Proteins

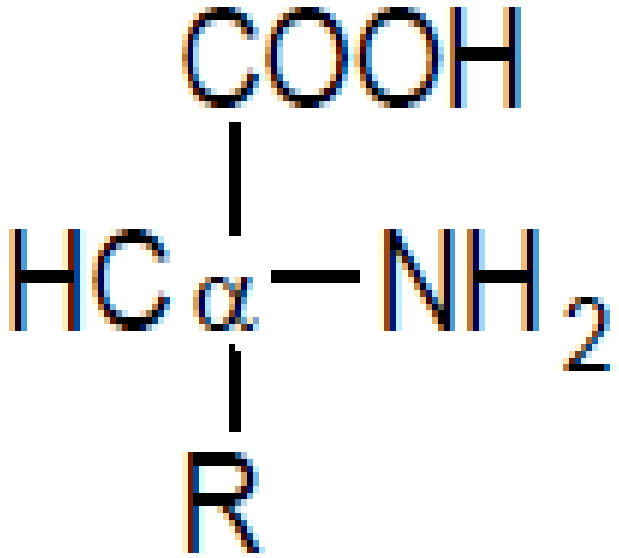
α-amino acid

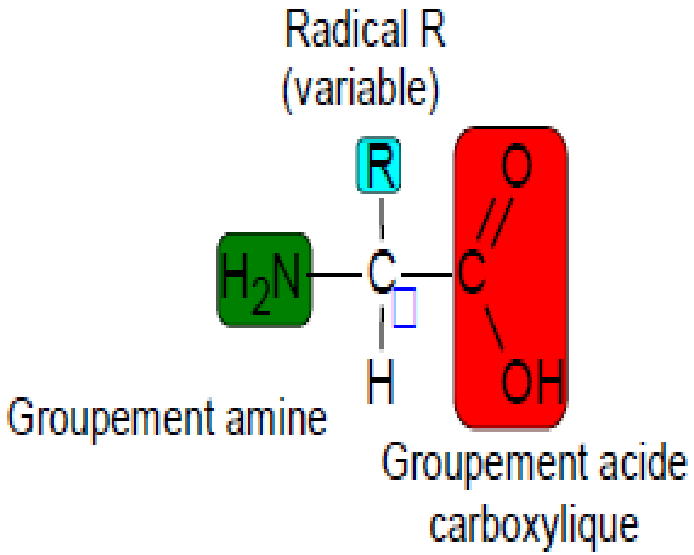
α-amino acid
Definition
Classification of α-amino acids
Classification based on the chemical nature of the side chain
Classification based on side-chain polarity
Asymmetric carbon
Ionization of α-amino acids
Net charge of amino acids
Titration curve of α-amino acids
Example 1: Titration curve of glycine
Proteins
Definition
Biological function
Formation of the peptide bond
Electrical properties of peptides
Levels of protein organization
Primary structure
Secondary structure
α-helix
β-pleated sheet
β-turn
Tertiary structure
Quaternary structure
Protein denaturation
Study of protein primary structure (protein sequencing)
Determination of the number of polypeptide chains
Identification of the N-terminal residue using the Sanger method
Identification of the N-terminal residue by the Edman method
Identification of the C-terminal residue
Cleavage of disulfide bonds (inter- and intramolecular)
Determination of the α-amino acid composition of peptide chains
Acid hydrolysis
Alkaline hydrolysis
Sequence determination via differential cleavage of the peptide chain

Definition

- An α-amino acid is a molecule that possesses a carboxyl group (-COOH), an amino group (NH2), and a side chain (also known as an R group).

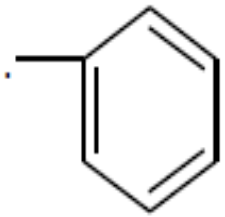
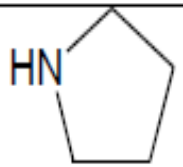
- There are 20 α-amino acids that serve as the building blocks for all proteins found in nature.





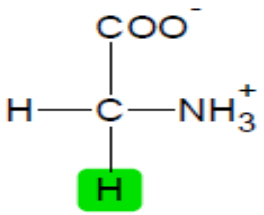
Classification of α-amino acids

Classification based on the chemical nature of the side chain

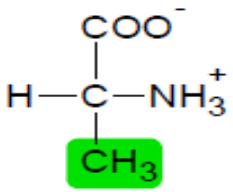
La famille	Chaîne R constituée de :	Aminoacides
Hydrocarbonée	C et H	Glycine*, Alanine, Valine, Leucine, Isoleucine.
Hydroxylée	-OH	Serine, Thréonine
Soufrée	-S-	Cystéine, Méthionine
Carboxylique	-COOH	Acide glutamique, Acide aspartique
Amide	$-CONH_2$	Glutamine, Asparagine
Basique	-NH-	Lysine, Arginine, Histidine
Aromatique		Phénylalanine, Tyrosine, Tryptophane
Hétérocyclique		Proline **(α-iminoacide)**

Classification based on side-chain polarity

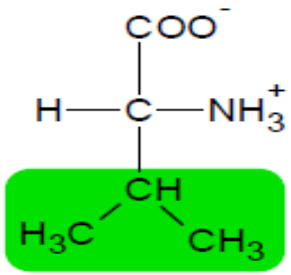
❑Non-polar α-amino acids: These are glycine, alanine, valine, leucine, isoleucine,methionine, and proline. The side chains of all these amino acids contain only carbon and hydrogen; they are hydrophobic amino acids.Glycine contains only a hydrogen atom.



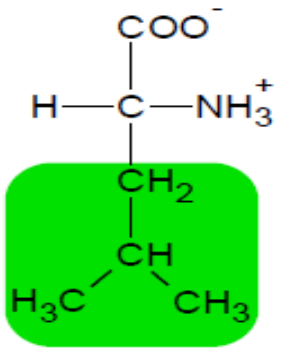
Glycine



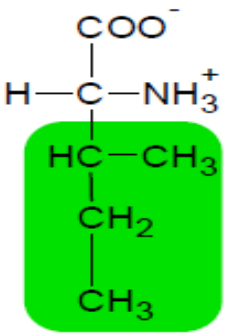
Alanine



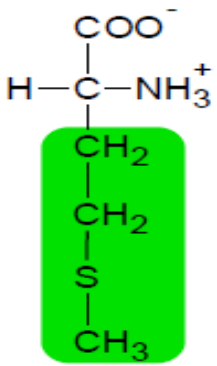
Valine



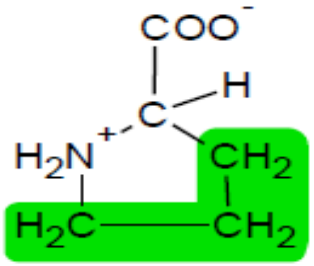
Leucine



Isoleucine

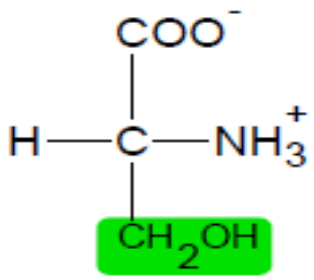


Methionine

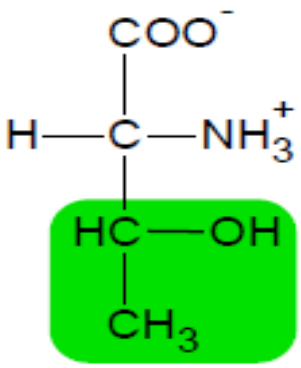


Proline

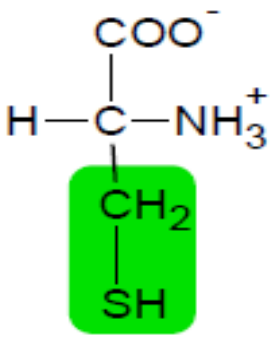
❑Uncharged polar α-amino acids: These are serine, threonine, cysteine, asparagine, and glutamine. The side chain of these amino acids consists of polar groups capable of forming hydrogen bonds with water.



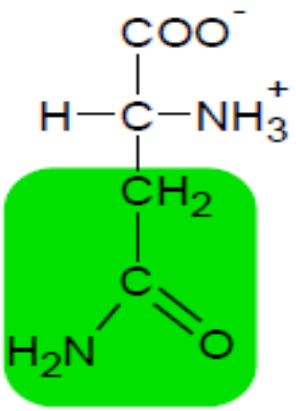
Serine



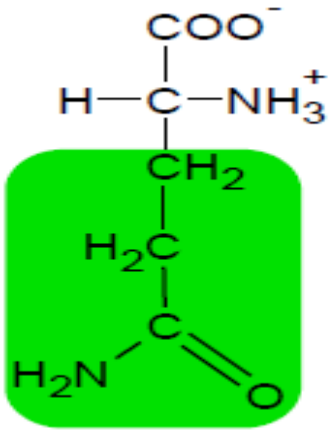
Thréonine



Cystéine

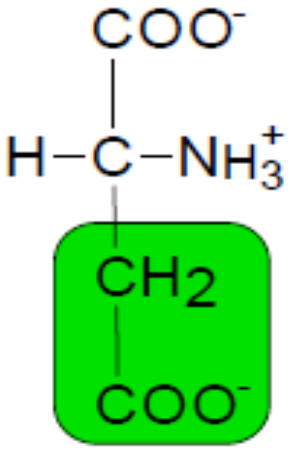


Asparagine

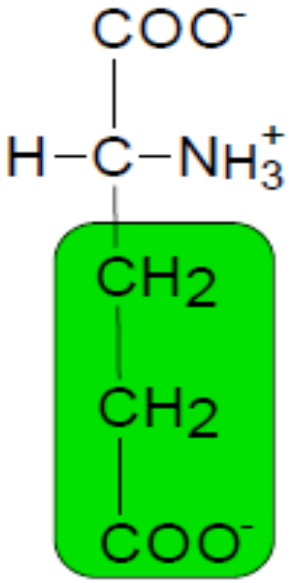


Glutamine

❑Negatively charged polar α-amino acids: These are aspartic acid and glutamic acid. Both of these amino acids contain a carboxylic acid group in their side chains, which ionizes at pH 7 and becomes negatively charged.

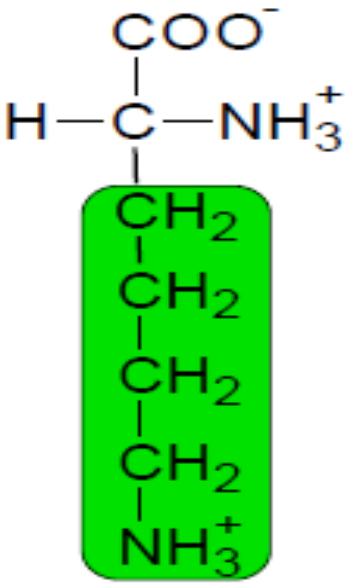


Aspartate

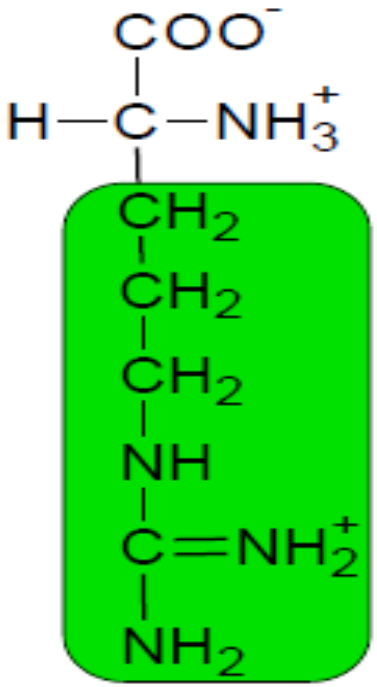


Glutamate

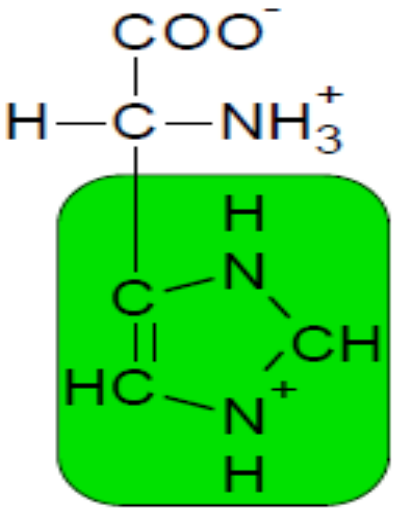
❑The positively charged polar α-amino acids: These are lysine, arginine, and histidine. These amino acids contain a positively charged group at pH 7: lysine an amine group, arginine a guanidino group, and histidine an imidazole group. This positive charge plays an important role in electrostatic interactions



Lysine

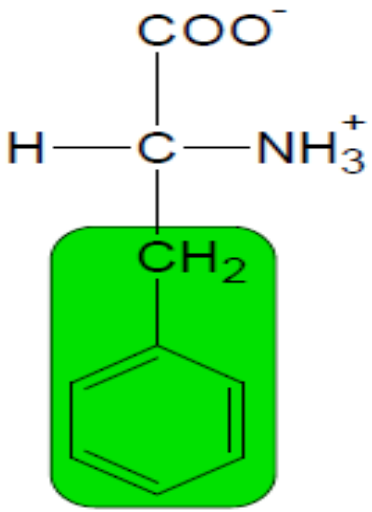


Arginine

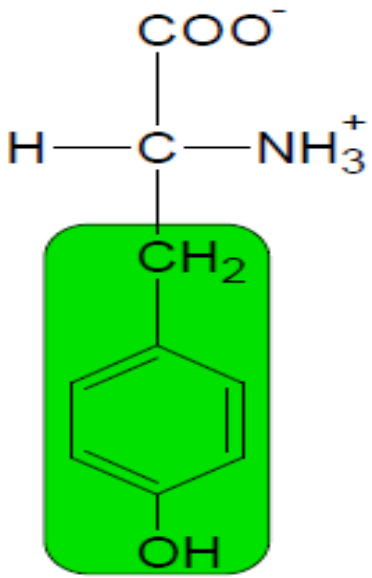


Histidine

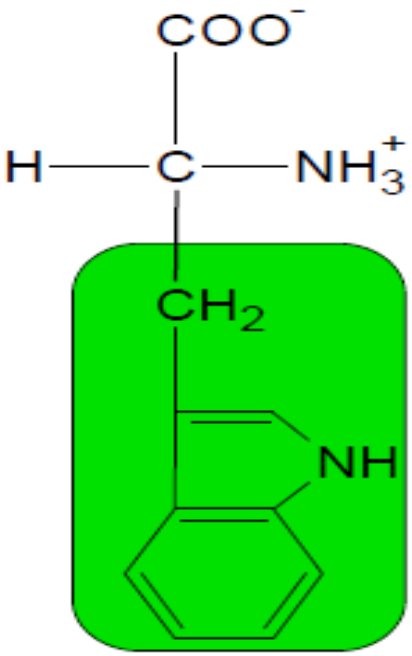
❑Aromatic α-amino acids: These are phenylalanine, tyrosine, and tryptophan. Phenylalanine contains a hydrophobic aromatic group and can be classified as a non-polar amino acid. Tyrosine and tryptophan contain polar groups (an –OH group for tyrosine and an –NH group for tryptophan), which can form hydrogen bonds with water.



Phenylalanine



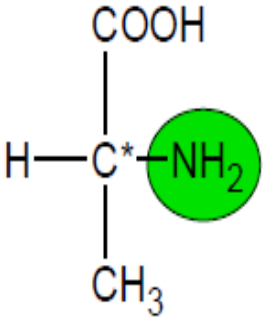
Tyrosine



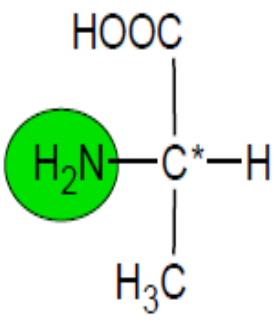
Tryptophane

Asymmetric carbon

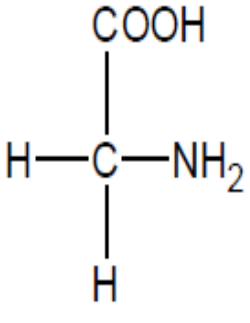
- With the exception of glycine, all amino acids contain at least one asymmetric carbon atom. As with carbohydrates, a distinction is made between the two series, D and L, but naturally occurring amino acids belong to the L series.
- For the same reasons, these molecules possess optical activity



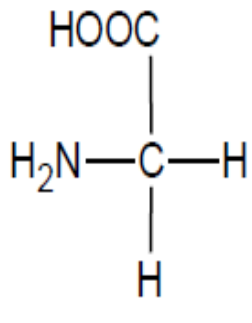
D- Alanine



L- Alanine



Glycine

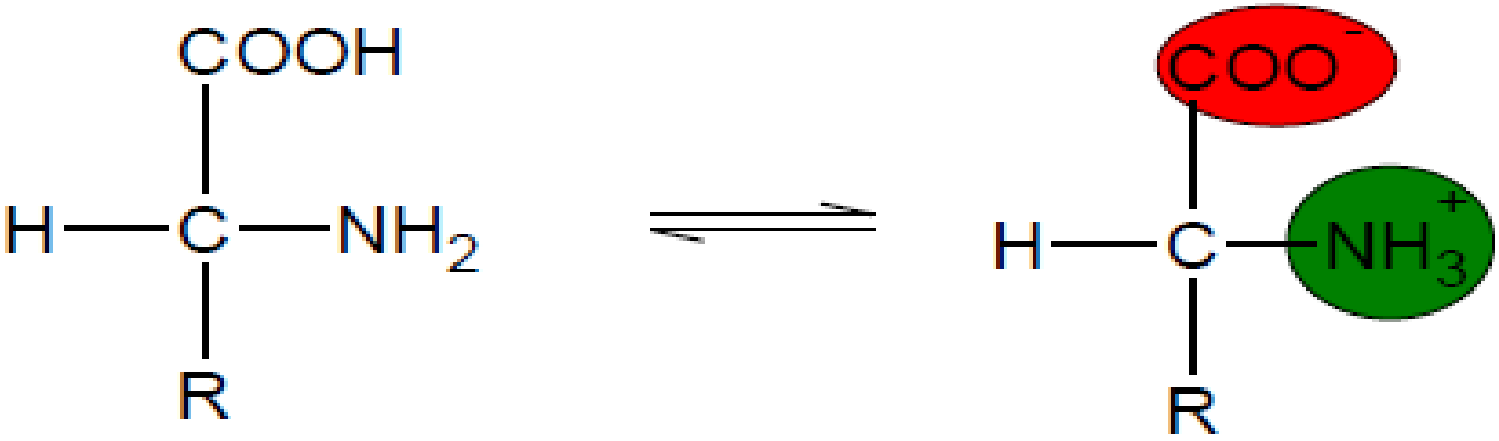


Glycine

Ionization of α-amino acids

Net charge of amino acids

- In aqueous solution, α-amino acids are electrically charged molecules:
- The α-COOH group acts as an acid, donating an H+ proton to become α-COO^- (the negatively charged deprotonated form)
- The α-NH2 group acts as a base, accepting a proton to become α-$NH3^+$ (the positively charged protonated form).
- The amino acid thus exists as a dipole, or zwitterion.



- In certain α-amino acids, the R side chain can also be charged, either positively or negatively.
- The overall charge of the amino acid is the sum of the electrical charges of the α-COO^- and α-NH_3^+ groups and the side chain, and it depends on the pH of the medium.

Titration curve of α-amino acids

❑All α-amino acids possess two ionizable groups (α-NH_3^+ and α-COOH); some also have a third ionizable group located in their side chains.

❑These groups, characterized by pKa values (pH at half-dissociation), become ionized depending on the pH of the medium and thus determine the overall charge of the α-amino acid

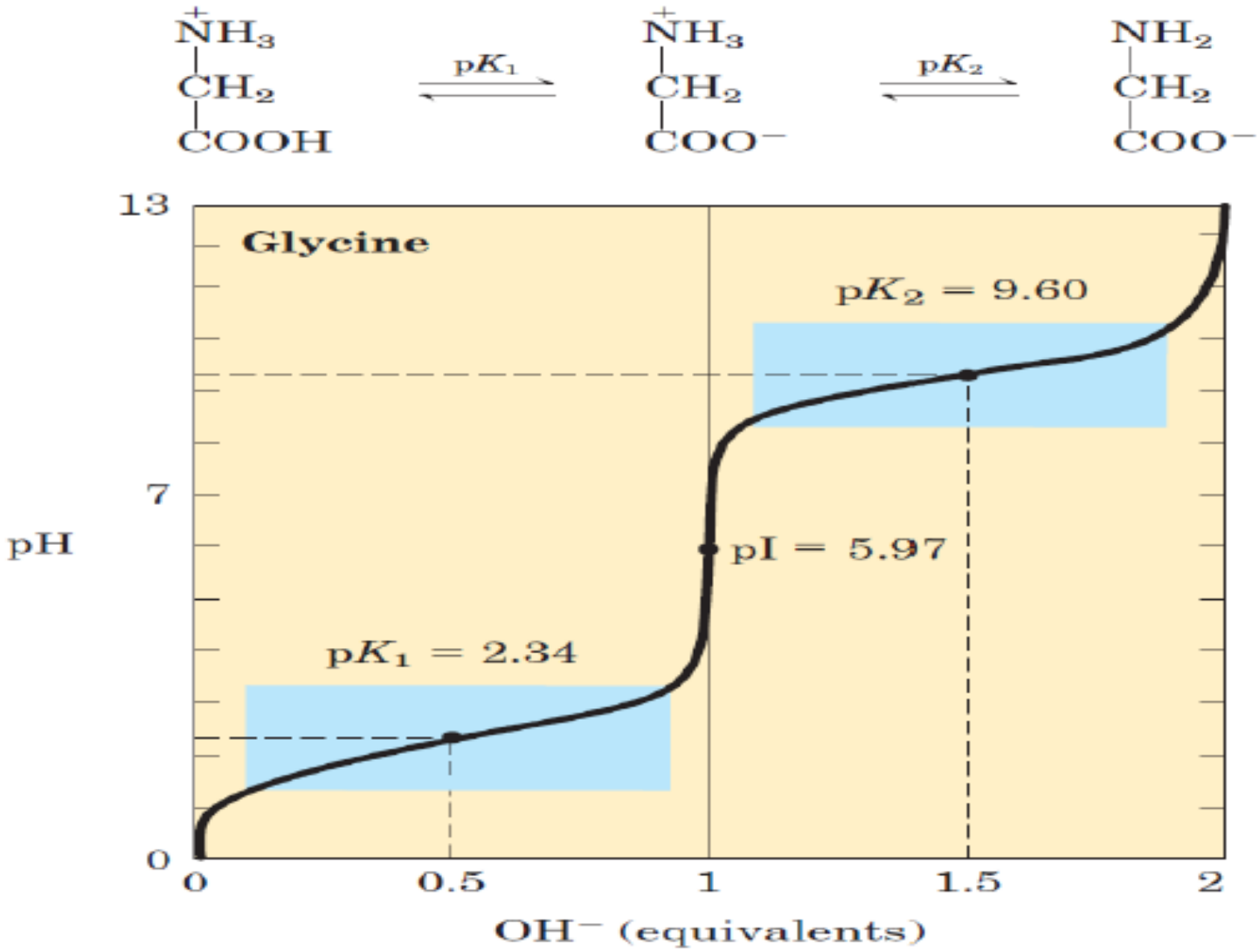
Titrating an amino acid with a strong base or a strong acid allows us to plot its titration curve and derive the following information:

- Quantitative determination of the pKa values for each ionizable group of the amino acid.
- Identification of regions with buffering capacity; the pH in such a region can be calculated using the Henderson-Hasselbalch equation:

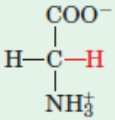
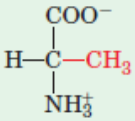
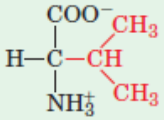
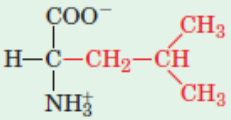
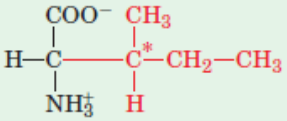
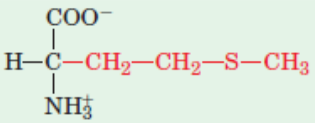
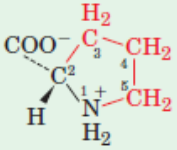
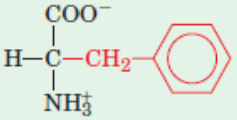
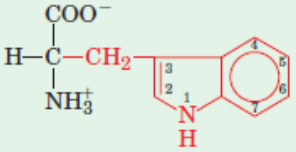
$$pH = pKa + log\frac{[\text{Base conjuguée}]}{[\text{acide}]}$$

- Determination of the net overall charge of the amino acid as a function of the pH of the medium.

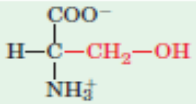
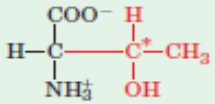
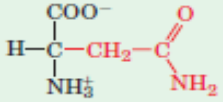
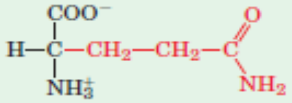
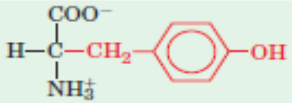
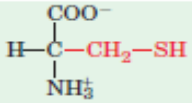
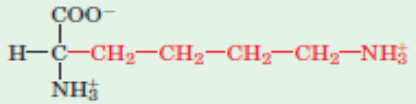
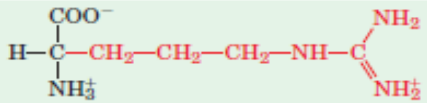
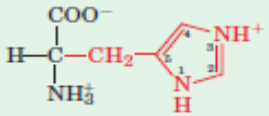
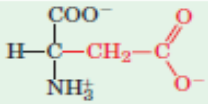
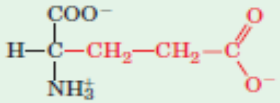
Example 1: Titration curve of glycine



❑1) Low pH: Protonation of all ionizable groups. Glycine has a net overall charge of +1.
❑2) pH = pK1 = 2.34: Half-dissociation of the α-COOH group. Glycine has an overall charge of +1/2.
❑3)pH = pI: End of dissociation of the α-COOH group and start of dissociation of the α-NH3+ group. Glycine has a net overall charge of zero.
❑4)pH = pK2: Half-dissociation of the α-NH3+ group. Glycine has an overall charge of -1/2.
❑5)High pH: End of dissociation of the α-NH3+ group. Glycine has a net overall charge of -1.

Name, Three-Letter Symbol, and One-Letter Symbol	Structural Formula[a]	Residue Mass (D)[b]	Average Occurrence in Proteins (%)[c]	pK_1 α-COOH[d]	pK_2 α-NH_3^+[d]	pK_R Side Chain[d]
Amino acids with nonpolar side chains						
Glycine Gly G		57.0	7.1	2.35	9.78	
Alanine Ala A		71.1	8.3	2.35	9.87	
Valine Val V		99.1	6.9	2.29	9.74	
Leucine Leu L		113.2	9.7	2.33	9.74	
Isoleucine Ile I		113.2	6.0	2.32	9.76	
Methionine Met M		131.2	2.4	2.13	9.28	
Proline Pro P		97.1	4.7	1.95	10.64	
Phenylalanine Phe F		147.2	3.9	2.20	9.31	
Tryptophan Trp W		186.2	1.1	2.46	9.41	

(*continued*)

Name Three-Letter Symbol, and One-Letter Symbol	Structural Formula[a]	Residue Mass (D)[b]	Average Occurrence in Proteins (%)[c]	pK_1 α-COOH[d]	pK_2 α-$^{+}NH_3$[d]	pK_R Side Chain[d]
Amino acids with uncharged polar side chains						
Serine Ser S		87.1	6.5	2.19	9.21	
Threonine Thr T		101.1	5.3	2.09	9.10	
Asparagine[f] Asn N		114.1	4.0	2.14	8.72	
Glutamine[f] Gln Q		128.1	3.9	2.17	9.13	
Tyrosine Tyr Y		163.2	2.9	2.20	9.21	10.46 (phenol)
Cysteine Cys C		103.1	1.4	1.92	10.70	8.37 (sulfhydryl)
Amino acids with charged polar side chains						
Lysine Lys K		128.2	5.9	2.16	9.06	10.54 (ε-NH_3^+)
Arginine Arg R		156.2	5.5	1.82	8.99	12.48 (guanidino)
Histidine[e] His H		137.1	2.3	1.80	9.33	6.04 (imidazole)
Aspartic acid[f] Asp D		115.1	5.4	1.99	9.90	3.90 (β-COOH)
Glutamic acid[f] Glu E		129.1	6.8	2.10	9.47	4.07 (γ-COOH)

Proteins

Definition

Proteins are biological macromolecules formed by the union of α-amino acids linked together by covalent bonds known as peptide bonds. A vast number of proteins exist in nature, differing in size, structure, and function

Biological function

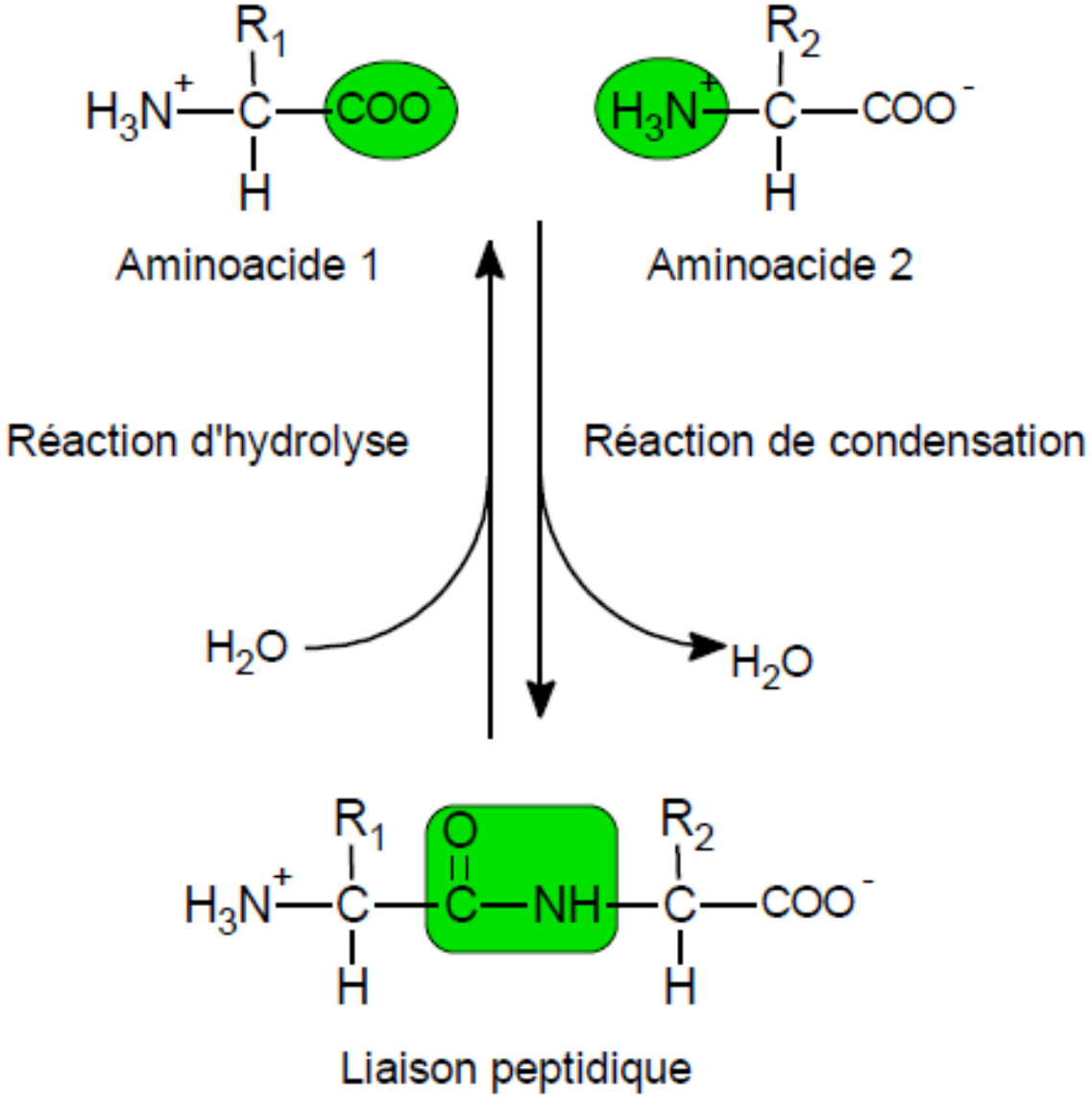
Proteins are involved in almost all biological processes, acting as:

- Biochemical catalysts: Enzymes are proteins that catalyze biochemical reactions.
- Transporters: Hemoglobin transports oxygen and carbon dioxide molecules; serum albumin transports fatty acids.
- Regulators: Transcription factors are proteins that regulate gene expression.
- Communication agents: Peptide hormones and receptors.
- Protective agents: Immunoglobulins.
- Structural components: Bone collagen and hair keratin are two proteins that provide structure and rigidity

Formation of the peptide bond

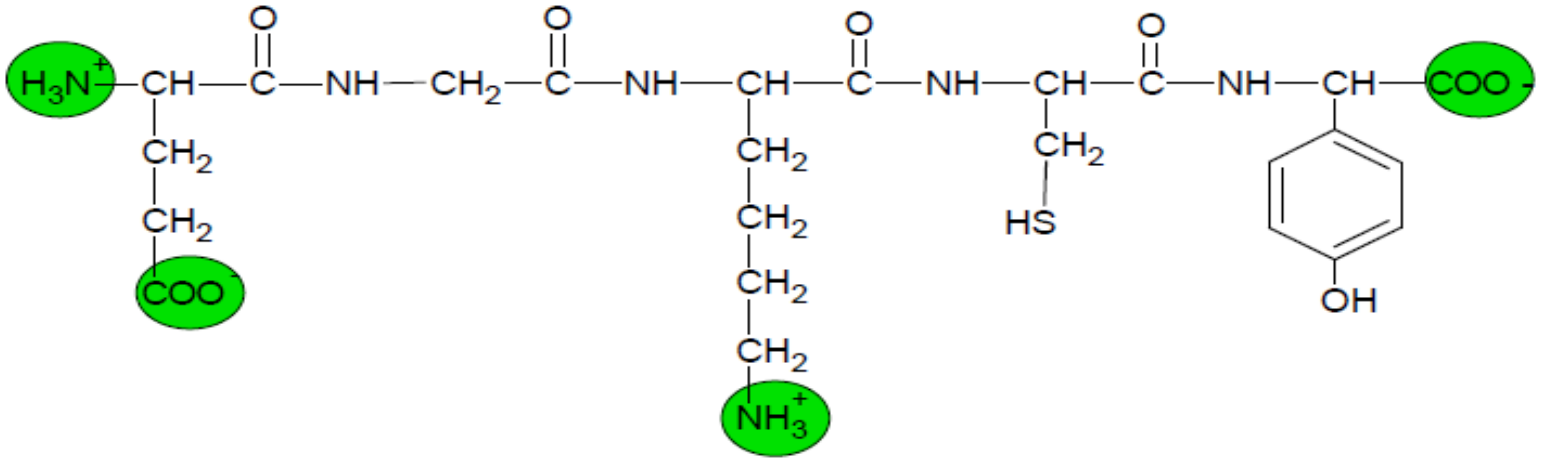
α-Amino acids can link together via covalent bonds known as peptide bonds or substituted amide bonds. Depending on the number of linked amino acids, a distinction is made between:

- **Oligopeptides**: formed by the union of two to a few α-amino acids, such as dipeptides (2 α-amino acids), tripeptides (3 α-amino acids), tetrapeptides (4 α-amino acids), and pentapeptides (5 α-amino acids).
- **Polypeptides**: formed by the union of multiple α-amino acids (10 to 100 amino acid residues) and having a molecular weight of less than that range from 1000 to 10000 Da.
- **Proteins**: formed by the union of multiple α-amino acids and having a molecular weight greater than 10 000 Da.


Aminoacide 1
Aminoacide 2
Réaction d'hydrolyse
Réaction de condensation
H2O
H2O
Liaison peptidique

Electrical properties of peptides

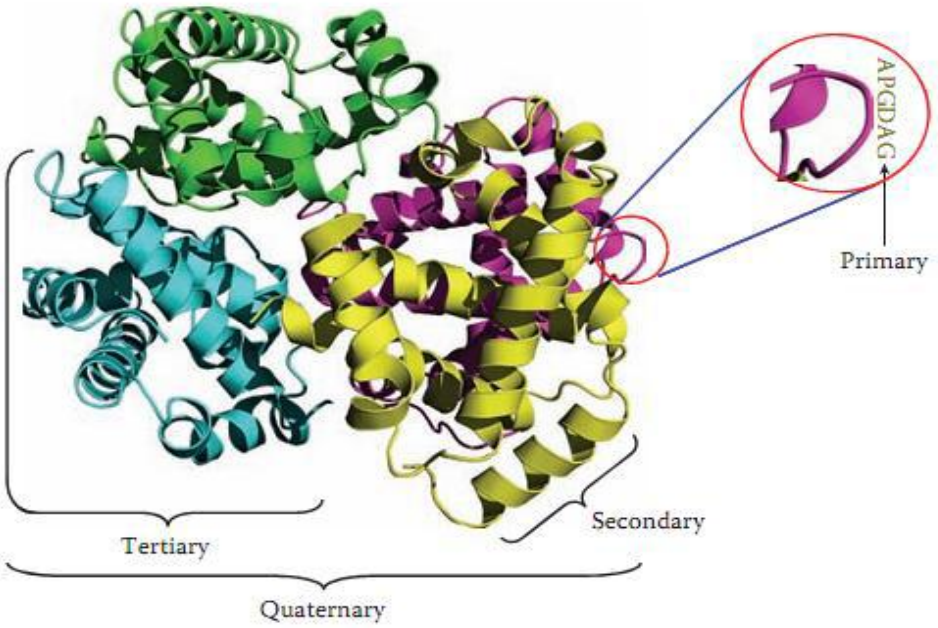
- Like free α-amino acids, a peptide can be charged and has an isoelectric pH (pI) at which the peptide's net electrical charge is zero; consequently, it does not migrate in an electric field.
- The net charge of a peptide is the sum of the charges of all the free ionizable groups of the α-amino acids.
- The ionization of these groups depends on the pH of the medium.
- This property can be used to separate peptides based on their electrical charge.



Levels of protein organization

A native protein is organized into a specific structure (a stable, functional three-dimensional structure); there are four levels of organization:

- ❑Primary structure
- ❑Secondary structure
- ❑Tertiary structure
- ❑Quaternary structure



Primary structure

- This is the sequence of α-amino acid residues that make up the protein; thus, for each protein, there is a specific number and arrangement of α-amino acids.

Secondary structure

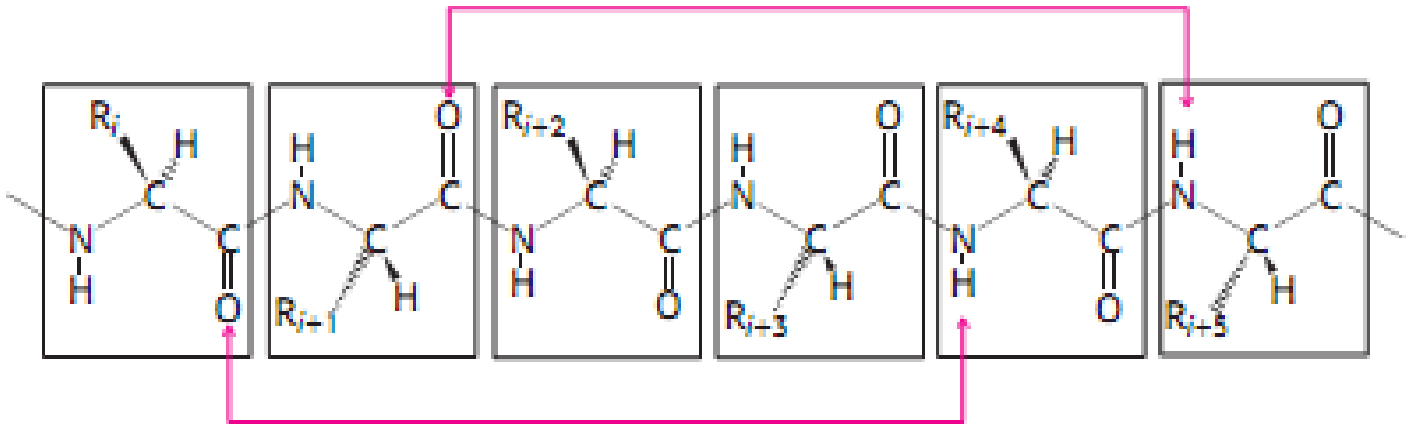
- This is an arrangement of the polypeptide chain stabilized by hydrogen bonds.
- The two most common stable structures found in proteins are the α-helix and the β-sheet.
- The β-turn also exists; it is a secondary structure required for a change in direction.

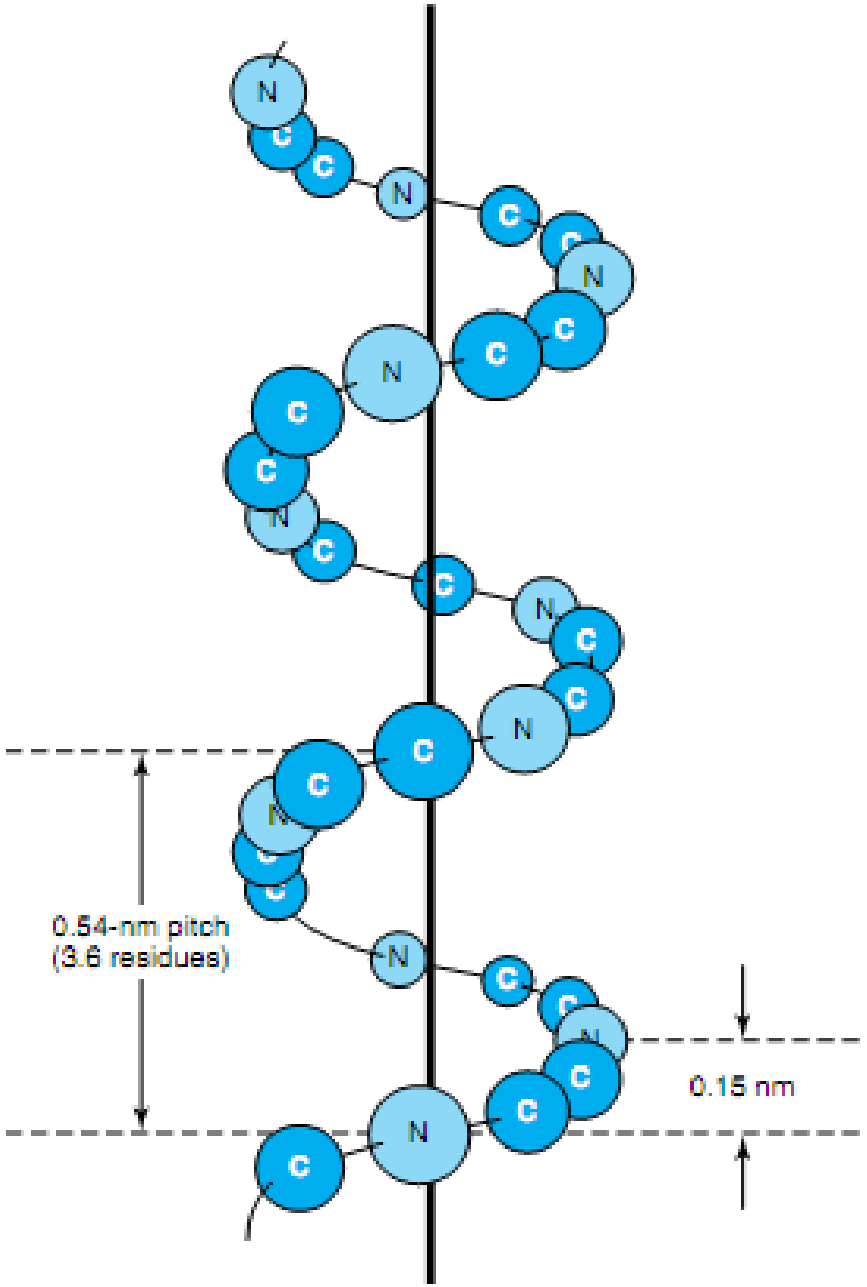
α-helix

❑This is a helical coiling of the polypeptide chain; the structure is stabilized by hydrogen bonds formed between the -CO group of the peptide bond of amino acid « i » and the -NH group of the peptide bond of amino acid « i+4 ».

❑One turn of the helix consists of 3.6 amino acid residues-referred to as the pitch-and measures 5.4 Å (0.54 nm) in length.

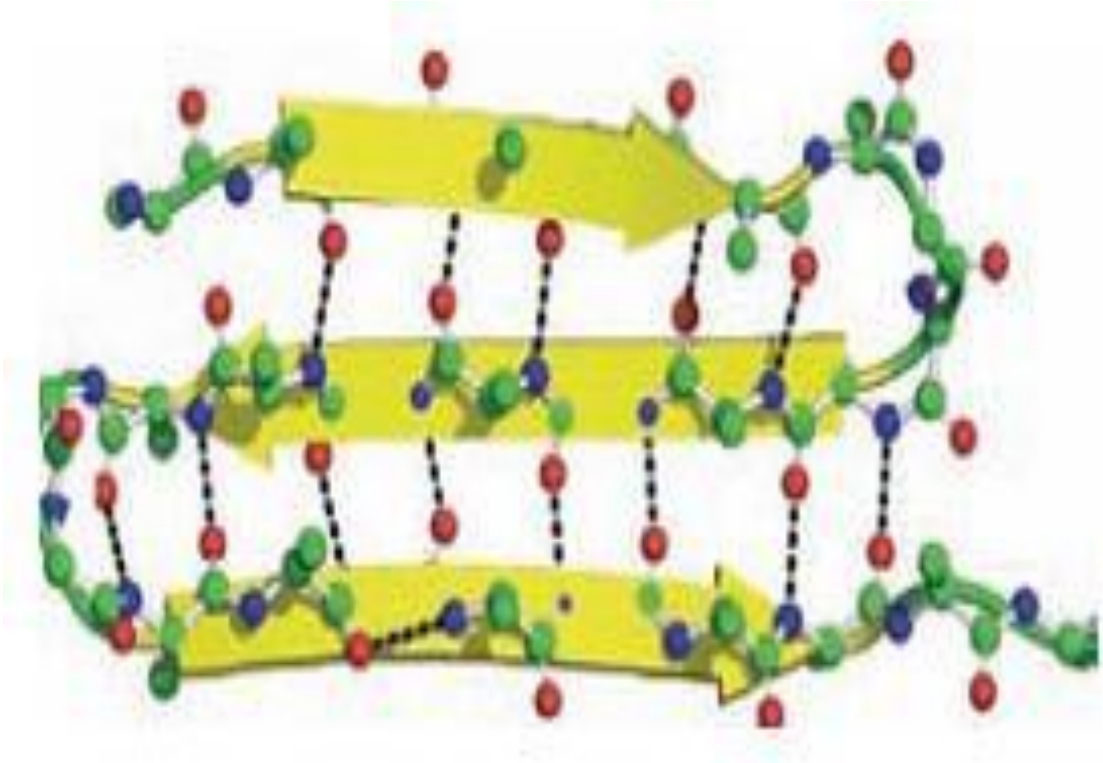
❑In the helix, the side chains of each amino acid residue are oriented outwards




0.54-nm pitch
(3.6 residues)
0.15 nm

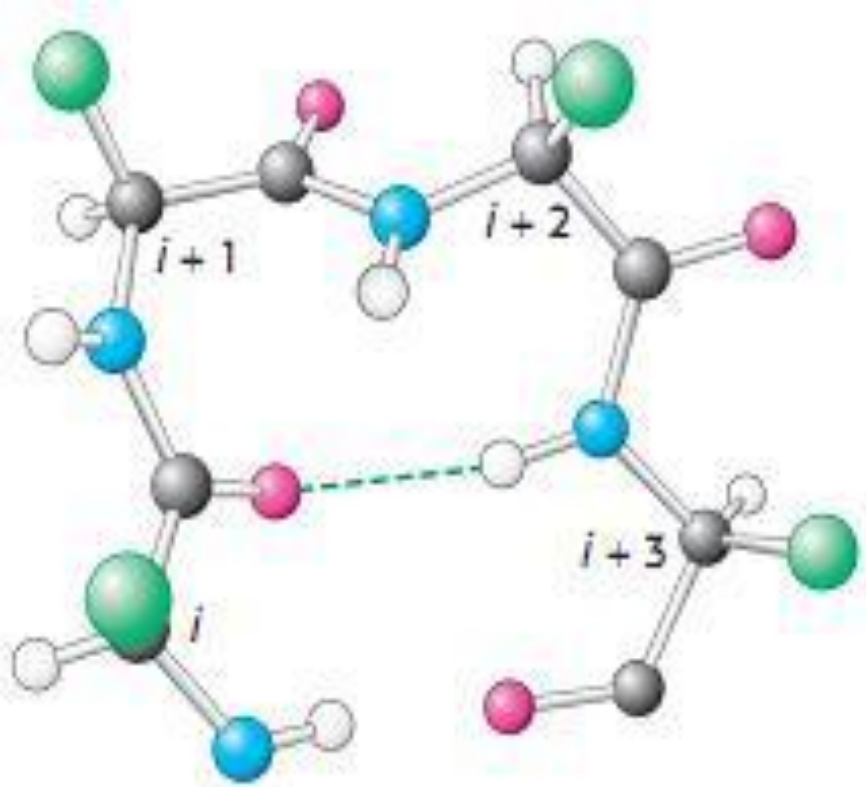
β-pleated sheet

- This is an arrangement of the polypeptide chain into a zig-zag structure, stabilized by the formation of hydrogen bonds between two peptide strands; the atoms participating in these hydrogen bonds belong to the peptide bonds.
- The side chains of each residue are positioned alternately on either side of the plane of the sheet



β-turn

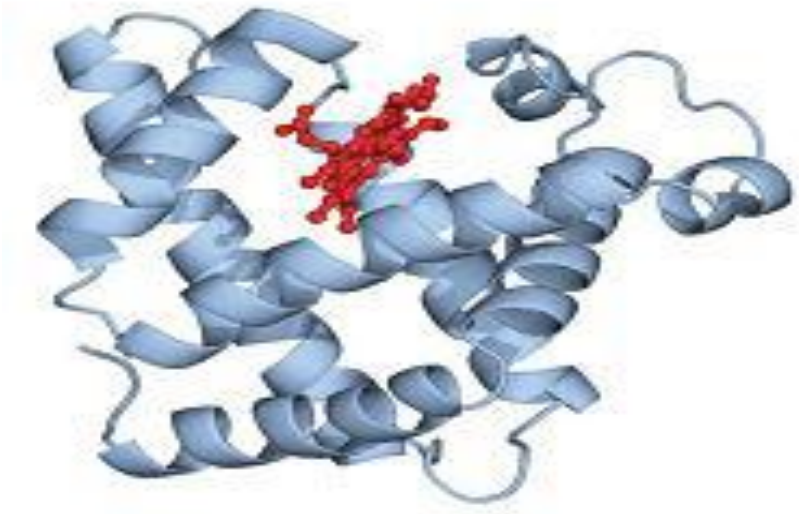
- Changes in the direction of the polypeptide chain are made possible by turns or loops.
- The β-turn is very common; it consists of four amino acid residues, with glycine and proline occurring very frequently.



Tertiary structure

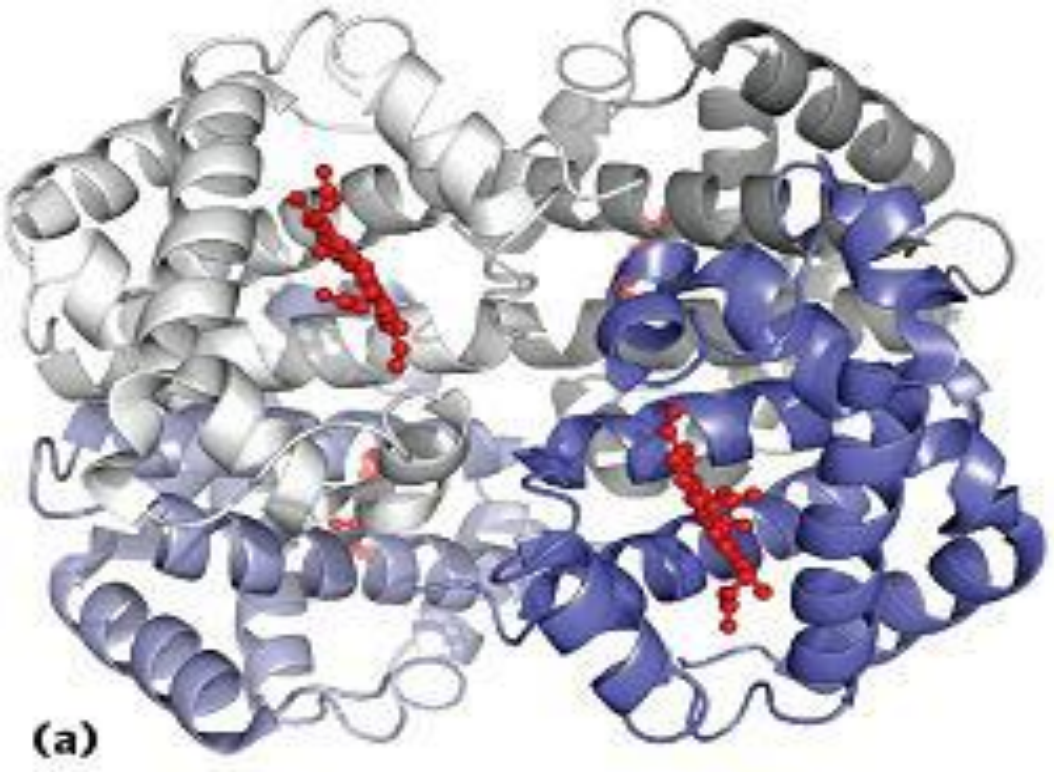
This is the folding of the polypeptide chain into a more compact three-dimensional shape; this structure is stabilized by bonds between the side chains of amino acid residues, such as

- Ionic bonds,
- Hydrogen bonds,
- Hydrophobic interactions,
- van der Waals forces



Quaternary structure

- Many proteins are formed by two or more polypeptide chains (called subunits) that associate to give the protein a quaternary structure



Protein denaturation

- Protein denaturation is the disruption of a protein's three-dimensional structure, leading to a loss of function (the primary structure remains unaffected).

- Denaturing agents: temperature (can cause the breaking of hydrogen bonds), pH changes (disruption of ionic interactions), organic solvents (alcohols, acetone), urea, etc.

Study of protein primary structure (protein sequencing)

To study proteins, they must be obtained in the purest possible state; several purification methods have been developed for this purpose, primarily electrophoresis and chromatography.

Once the protein is purified, it is essential to determine its amino acid composition as well as the exact order of the amino acids within the peptide chain.

Two methods exist for this:

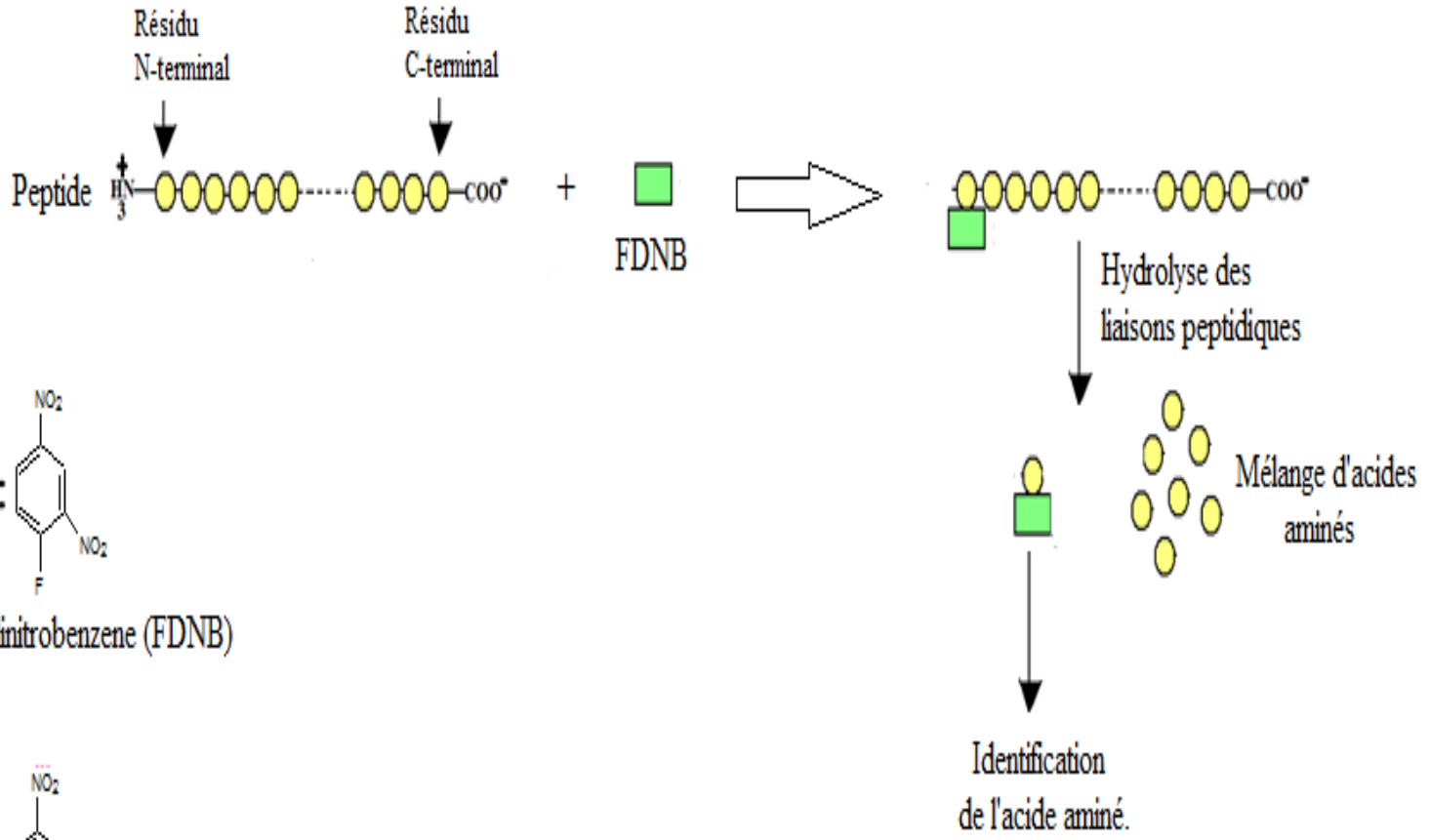
- Sequencing the protein itself;
- Sequencing the DNA of the gene encoding the protein.

Determination of the number of polypeptide chains

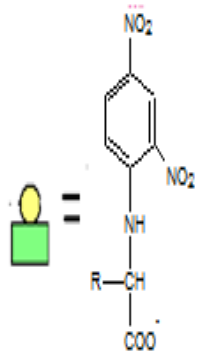
- Determining the number of polypeptide chains that make up a protein involves determining the number of terminal residues, since each chain possesses a single N-terminal residue and a single C-terminal residue.
- For example, if a protein is found to have two N-terminal residues, this means it is composed of two polypeptide chains.

Identification of the N-terminal residue using the Sanger method

- In the Sanger method, 1-fluoro-2,4-dinitrobenzene (FDNB) is used;
- This reagent forms a chemical bond exclusively with the N-terminal residue, which-following hydrolysis of the peptide bonds, is released as a dinitrophenyl derivative that is easily identified
- Dansyl chloride also reacts with the N-terminal residue to yield a fluorescent derivative of the N-terminal amino acid, which is easily identified.



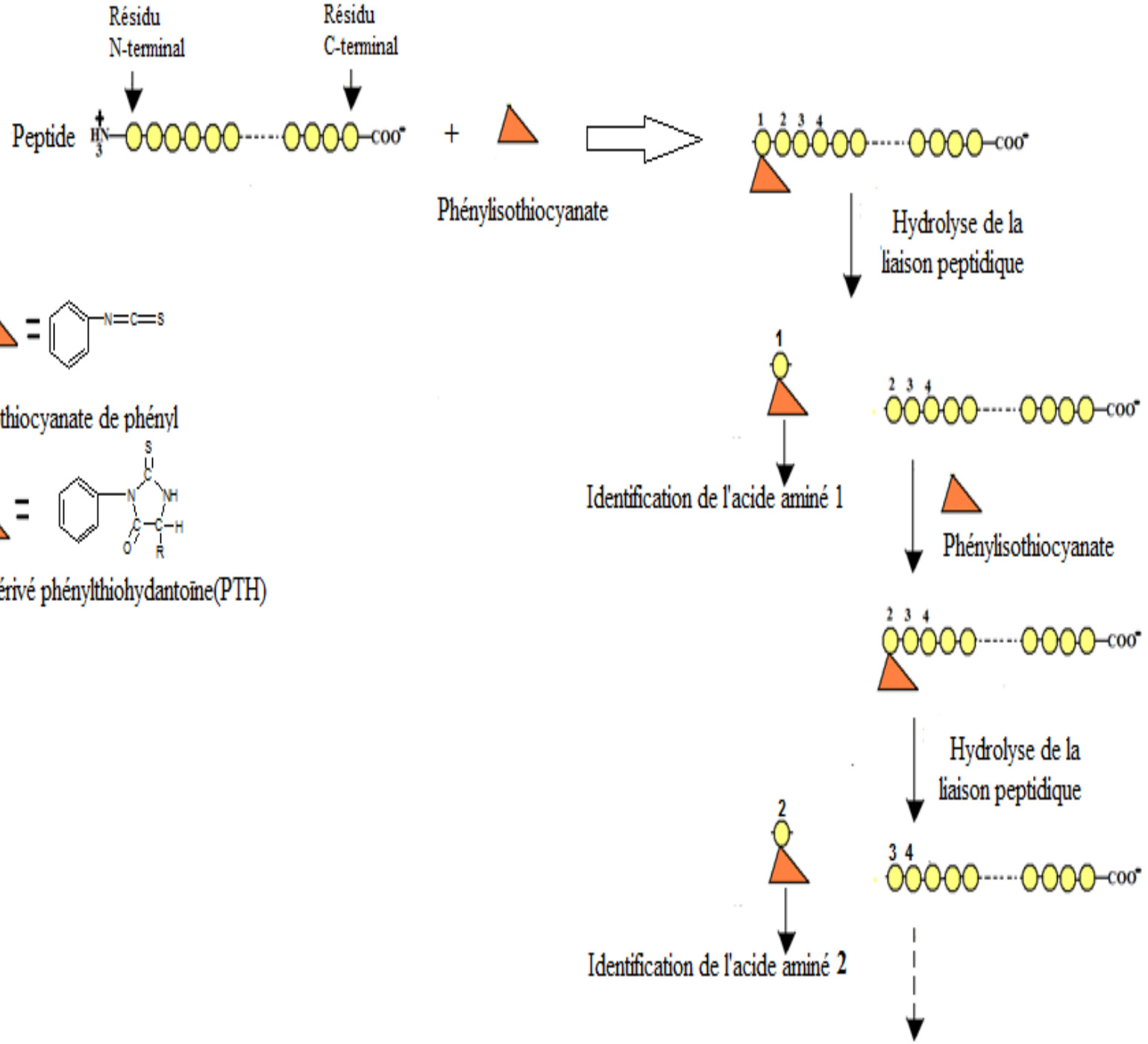
1-fluoro-2,4 – dinitrobenzene (FDNB)



2-4 dinitrophényl dérivé
de l'acide aminé N-terminal

Identification of the N-terminal residue by the Edman method

- This method uses phenylisothiocyanate, which reacts with the N-terminal amino acid of the peptide chain;
- However, unlike the Sanger method-which identifies only a single amino acid-the Edman method can identify amino acids one by one starting from the N-terminus, making it a sequential method

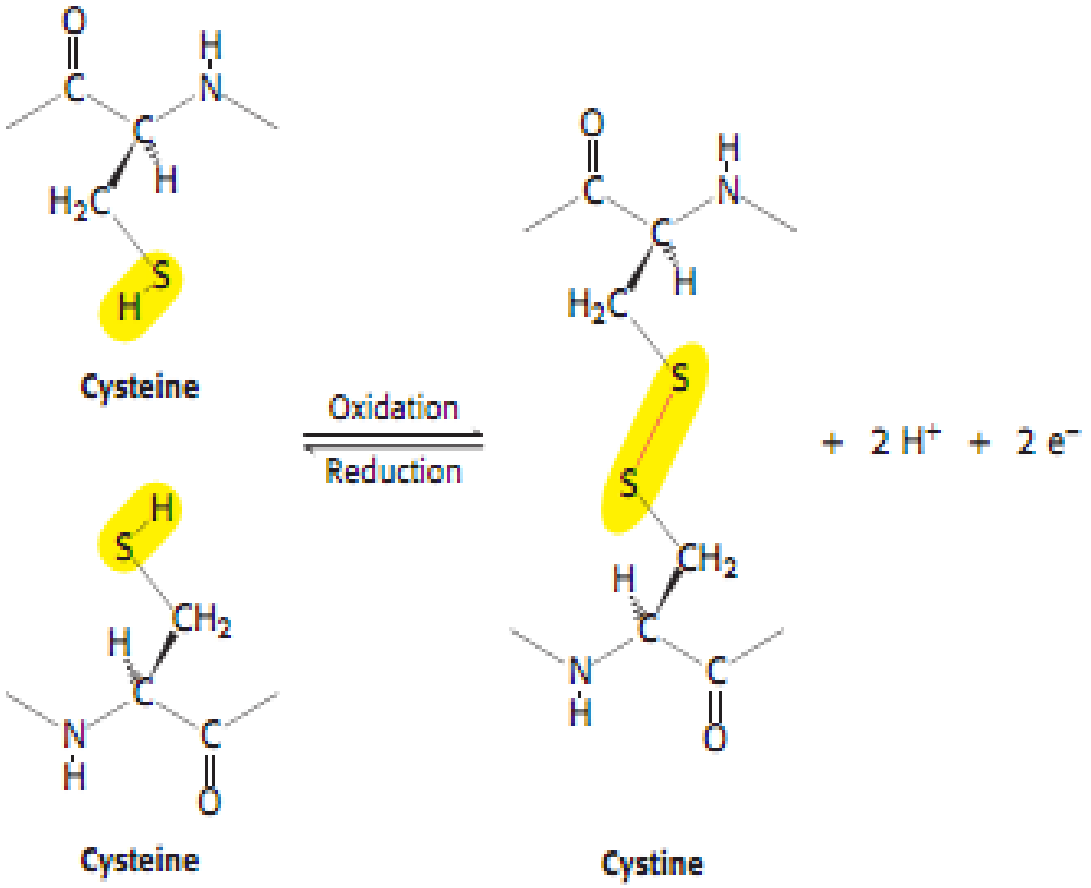

Résidu
N-terminal
Résidu
C-terminal
Peptide
Phénylisothiocyanate
Hydrolyse de la
liaison peptidique
Isothiocyanate de phényl
Dérivé phénylthiohydantoïne(PTH)
Identification de l'acide aminé 1
Phénylisothiocyanate
Hydrolyse de la
liaison peptidique
Identification de l'acide aminé 2

Identification of the C-terminal residue

- The C-terminal amino acid can be identified using an enzymatic method involving carboxypeptidases (exopeptidases) enzymes that act specifically on the C-terminal amino acid.
- Alternatively, a chemical method may be used, which involves treating the peptide with hydrazine at 100°C; this converts all amino acids into hydrazides, except for the C-terminal residue, which remains intact and free, making it easy to isolate and identify

Cleavage of disulfide bonds (inter- and intramolecular)

- 2-mercaptoethanol (HSCH2CH2OH) is used to cleave the -S-S- bonds



Determination of the α-amino acid composition of peptide chains

- To identify the α-amino acid composition of a peptide chain, it is necessary to cleave all the peptide bonds and release the amino acids, which are subsequently identified using chromatographic methods.

- There are two methods for the hydrolysis of peptide bonds

Acid hydrolysis

- This is performed in an acidic medium (6N HCl at 105°C) for 24 hours in tubes sealed under vacuum or nitrogen; the sample concentration must be low (0.5 to 1%).
- The disadvantage of acid hydrolysis is the conversion of glutamine (Gln) into glutamic acid (Glu) and asparagine (Asn) into aspartic acid (Asp), as well as the destruction of tryptophan (Trp).

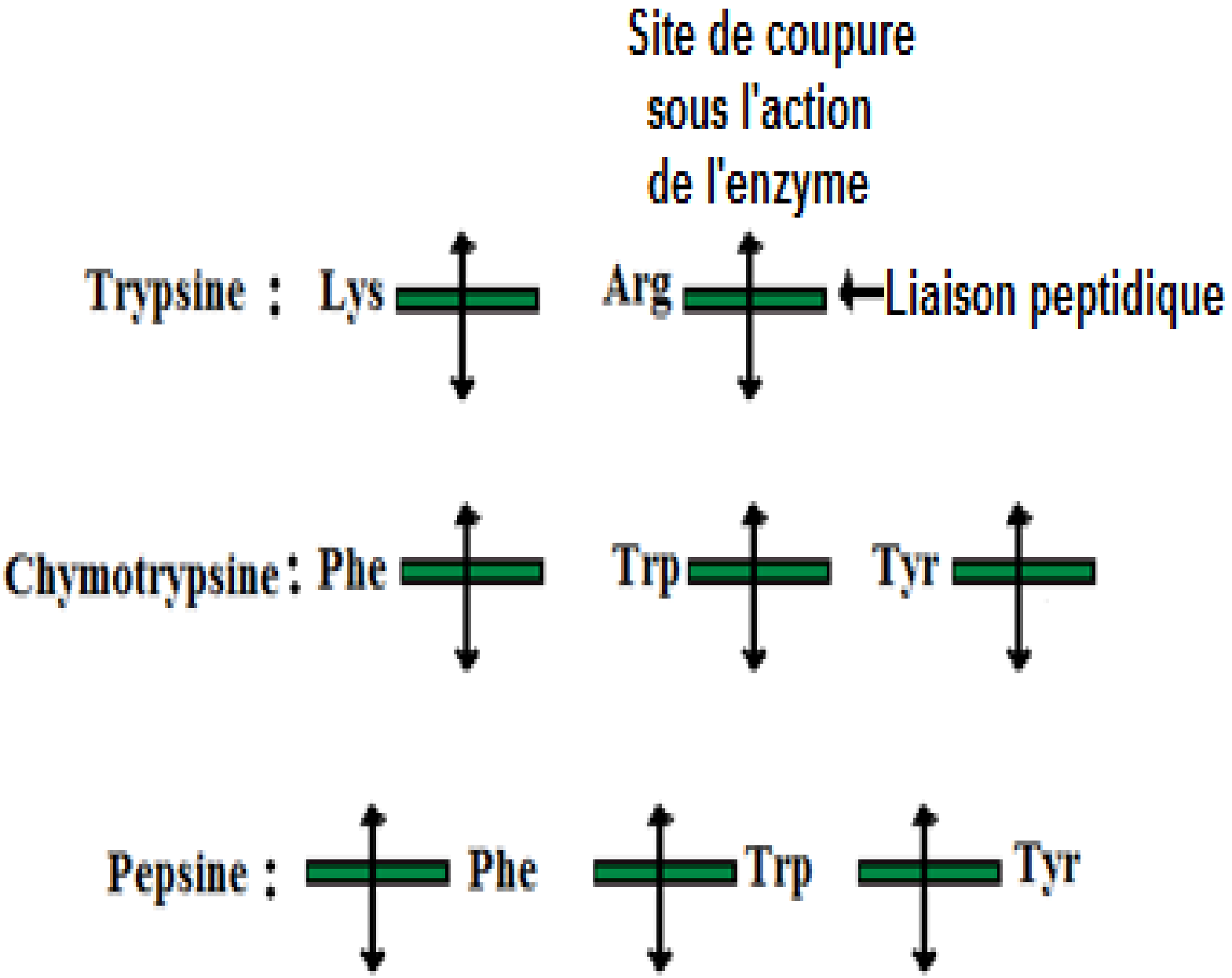
Alkaline hydrolysis

- Performed in a basic medium (6 M NaOH at 100°C) for 4–8 hours, it alters many amino acids but does not destroy tryptophan (Trp); it can therefore be used—alongside acid hydrolysis—to verify the presence or absence of tryptophan

Sequence determination via differential cleavage of the peptide chain

- A complete peptide can be cleaved at various sites; depending on the enzyme used.
- A set of shorter peptides is obtained, which must be reassembled to determine the exact sequence of the original peptide.
- The enzymes employed are proteolytic enzymes-specifically peptidases-such as trypsin, chymotrypsin, and pepsin.

- Trypsin hydrolyzes peptide bonds in which lysine (Lys) and arginine (Arg) are involved via their carboxyl groups.
- Chymotrypsin hydrolyzes peptide bonds in which phenylalanine (Phe), tryptophan (Trp), and tyrosine (Tyr) are involved via their carboxyl groups.
- Pepsin hydrolyzes peptide bonds in which phenylalanine (Phe), tryptophan (Trp), and tyrosine (Tyr) are involved via their amino groups.
- Cyanogen bromide (CNBr) is a reagent that specifically hydrolyzes peptide bonds after the methionine residue.


Site de coupure
sous l'action
de l'enzyme
Trypsine : Lys
Arg
Liaison peptidique
Chymotrypsine : Phe
Trp
Tyr
Pepsine : Phe
Trp
Tyr