Protein Engineering:

Structural & Functional Analysis

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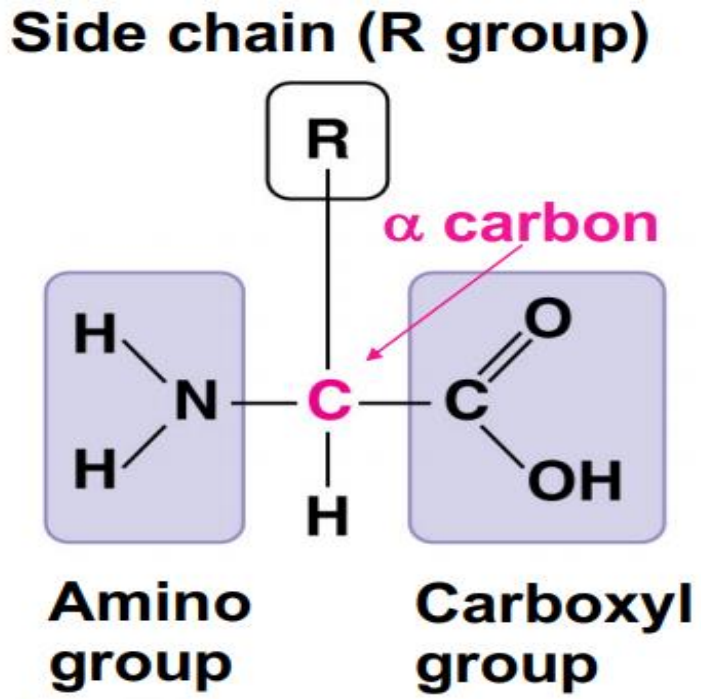
Introduction:

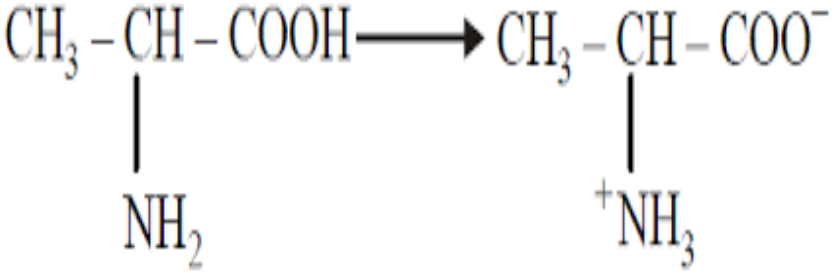
- Proteins are biologically functional molecules and polymers of structural units called **amino acids** i.e. amino acids are the building blocks (monomers) of proteins.

- Amino acids are organic molecules with **carboxyl** and **amino groups** and differ in their properties due to differing side chains, called **R groups**

- The **α-carbon** is the central point in the backbone of every amino acid. i. e. before the carbonyl carbon atom in the molecule.



- 20 different types of amino acid exist as zwitter ion form contain one functional group have a +ive charge and the other have a negative (-ive) charge i.e. overall charge is zero.



Zwitter ion

Classification of Amino acid

- They are classified on the basis of :
  - R group that is attached to α-carbon.
  - Source of energy for the body.

R group attached to α-carbon.

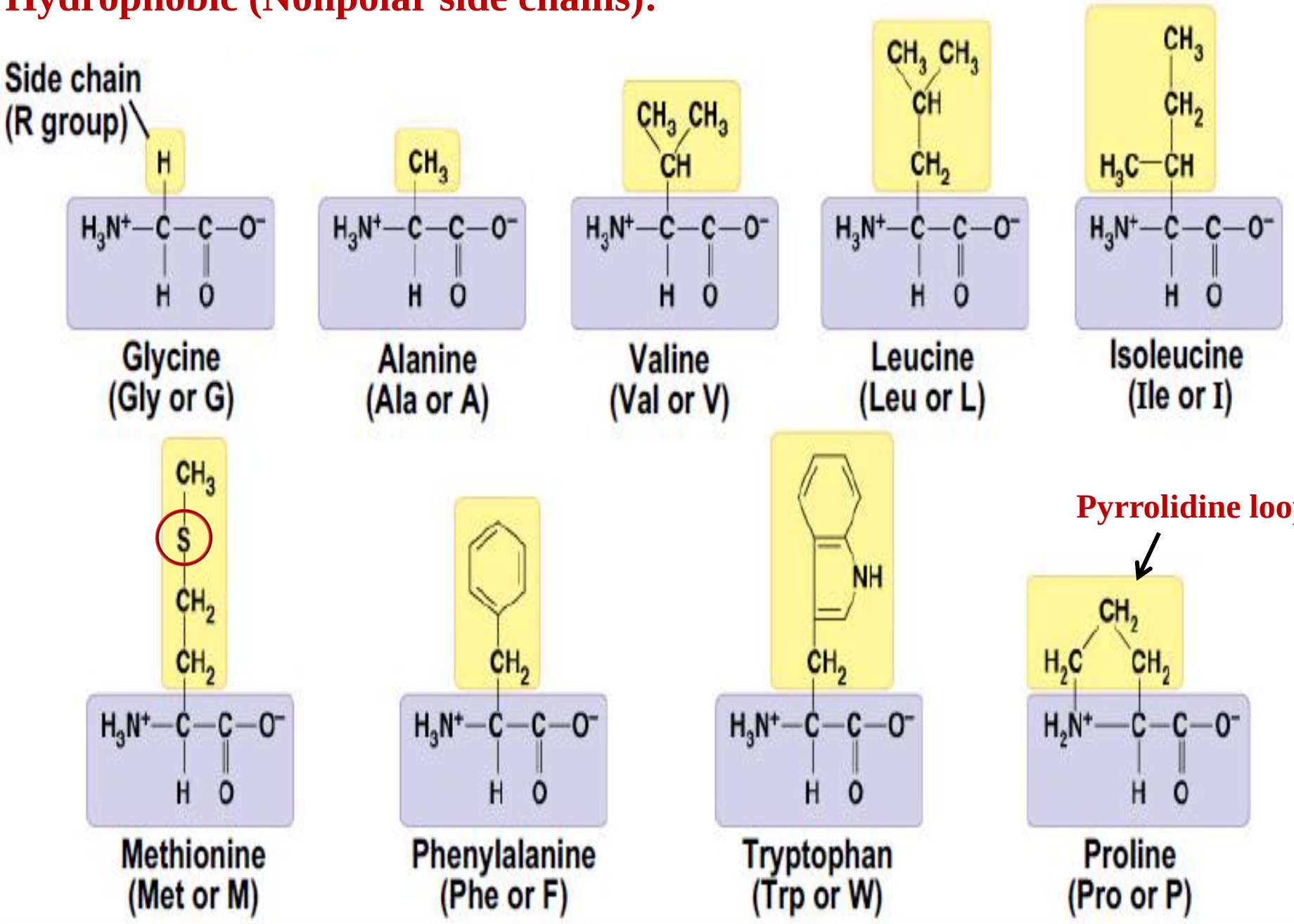
Hydrophobic (Nonpolar side chains)

Hydrophilic (Polar side chains)

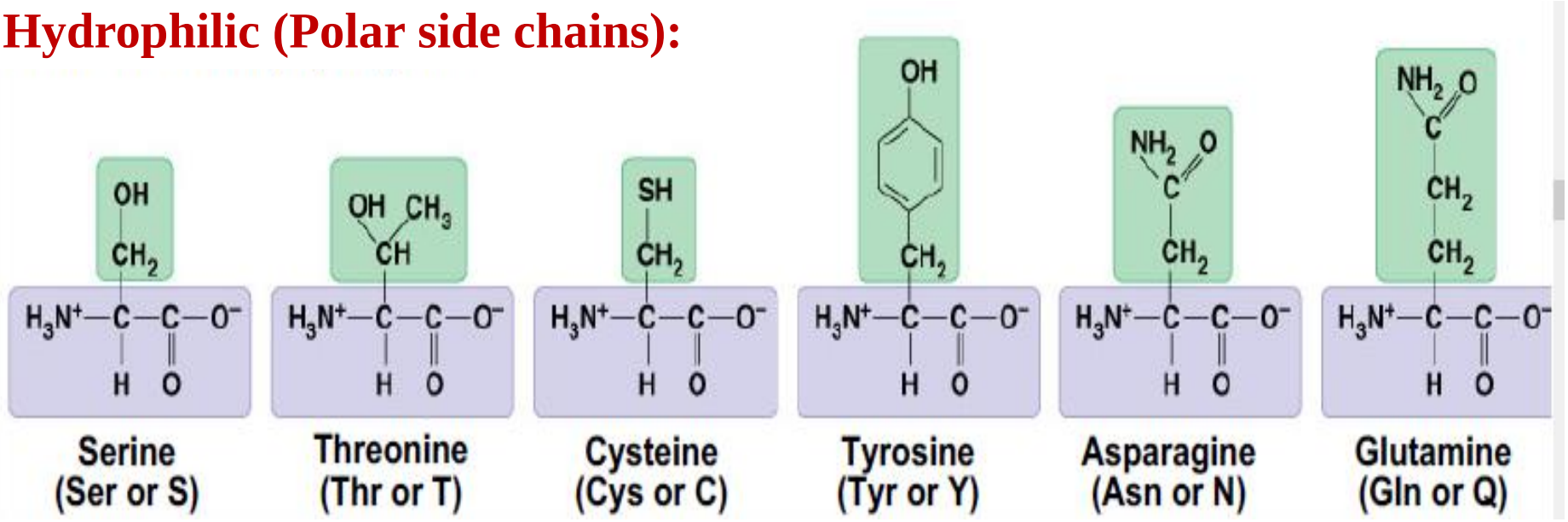
Acidic (Negatively charged)

Basic (Positively charged)

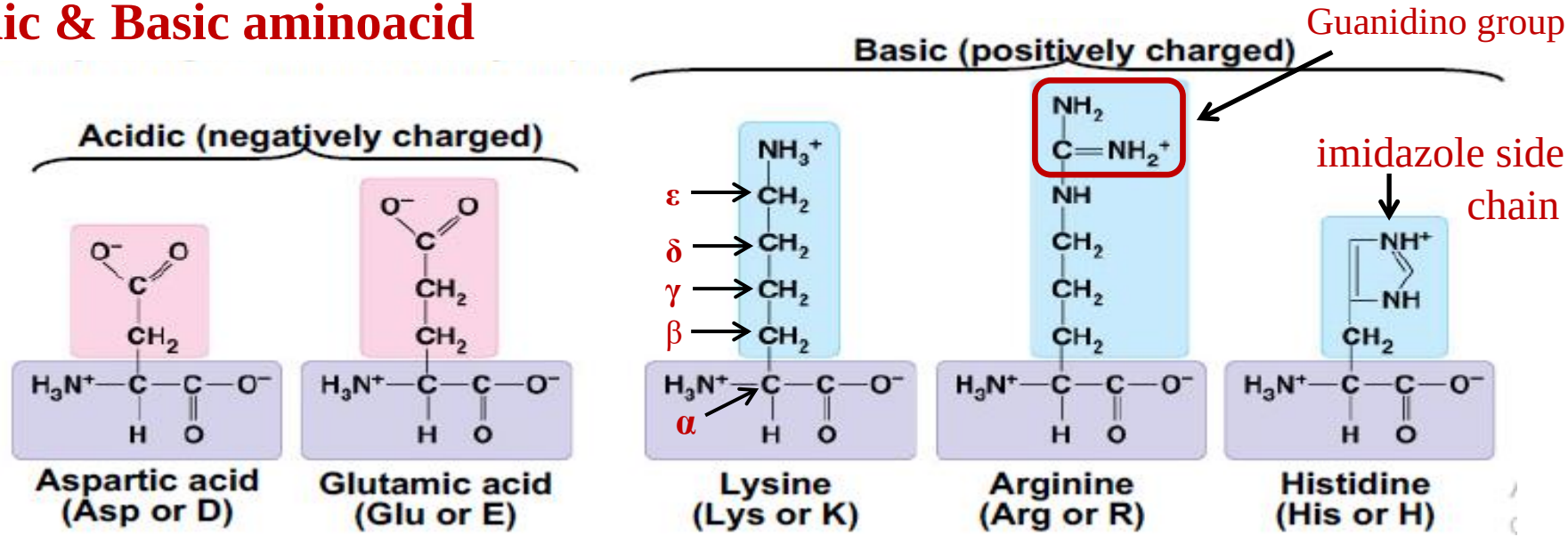
Hydrophobic (Nonpolar side chains):



Hydrophilic (Polar side chains):



Acidic & Basic aminoacid



2. On the basis of source of energy for the body.

Three Types:

- Essential amino acids
- Nonessential amino acids
- Conditional amino acids

- **Essential amino acids** cannot be made by the body. They must come from food.

  Example: V I F H R K L M e T W (1 0)

- **Nonessential amino acids** can be produced by our body, even if we do not get it from the food.

  Examples: D N A S E G Y (7)

- **Conditional amino acids** usually not essential, except in times of illness and stress.

  Examples: P C Q (3)

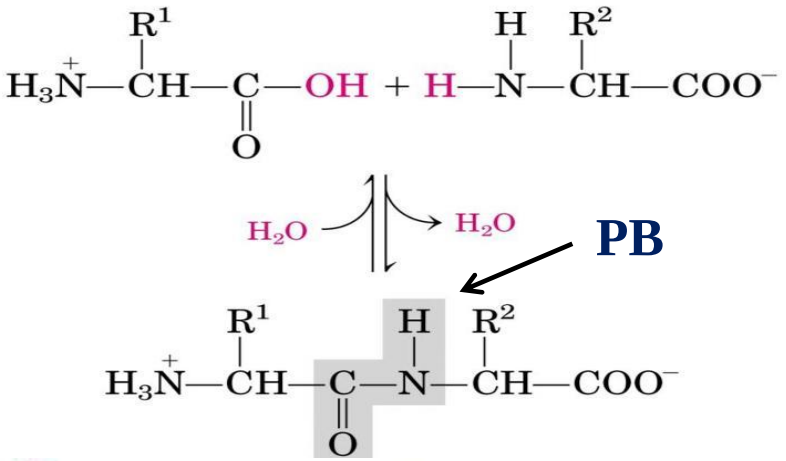
Peptide bonds & Its Formation:

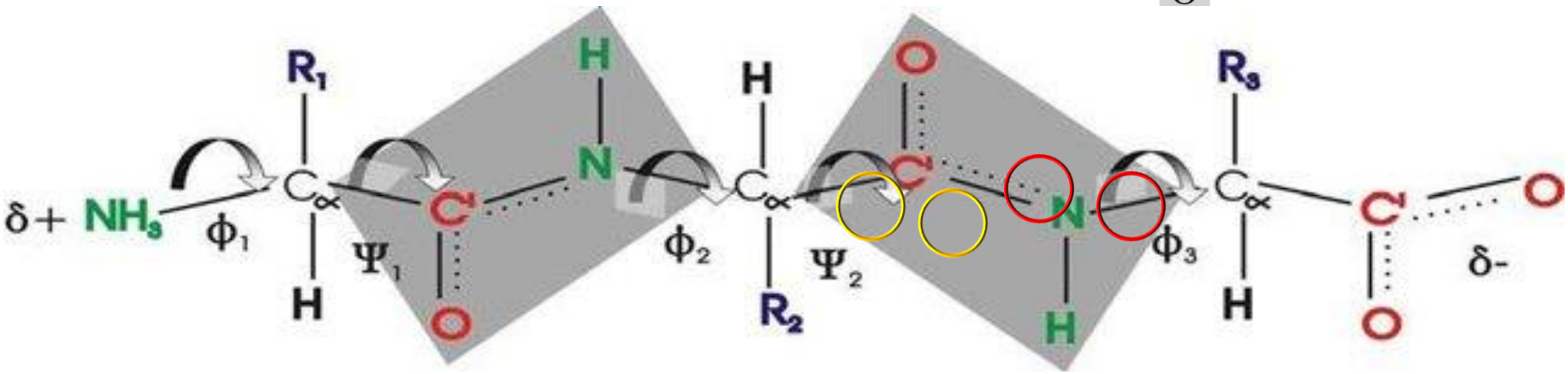
- Amino acids are linked by **Peptide Bonds** to form a polypeptide chain i.e. each polypeptide has a unique linear sequence of amino acids, with a carboxyl end (C-terminus) and an amino end (N-terminus).

- In a polypeptide chain dihedral angles are as follows:

  φ (phi) is the angle between **Cα – N**

  ψ (psi) is the angle between **Cα – C**

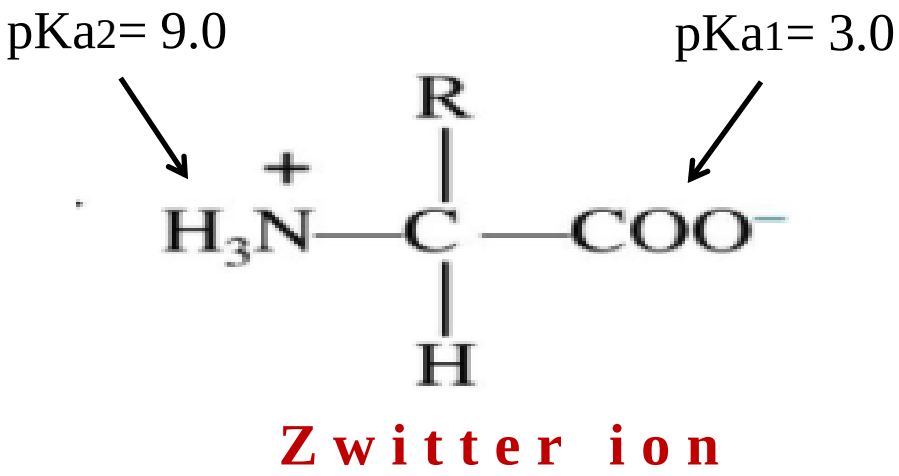




Isoelectric Point (pI)

- It is the pH at which a amino acid carries no net electrical charge.
- The net charge on the molecule is affected by the pH and can become more positive or negative due to the gain or loss of protons, respectively.

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$



Zwitter ion

pKa describes acid dissociation constant

pKa = -log10 Ka

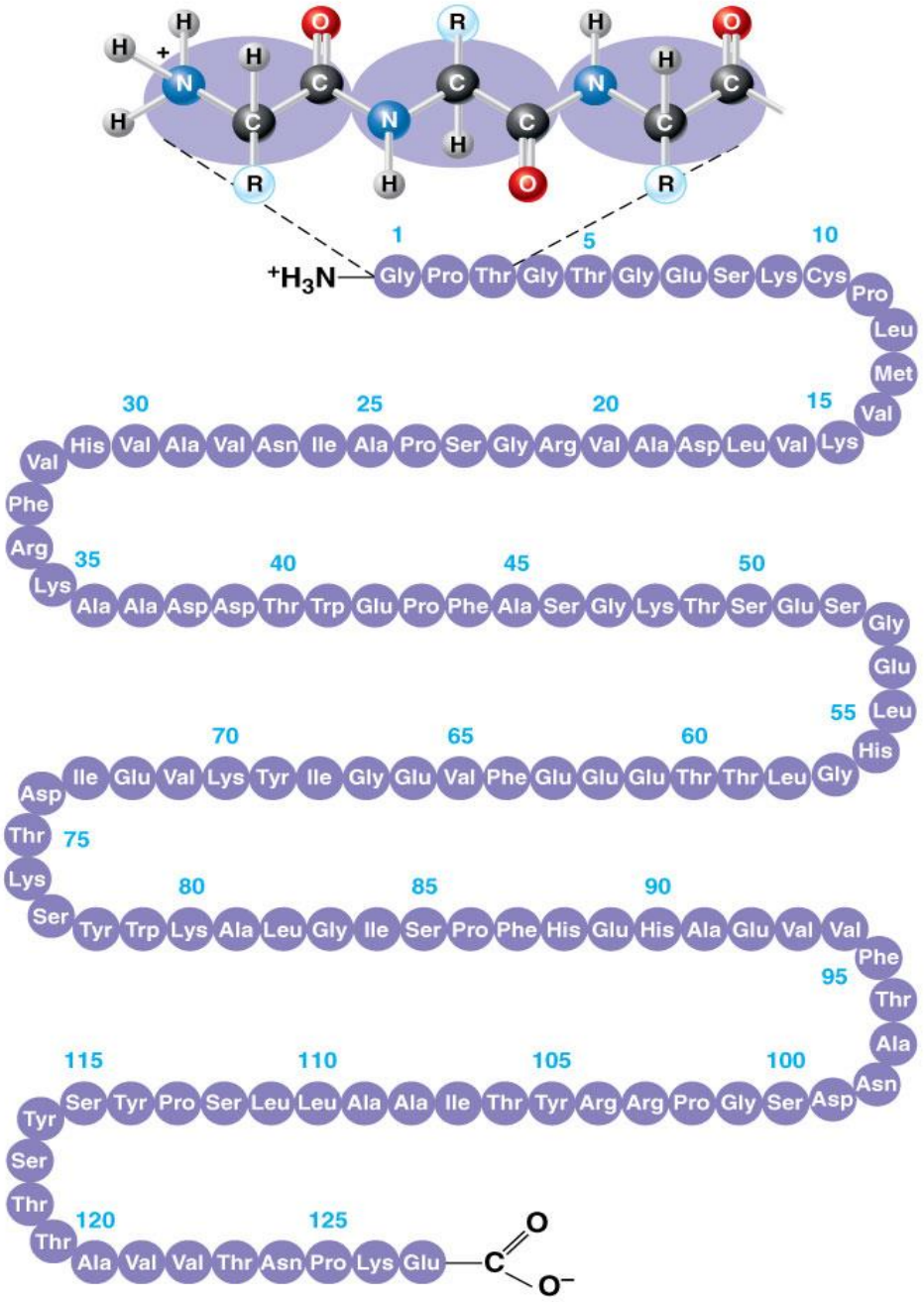
Note: The lower the pKa value, the stronger the acid.

If P^H> pKa (Deprotonation); If P^H< pKa (Protonation)

If P^H> pKa (Deprotonation); If P^H > pKa (Deprotonation)

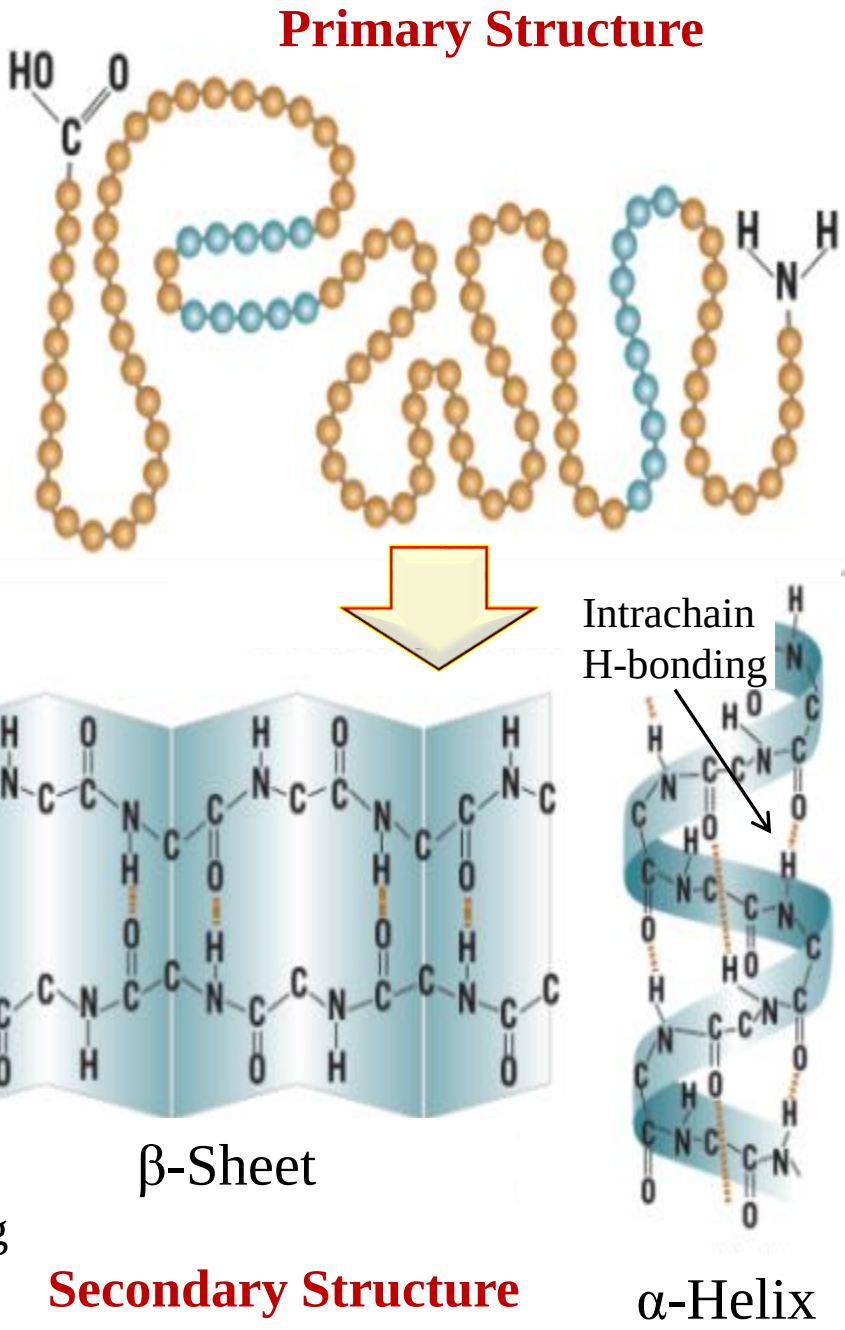
❖ Types of Protein Structure:

- ***Primary Structure***
  - It is the simplest level of protein structure.
  - The linear sequence of amino acids within a protein is considered the primary structure of the protein.
  - It is stabilized by Peptide bond (Covalent bond)



❖ *Secondary Structure*

- It is regular folding of polypeptide backbone of primary structure.
- The most common types of secondary structures are the **α helix** and the **β pleated sheet.**
- Both structures are held in shape by hydrogen bonds, formed between the carbonyl O of one amino acid and the H of other amino acids.



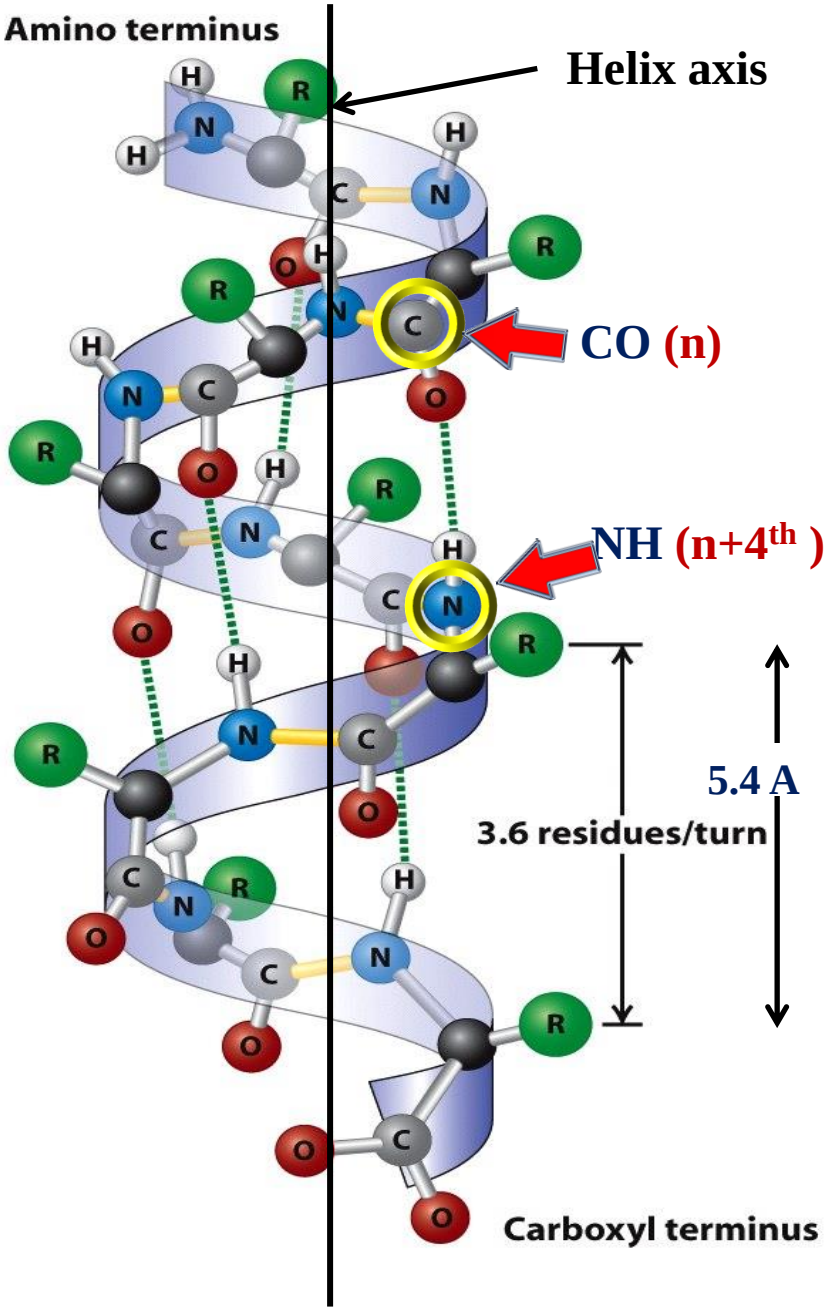
- In an **α helix,** C=O and N-H group is hydrogen-bonded to a peptide bond 4 residues away i.e.

  CO (n) to **NH (n+4th)**

- The structure repeats (turn) itself every 5.4 A along the helix axis (pitch).

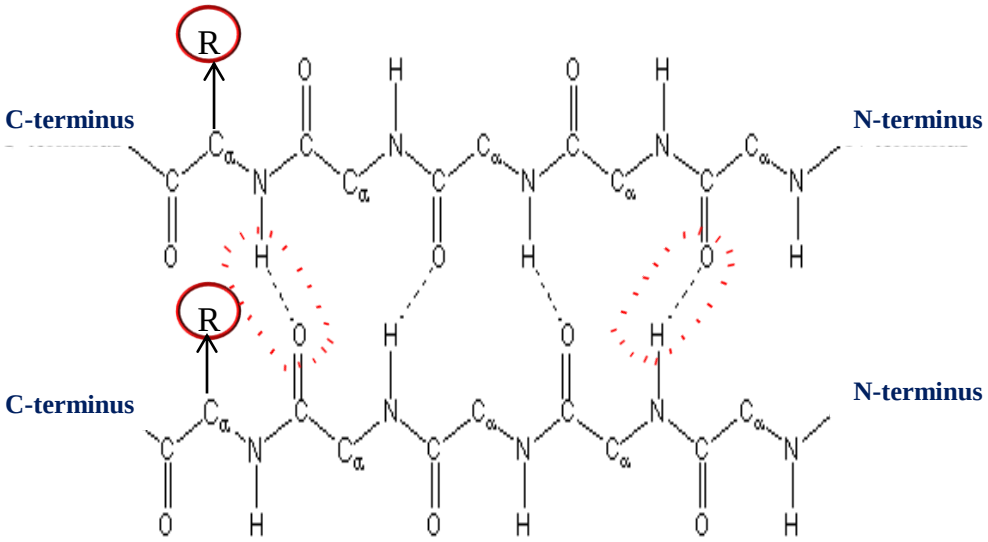
- **α helix** have **3.6** amino acid residues/turn, i.e. a helix 36 amino acids long would form 10 turns.

- The separation of each amino acids along the helix axis is 5.4/3.6 or 1.5 A i.e the **α helix** has a rise/aa of **1.5** A.

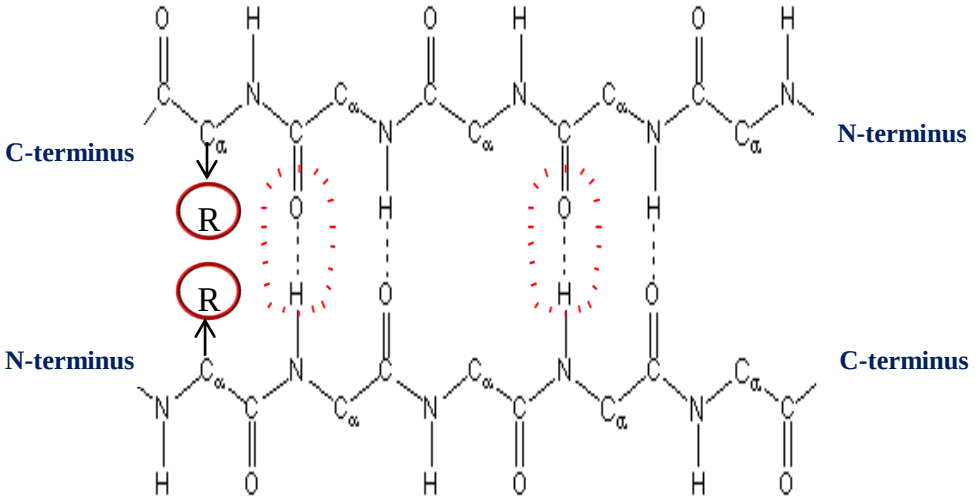


β-sheets

- When two adjacent β-strands line up they can form bridges of hydrogen bonds. They can be in either **parallel** or **anti-parallel** orientation.
- **In parallel** orientation, the carbonyl oxygen and the amide hydrogens are staggered.
- **In anti-parallel** the carbonyl oxygen and the amide hydrogen are placed directly opposite to form hydrogen bond i.e. anti-parallel is more stable.



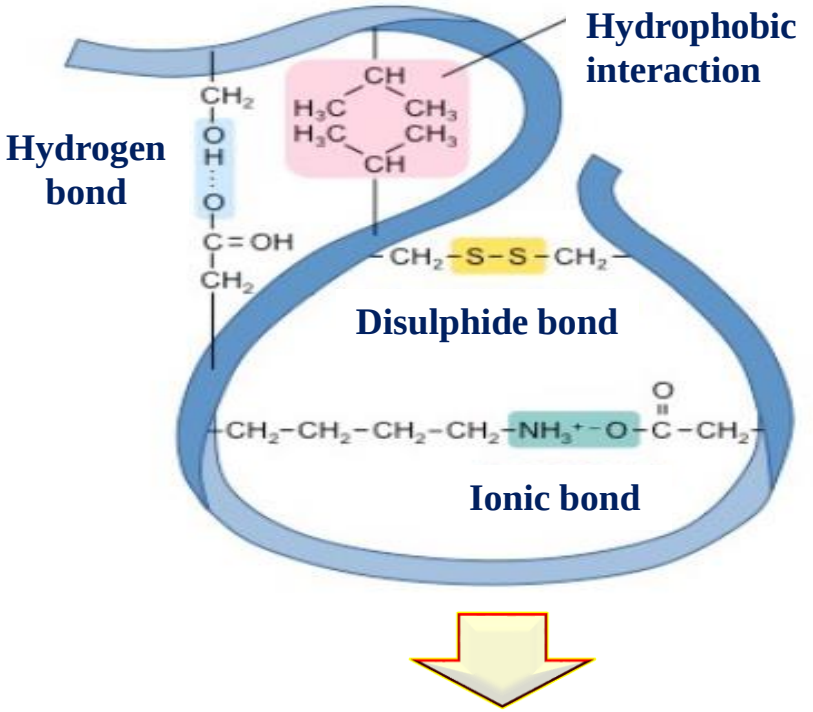
Parallel β Sheets



Antiparallel β Sheets

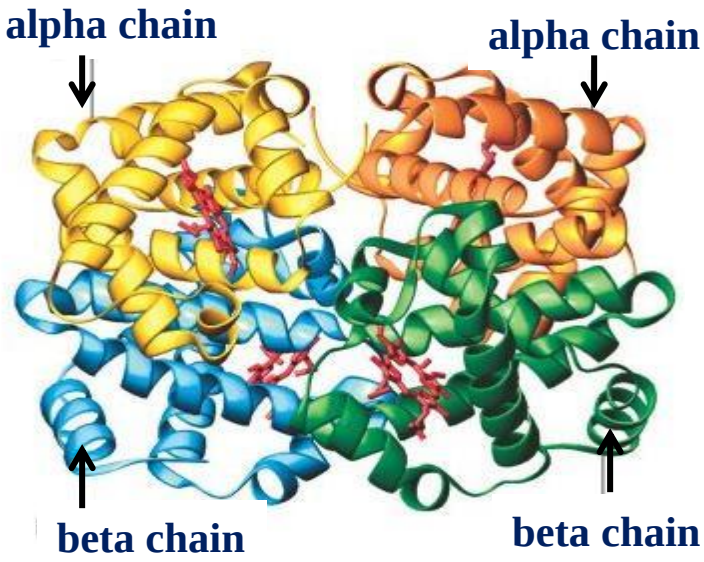
Tertiary Structure

- The tertiary structure is the way the polypeptide chain coils and turns to form a complex molecular shape (i.e. the 3D shape)
- It is caused by interactions between R groups; including H-bonds, S-S bridges, ionic bonds and hydrophobic interactions



Quaternary Structure

- Multiple polypeptides may interact to form a single, larger, biologically active protein (quaternary structure)
- It also held together by a variety of bonds (similar to tertiary structure)

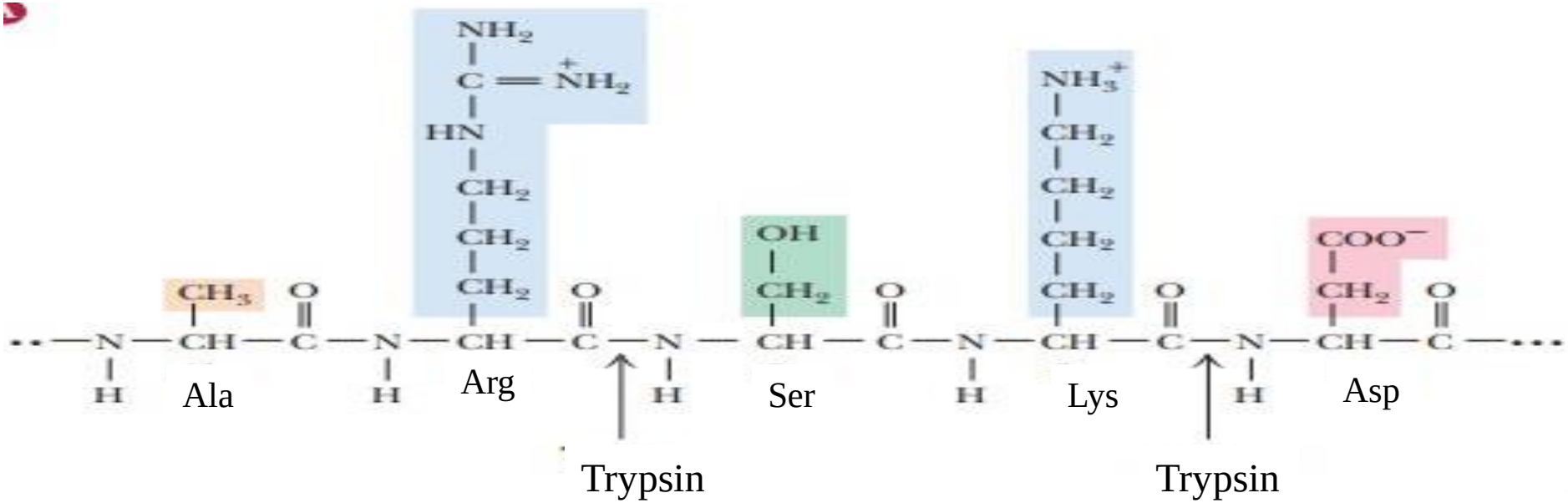


Fourth level of protein structure; (Quaternary structure)

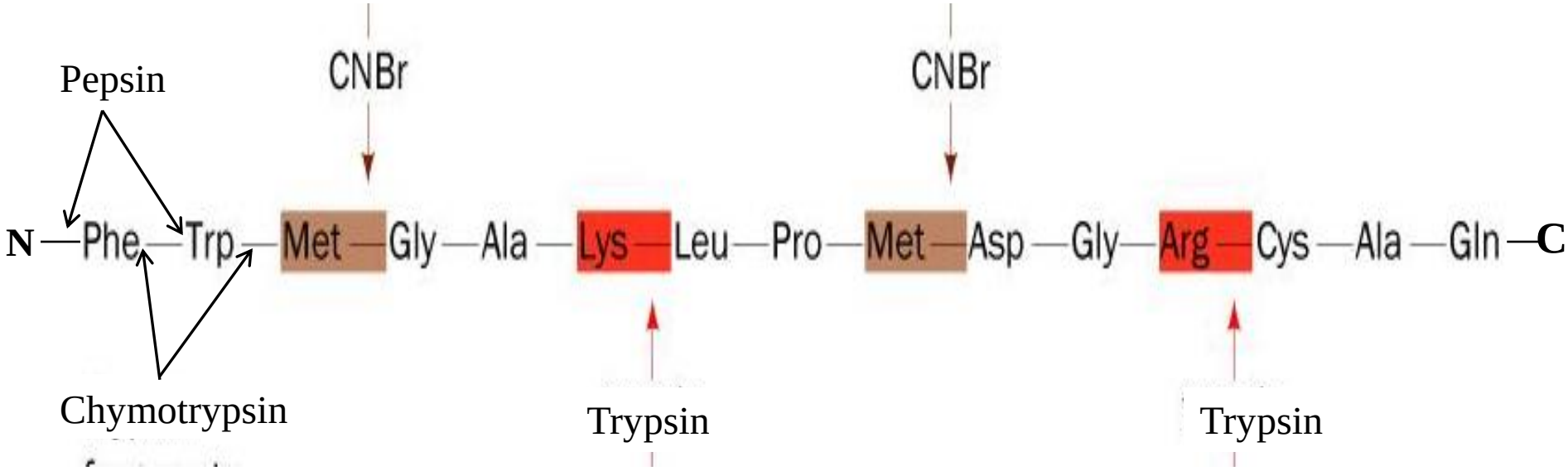
Proteolytic cleavage

- It is important in laboratory experiments where it is often useful to work with specific peptide fragments instead of entire proteins.
- Therefore**,** **proteases** often have a specific recognition site where the peptide bond is cleaved.

Example: Trypsin cleaves at C terminal of arginine (R) or lysine (K) residues



Proteases	Cleavage points	Amino acid
Trypsin	(C)	Lys (K), Arg (R)
Chymotrypsin	(C)	Phe (F), Trp (W), Tyr (Y)
Pepsin	(N)	Phe (F), Trp (W), Tyr (Y)
Cyanogen bromide (CNBr)	(C)	Met (M)



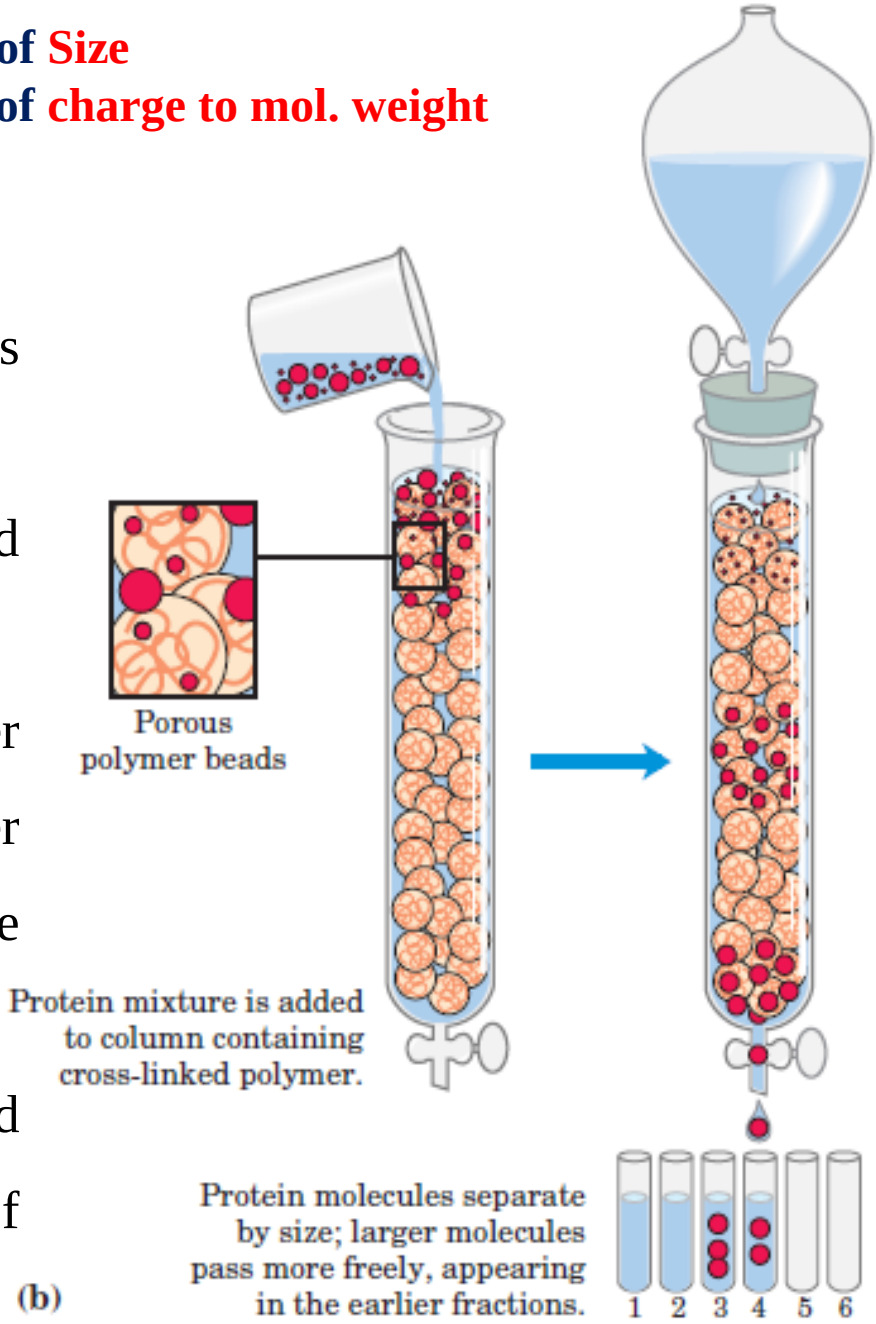
Protein purification:

1. On the basis of Size
2. On the basis of charge to mol. weight

1. On the basis of Size:

Size-exclusion chromatography

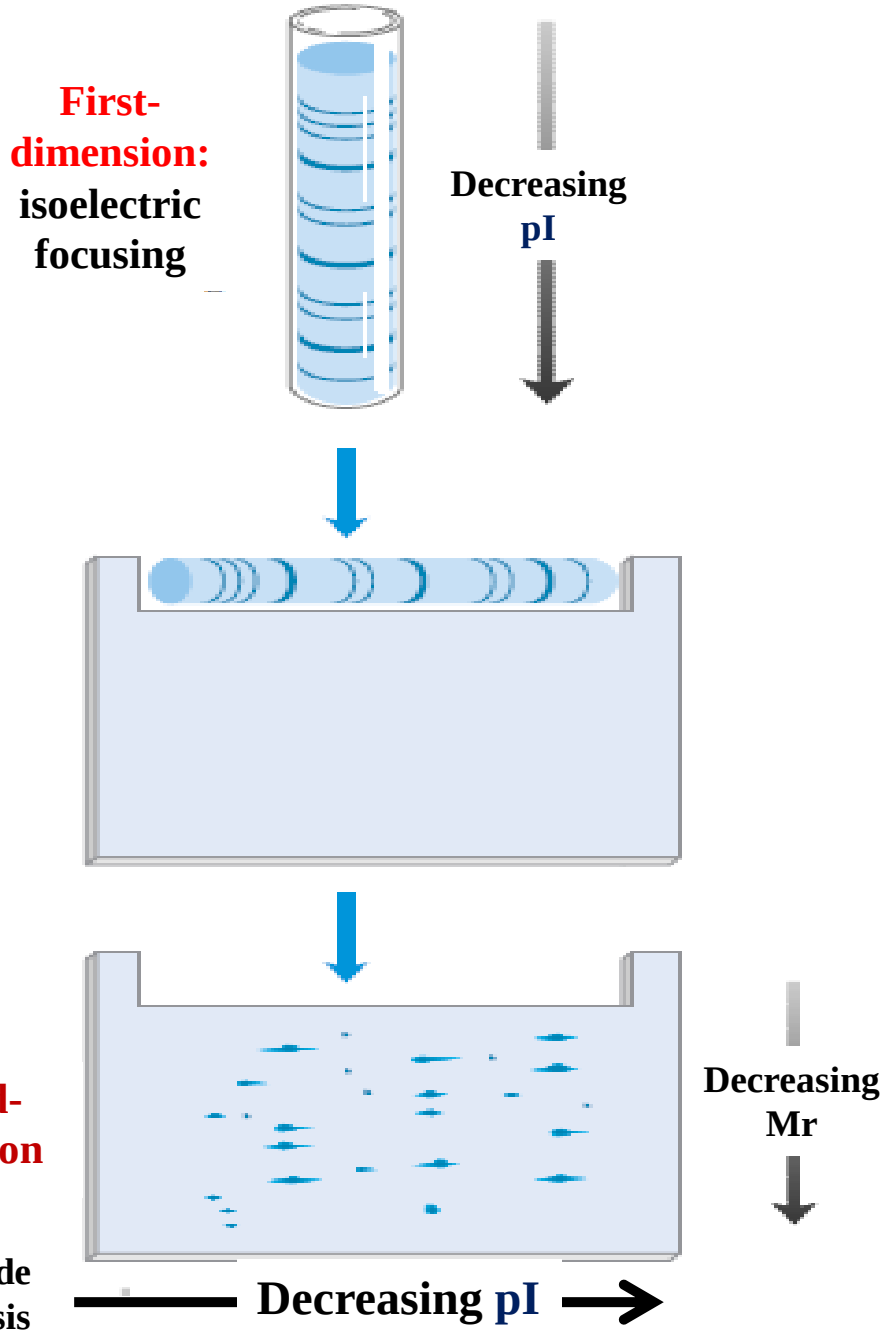
- Also called gel filtration, separates proteins according to size.
- The column matrix is a cross-linked polymer with pores of selected size.
- Larger proteins migrate faster than smaller ones, because they are too large to enter the pores and hence take a direct route through the column.
- The smaller proteins enter the pores and are slowed as they enter into the pores of the column.

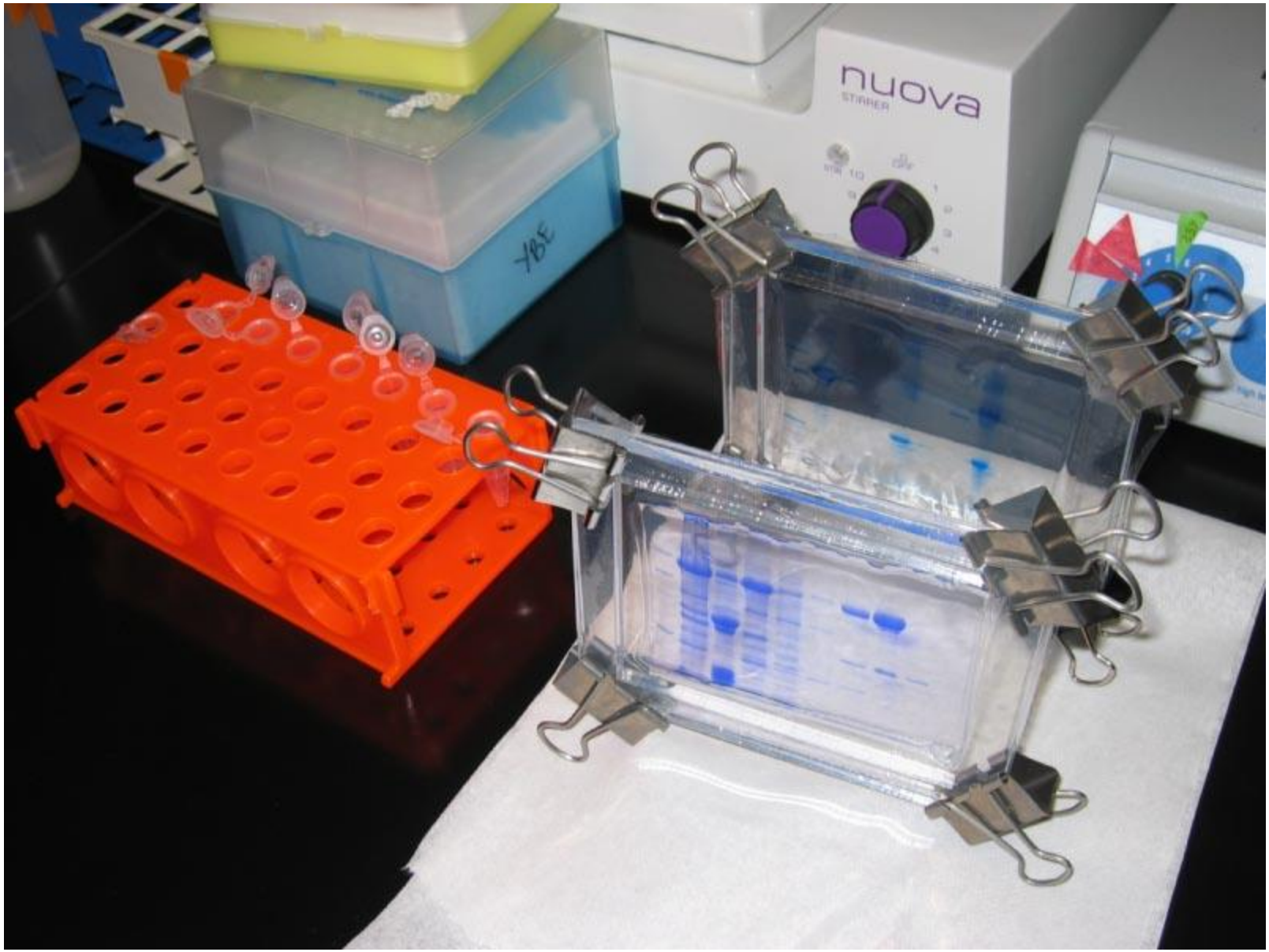


2. On the basis of charge to mol. weight

Two-dimensional electrophoresis

- Proteins are first separated by **isoelectric focusing** in a cylindrical gel. The gel is then laid horizontally on a second, slab-shaped gel, and the proteins are separated by SDS-PAGE.
- Horizontal separation reflects differences in IP & vertical separation reflects differences in molecular weight.

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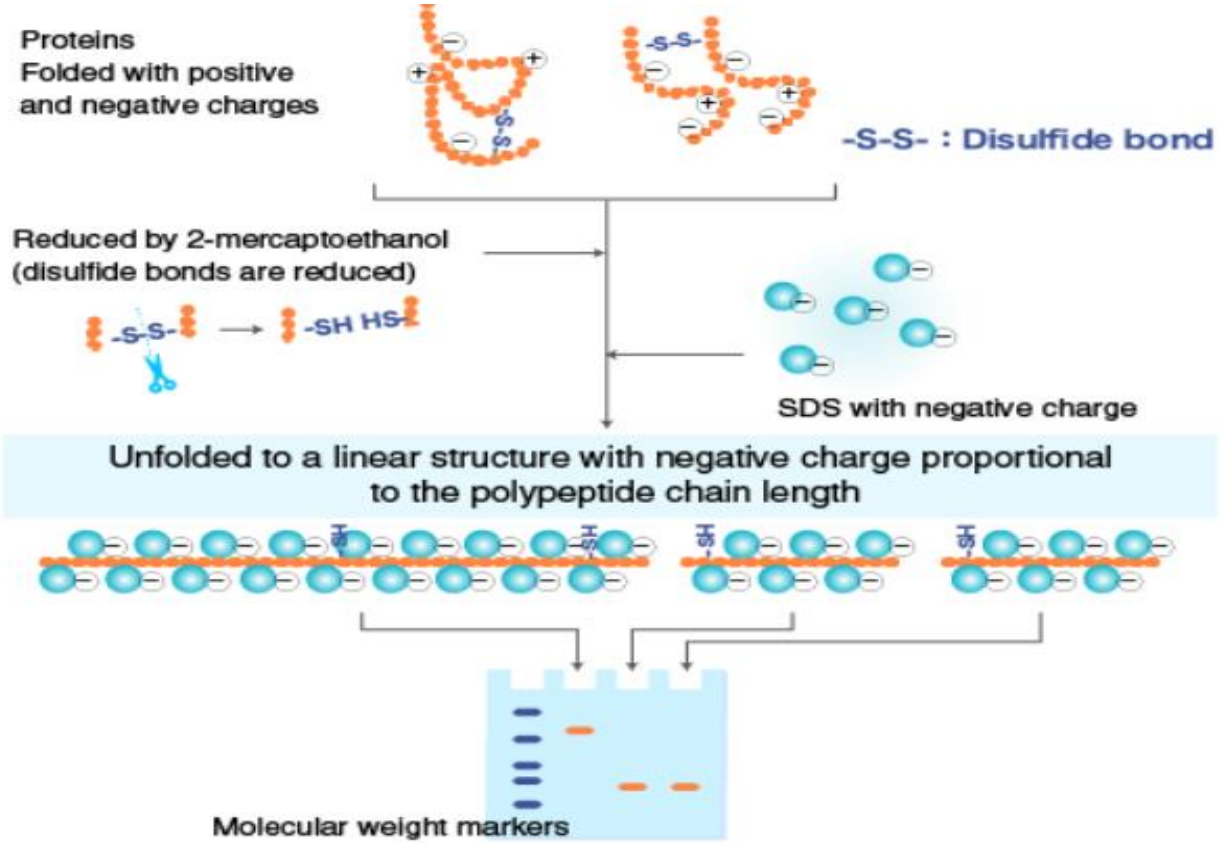
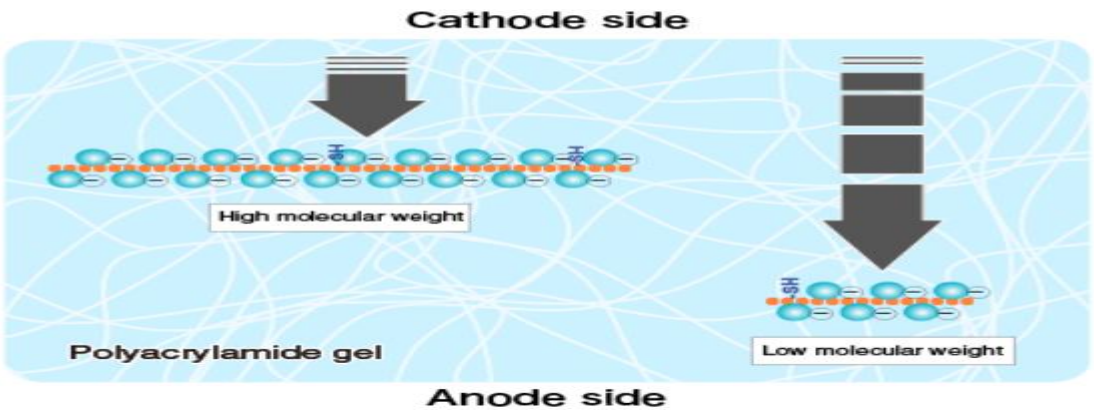


Proteins are separated based on their polypeptide chain length by electrophoresis in a polyacrylamide gel with an appropriate mesh size.



Questions and Answer

Q. **The peptide Ala-Arg-Gln-Met-Thr-Lys-Val, was digested with Cyanogen bromide to produce** **(IISc Banglore 2005, 2019)**

A. Ala-Arg-Gln + Met-Thr-Lys-Val,

B. Ala-Arg-Gln-Met-Thr + Lys-Val,

C. Ala-Arg-Gln-Met + Thr-Lys-Val,

D. Ala-Arg + Gln-Met-Thr-Lys-Val,

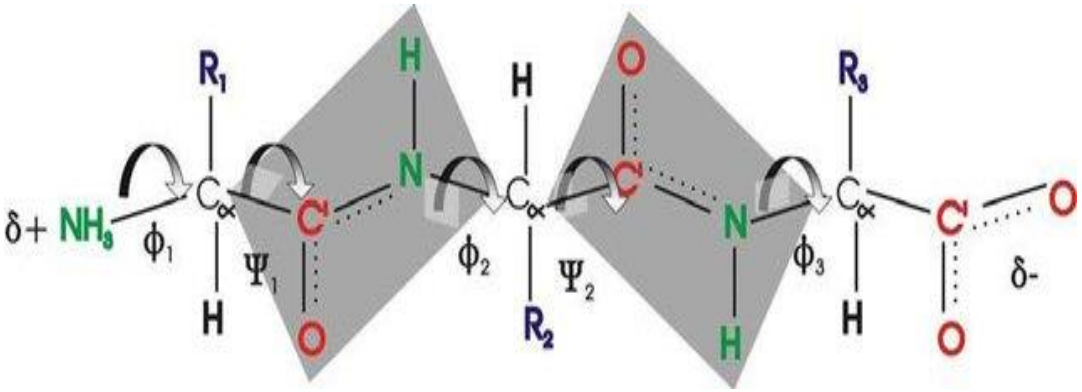
Q. Hydrolysis of a peptide involves cleavage of the bond between the atoms GATE-2016

A. N and Cα

B. C and O

C. Cα and C

D. N and C



Thank You