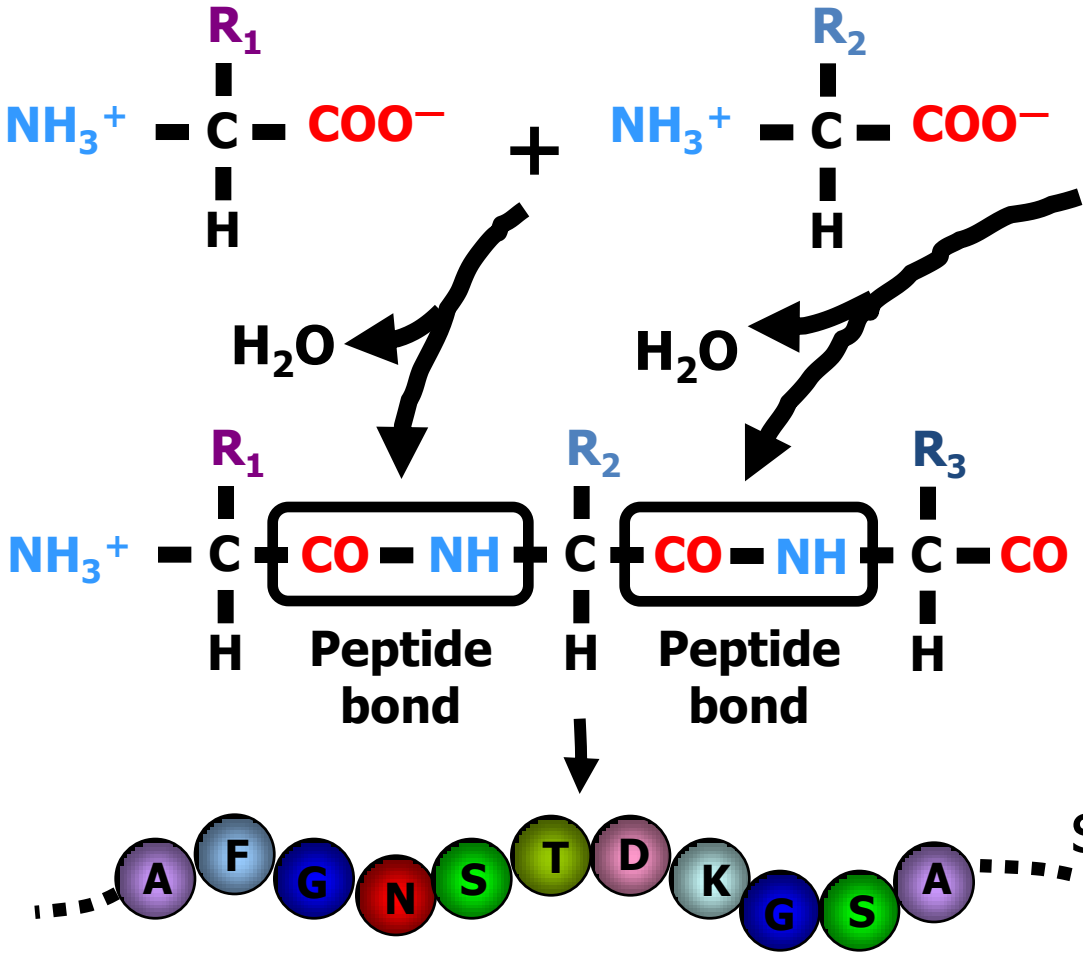
# Different level of structures in protein and its characterization

Rizwan Hasan Khan

# Proteins are linear polymers of amino acids




A carboxylic acid condenses with an amino group with the release of a water

The amino acid sequence is called as primary structure

# Hierarchical nature of protein structure

Primary structure (Amino acid sequence)

↓

Secondary structure (α-helix & β-sheet)

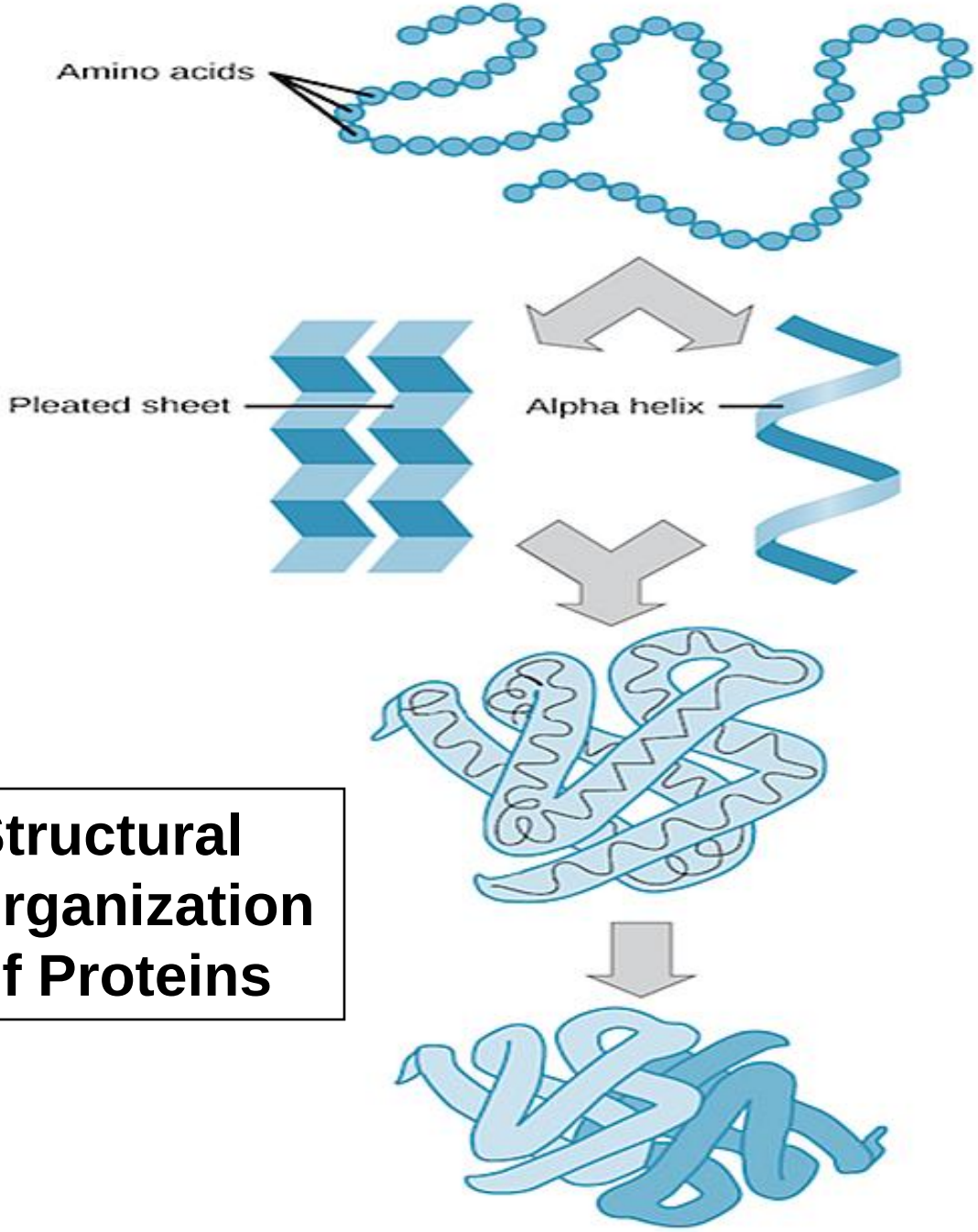
↓

Tertiary structure (Three-dimensional structure formed by assembly of secondary structures)

↓

Quaternary structure (Structure formed by more than one polypeptide chains)

# Structural organization of Proteins

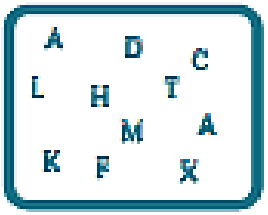



**Primary protein structure**
sequence of a chain of animo acids

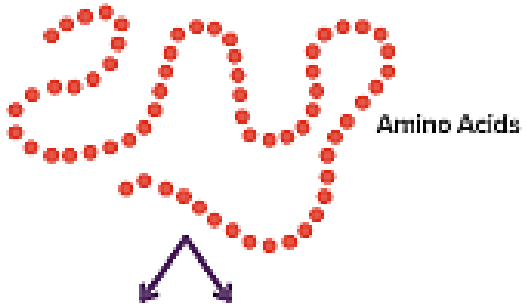
**Secondary protein structure**
hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern

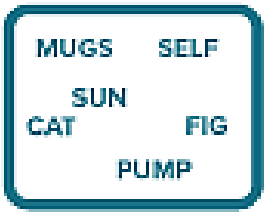
**Tertiary protein structure**
three-dimensional folding pattern of a protein due to side chain interactions

**Quaternary protein structure**
protein consisting of more than one amino acid chain

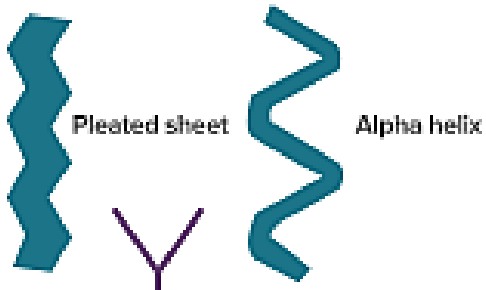


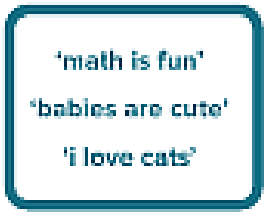

Alphabets ⟷ Primary structure



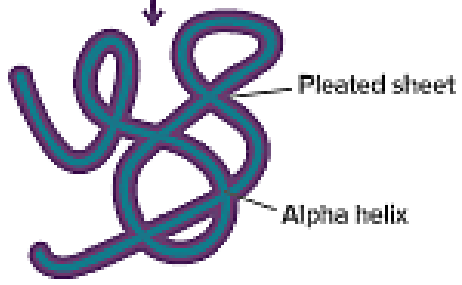



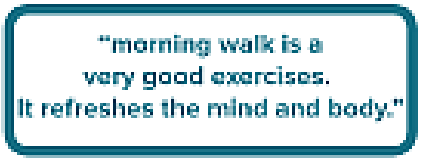

Words ⟷ Secondary structure




Sentences ⟷ Tertiary structure

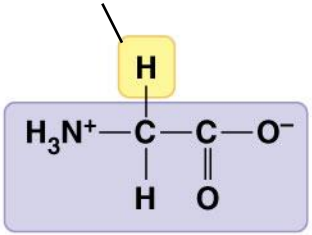


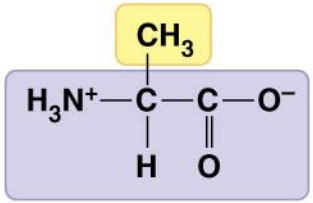

Paragraph ⟷ Quaternary structure

**Nonpolar side chains; hydrophobic**

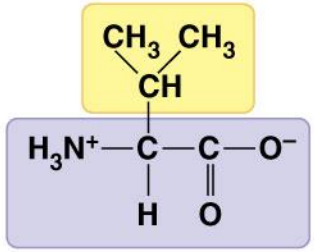
**Side chain**



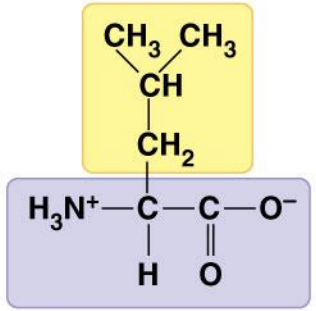
**Glycine (Gly or G)**



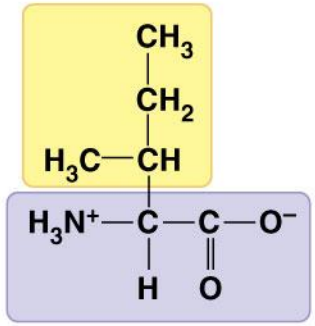
**Alanine (Ala or A)**



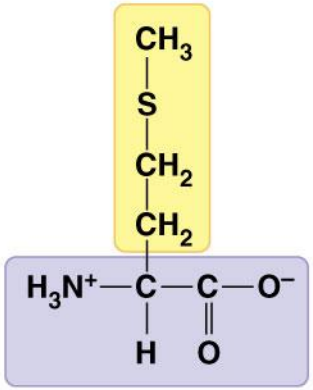
**Valine (Val or V)**



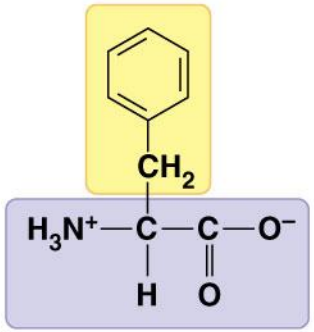
**Leucine (Leu or L)**



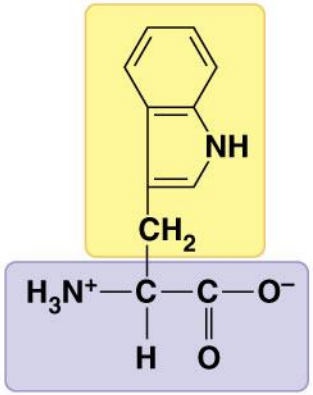
**Isoleucine (Ile or I)**



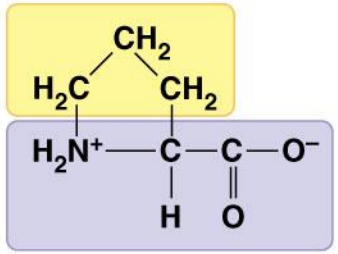
**Methionine (Met or M)**



**Phenylalanine (Phe or F)**

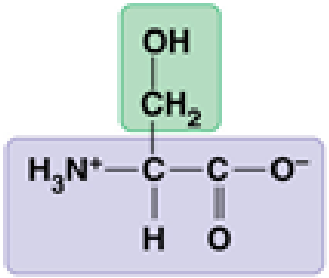


**Tryptophan (Trp or W)**

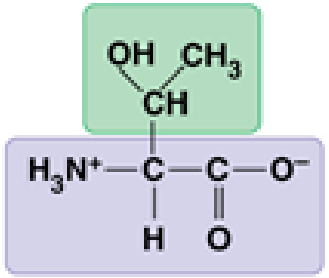


**Proline (Pro or P)**

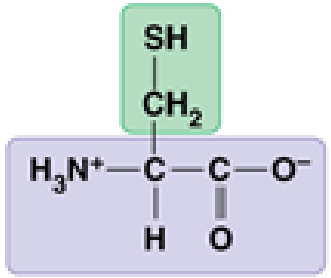
## Polar side chains; hydrophilic



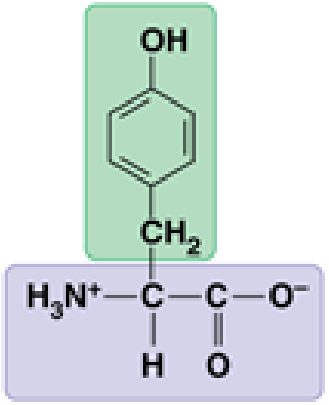

Serine
(Ser or S)



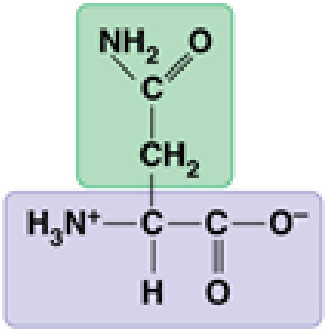

Threonine
(Thr or T)



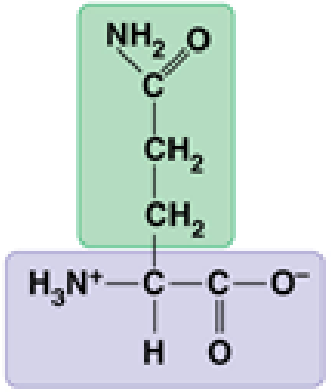

Cysteine
(Cys or C)




Tyrosine
(Tyr or Y)




Asparagine
(Asn or N)

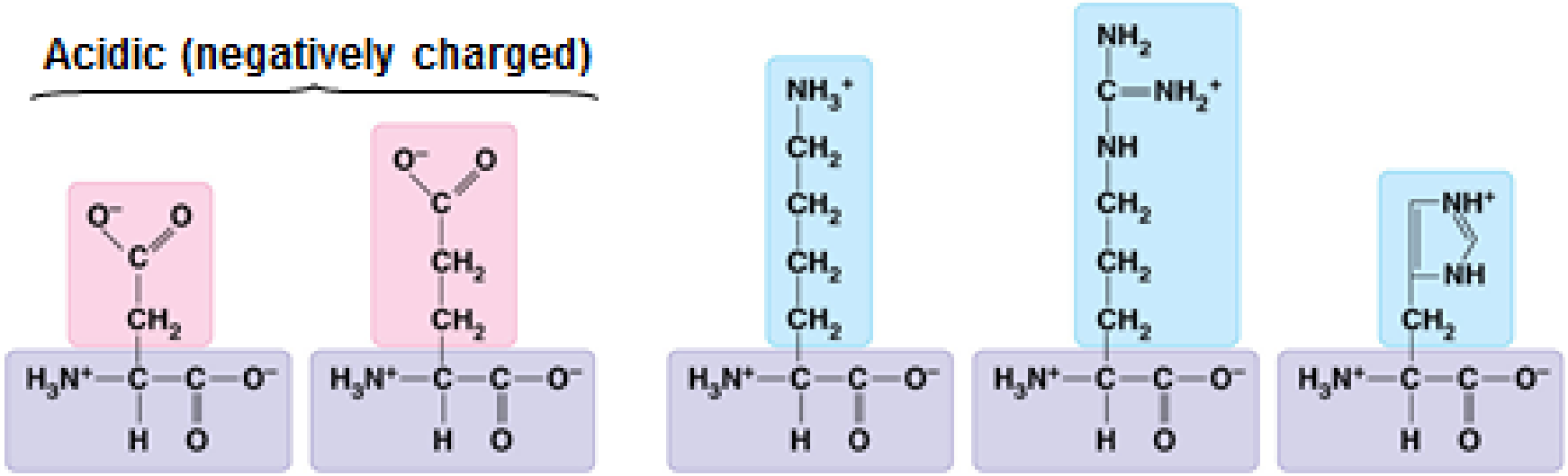



Glutamine
(Gln or Q)

## Electrically charged side chains; hydrophilic

Acidic (negatively charged)

Basic (positively charged)



Aspartic acid (Asp or D)

Glutamic acid (Glu or E)

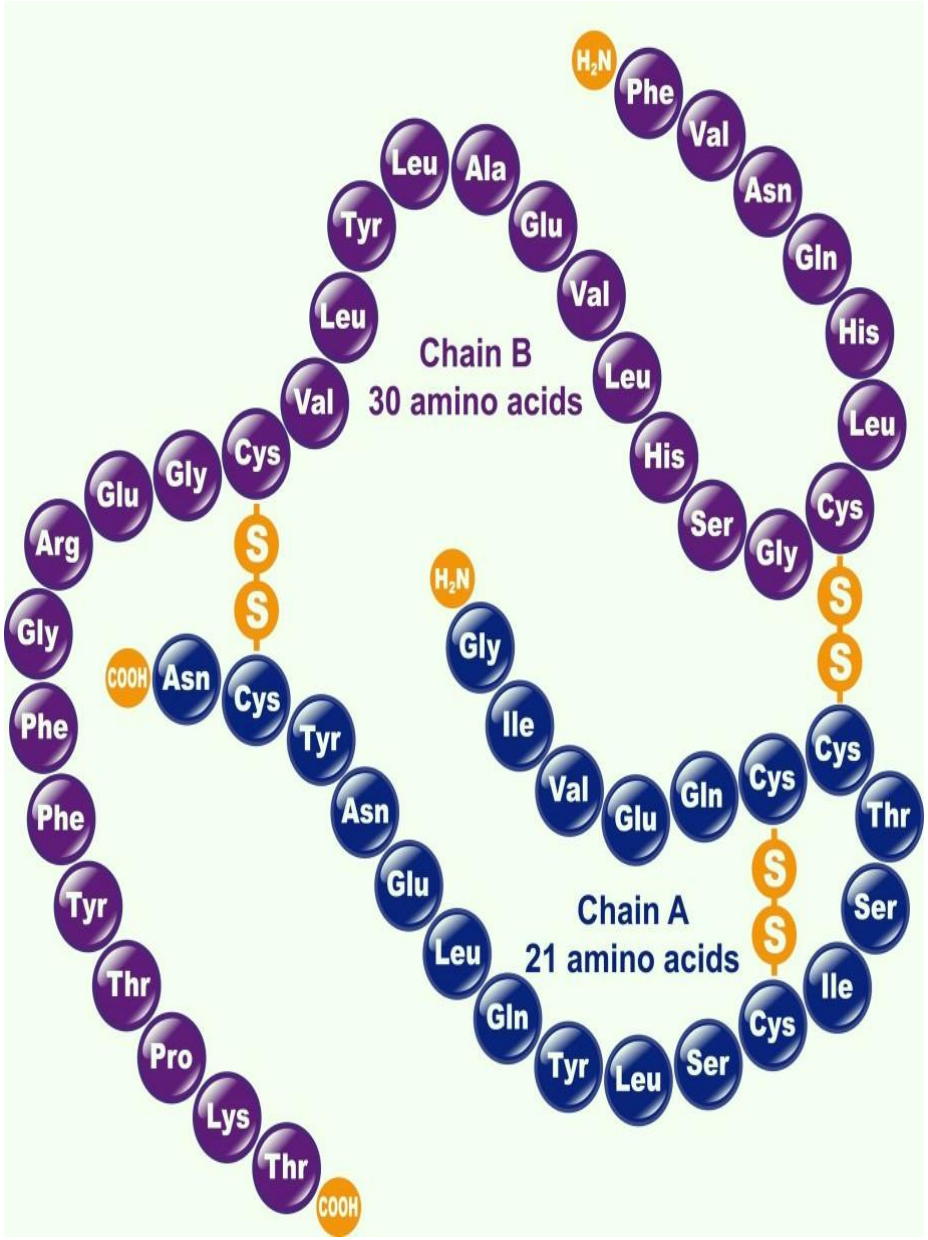
Lysine (Lys or K)

Arginine (Arg or R)

Histidine (His or H)

# LEVELS OF PROTEIN ORGANISATION-

## PRIMARY STRUCTURE

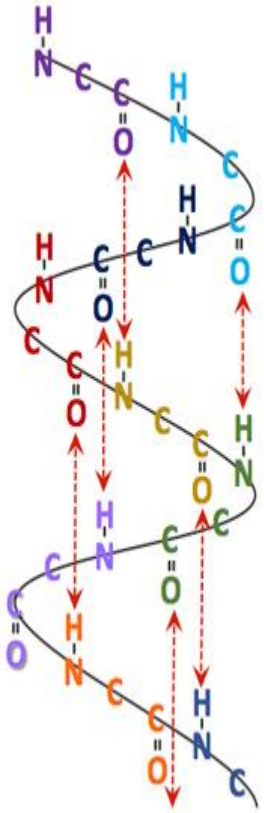


# Polypeptide Chain

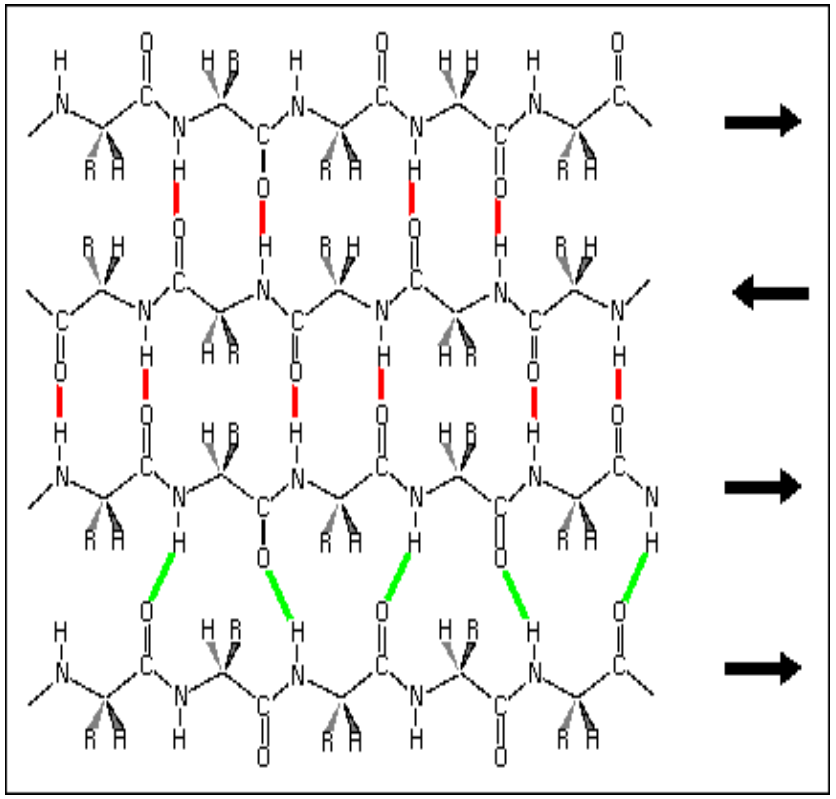
**Engine as N-terminal**

**Guard coach as C-terminal**

# SECONDARY STRUCTURE IN PROTEINS



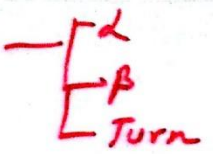
α helix



β pleated sheet

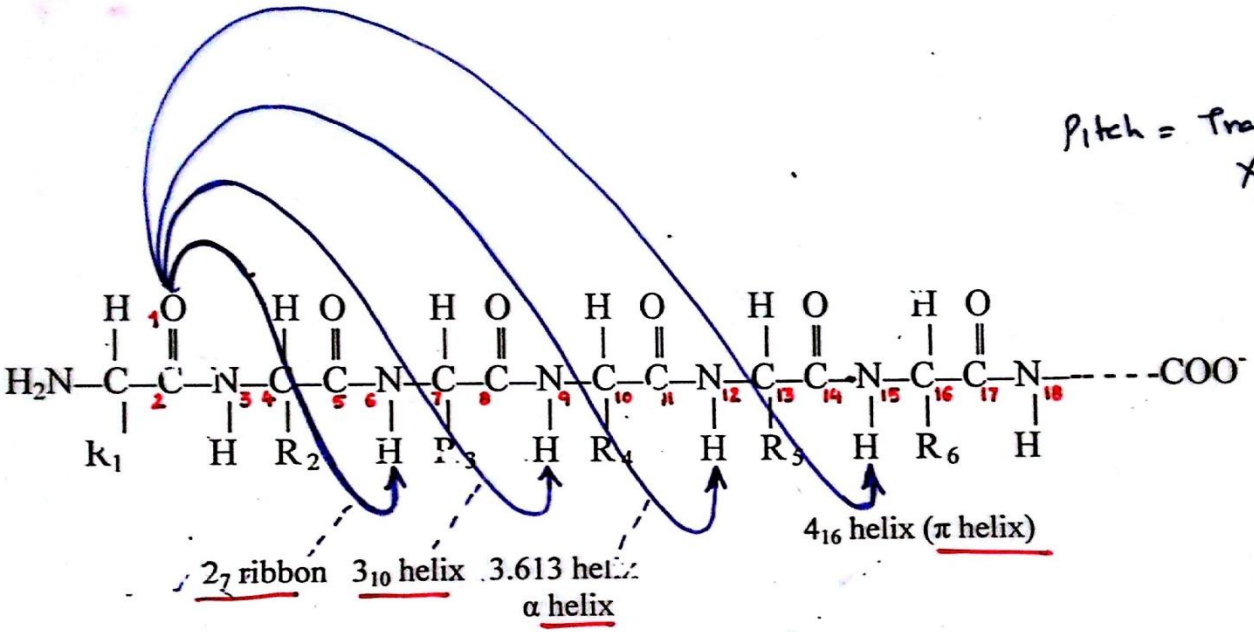
# SECONDARY STRUCTURE

- α
- β
- Turn




Rise 1.5A°
100 degree rt

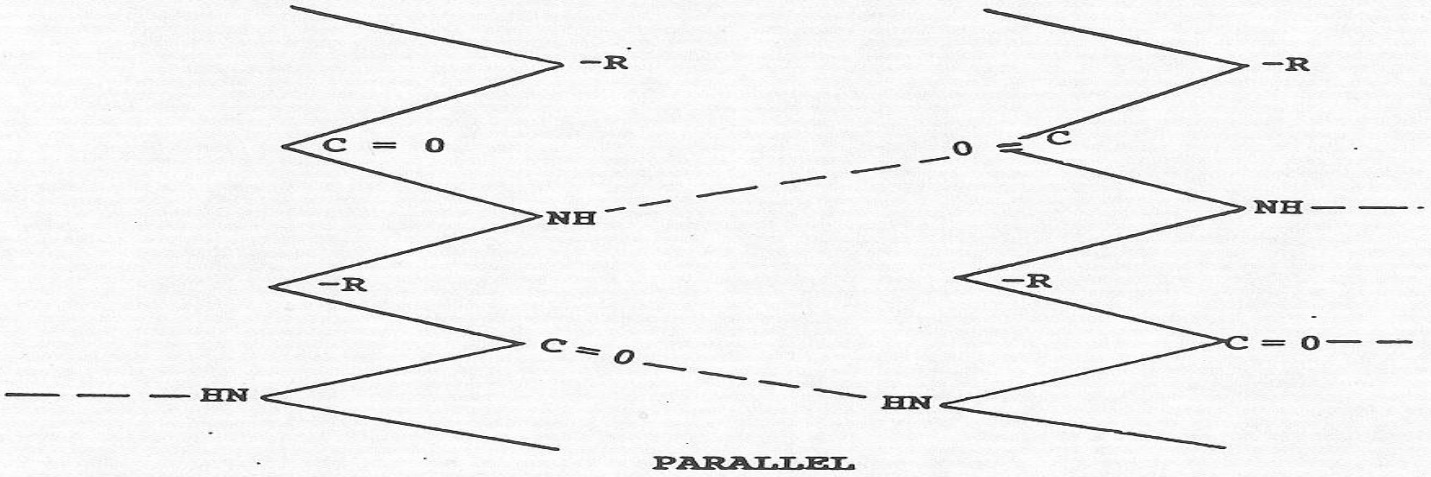
Pitch = Translation (1.5) X Numb of res per turn S.C = 5.4



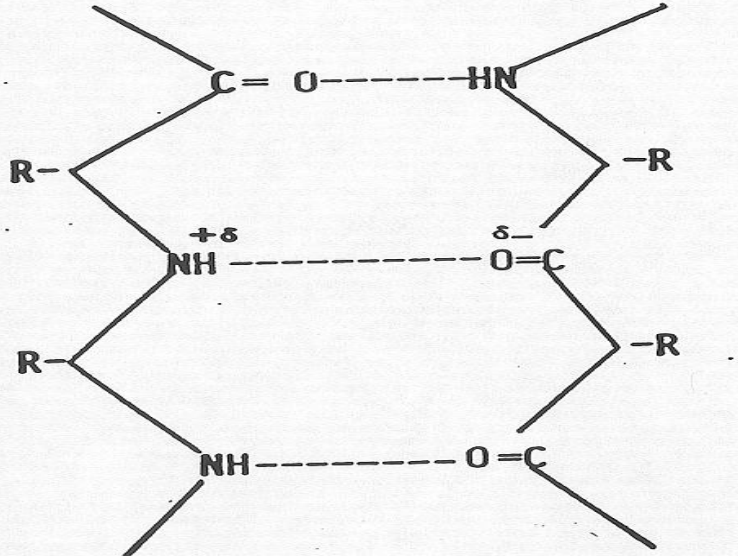

no of residues in one turn

| | |
|---|---|
| $2_7$ ribbon (7) amino hydrogen of 3rd aa | 2 |
| $3_{10}$ helix (10) amino hydrogen of 4th aa | 3 |
| 3.613 helix (α helix) (13) amino hydrogen of 5th aa | 3.6 |
| $4_{16}$ helix (π helix) (16) amino hydrogen of 6th aa | 4 |

Pleated sheets structure --- hydrogen bonding between adjacent ploypeptide.

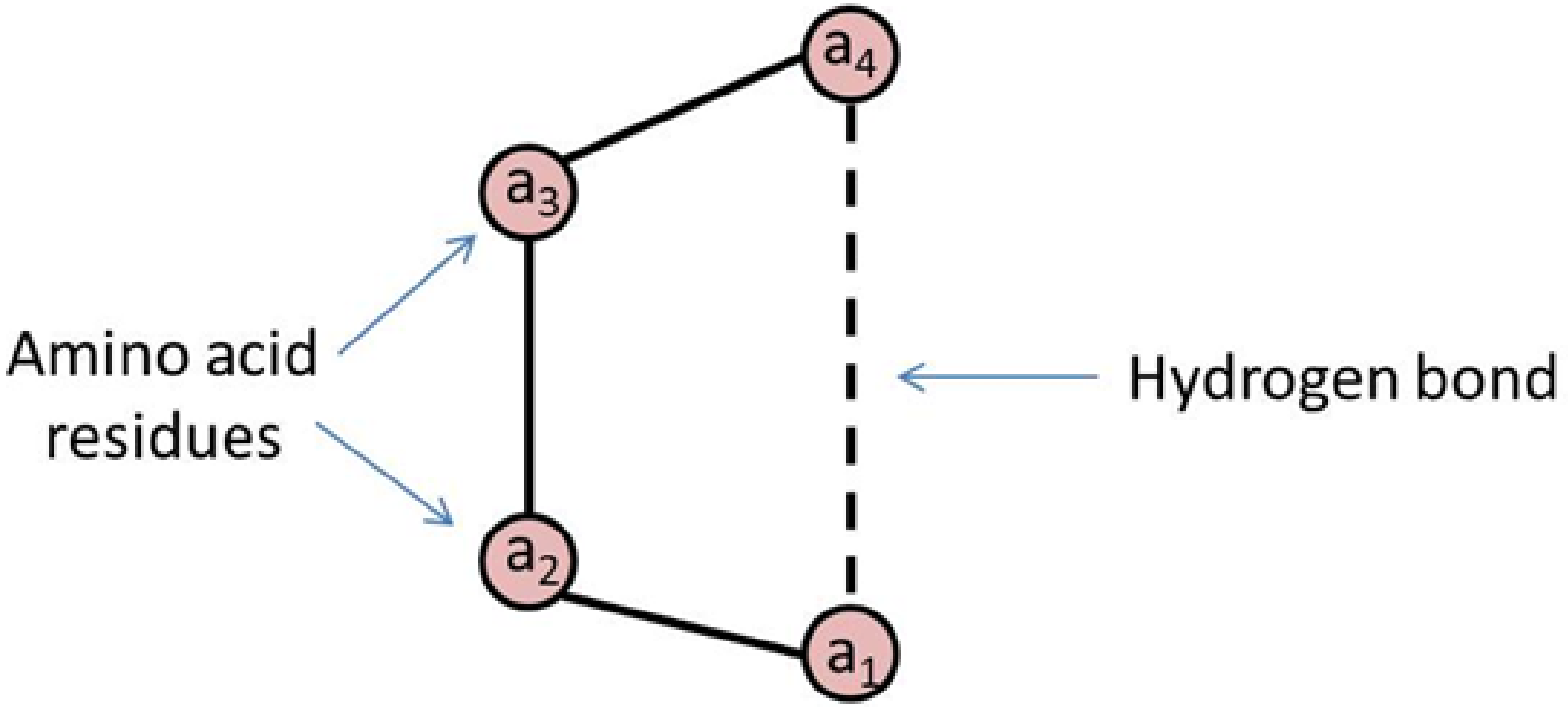



Anti-parallel pleated sheets structure --- hydrogen bonding between adjacent polypeptides.
(β – Keratin or silk fibroin)



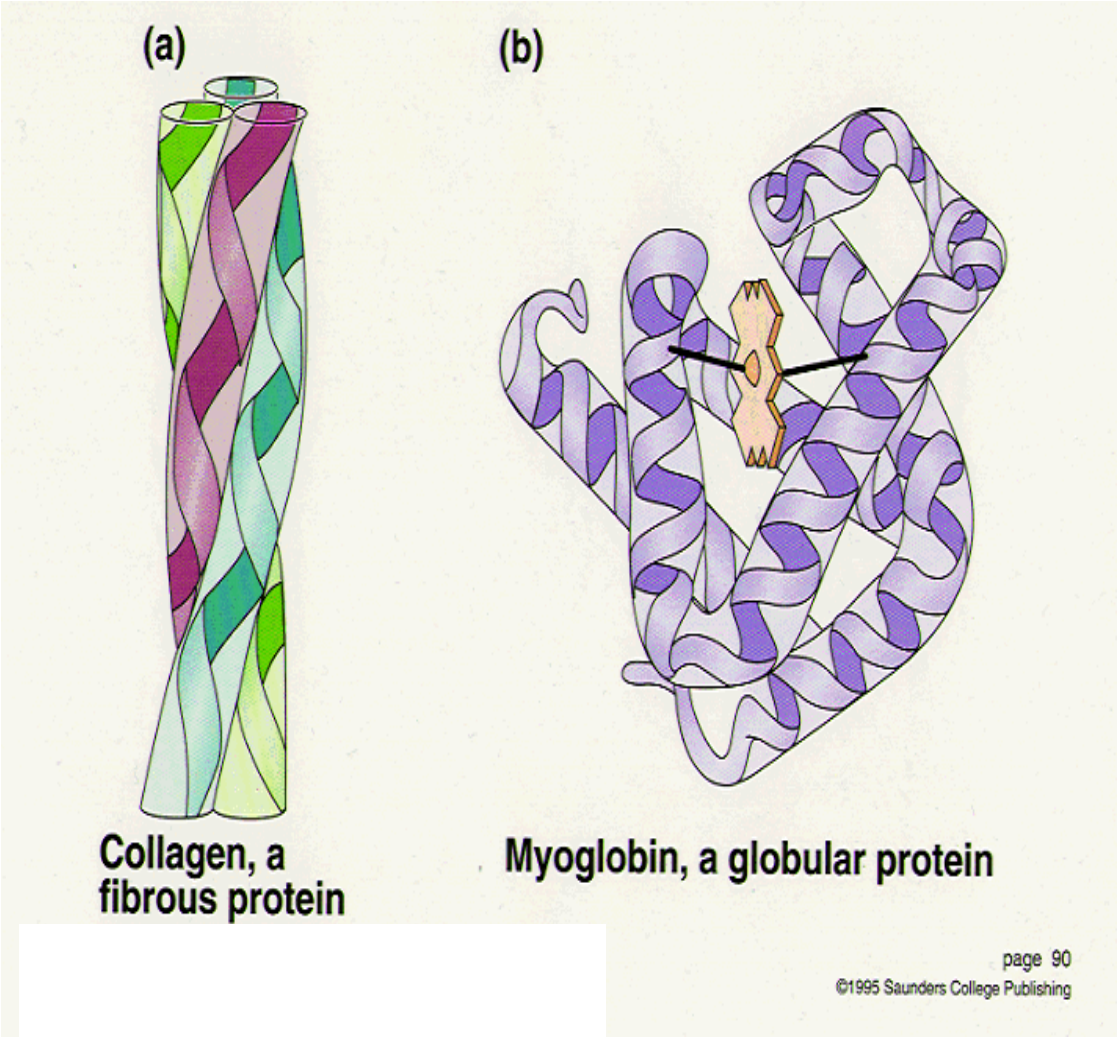

**β-sheet**

# Beta turn



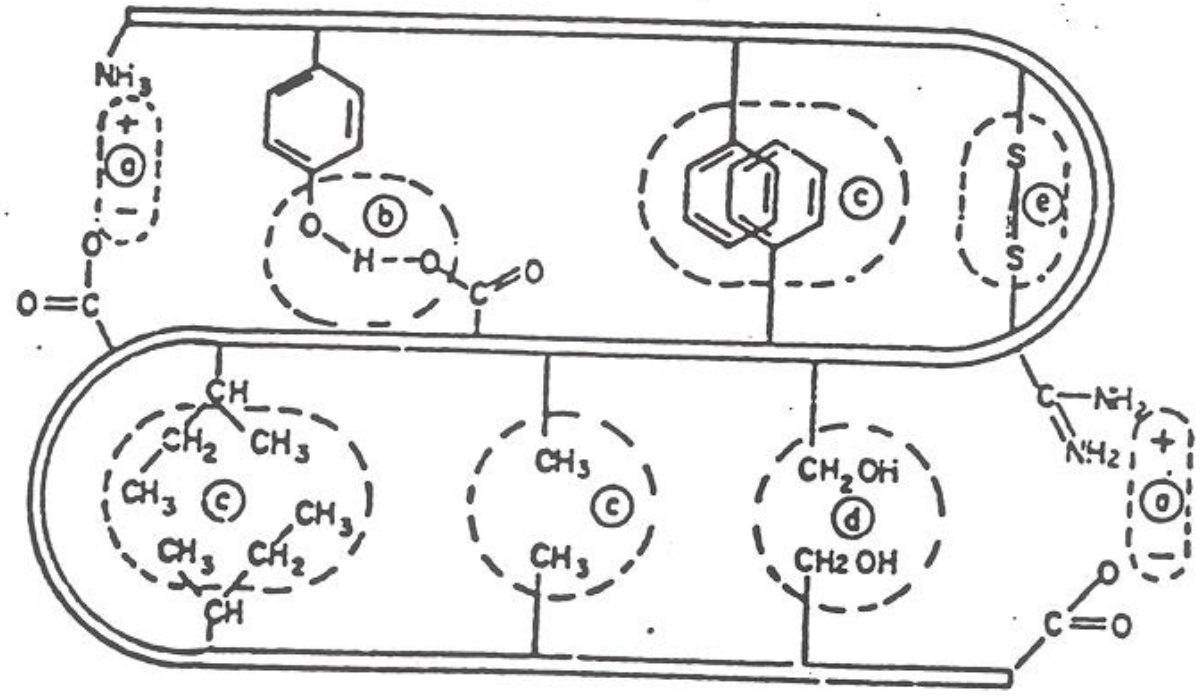

Beta turn

# TERTIARY STRUCTURE

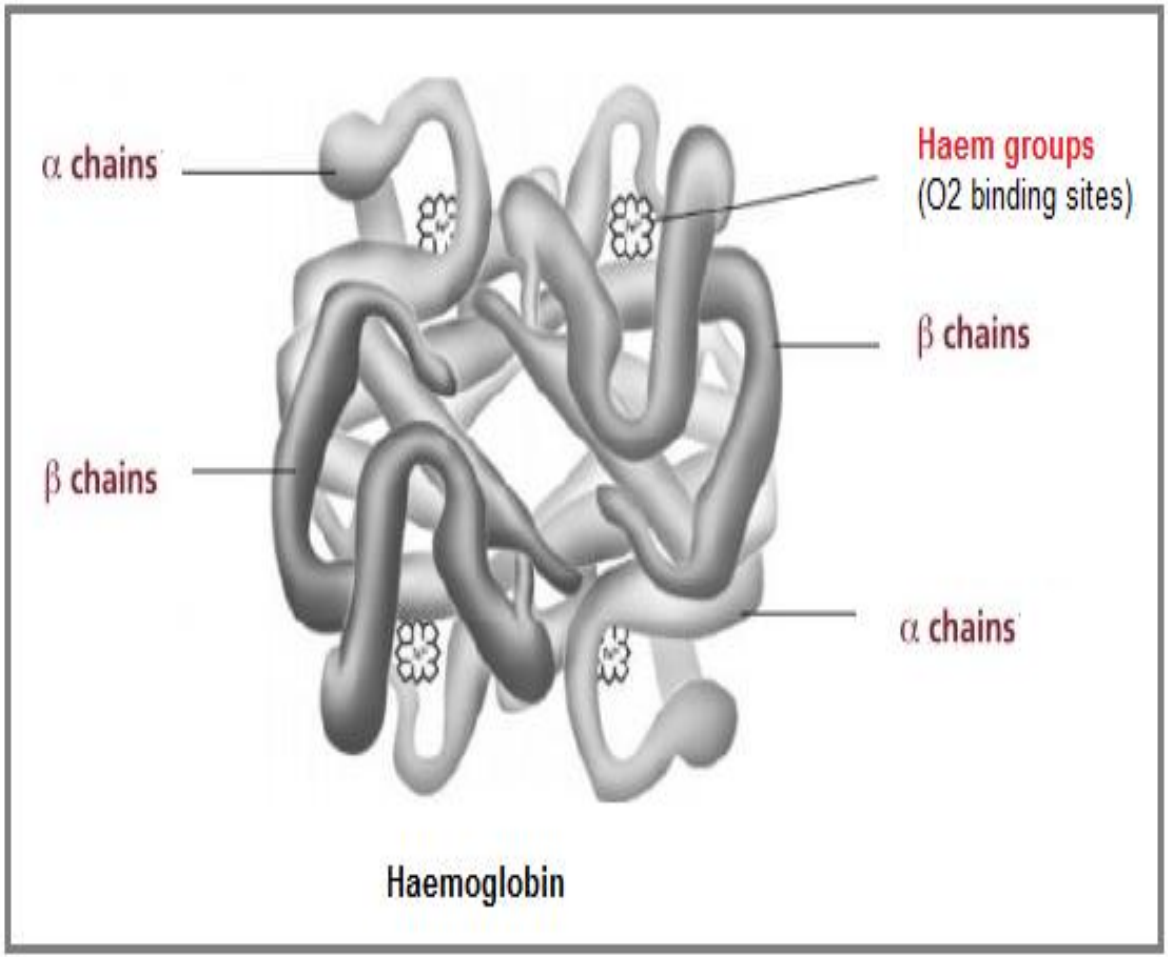


Tertiary Structure

Bonds which stabilize tertiary and quaternary structures of proteins: (a) electrostatic interaction; (b) hydrogen bonding; (c) hydrophobic interaction; (d) deplore-dipole interaction; and (e) disulfide linkage.



# QUATERNERY STRUCTURE

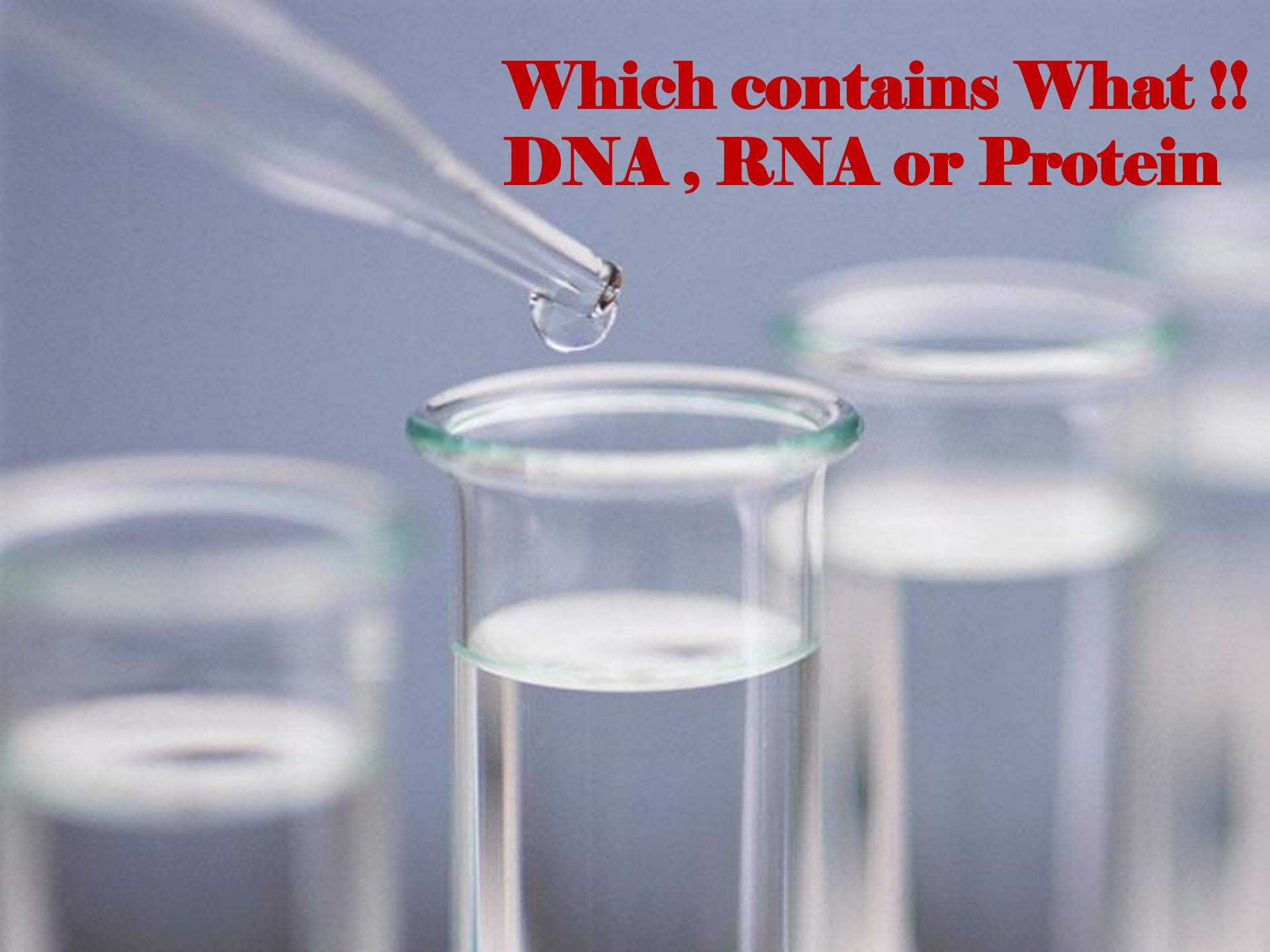


# PROTEIN DETERMINATION METHODS

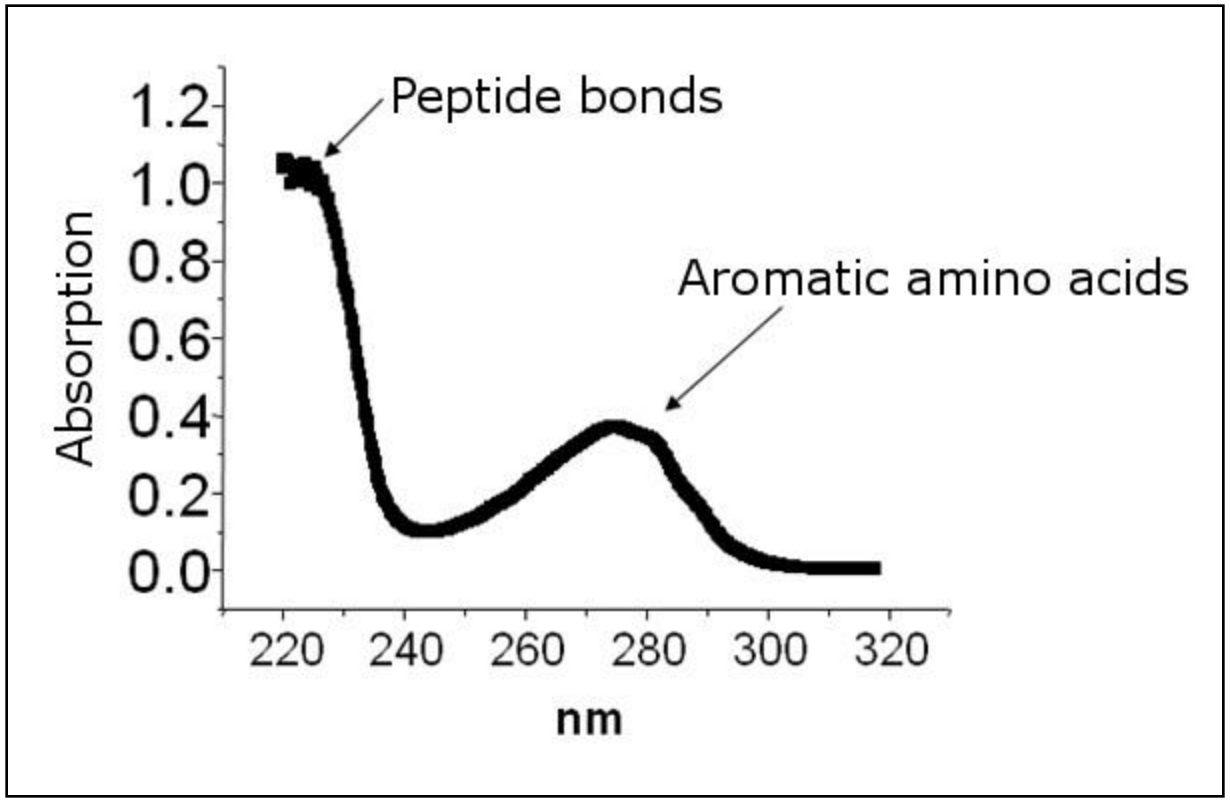
Ultraviolet Method.

Fluorescence Method.

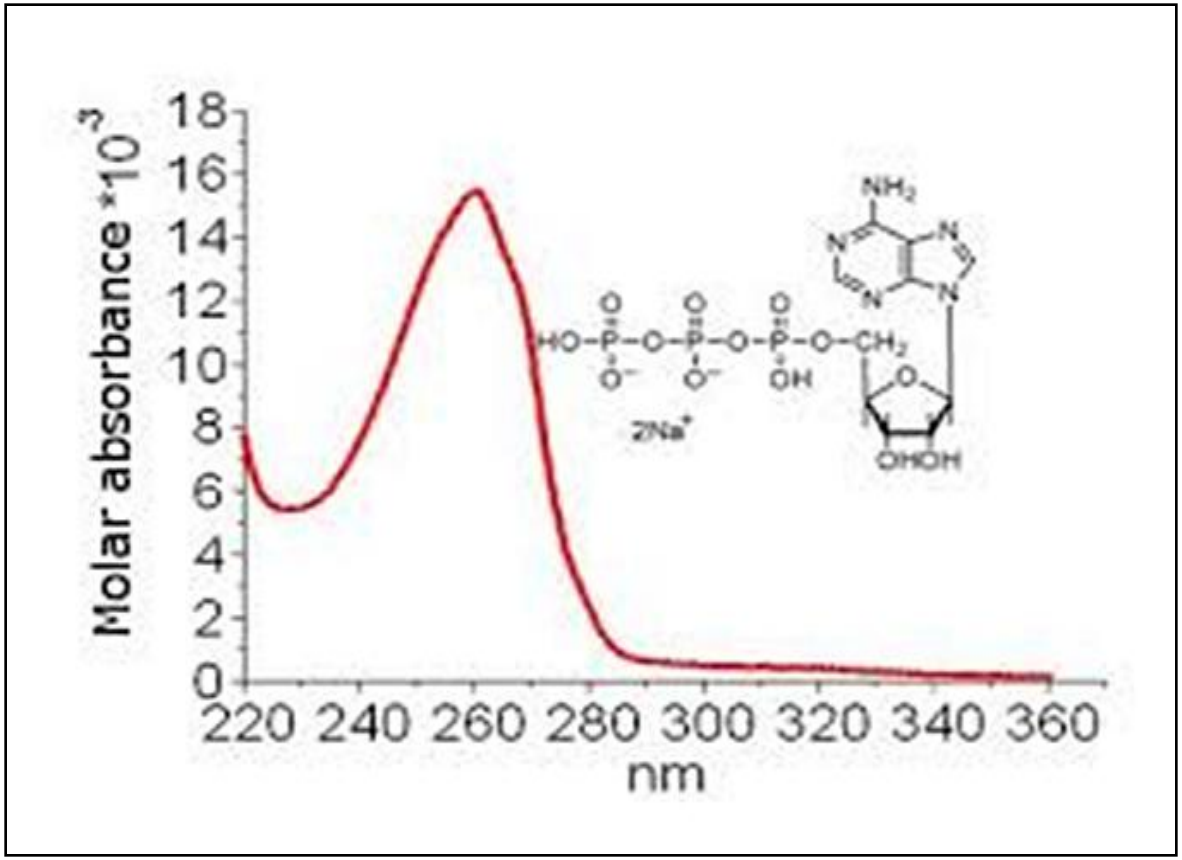
Lowry Method.


Which contains What !!
DNA , RNA or Protein

# Absorption spectrum of protein



# Absorption spectrum of NA



# Ultra-violet Absorption (UV) at 280 nm

1. Chromophoric / aromatic amino acids (Trosine, Tryptophan,Phe).
2. Absorption at 280 nm.
3. detect proteins in the range of 50-100 µg
4. “Non-destructive means to determine protein”.
5. Calculation protein conc. based upon absorption.

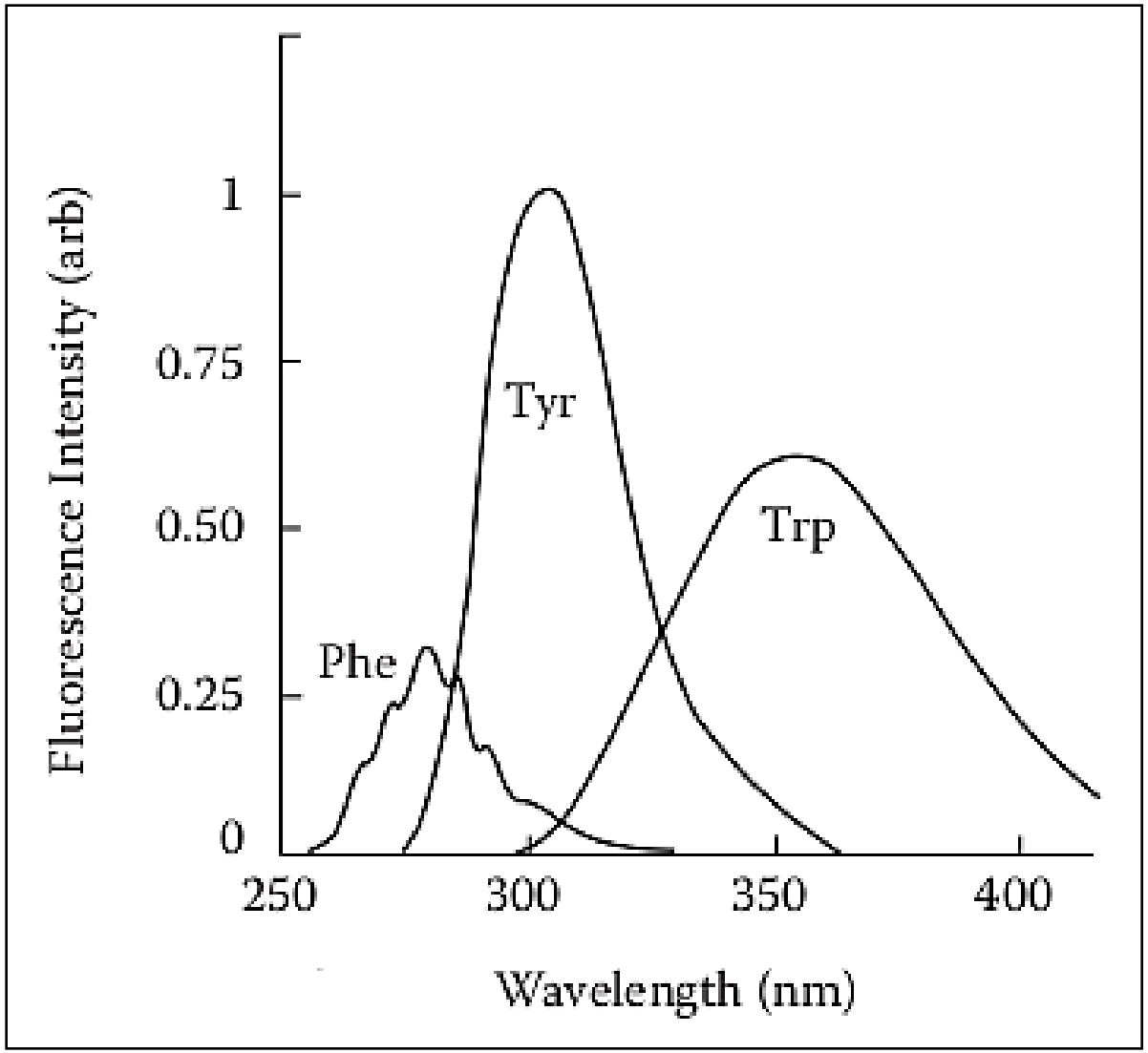
# Fluorescence Method

Tyrosine, tryptophane and phenylalanine are fluorescent compound.

Excite the amino acids at 280 nm.

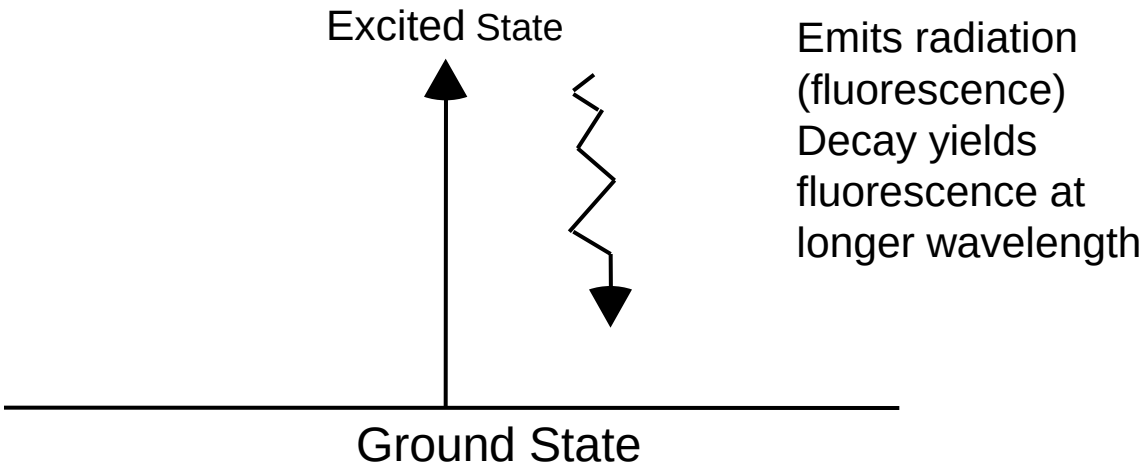
Measure emission at 348 nm.

Advantage: more sensitive than UV absorption.


Fluorescence Intensity (arb)
1
0.75
0.50
0.25
0
Tyr
Trp
Phe
250
300
350
400
Wavelength (nm)

# Fluorescence Method

What is fluorescence and how to measure it?

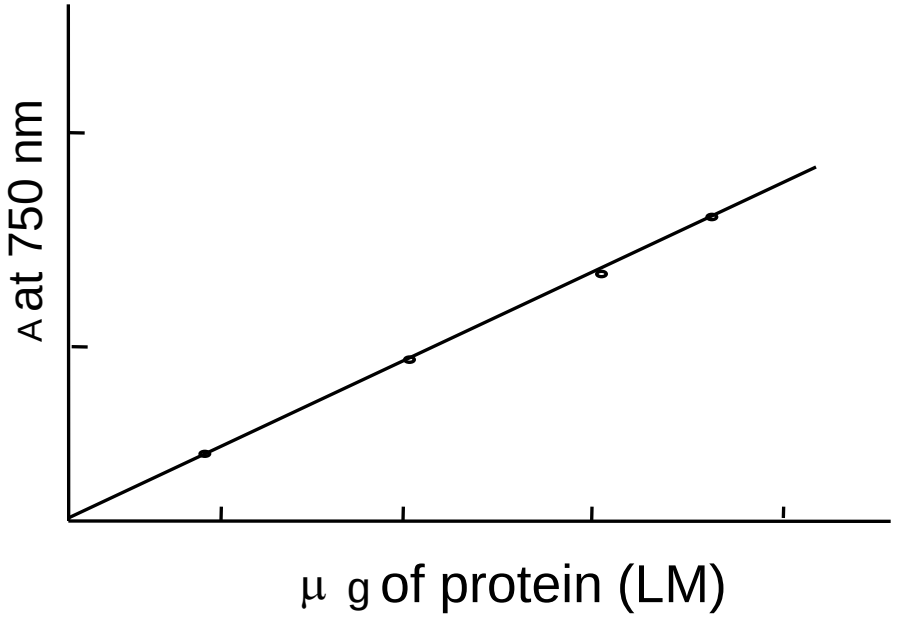



By using specific λ (wavelength) to excite and measure output at a specific λ. It is rather specific.

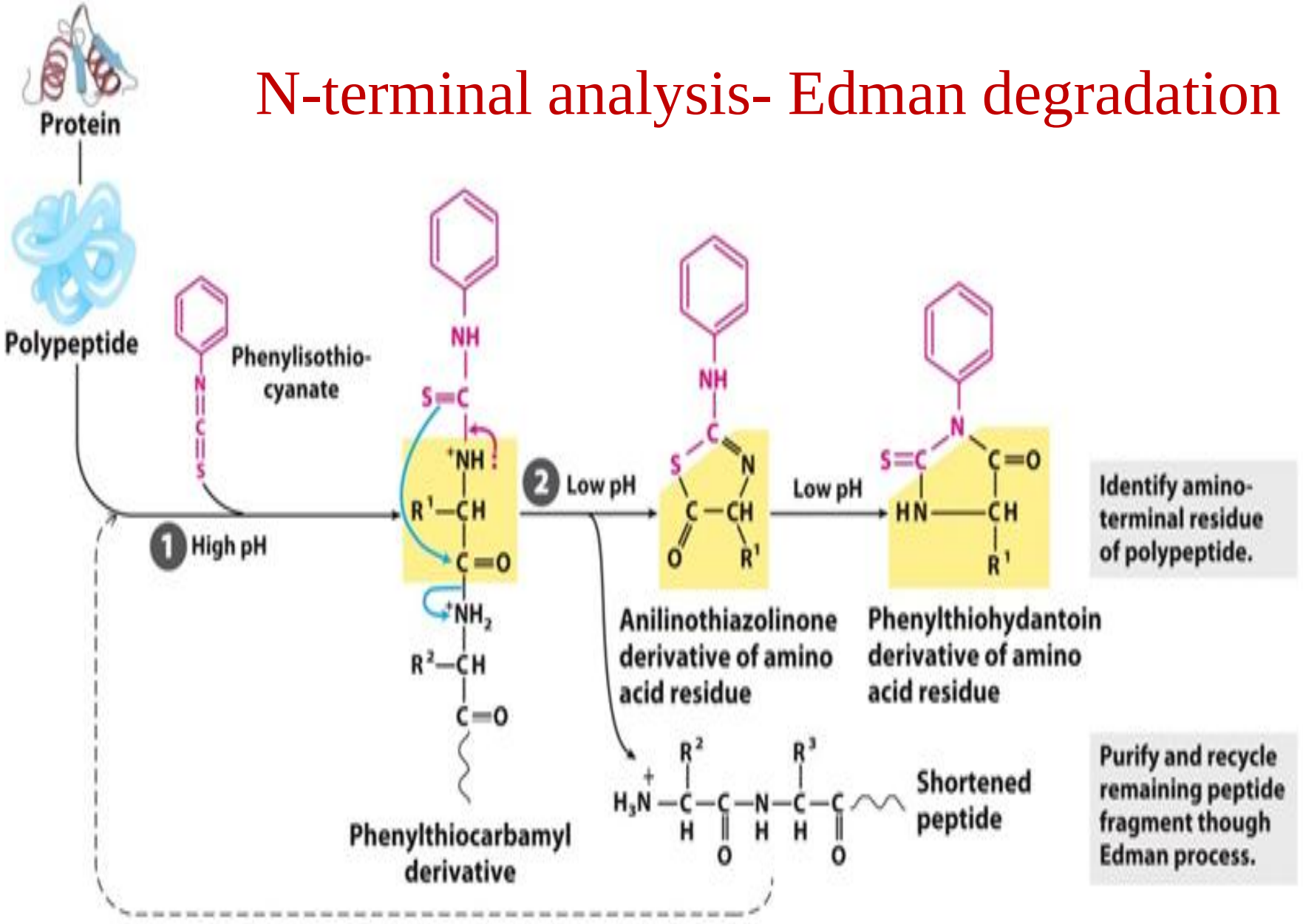
Problems: Turbidity/Quenching (self or others)/Expensive/ Quantitation is difficult.

# Lowry Method: (one of most sensitive methods)

- $Cu^{++}$ in alkaline solution to form complexity with protein.
- Cu++ catalyses oxidation of phenol group of tyrosine with phosphomolybdic-phosphotungstic acid.

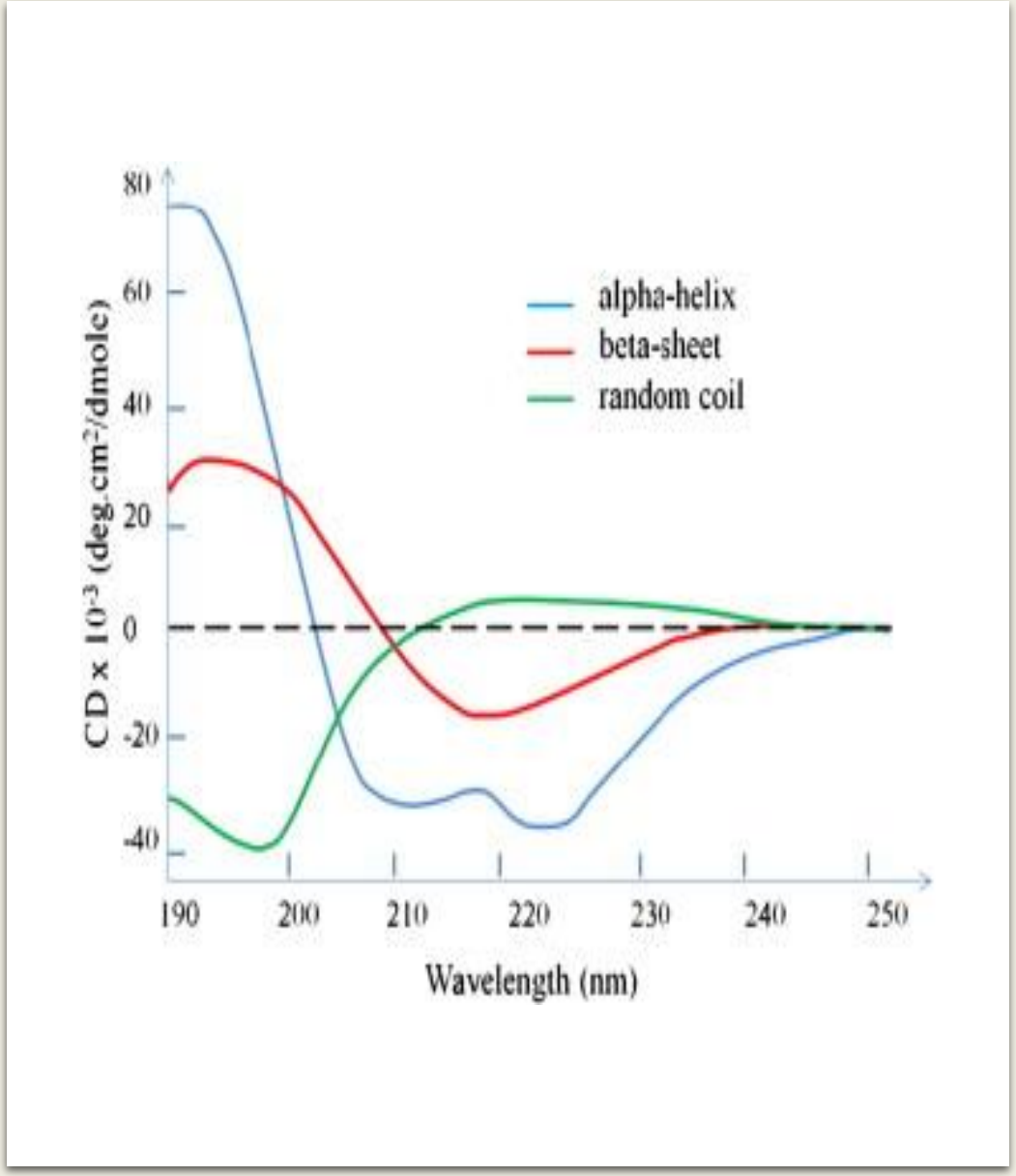


# N-terminal analysis- Edman degradation



# Determination of secondary structure of proteins

| | λ max | MRE |
|---|---|---|
| α helix | 191 | +77,000 |
| | 208 | -33000 |
| | 222 | -36,000 |
| | | |
| β sheet | 195 | +32,000 |
| | 217 | -18,000 |
| | | |
| Random coil | 197 | -42,000 |
| | 217 | +46,00 |



# Helical content of proteins determined by CD compared with X ray analysis

| Protein | % α helix by CD | % α helix by X ray diffraction |
|---|---|---|
| Myoglobin | 77 | 77 |
| Lysozyme | 29 | 29 |
| Ribonuclease | 18 | 19 |
| Lactate dehydrogenase | 31 | 29 |
| Chymotrypsinogen | 9 | 6 |

# Structure determination method X-ray crystallography


Thank
You!