الجمهورية الجزائرية الديمقراطية الشعبية
PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
وزارة التعليم العالي والبحث العلمي
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
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كلية العلوم
FACULTY OF SCIENCES
قسم البيوكيمياء
DEPARTMENT OF BIOCHEMISTRY

Stuctural and Metabolic Biochemistry

TCSNV 2nd Fondamental unit S3

AMINO ACIDS / PROTEINS

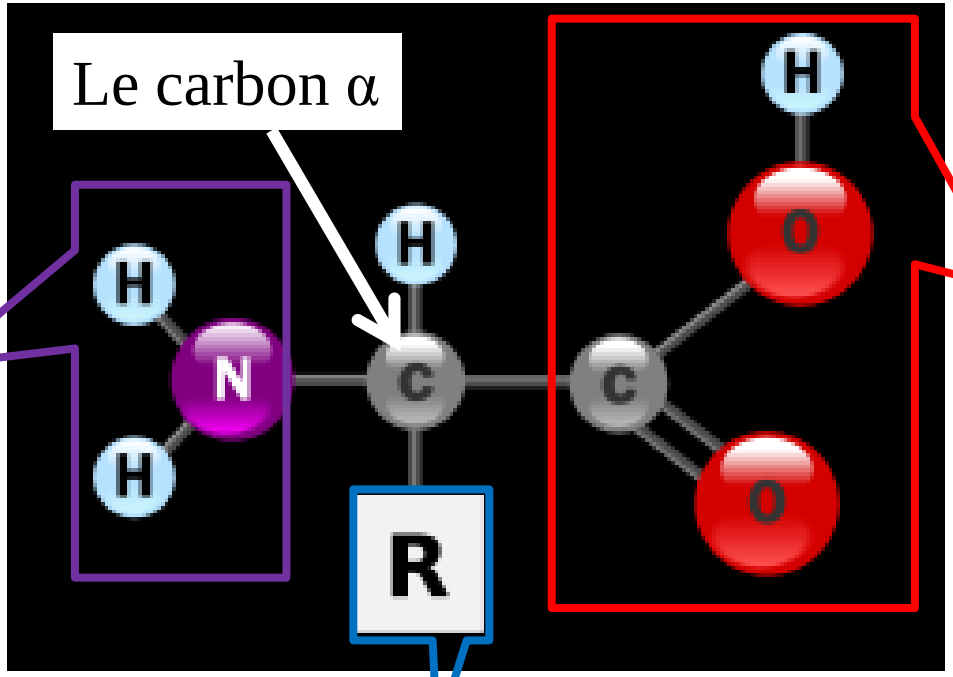
Pr. KADI-BIREM

Protein → proteide → peptide → amino acid

To date, 22 amino acids have been discovered in the proteins of living organisms. Among these, 20 are included in the genetic code of animals. Of these 20 amino acids, 8 are essential: they cannot be synthesized de novo by the organism and must therefore be obnained through the diet.

Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophane, Valine

General structure of an amino acid



Amine group

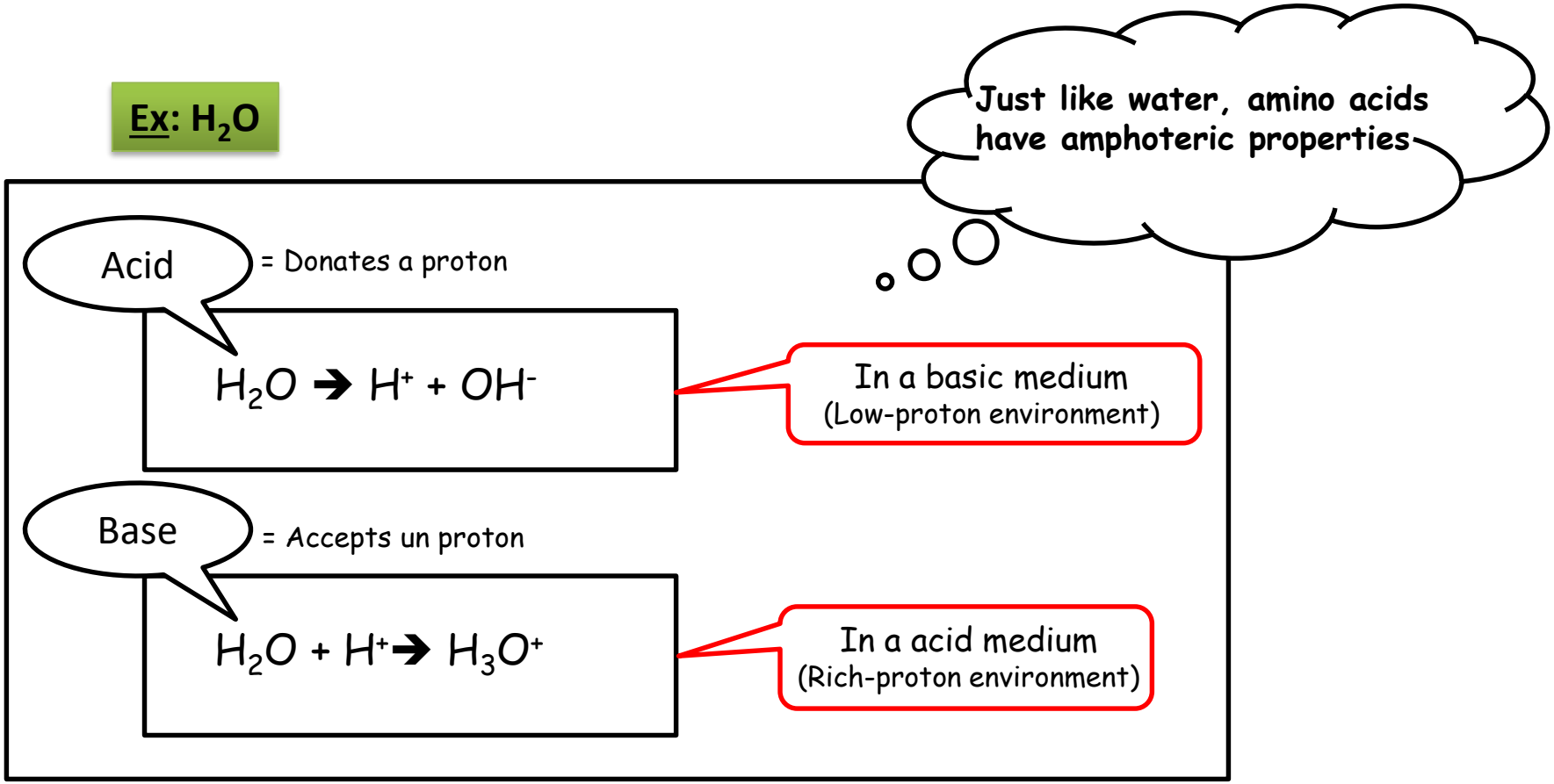
Carboxylic group

Variable side chain from one AA to another

Properties of amino acids

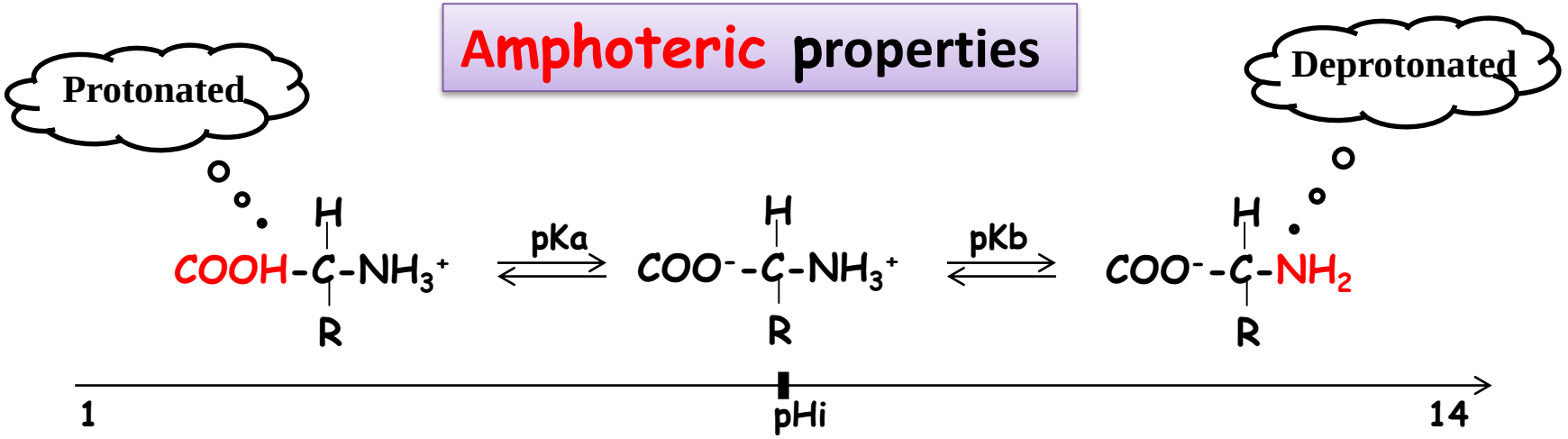
Amphoteric properties

➔ **Substance that can act as un acid and as a base**



Properties of amino acids

Amphoteric properties



- pK ➔ a specific pH

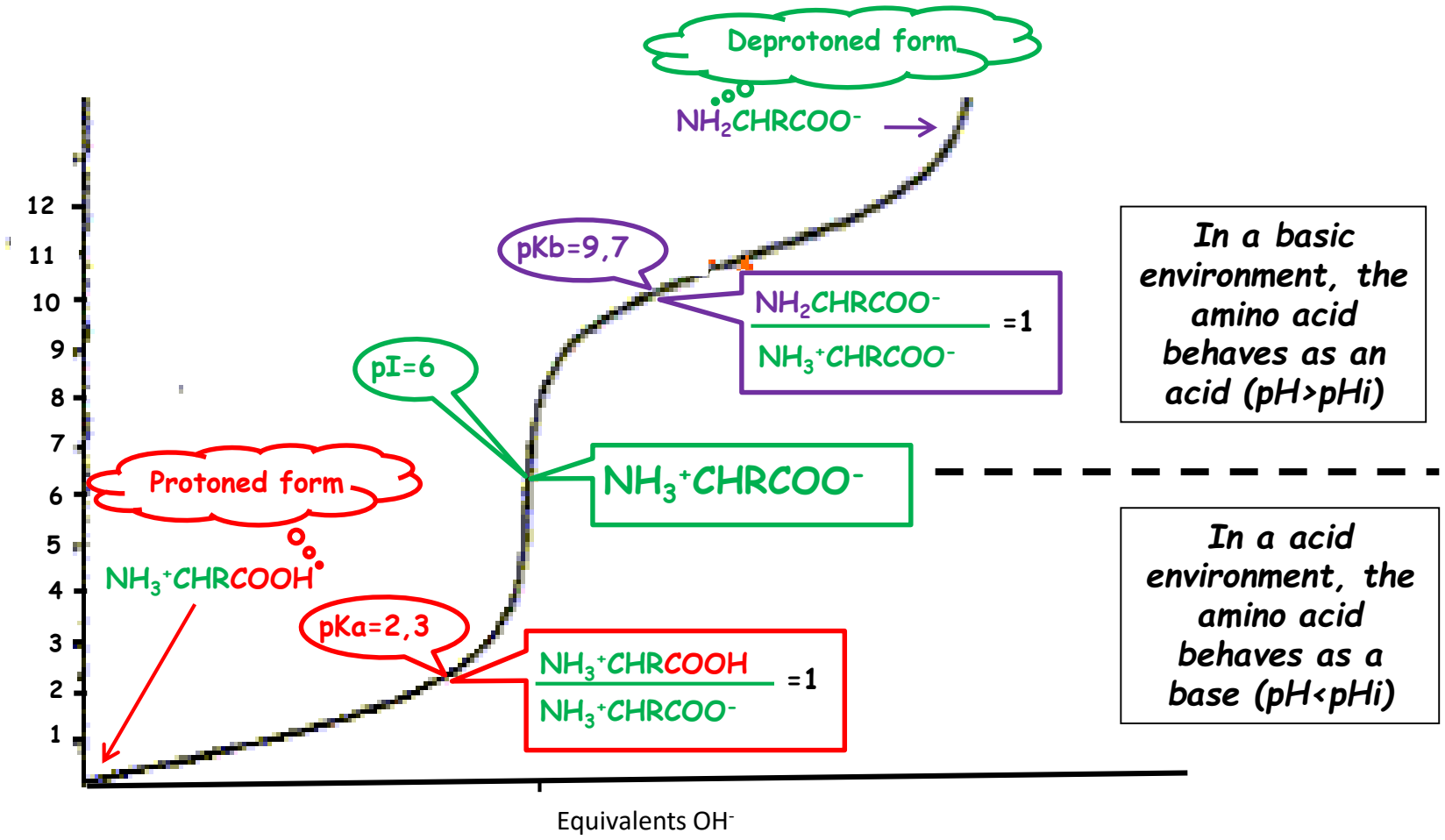
  Every amine and carboxylic group have a pK (pKa / pKb)

  pH=pK ➔ 50 % protoned form and 50 % deprotoned form

- Isoelectric point (pHi)

  $pH = (pK_A + pK_B) / 2$

Titration of amino acids



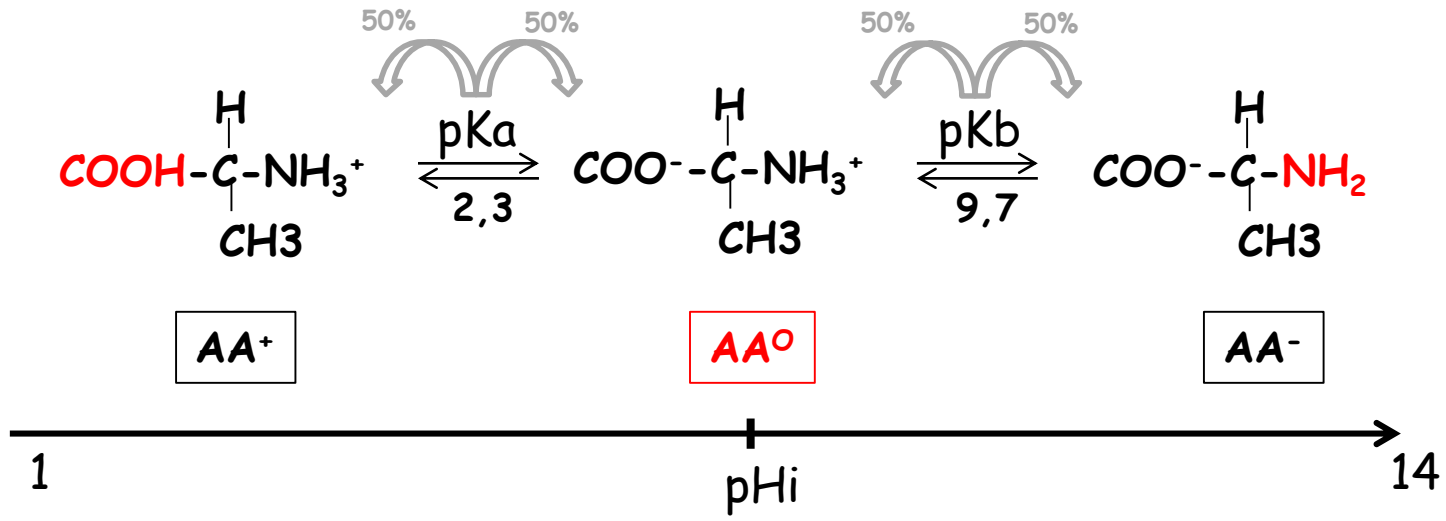
Properties of amino acids

Amphoteric properties

For an amino acid with a non ionizable side chain

Ex: Alanine (ALA) (A)

pKa=2,3 pKb=9,7



pHi = pKa + pKb / 2

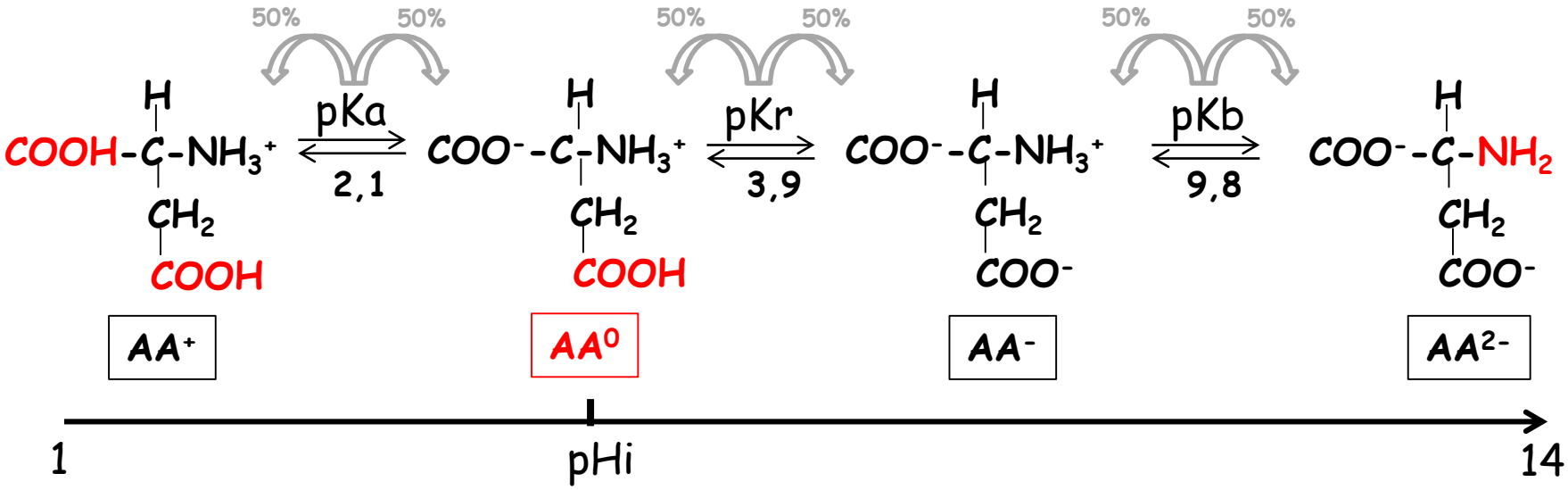
Properties of amino acids

Amphoteric properties

For an amino acid with ionizable side chain

Ex1: Acide aspartique (ASP) (D)

pKa=2,1 pKb=9,8 pKr=3,9



pHi = pKa + pKr / 2

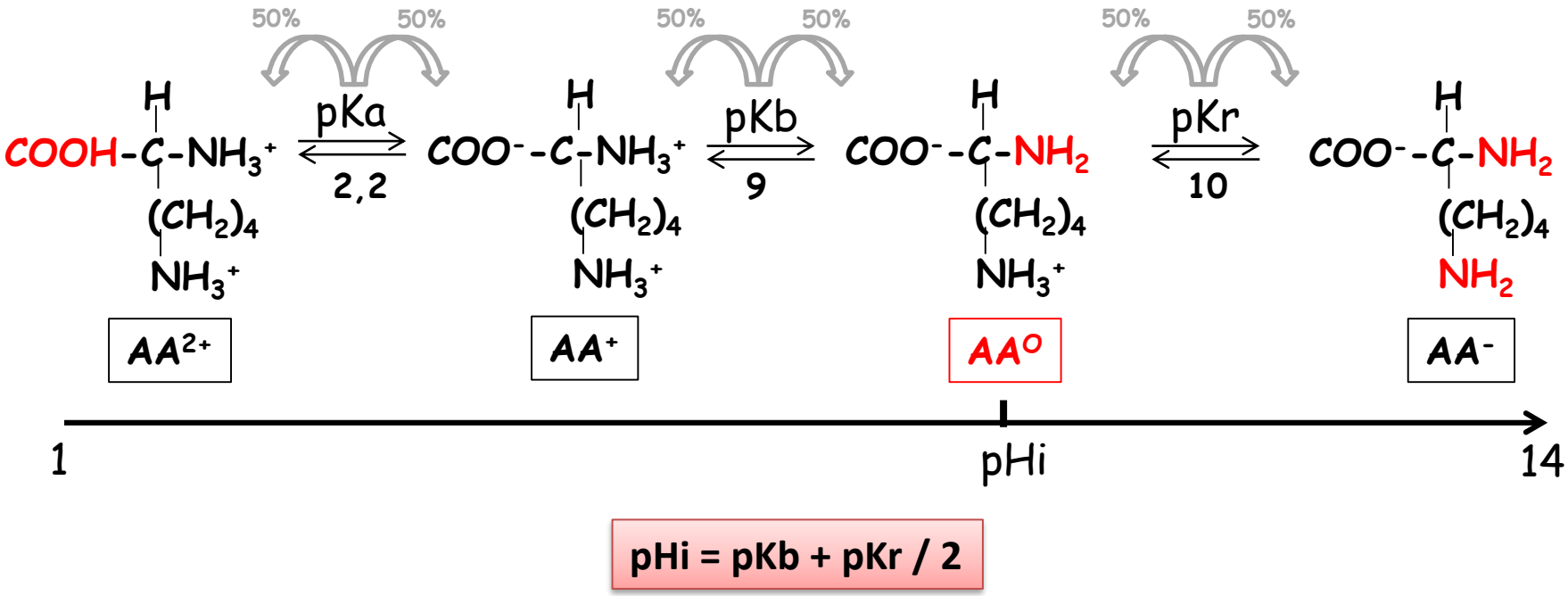
Properties of amino acids

Amphoteric properties

For an amino acid with ionizable side chain

Ex2: Lysine (LYS) (K)

pKa=2,2 pKb=9 pKr=10



pHi = pKb + pKr / 2

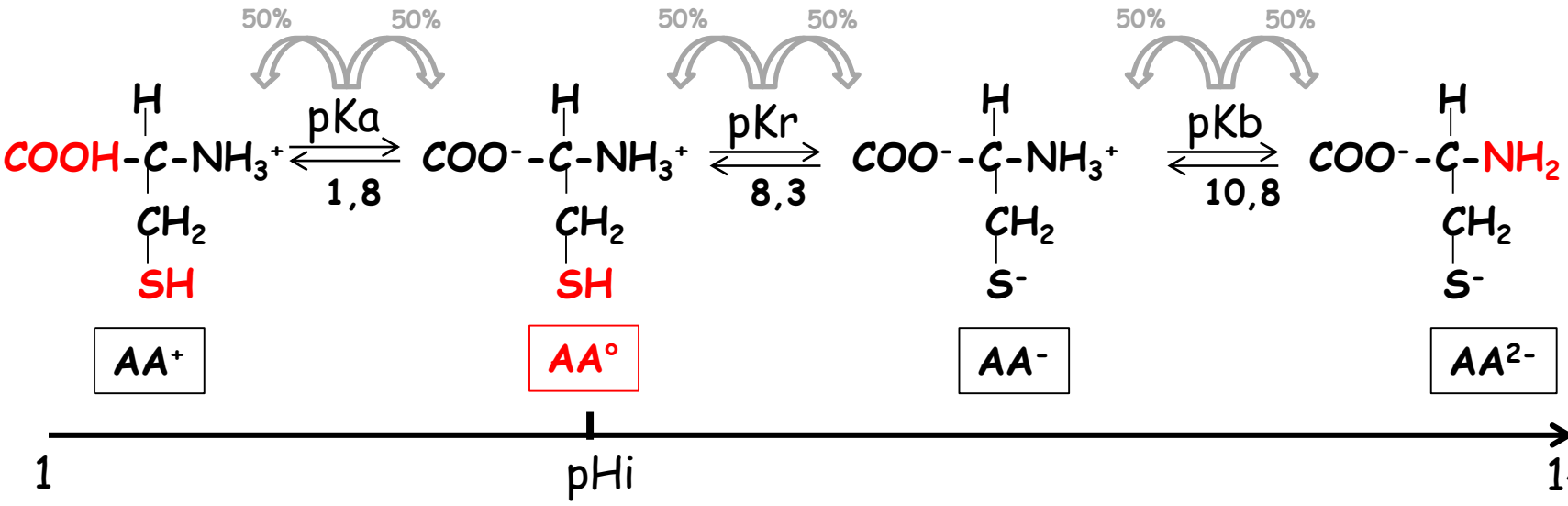
Properties of amino acids

Amphoteric properties

For an amino acid with ionizable side chain

Ex3: Cystéine (CYS) (C)

pKa=1,8 pKb=10,8 pKr=8,3



pHi = pKa + pKr / 2

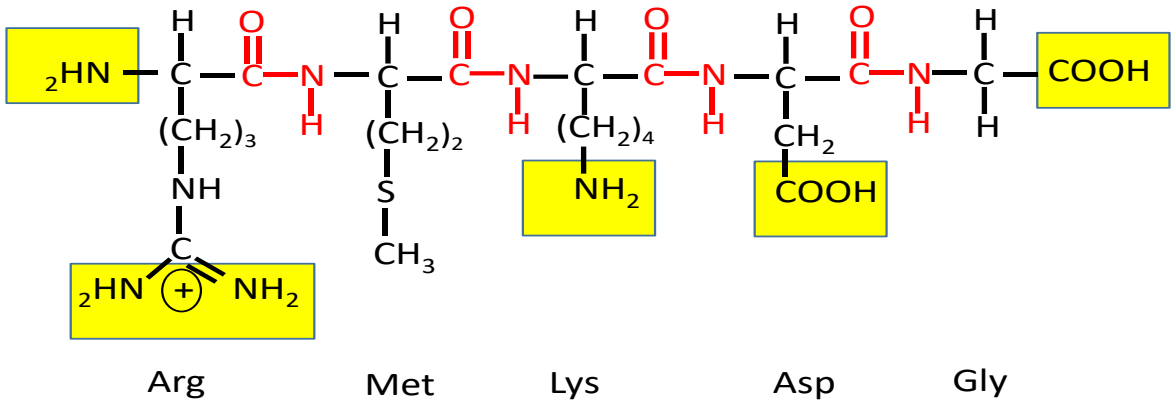
Properties of amino acids

Amphoteric properties

Ionisation of a peptide

Example : *at pH = 10*

To determine the ionization state of a peptide, we must first identify the ionizable groups.



This peptide has 5 ionizable groups.

When studying the ionization of amino acids, the rule is:

- **When pH > pI → the amino acid is negatively charged (AA^-)**
- **When pH < pI → the amino acid is positively charged (AA^+)**
- **When pH = pI → the amino acid is neutral (AA^0)**

In the case of a peptide, we consider the pKa, pKb, and pKr values.

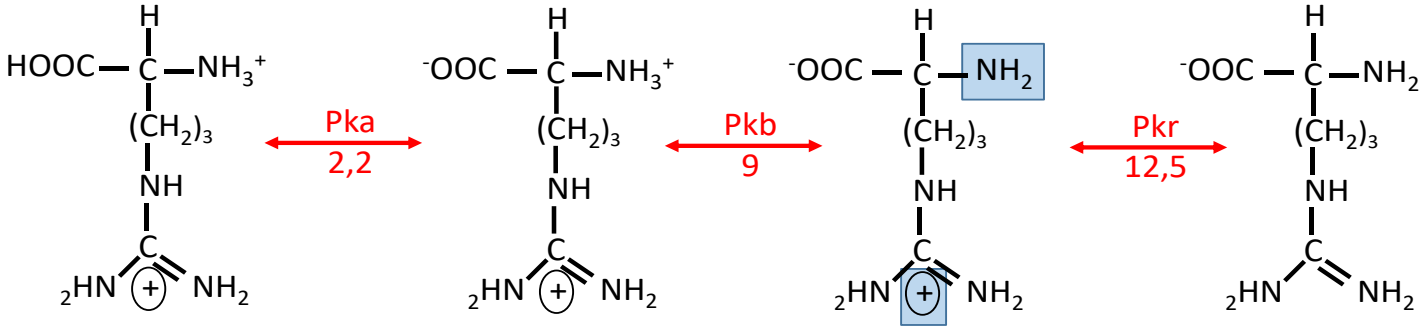
Properties of amino acids

Amphoteric properties

Ionisation of a peptide

We analyze each amino acid individually:

Arginine



- **pH 10 > pKb,** the α-amino group is deprotonated → becomes **NH_2**.
- **pH 10 < pKr**: the guanidinium side chain is **still protonated** → remains positively charged **(Arg $^+$).**

Properties of amino acids

Amphoteric properties

Ionisation of a peptide

We analyze each amino acid individually:

Methionine

- The α-amino and α-carboxyl groups are involved in the peptide bond.
- The side chain is **not ionizable**.
- This amino acid is **neutral at any pH**.

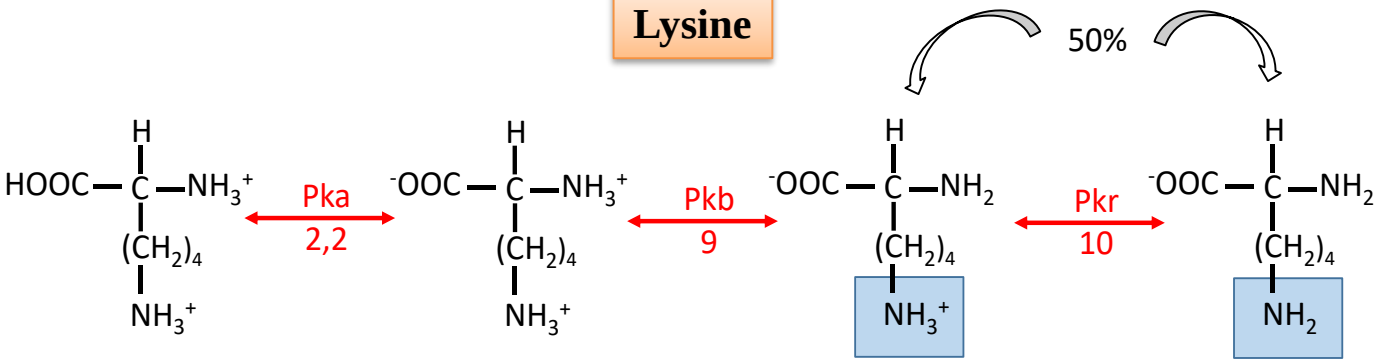
Properties of amino acids

Amphoteric properties

Ionisation of a peptide

We analyze each amino acid individually:

Lysine



- The α-amino and α-carboxyl groups are involved in the peptide bond.
- Only the **side chain ($-NH_3^+$)** is considered.
- **pH = pKr** → 50% of side chains are **protonated (+)**, 50% are **deprotonated (neutral)**.

So, we consider **both cases**:

- Neutral: **$-NH_2$**
- Positive: **$-NH_3^+$**

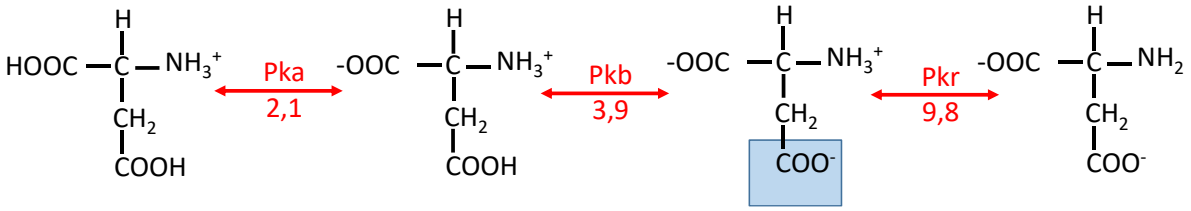
Properties of amino acids

Amphoteric properties

Ionisation of a peptide

We analyze each amino acid individually:

Aspartate



- **pH 10 > pKr** : the carboxyl group is **deprotonated ➔ COO^-**

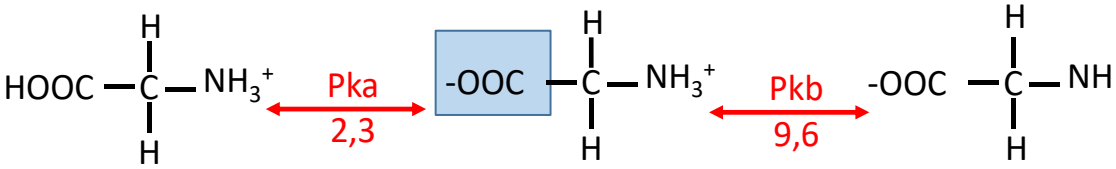
Properties of amino acids

Amphoteric properties

Ionisation of a peptide

We analyze each amino acid individually:

Glycine



- **pH 10 > pKa,** the group is **deprotonated ➔ COO^-**

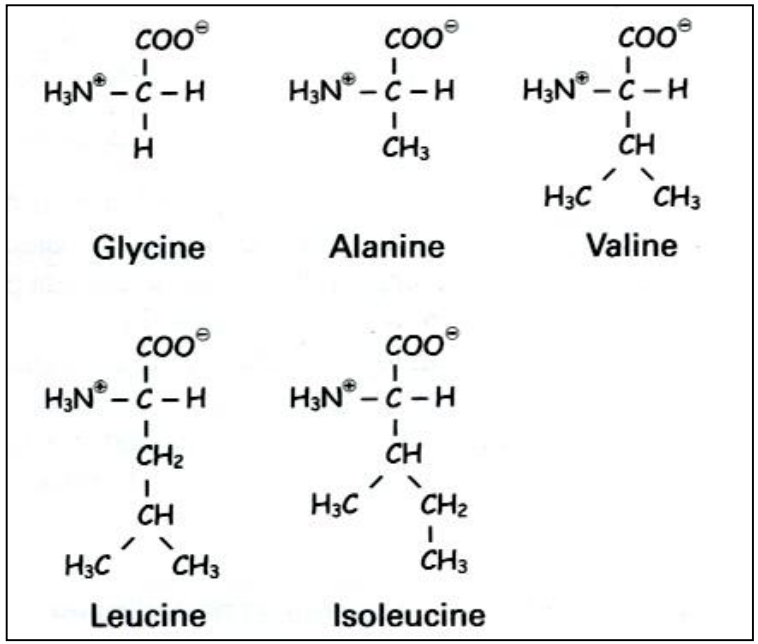
Final result at pH 10:

NH_2 – Arg(+) – Met(0) – Lys(0/+) – Asp(–) – Gly – COO^-

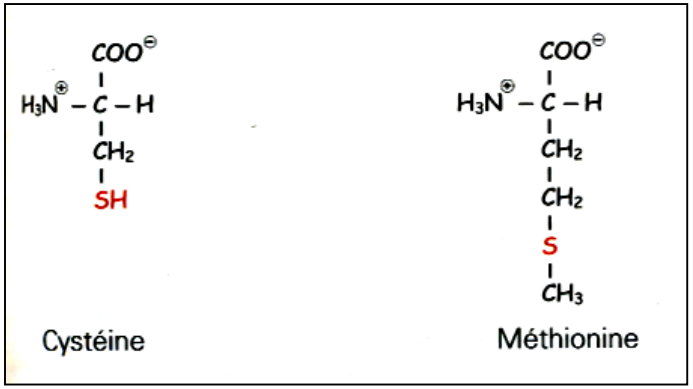
So, the **overall charge** is: **–1 (or 0 if Lys is positively charged)** But assuming the average state, the charge is **approximately –1**.

Nonpolar amino acids (hydrophobes)

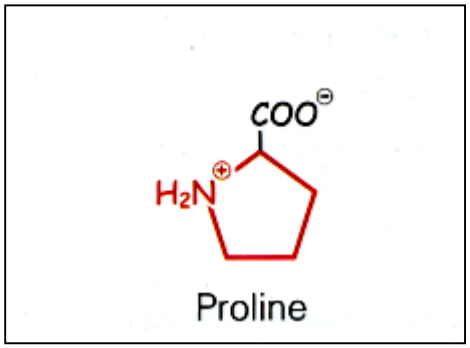
Aliphatic amino acids



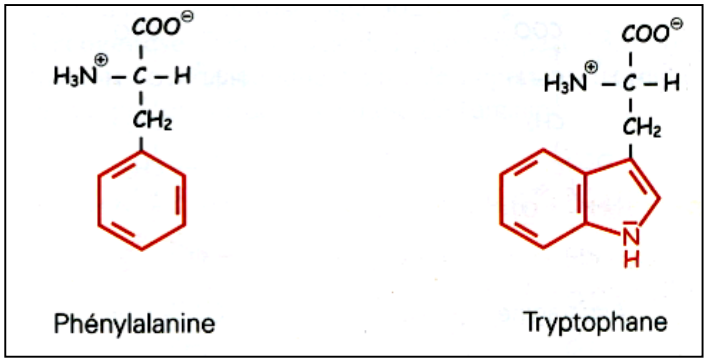
Sultur-containing amino acids



Aliphatic cyclic amino acid



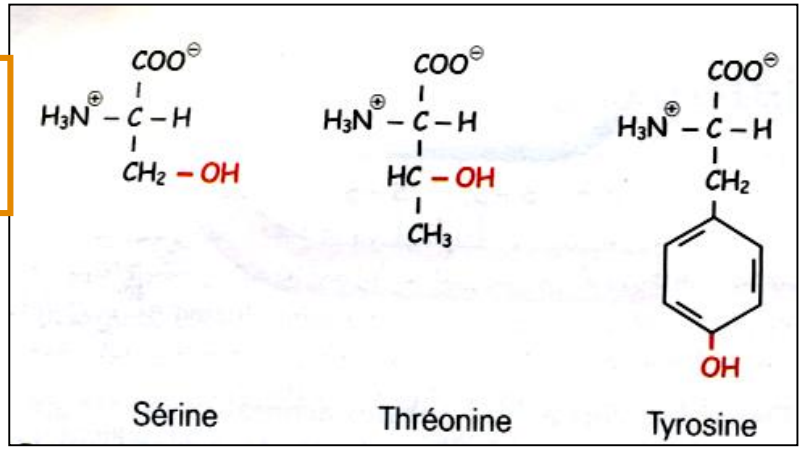
Aromatic amino acid



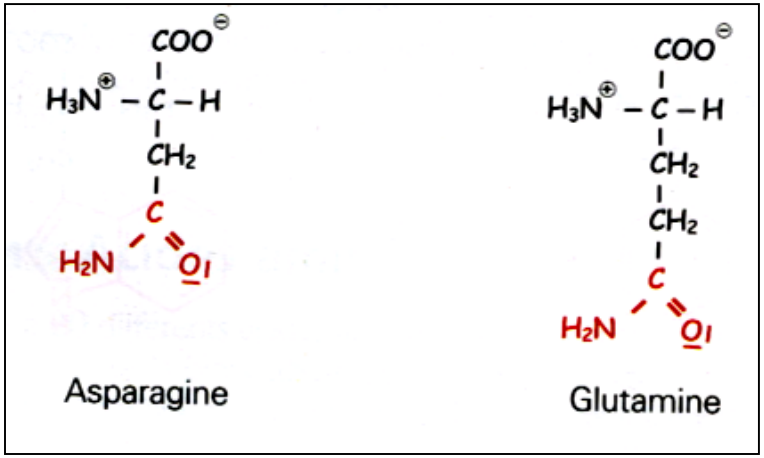
Polar amino acids (hydrophiles)

Uncharged amino acids

Hydroxyl amino acids

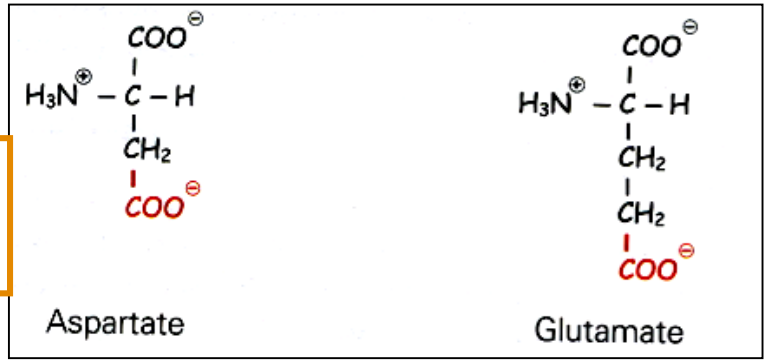


Amide amino acids

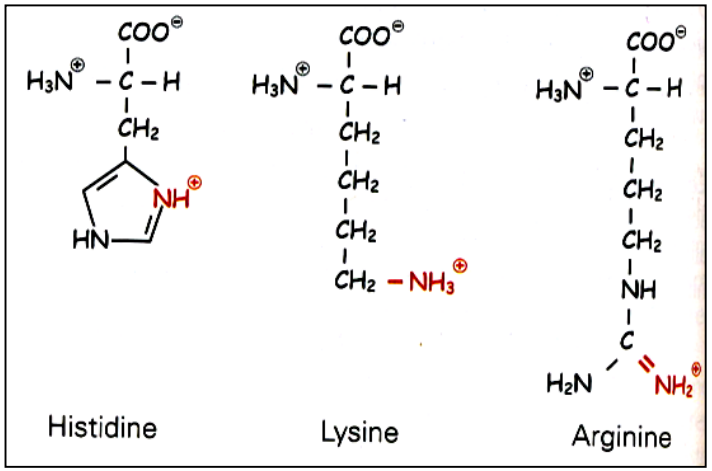


Charged amino acids

Dicarboxylic amino acids



Basic amino acids



Fractionation and dosage of amino acids

✓ **Certain amino acids have specific reactions (coloration or spectrum) that allow for their quantification.**
✓ **Here are techniques for identifying amino acids:**

Thin-Layer Electrophoresis (TLE)

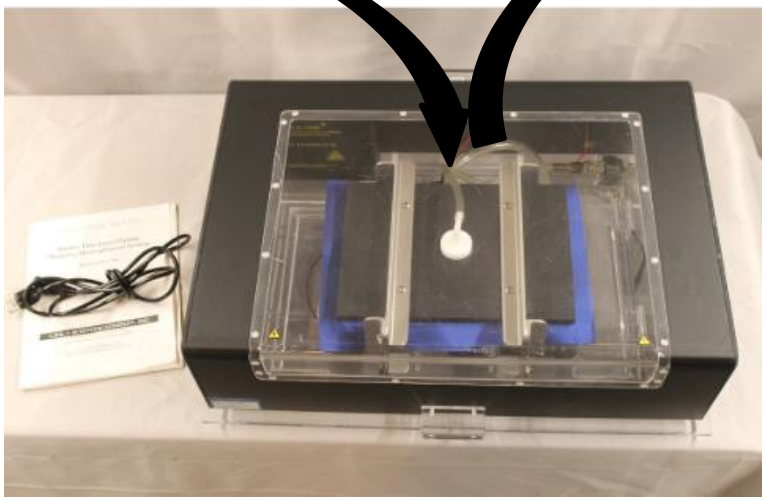
Ion exchange column chromatography

Separation and quantification of amino acids

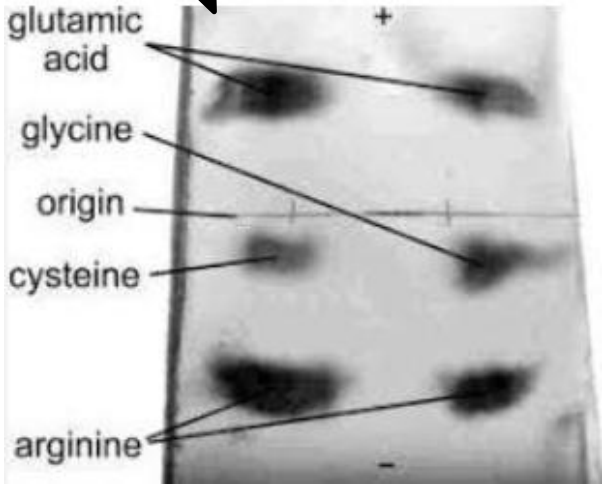
Thin layer Electrophoresis (TLE)

Allows for the separation of amino acids on a stationary phase (membrane) using an electric field based on their charges, with different solvents (mobile phase) and varying pH levels.

Cellulose layer



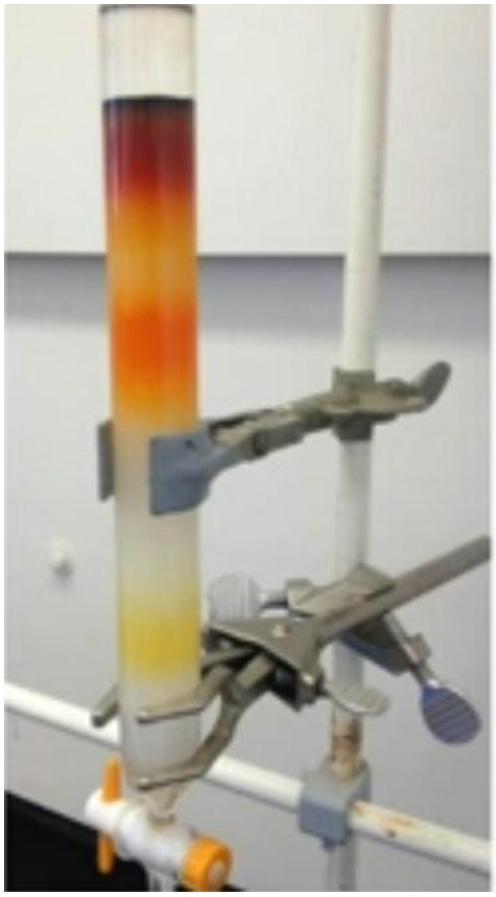
Electrophoresis chamber



Amino acids visualization

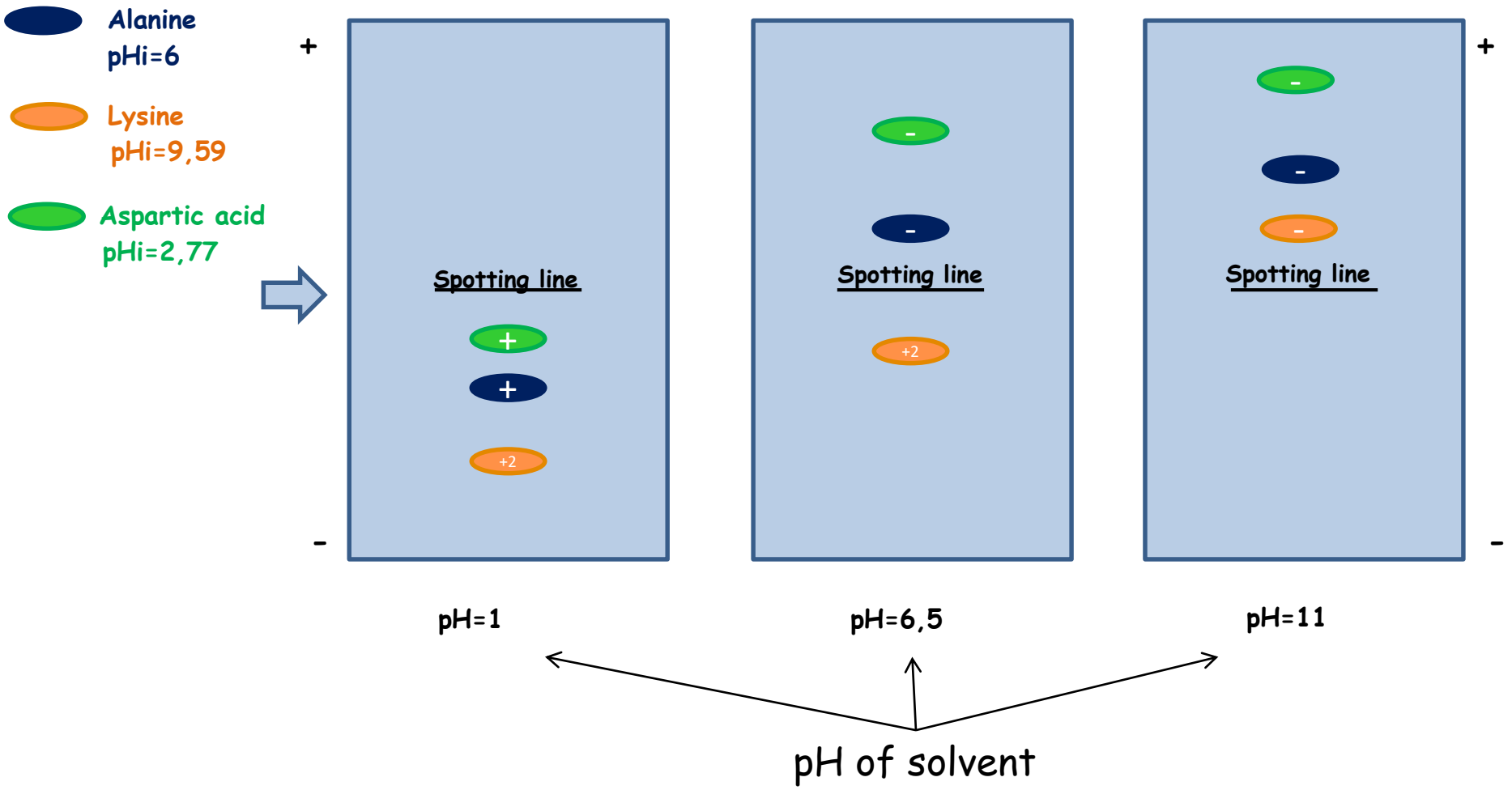
Separation and quantification of amino acids

Ion exchange chromatography
(chromatographie sur colonne de résine échangeuse d'ions)

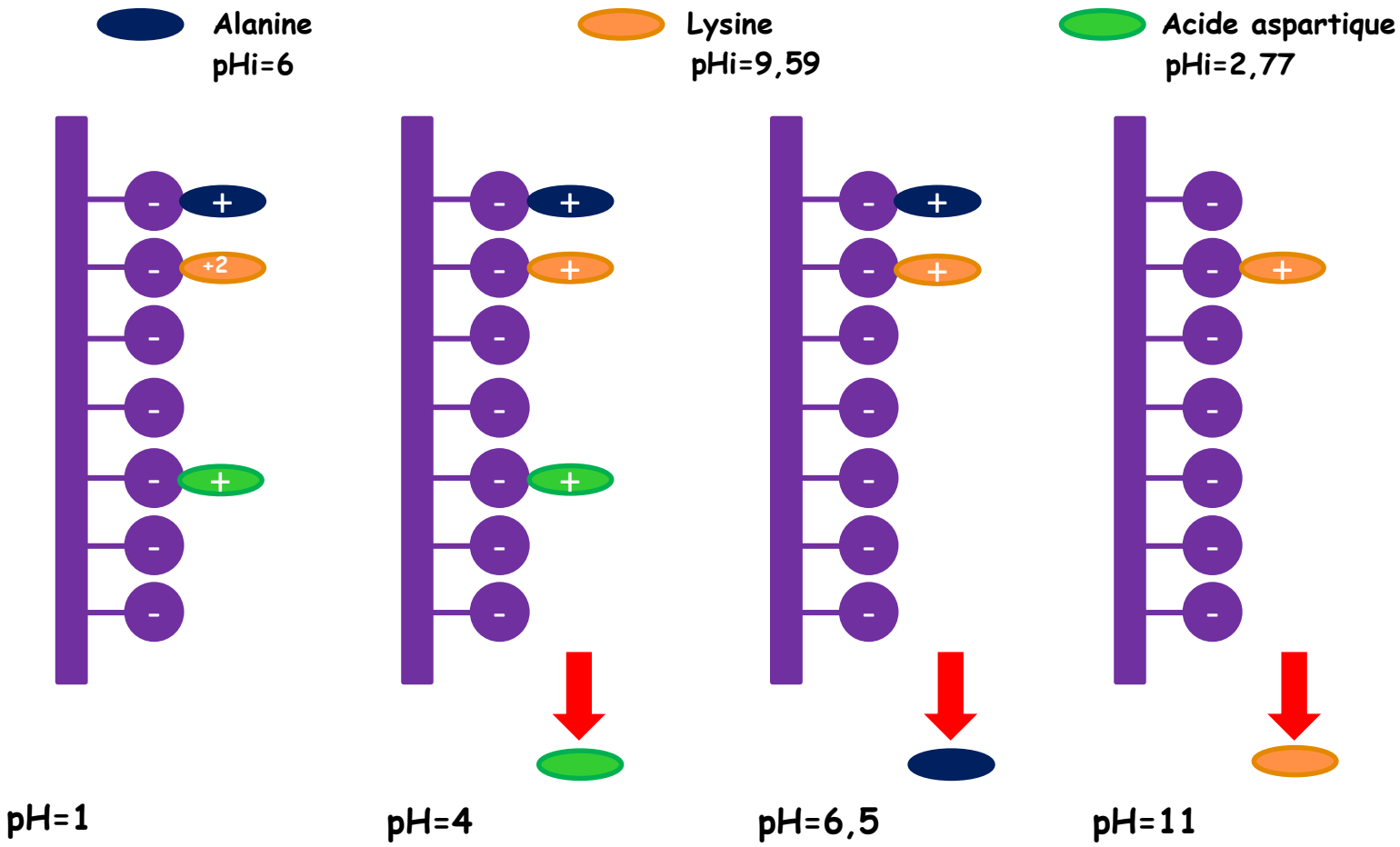


- The column contains a resin with charged groups SO_3H^- Na^+capable of exchanging ions with those in the solution.
At an acidic pH amino acids behave as bases (charge +). They displace some of the Na^+ ions, replacing them and remaining attached to the column via electrostatic forces.
- a buffer with increasing pH is passed through the column. When the pH of the eluent exceeds the pI of the amino acid, its net charge changes, causing it to detach and move through the column. This process is called elution.

Separation with thin layer chromatography (CCM)



Separation with ion exchange chromatograpy

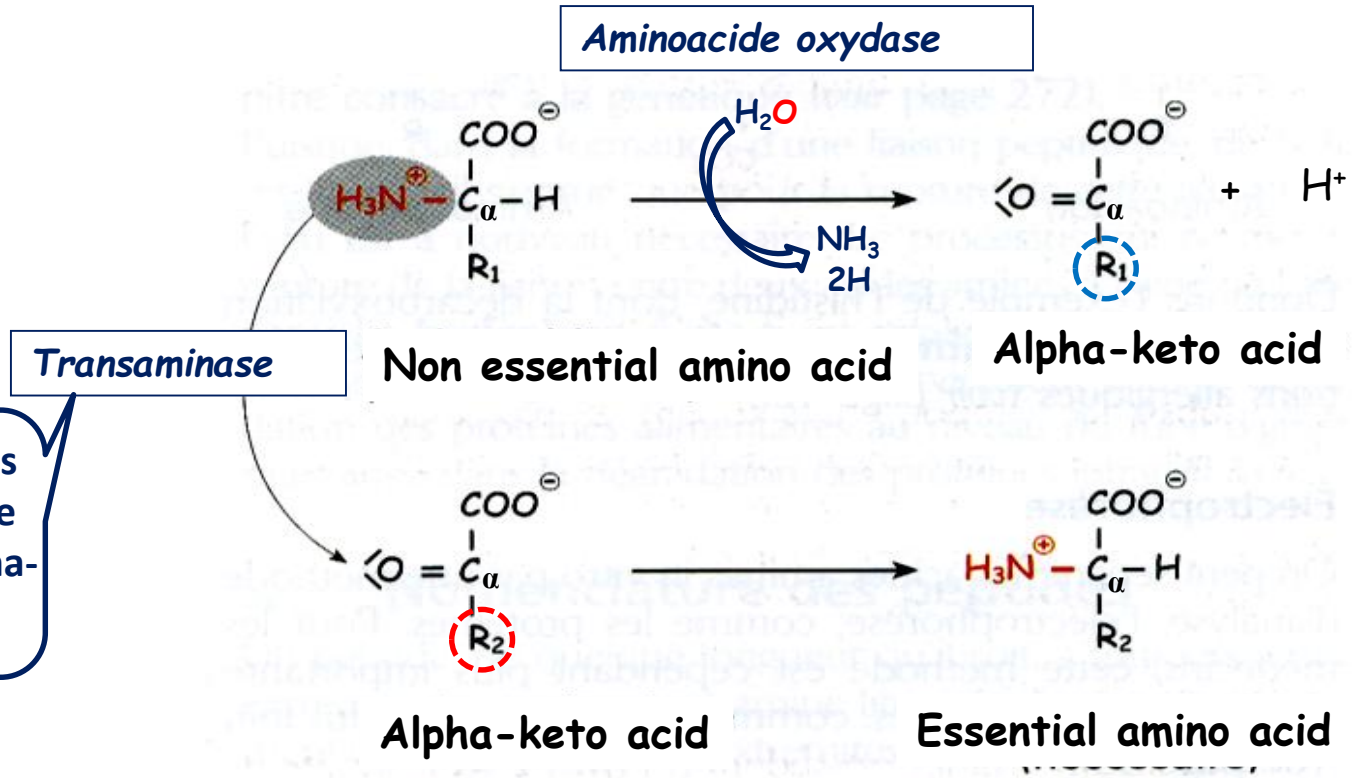


Reactions of amino acids

Desamination and transamination

Crucial for the degradation of amino acids

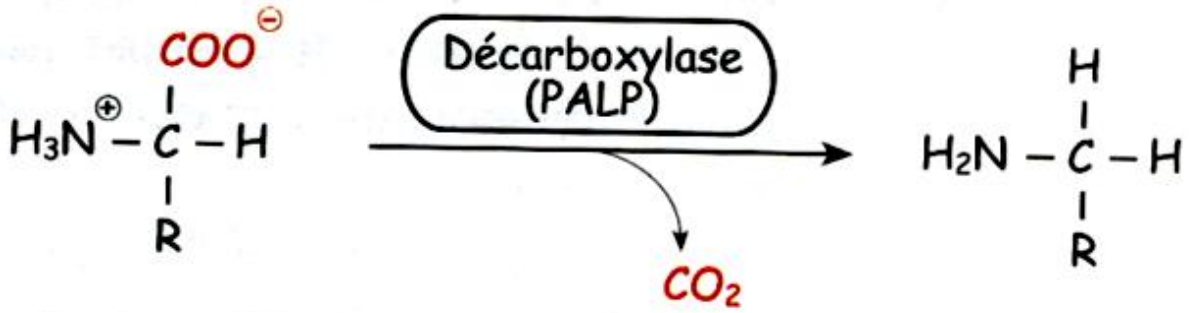
Essential for amino acid biosynthesis



Amino group (NH_2) is transferred from one amino acid to an alpha-keto acid

Reactions of amino acids

Decarboxylation



Amino acid **Biogenic amine**

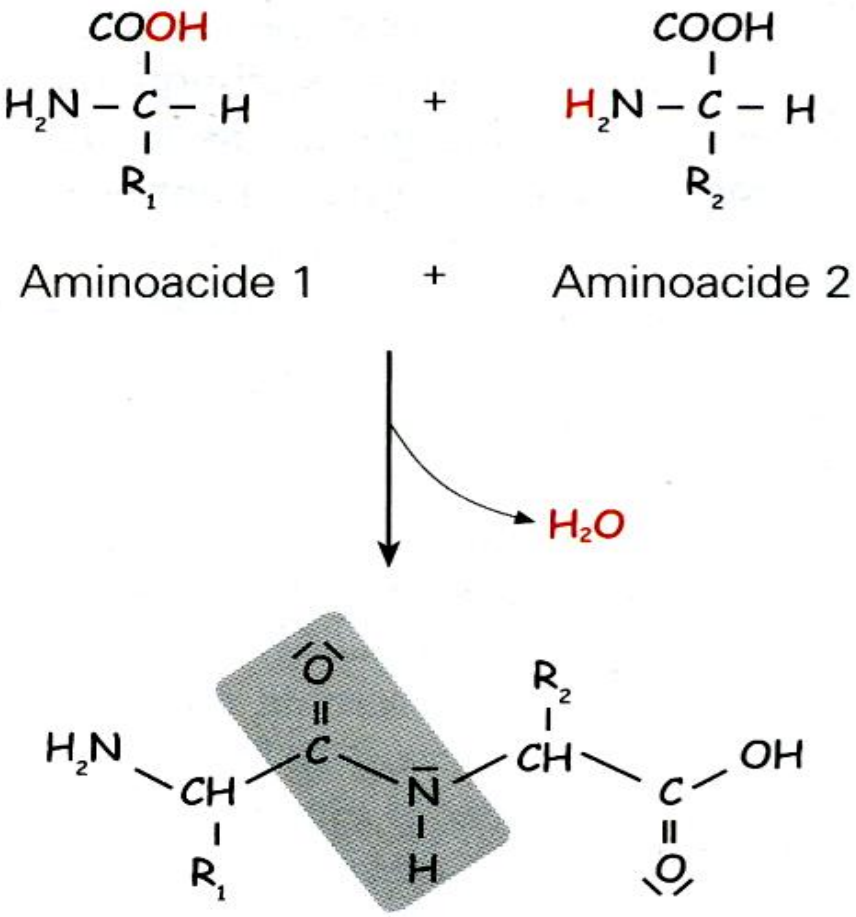
Ex : histidine ➔ histamine acts as a chemical mediator (neurotransmitter)

Characteristic constants of amino acids

Name	Code	Letter code	pKa of COOH	pKb of NH3	pKr of Lateral chain	MW
Alanine	ALA	A	2,3	9,7		89,09
Arginine	ARG	R	2,2	9	12,5	174,2
Asparagine	ASN	N	2	8,8		132,12
Acide aspartique	ASP	D	2,1	9,8	3,9	133,1
Cystéine	CYS	C	1,8	10,8	8,3	121,15
Glutamine	GLN	Q	2,2	9,1		146,15
Acide glutamique	GLU	E	2,2	9,7	4,2	147,13
Glycine	GLY	G	2,3	9,6		75,07
Histidine	HIS	H	1,8	9,2	6	155,16
Isoleucine	ILE	I	2,4	9,7		131,17
Leucine	LEU	L	2,4	9,6		131,17
Lysine	LYS	K	2,2	9	10	146,19
Méthionine	MET	M	2,3	9,2		149,21
Phénylalanine	PHE	F	1,8	9,1		165,19
Proline	PRO	P	2	10,6		115,13
Sérine	SER	S	2,2	9,2		105,09
Thréonine	THR	T	2,6	10,4		119,12
Tryptophane	TRP	W	2,4	9,4		204,23
Tyrosine	TYR	Y	2,2	9,1	10,1	181,19
Valine	VAL	V	2,3	9,6		117,15

Protreins

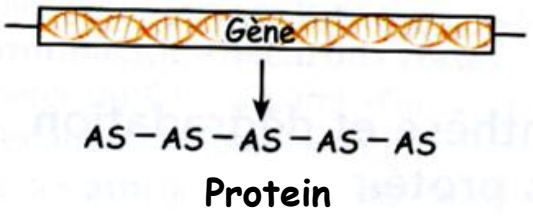
The peptide bond



Peptide bond

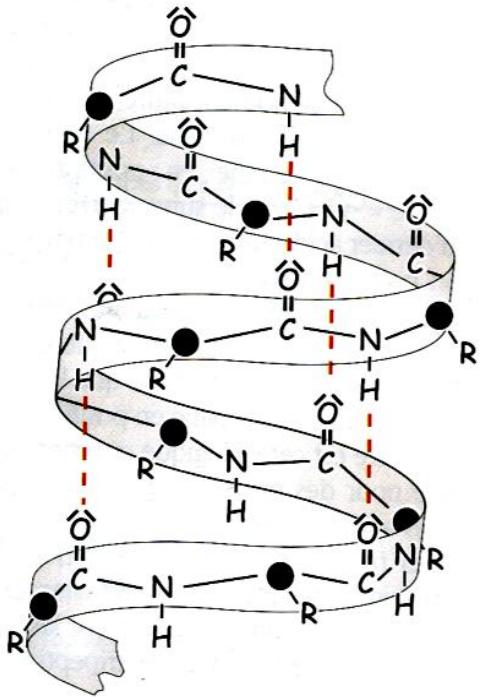
Spatial structure of proteins

Primary structure

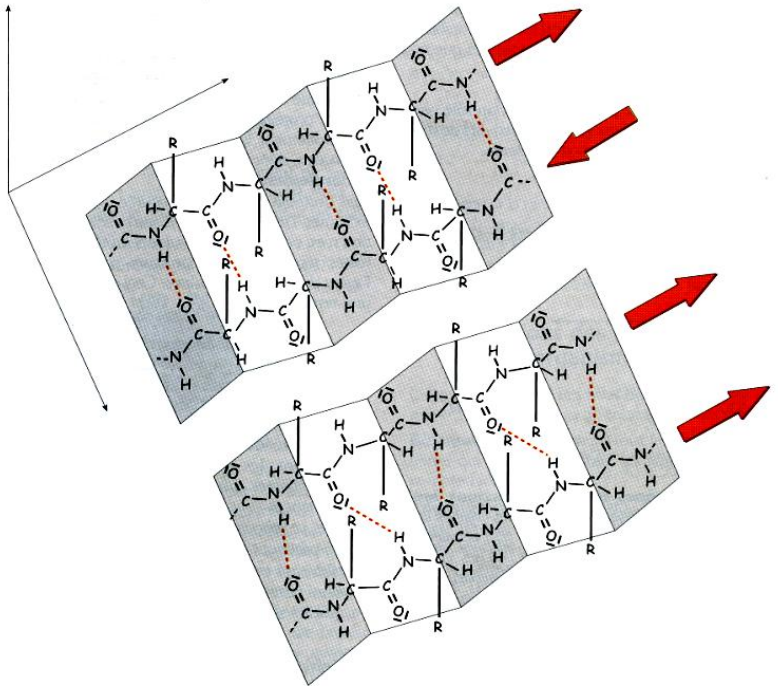


Secondary structure

- α helix

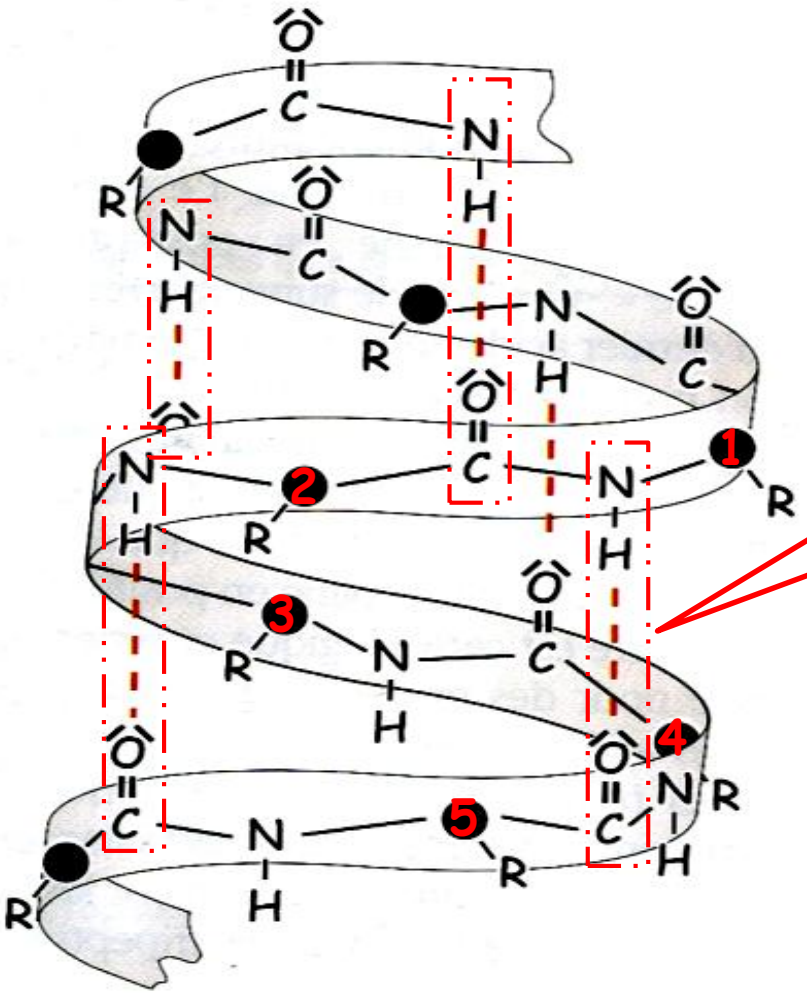


- β sheet



Spatial structure of proteins

Structure of α helix

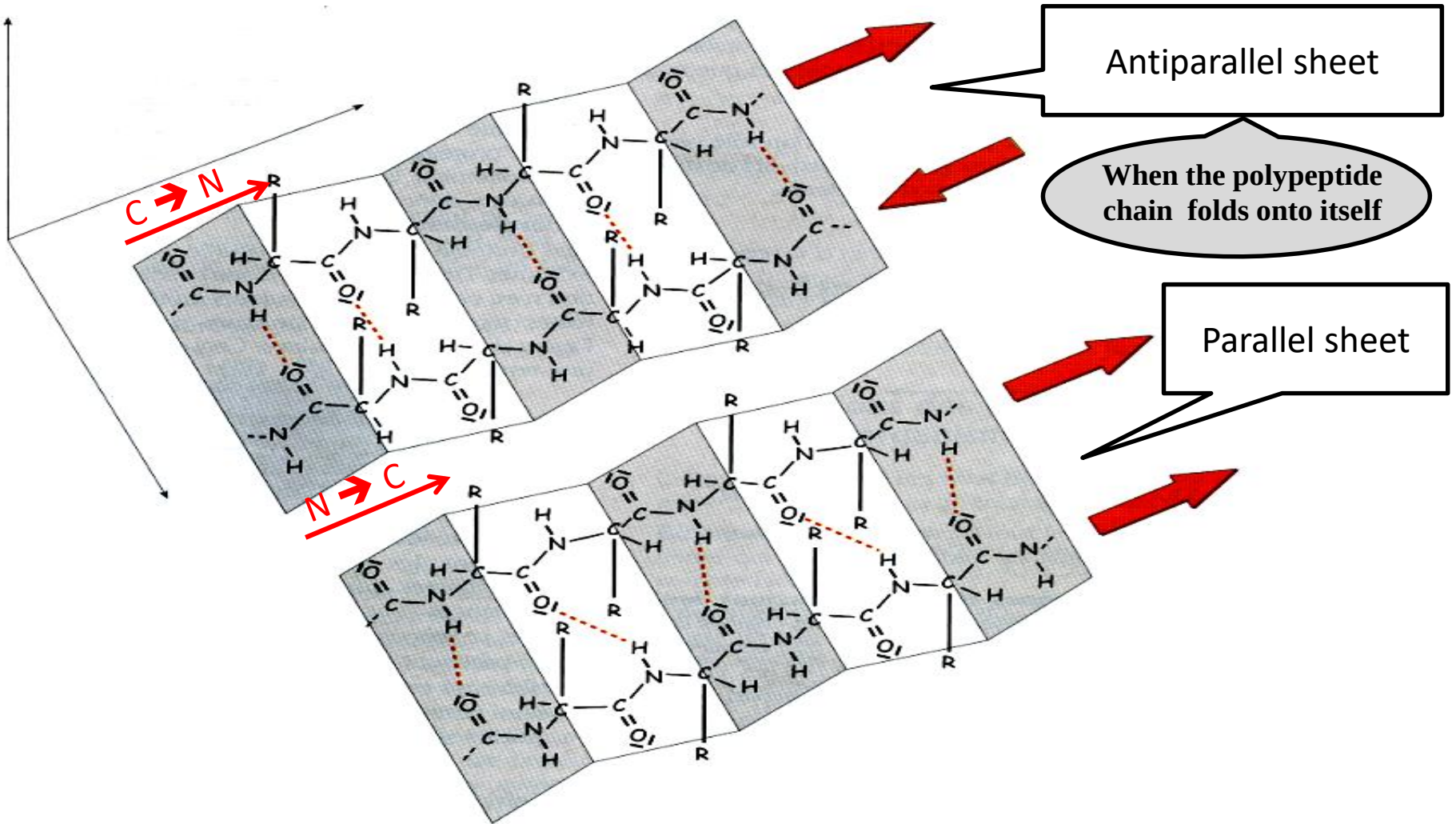


Contain a minimum of 5 residues and a maximum of 40

Hydrogen bonds form between amino acids n and n+4

Spatial structure of proteins

Structure of β sheet



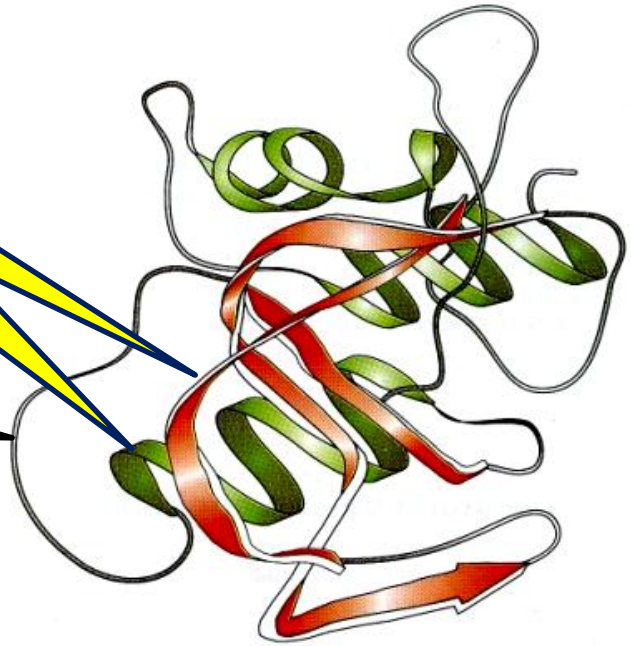
Spatial structure of proteins

Tertiary structure

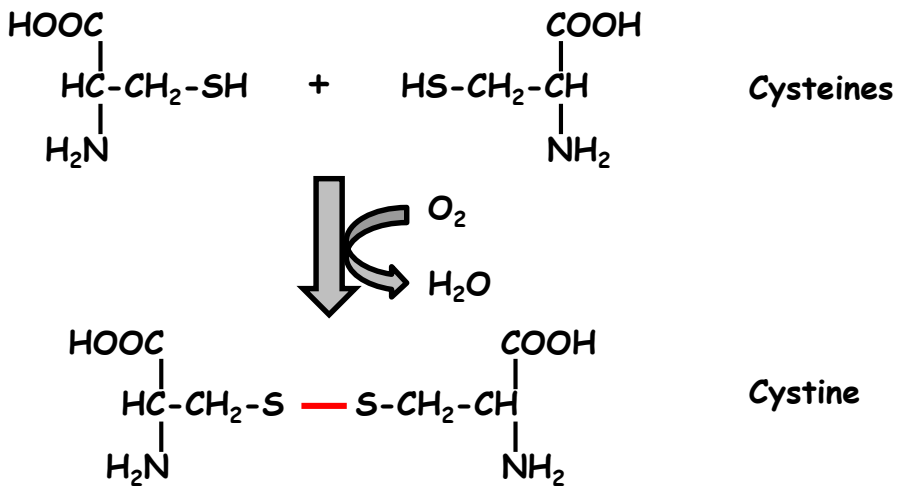
A helix + β sheet

Stable structure due to disulfide bridges (covalent bonds) formed between the thiol groups of sulfur-containing amino acids, particulary Cys

$R\text{-}SH + SH\text{-}R' \rightarrow R\text{-}S\text{-}S\text{-}R' + 2H^+ + 2e^-$



Oxidation of cysteine and the formation of cystine

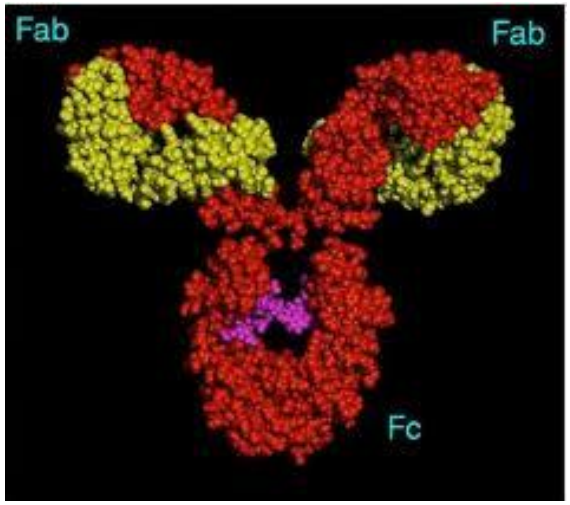


Spatial structure of proteins

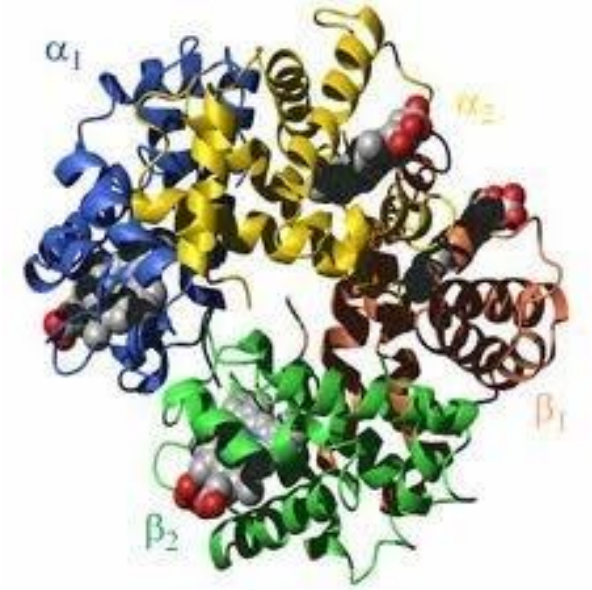
Quaternary structure

Assembly of tertiary structures
➔ Protein complex

Antibody



Hemoglobin



Denaturation of protein

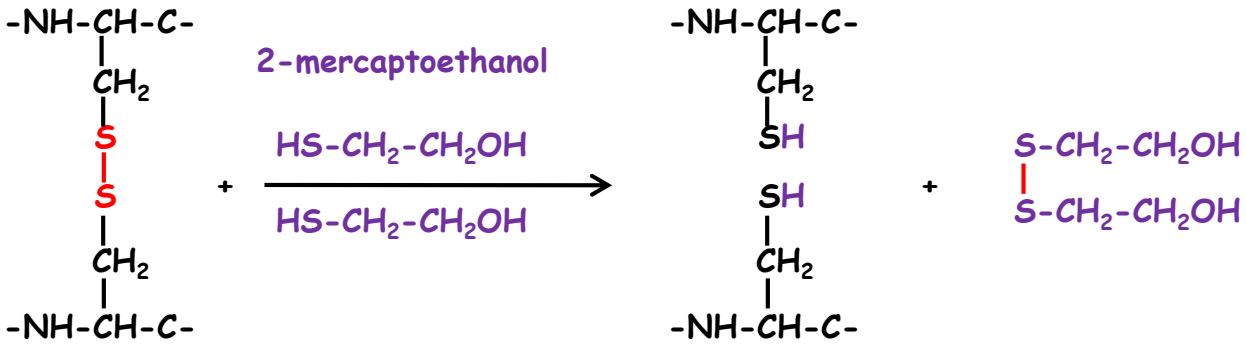
The denaturation of protein requires the breaking of:

➔Disulfide bonds: through reduction (unfolding of the tertiary structure)

➔Hydrogen bonds: through heat (unfolding of the secondary structure)

The denatured primary structure migrates in electrophoresis through the pores of a polyacrylamide gel.

Reduction of disulfide bonds



Determination of the amino acid composition of a polypeptide chain

- Fractionated polypeptide chains are separated by electrophoresis or chromatography.

- For each isolated chain, the amino acid composition is determined.

  → Determination of the N-terminal amino acid
  → Determination of the C-terminal amino acid

Determination of the amino acid composition of a polypeptide chain

Determination of the N-terminal amino acid

Main reagents:

- **FDNB** (1-fluoro 2,4-dinitrobenzene) (1945): **Sanger reagent**
- **PTC** (phenylisothiocyanate): **Edman reagent**
- **Chlorure de dansyl** (acide chloride 1-dimethylaminonaphtalene-5-sulfonique)

Enzymatic method :

- **Aminopeptidase:** acts only on the last peptid bond.

Determination of the C-terminal amino acid

Enzymatique method:

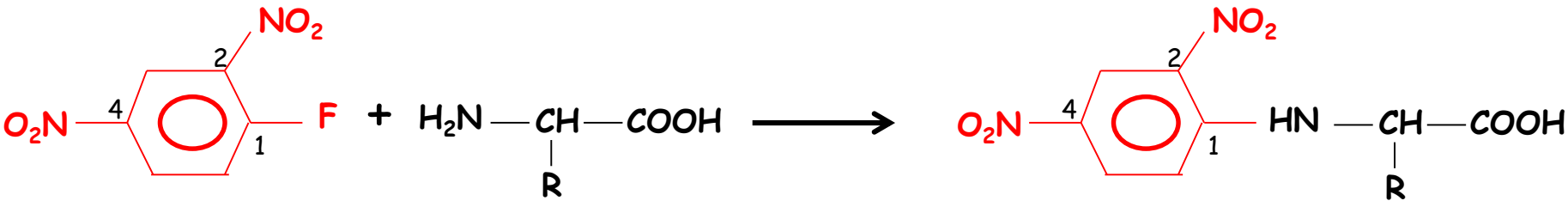
- **Carboxypeptidase:** acts only on the last peptid bond.

Determination of the amino acid composition of a polypeptide chain

Determination of the N-terminal amino acid

FDNB (1-fluoro 2,4-dinitrobenzene) (1945): Sanger reagent
Non-recurrent method

This method identifies N-terminal amino acid, while the rest of the chain is degraded and thus not reusable.



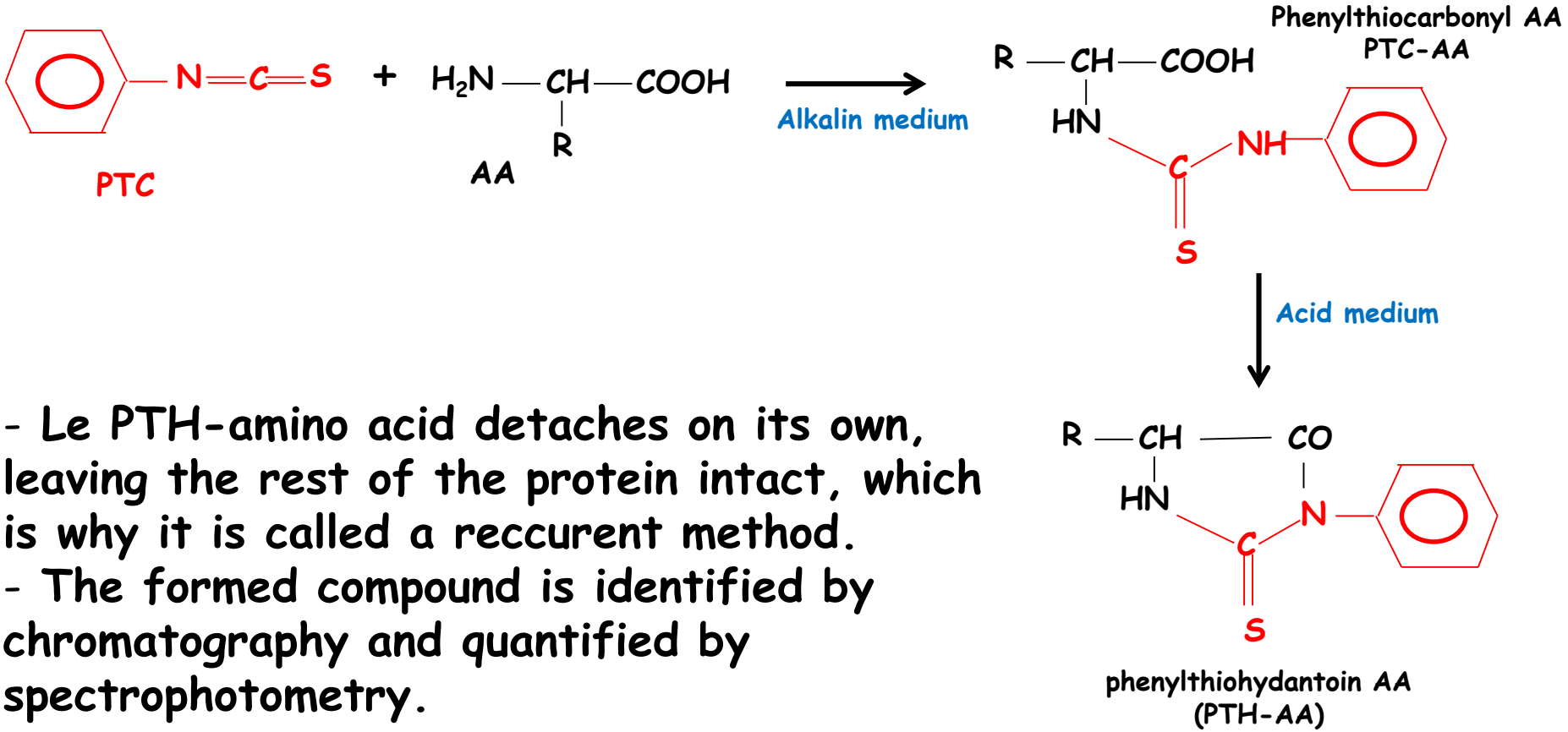
FDNB AA N-2,4-dinitrophenyl derivative

The formed compound is identified by chromatography and quantified by spectrophotometry.

Determination of the amino acid composition of a polypeptide chain

Determination of the N-terminal amino acid

PTC (phenylisothiocyanate): Edman reagent
Recurrent method



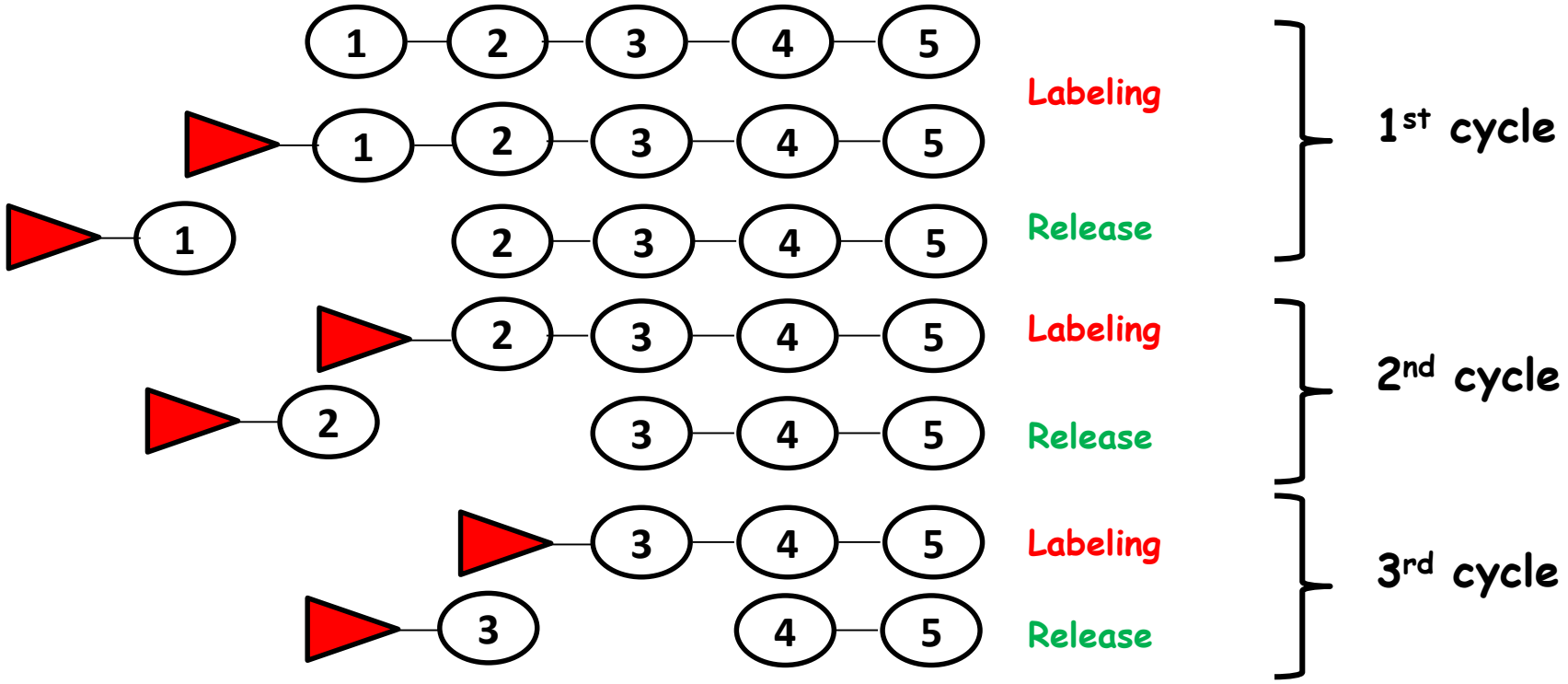
- Le PTH-amino acid detaches on its own, leaving the rest of the protein intact, which is why it is called a reccurent method.
- The formed compound is identified by chromatography and quantified by spectrophotometry.

Determination of the amino acid composition of a polypeptide chain

Determination of the N-terminal amino acid

Mechanism

PTC (phenylisothiocyanate): Edman reagent
Recurrent method

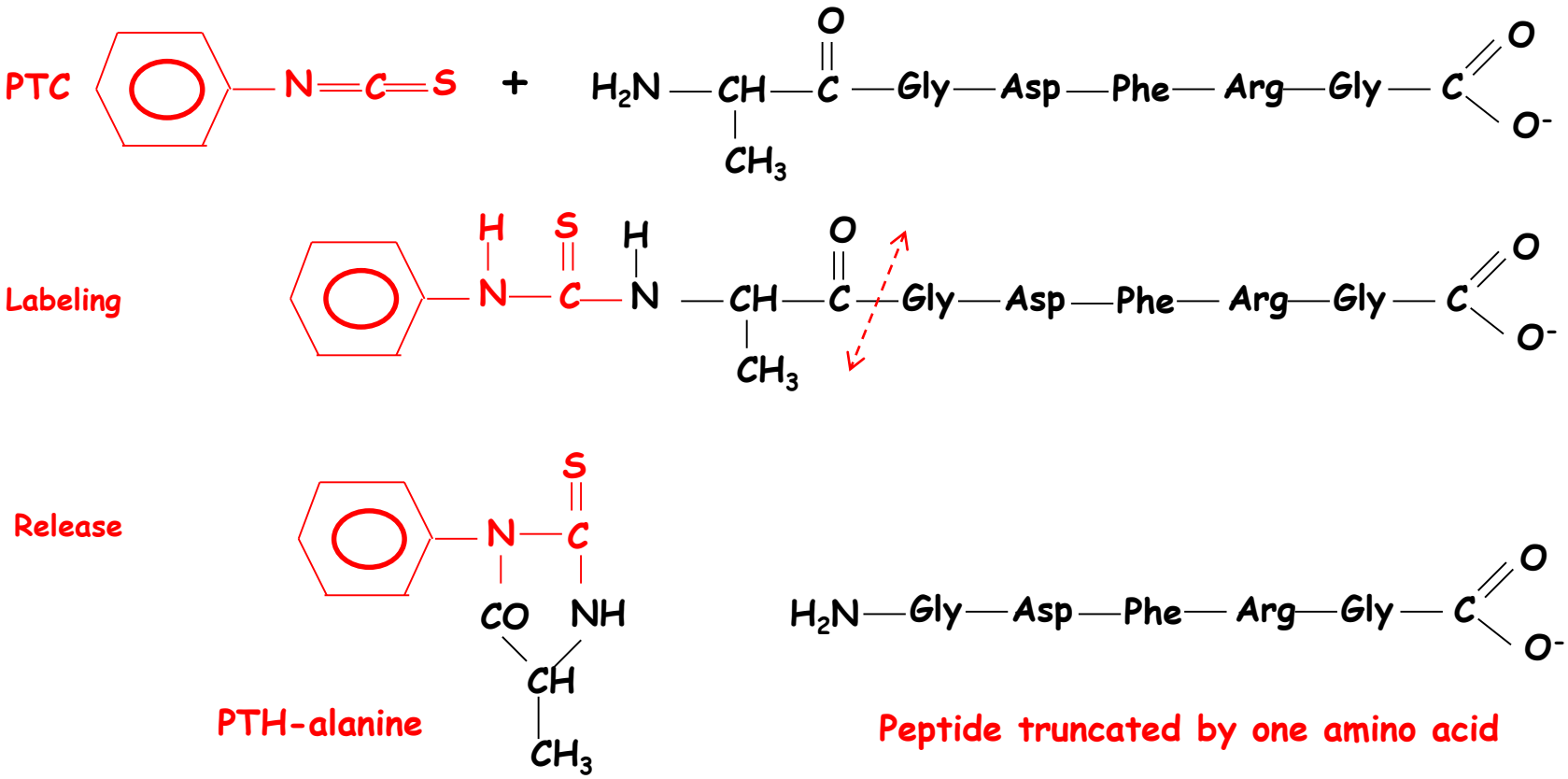


Determination of the amino acid composition of a polypeptide chain

Determination of the N-terminal amino acid

Mechanism

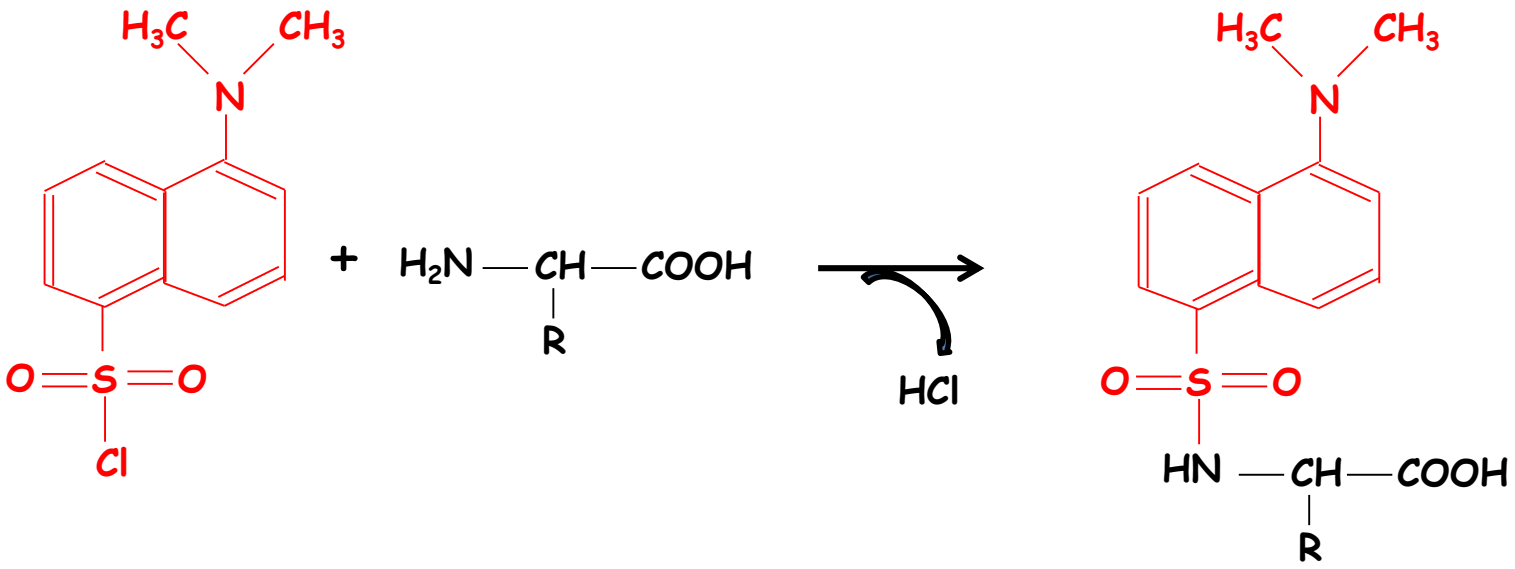
PTC (phenylisothiocyanate): Edman reagent
Recurrent method



Determination of the amino acid composition of a polypeptide chain

Determination of the N-terminal amino acid

Dansyl chloride (acide 1-diméthylaminonaphtalène-5-sulfonique chloride)



Dansyl chloride AA Dansyl AA

The Dansyl-amino acide is highly fluorescent.
It is separated by Thin layen chromatography (TLC) and detected by fluorescence.

Determination of the amino acid composition of a polypeptide chain

Partial hydrolysis

- Obtaining peptides either by chemical agents or by enzymes.
- Enzymatic proteolysis using **endopeptidases**:

  ➔**The trypsin:** cuts the *CO*(*O*) of the lysine and arginine.

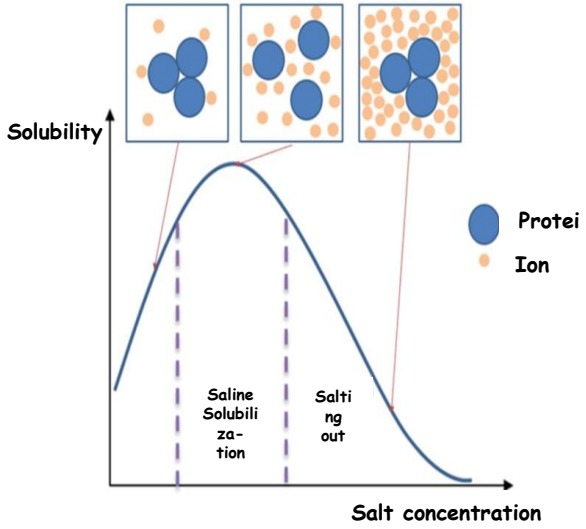
  ➔ **The chymotrypsin:** cuts the *CO*(*O*) of aromatic amino acides (phenylalanine, tryptophane) and leucine (which are nonpolar amino acids).

Properties of proteins

Solubility

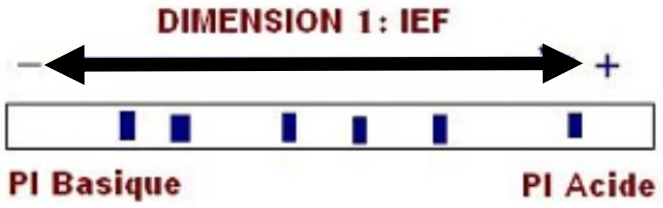
Depends on ionic strengh and pH.

- At low ionic strengh, monovalent ions promote solubilization by stabilizing the charged groups of proteins.
- At higher strengh, ions tend to precipitate proteins. There is competition between the ions and proteins for water molecules, a phenomenon known as « salting out ».



Electrolytic properties

- In a basic medium, proteins behave as anions and migrate toward the anod in an electric field.
- Ina acid medium, proteins behave as cations and migrate toward the cathode (-).
- At pI, the net charge is zero, and proteins do not migrate.



Antigenic properties

An antigen is a substance capable of provoking the synthesis of specific antibodies when injected into a living organism.

- The part of the protein involved in the formation of the complex with the antibody is called the antigenic determinant.
- Used in protein assays, such as affinity chromatography.