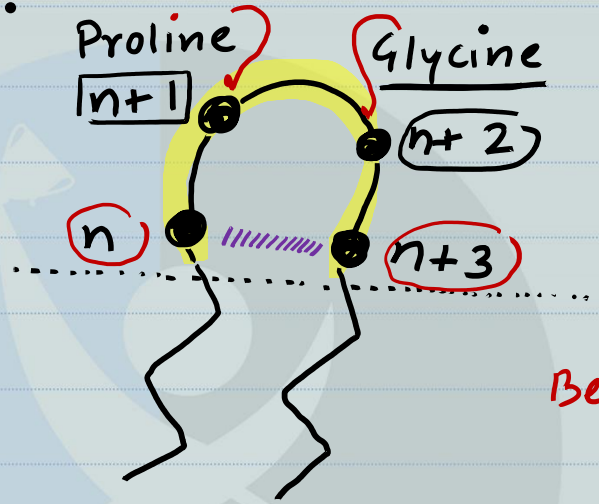
] — strong electrostatic repulsion

Most Common

- Isoleucine
- Valine
- Alanine ✓
- Glycine ✓

β Turn or β Bend or Hairpin Bend:



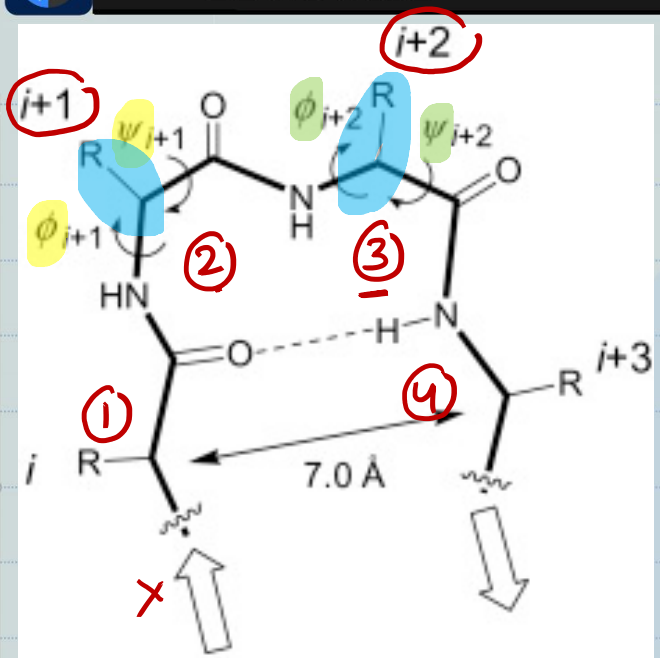


Bet^{n} H-bonded groups

(1) Formed by 4 aa & 10 atoms

(2) n ⟶ n+3 Hydrogen bonds

→ n+1 & n+2 donot participate in H-bonding



	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}
type I	-60	-30	-90	0
type I'	-60	120	80	0
type II	60	30	90	0
type II'	60	-120	-80	0

	Residue 2 (n+1)		Residue 3 (n+2)	
	ϕ	ψ	ϕ	ψ
Type-I	Pro -60	-30	-90	0
Type-I'	Asn +60	+30	+90	0
Type-II	-60	+120	-90	0
Type-II'	+60	-120	+90	0

[n+1 or i+1] Residue 2

[n+2 or i+2] Residue 3

- ϕ & ψ values of ② & ③ amino acid decides type of reverse turn

Collagen helix ← seen in collagen protein.

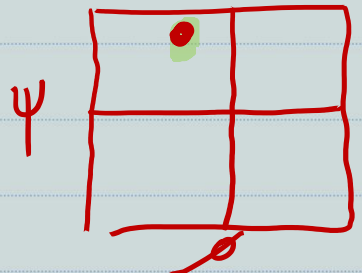
- Gly-X-Y – Gly – X – Y – Gly – (X) – Y
- Left-handed helix
- Peptide bond: trans
- Similar to poly-proline Type II
- 3.3 residues per turn
- Distance between 2 amino acids: 2.9 Å.
- ϕ, ψ = –51°, +150°
- No intra-chain hydrogen bonding

X = Proline

Y = Hydroxy Proline

– Pro – Pro – Pro – polyproline type-I
- cis-peptide bond
- Polyproline type-I
- Non-polar env^n

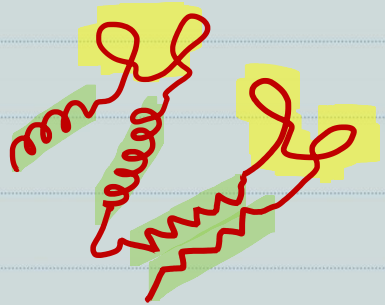
– Pro – Pro – Pro
- Polar env^n
- Trans peptide
- Polyproline type-2



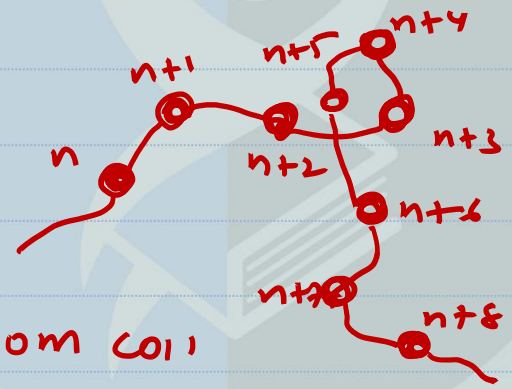
Random Coil

- Adjacent bulky residues such as isoleucine: High steric hindrance
- Adjacent charged residues such as glutamic acid and aspartic acid or lysine and arginine: Strong repulsion

The polypeptide donot have any defined secondary structure



Random coil configuration

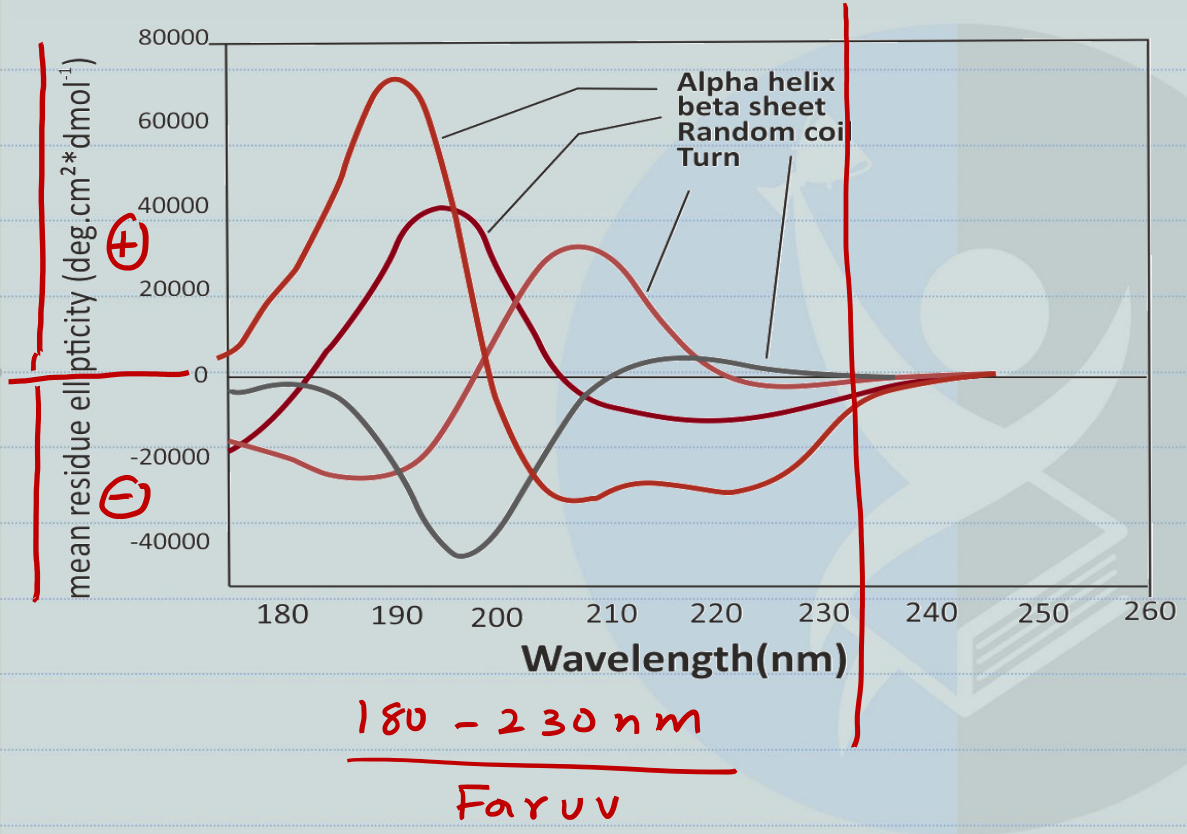


Techniques to study secondary structure of proteins

1. Far UV- Circular Dichroism (CD) Spectroscopy
2. H/D Proton Exchange NMR Spectroscopy
3. X-Ray crystallography
4. Fourier Transform Infra Red (FTIR) Spectroscopy

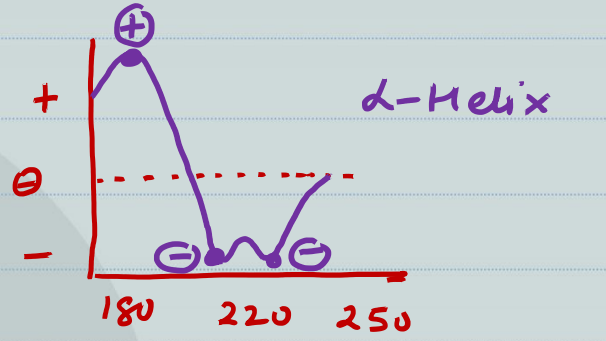
100 – 250 nm Far UV
250 – 400 nm Near UV
400 – 700 nm Visible
700 – 1000 nm IR

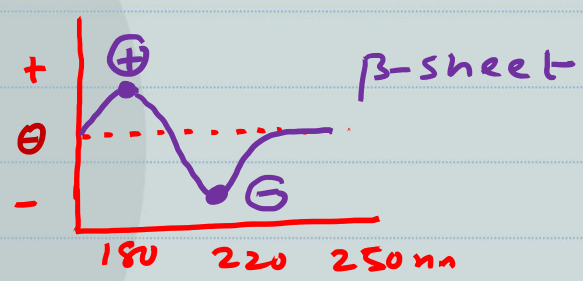
Far UV CD spectra of major secondary structure in proteins

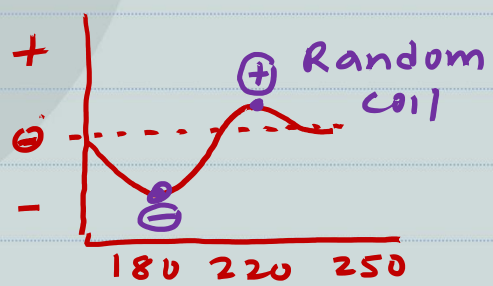


180 - 230 nm

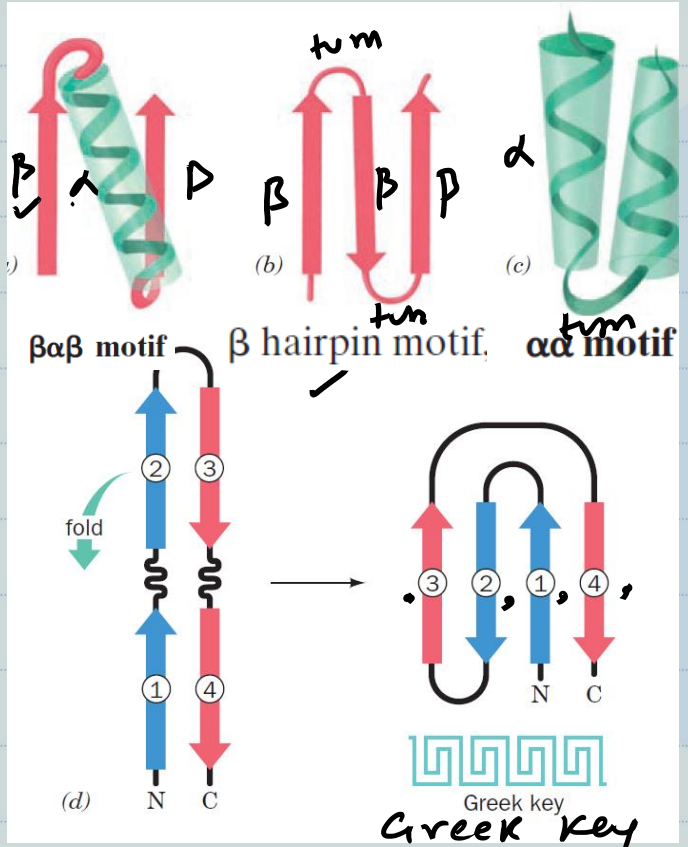
Far UV







Super secondary Structures (Motif): building blocks



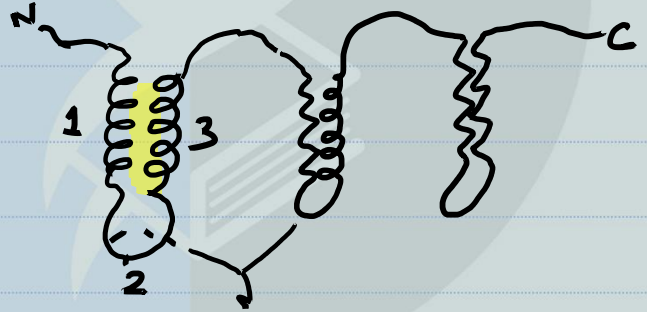
Greek key motif

1° → 2° → Super Secondary → fold or Domain → 3°

α helix

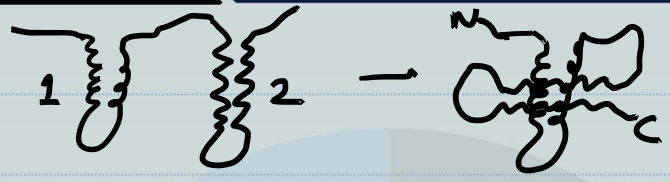
motif

Formed due to interaction bet^n more than 1 secondary structure through R-chain

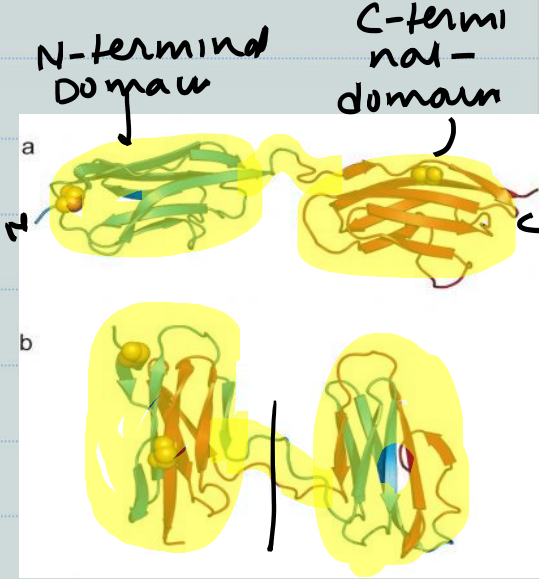


Helix-turn Helix

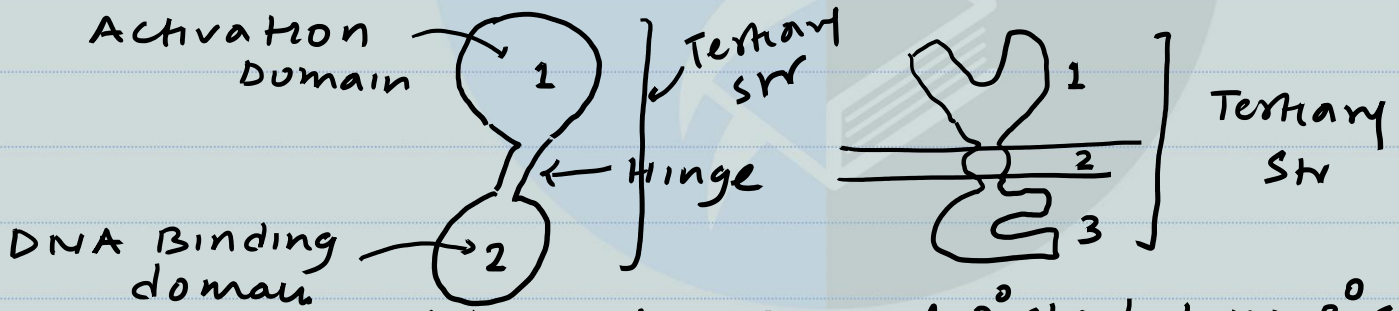
Domain or fold



Groups of motifs combine in overlapping and non-overlapping ways to form the tertiary structure of a domain, which is called a **fold or domain.**



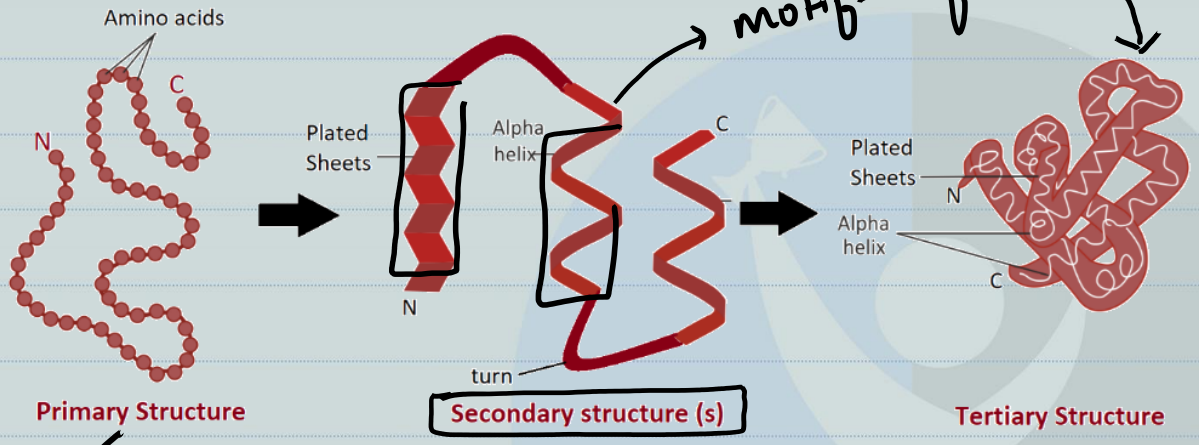
A protein domain is a conserved part of a given protein sequence and (tertiary) structure that can evolve, function, and exist independently of the rest of the protein chain.



molten globule : Abundance of 2° str but no 3° str.

TERTIARY STRUCTURE OF PROTEINS:

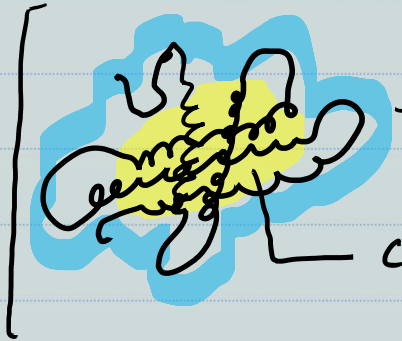
→ Single Subunit protein = myoglobin Albumin, Insulin.



motif → fold

- Driving force for protein folding is hydrophobic interaction
- main role is played by R-chain of aa.

molten Globule

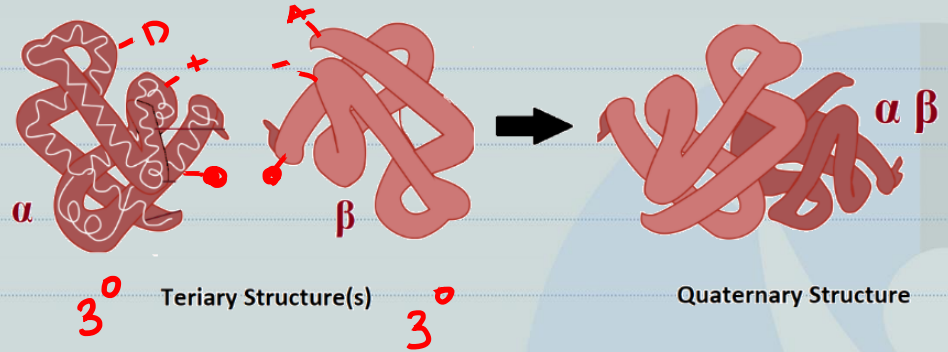


Polar R-chain at periphery (non-polar absent) → α-Helix

core: Non-Polar aa (common)
Polar aa (Rare), β-Sheet (more)

QUATERNARY STRUCTURE OF PROTEINS:

more than one subunit



Haemoglobin = 2α, 2β

IgG = 2L & 2H

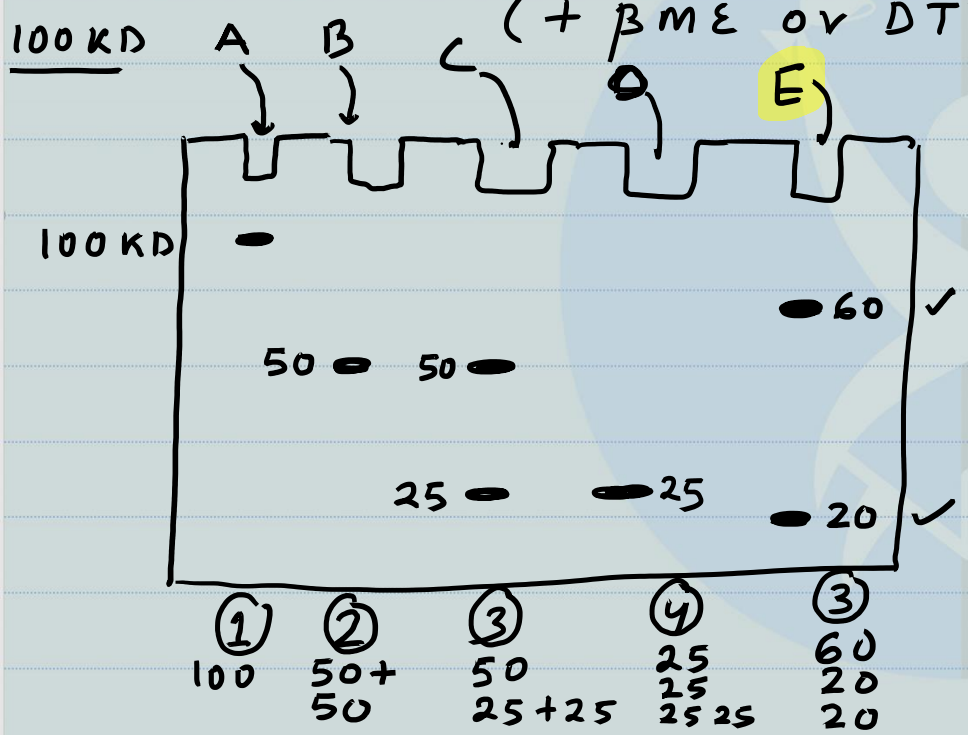
Nucleosome = $2H_2A$, $2H_2B$
$2H_3$, $2H_4$

Bacterial RNA polymerase
= 2α, β, β', ω

- Stabilized by non-covalent Interaction — Hydrogen bonding
  — Electrostatic interaction (salt bridge)
  — Hydrophobic interaction
  — vanderwall interaction
- May be covalent bond — Disulphide bond

SDS Analysis of Assessment of Oligomer Status

In presence of reducing condition (+ βME or DTT)



Protein = 100 KD

Gel permeation Chromatograph

Single subunit = ?

Multiple subunit = ?

Similar subunit : Homomer

Disimilar subunit : Heteromer

Tertiary / Quaternary str

Globular
- Non-polar aa & Polar aa
- Soluble protein

eg Myoglobin, Haemoglobin

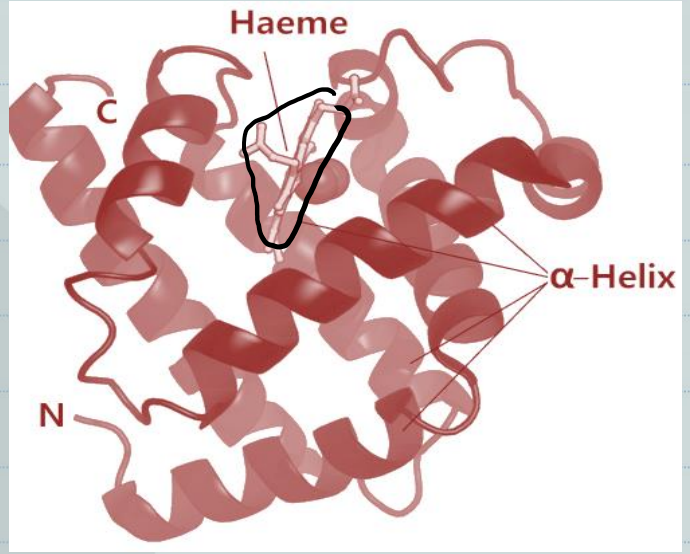


Fibrillar
- Abundance of non-polar aa
- Insoluble in water

eg Keratin, Fibroin, Collagen, Elastin



Globular Proteins:



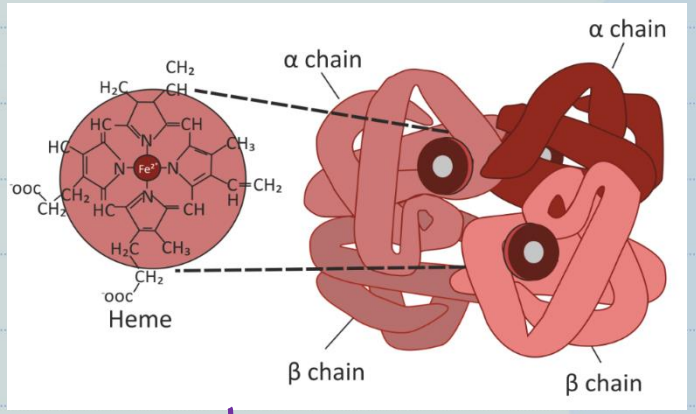
I. Myglobin — Haeme - Protein

- 153 amino acids (M_r 16.7 kDa)
- Secondary str: RH α-Helix
  loops
- O_2 binding (storage) protein of muscles
- Can bind to single O_2 molecule
- High affinity for O_2 as compare to haemoglobin
- Single subunit — Tertiary structure

Haemoglobin

Fe^{2+} — O_2

Hemoglobin (M_r 64,500), which contains four polypeptide chains and **four heme** prosthetic groups, in which the iron atoms are in the **ferrous (Fe^{2+}) state**.

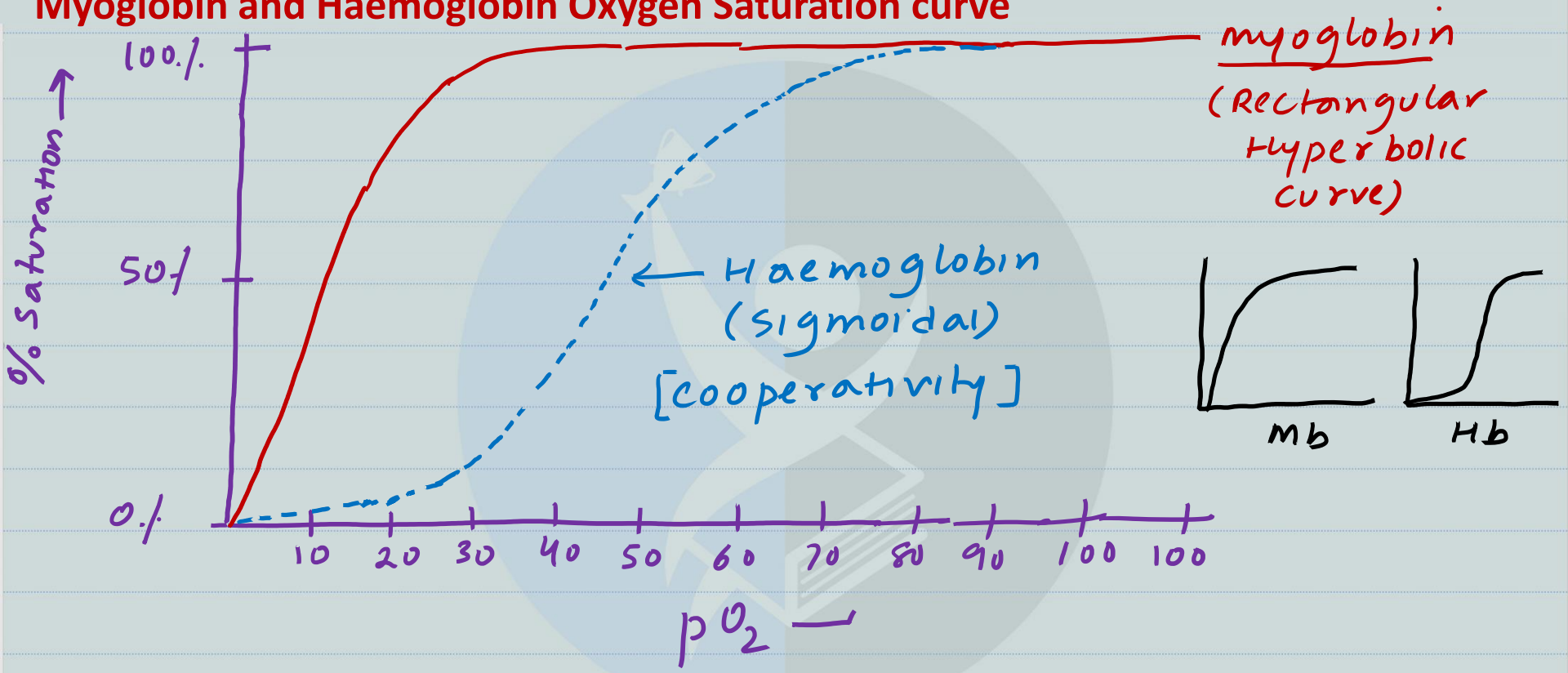


Adults : 2α + 2β (2S) — Rare
Embryo : 2α + 2ε
Fetus : 2α + 2γ

- Every subunit is similar to myoglobin — α-Helix
- Quaternary structure

- Cytosol of RBC
- Maximum can bind to 4 O_2 molecule.

Myoglobin and Haemoglobin Oxygen Saturation curve



Fibrous Protein

RH α-Helix → Coiled coil dimer (Left handed)

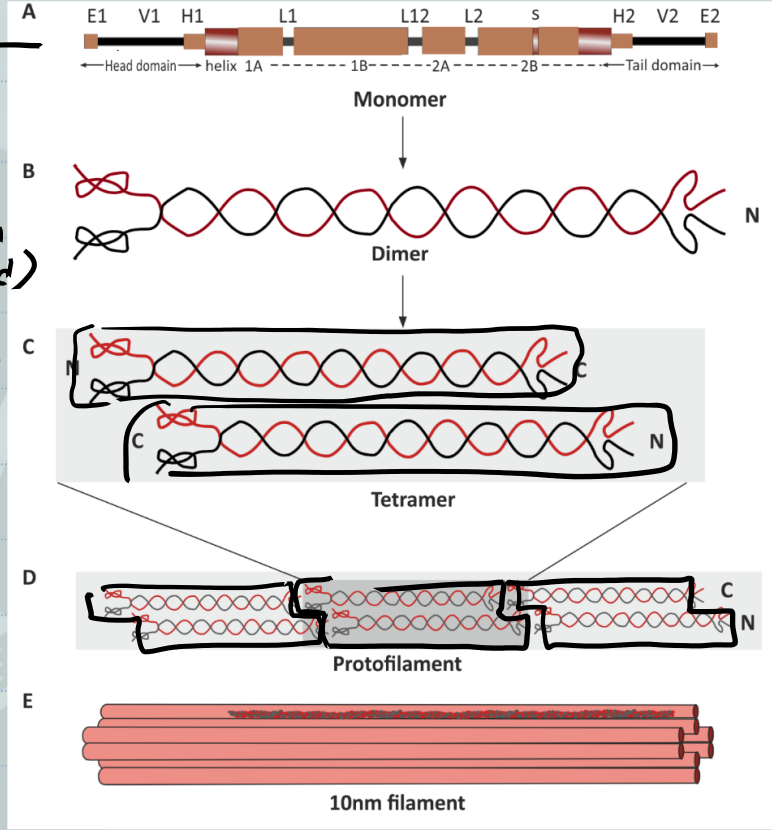
1. **Keratin** — Hair, Nail

- ✓ Monomer — RH α-Helix
- ✓ Dimer — LH coiled coil dimer
- ✓ Tetramer — Antiparallel coiled coil dimer
- ✓ Protofilament — length wise arrangement of tetramer
- ✓ 10 nm filament
  - → Bundles of Protofilament
  - → Keratin filament

- Extra stability : Disulphide Bond

Disorders

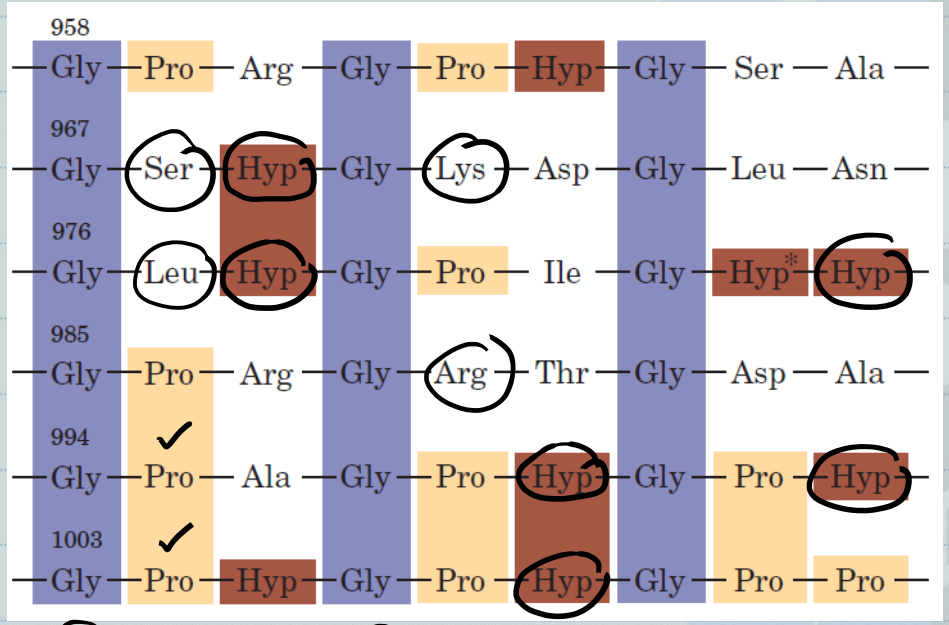
- **Epidermolysis bullosa simplex (EBS)**
- **Epidermolytic hyperkeratosis (EHK)**



2. Collagen : most abundant protein of animals

- Present in extracellular matrix (ECM) & Basal lamina

Primary Structure



– (Gly) – X – Y – (Gly) – X – Y – (Gly) – X – Y –

Prolyl hydroxylase requires

O_2, ascorbic acid (vitamin C) and Fe^{2+}.

Scurvy → vit C deficiency

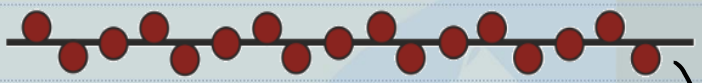
Gly – X – Y

↑ Pro ↑ Hydroxy Proline

Primary Structure

Gly-X-Y-Gly-X-Y-Gly-X-Y-Gly-X-Y- ✓

Secondary Structure



3 Polypeptide chains

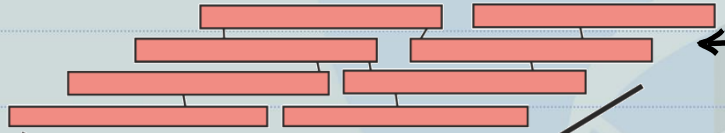


Tropocollagen ~300 nm

$\phi = -50$ $\Psi = +150$

Collagen Helix – left handed (NO H-bonding)

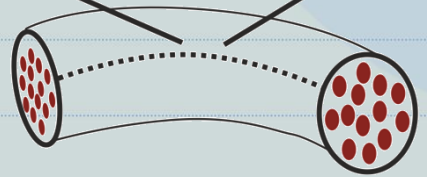
Tripple helix (Right handed) or Tropo collagen (H-bonding)



✓ **Fibrils ~1 µM**

← covalent Bond

– (merodesmosine bond) or Lysine nor leucine Bond



Collagen Fibers ~10 µM

Type of collagen depend on type of α polypeptide used for triple helix

Type	Polypeptide composition	Distribution
I	$\alpha 1(I)_2$ $\alpha 2(I)$	Skin, bone, tendon, blood vessels, cornea
II	$\alpha 1(II)_3$	Cartilage, intervertebral disk
III	$\alpha 1(III)_3$	Fetal skin, blood vessels
IV	$\alpha 1(IV)_2$ $\alpha 2(IV)$	Basement membrane
V	$\alpha 1(V)]_2$ α 2(V)	Placenta, skin



- more than 28 different types of collagen

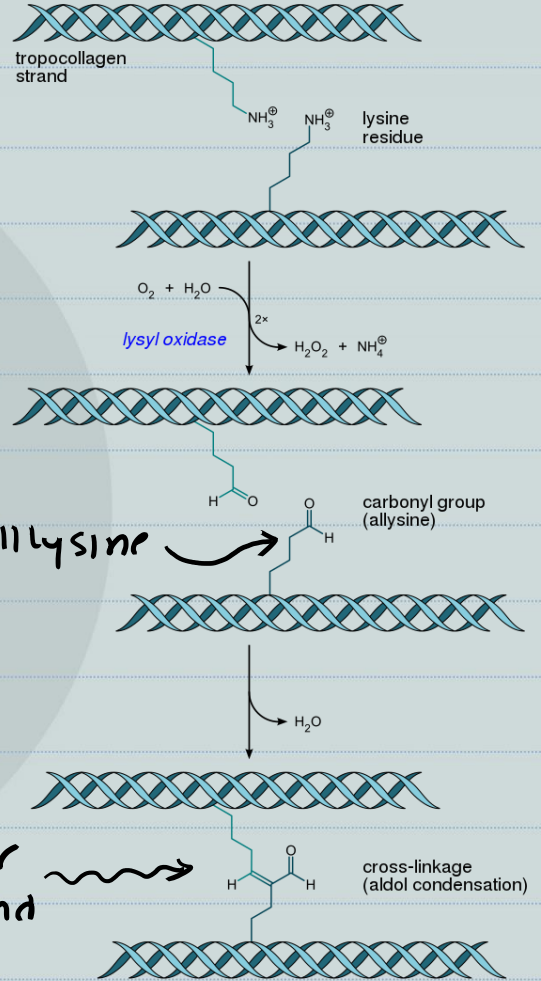
Collagen Fibrils Are Covalently Cross-Linked

Covalent bonds are derived from Lys and hydroxy lysine

✓**Lysyl oxidase**, (Cu-containing enzyme) converts Lys residues to those of the aldehyde **allysine**

Cross-linkage : Aldol condensation

Beta-amino propio nitrile (BAPN) inactivates lysyl oxidase and causes lathyrism



Collagen Defects

- Osteogenesis imperfecta (brittle bone disease): Mutation in type I collagen
- Ehlers–Danlos syndromes → mutation in various type of collagen

HAPPY LEARNING

THANKS

IfAS

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