Chemical Properties of Amino Acids

A. Reactions due to ***carboxylic group***:

1) amino acids form *salts* (-COONa) with bases, and *estres* (-COOR) with alcohols.

2)Deacarboxylation: AA undergo decarboxylation to produce amines.

$$H_2N\text{-}CH_2\text{-}COOH + Ba(OH)_2 \longrightarrow CH_3\text{-}NH_2 + BaCO_3 + H_2O$$

Glutamic acid ⟶ GABA

Histidine ⟶ Histamine

3) Reaction with ammonia : form amides

aspartic acid + NH_3 ⟶ aspargine

glutamic acid + NH_3 ⟶ glutamine

Cont.

B. Reactions due to ***NH2 group***:

1) Amino groups behave as bases and combine with acids (eg.HCl) to form salts.

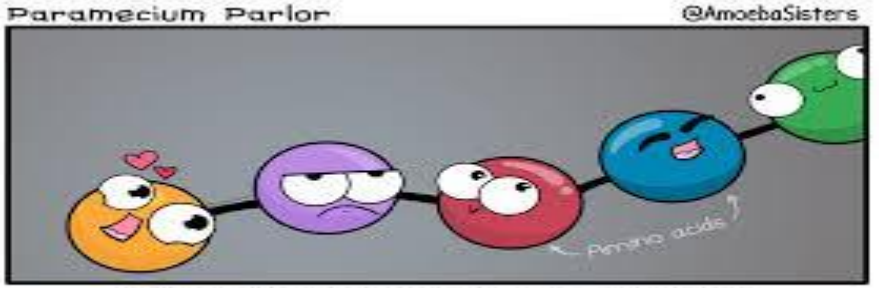
2) α-Amino acids react with nitric acid at room temperature to produce hydroxy acids and nitrogen gas

3) Reaction with ninhydrin: the α-amino acid react with ninhydrin to form a purple color (Ruhemanns`s purple). Ninhydrin reaction is used for the quantitative determination of amino acids and proteins.

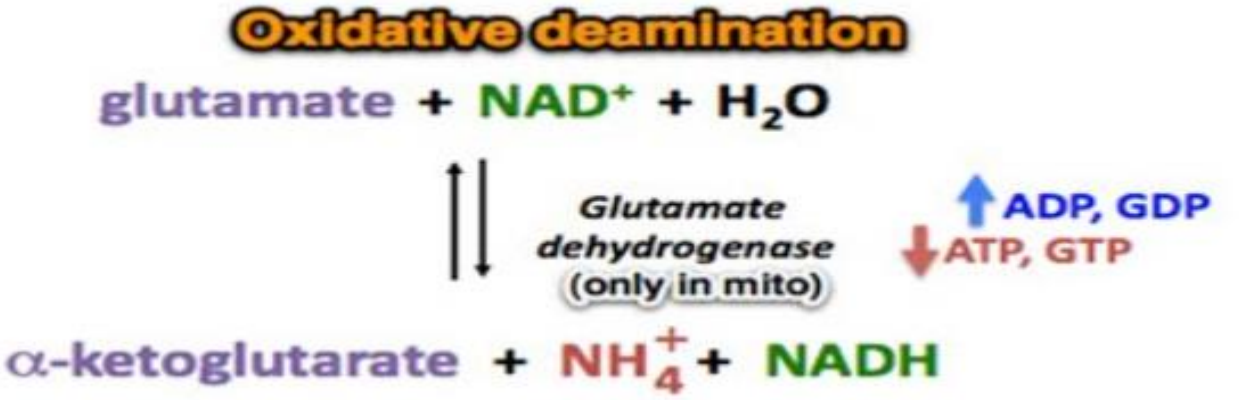
4) Tissue CO2 binds to α-amino acid on the globin chain of hemoglobin to form carbamino hemoglobin

5) Oxidative deamination– α amino group is removed and corresponding α-keto acid is formed. α-keto acid produced is either converted to glucose or ketone bodies or is completely oxidized.

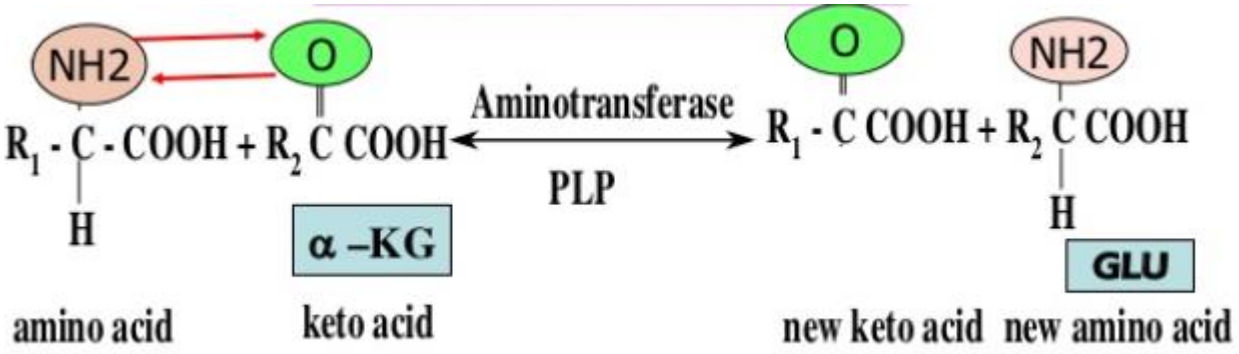
6) Transamination– Transfer of an α amino group from an amino acid to an α keto acid to form a new amino acid and a corresponding keto acid.



Oxidative deamination: The amino acids undergo oxidative deamination to liberate free ammonia.



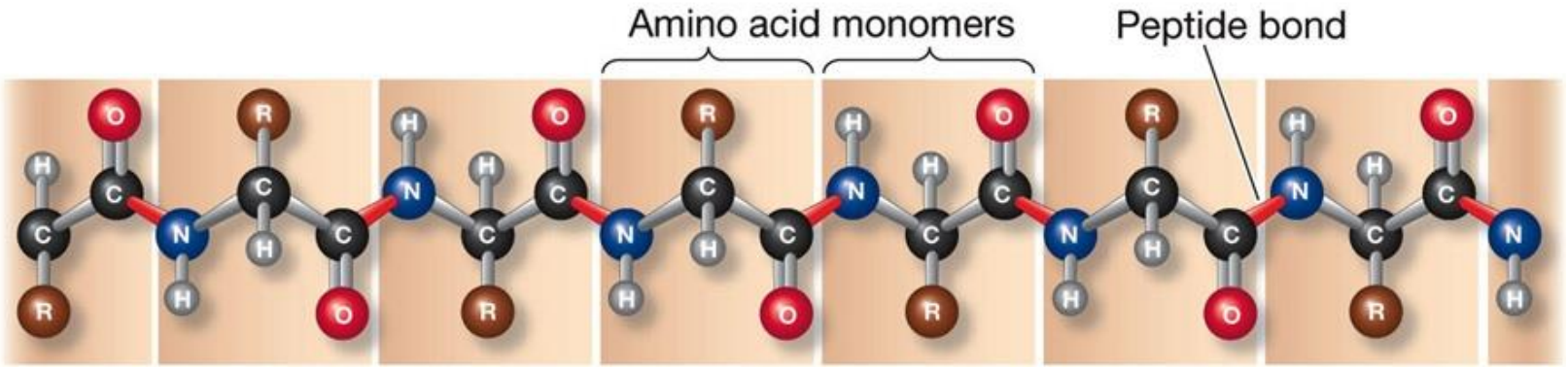
Transamination

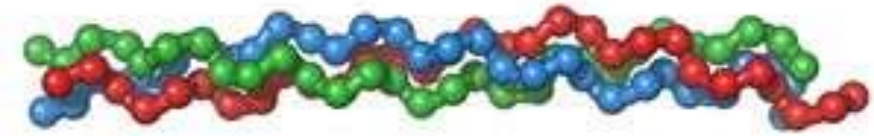


SGPT: Glutamate + Pyruvate -------> -Ketoglutarate + Alanine

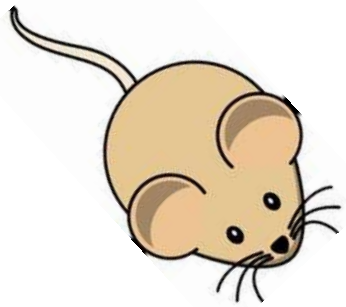
A protein molecule

- contains 100's and 1000's of amino acids joined together by peptide links into one or more chains

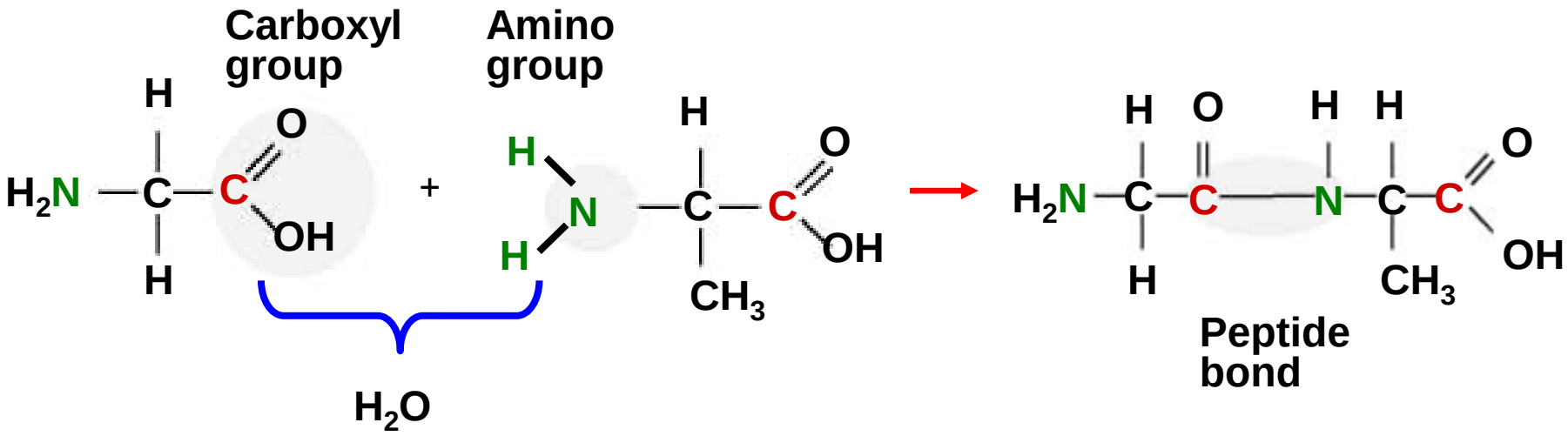




3 chains in collagen (in mouse tail)



Amino acids are joined together by a condensation reaction



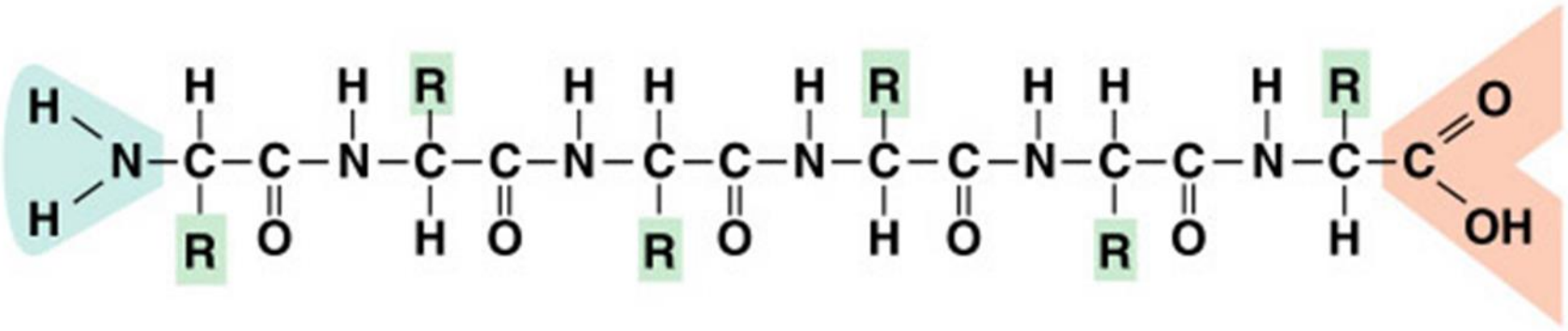
A peptide bond is a covalent C-N bond formed by condensation between the -NH_2 of one amino acid and -COOH of another

C-N atoms of the peptide bonds:

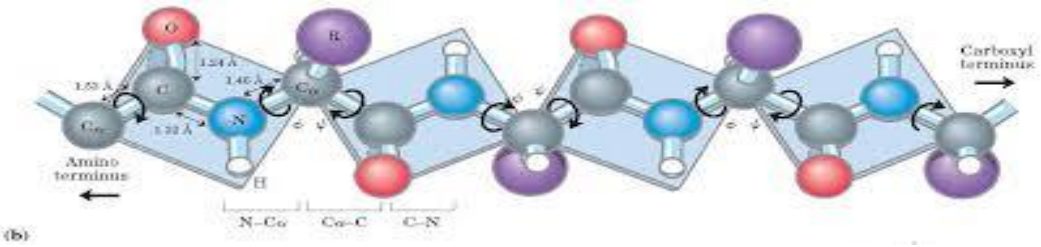
- lie in the same plane to form the backbone

Side chains of the individual amino acids:

- are arranged transversal to each other across the backbone – this confers *stability* to the molecule



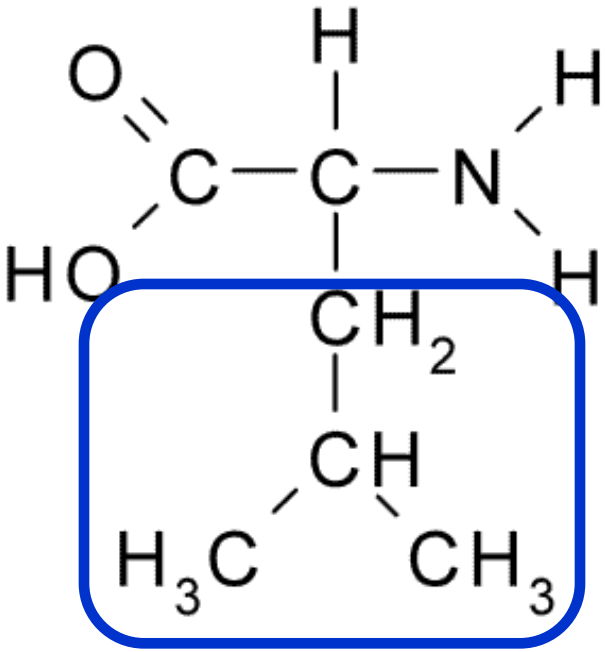
- Peptides bond are mainly planar.
- They have considerable double bond character. This prevent rotation about this bond
- Inability of the bonds to rotate account for planarity
- Trans and cis configuration is possible for a peptide bond. However, the most commonly observed is trans configuration.



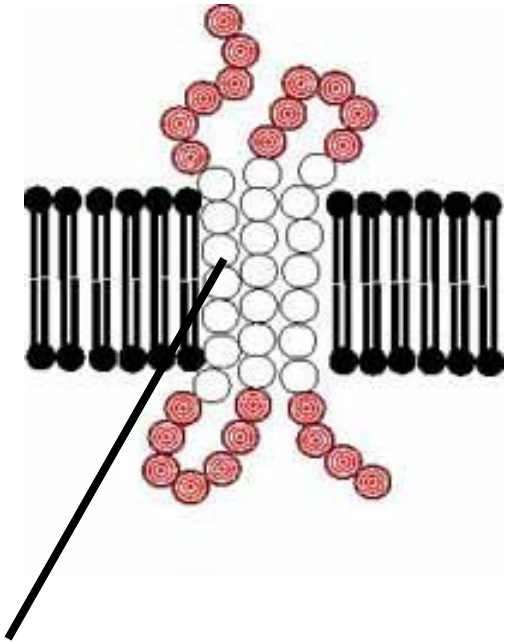
Amino acids can be grouped based on side chains

The various side groups of amino acids

NONPOLAR



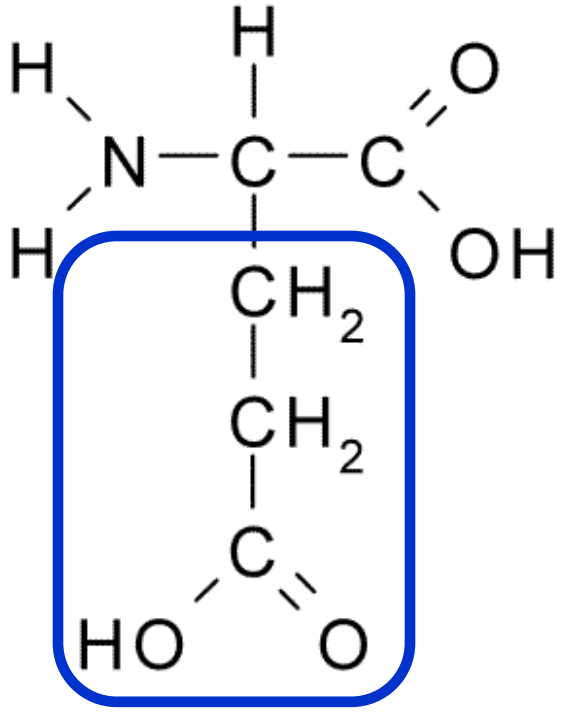
Leucine



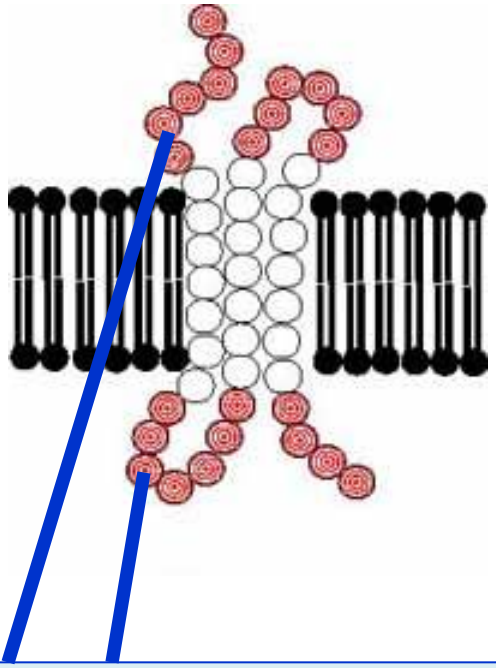
Amino acids are nonpolar.

The various side groups of amino acids

POLAR CHARGED



Glutamic acid



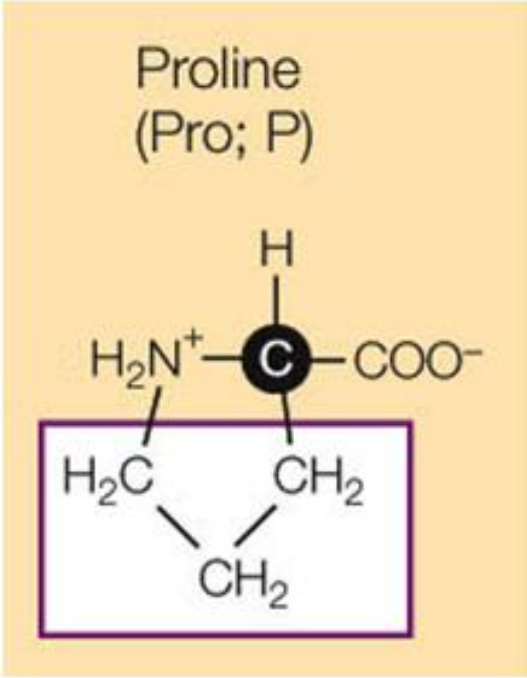
Amino acids are polar.

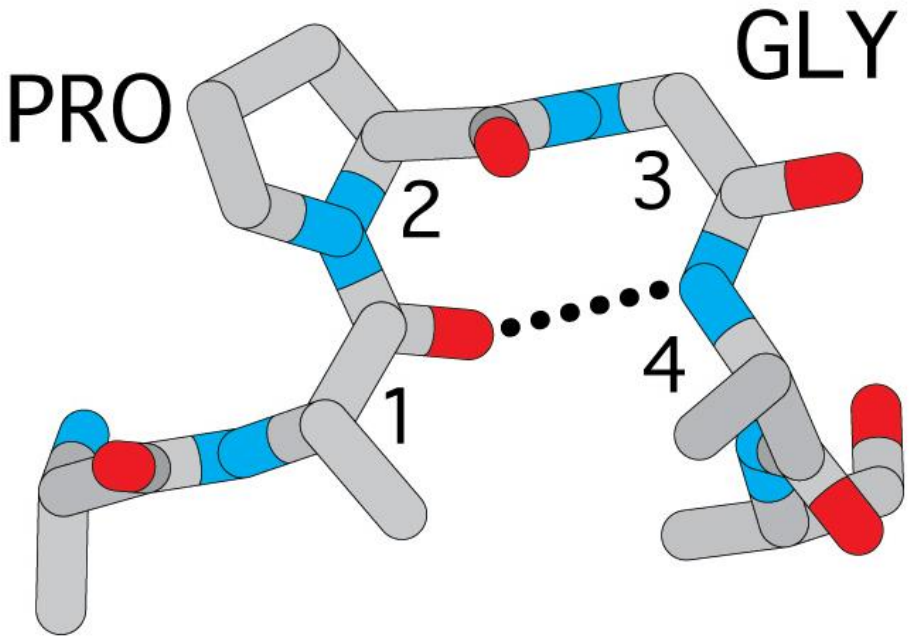
Let's mention three amino acids of special interest:

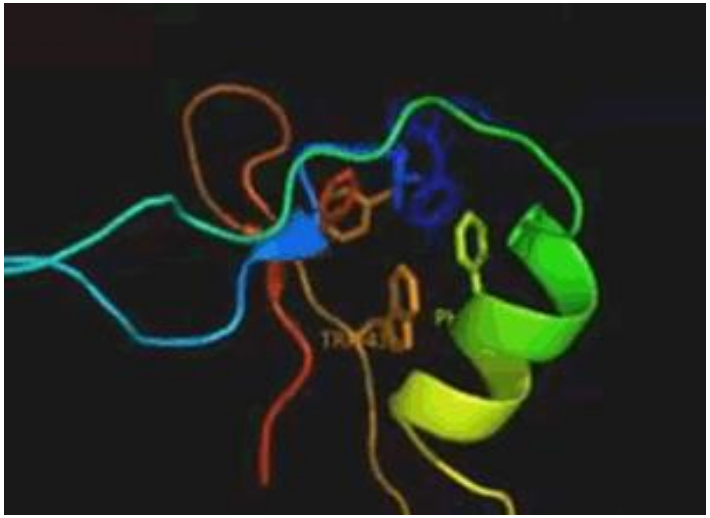
- ✓ **Proline**
- ✓ **Cysteine**

Proline:

causes kinks in chains



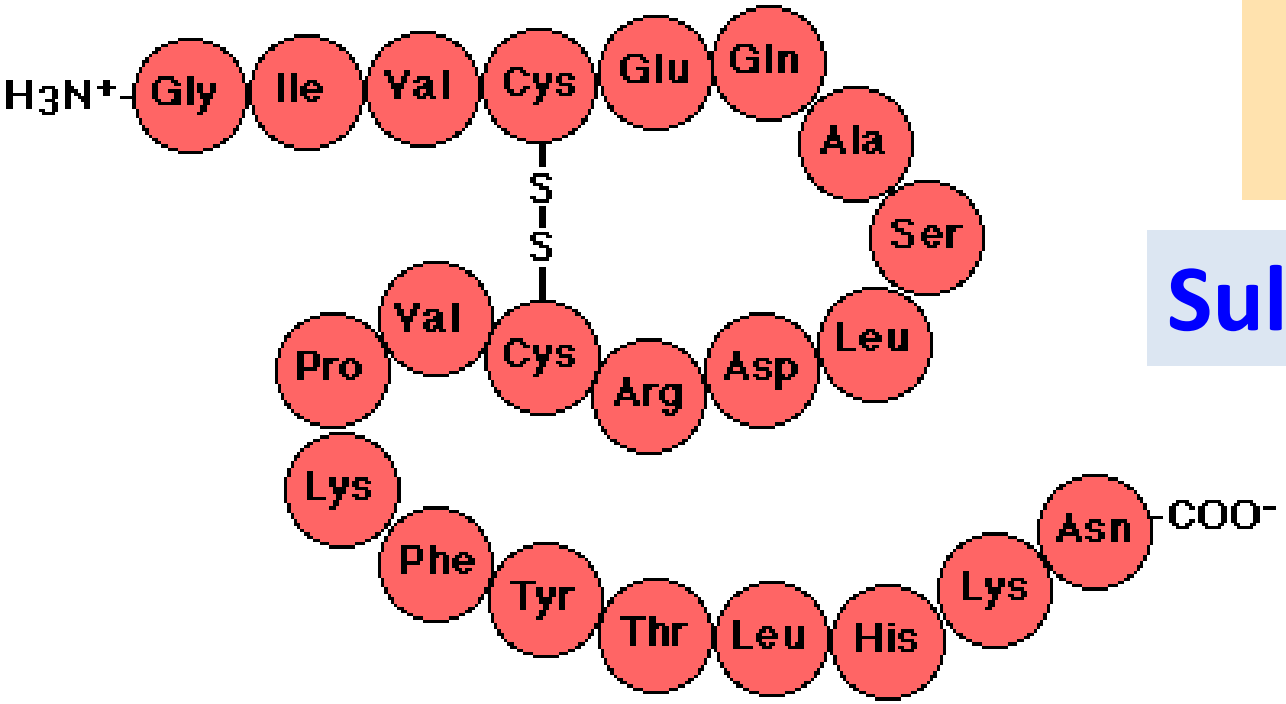


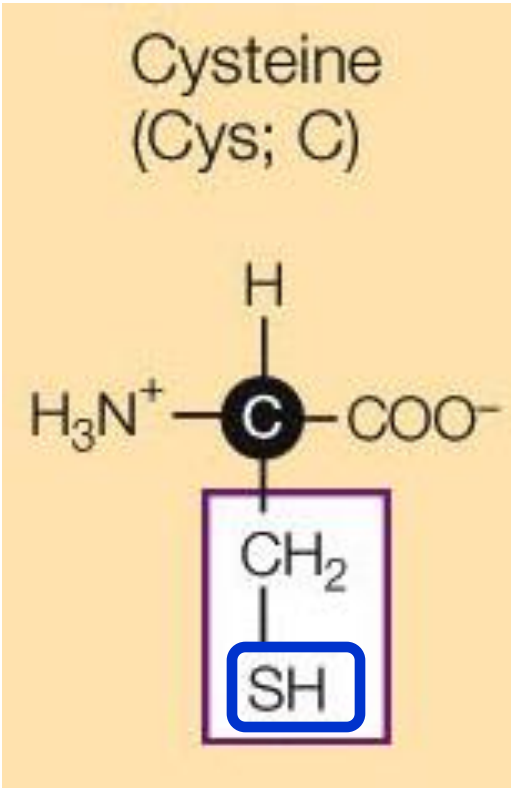


Non polar, non essential AA

Cysteine:

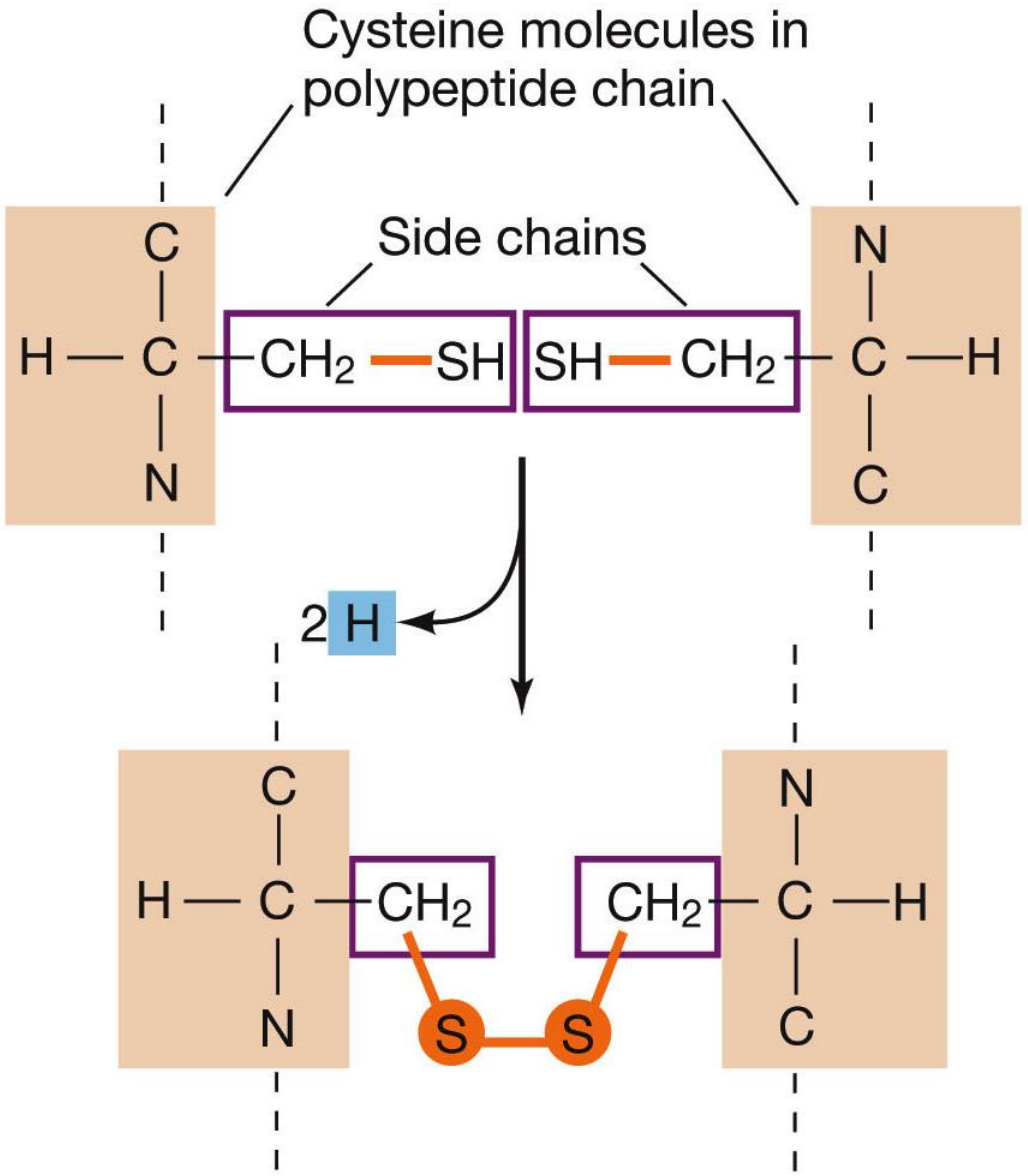
- contains sulfur
- can form disulfide bridges



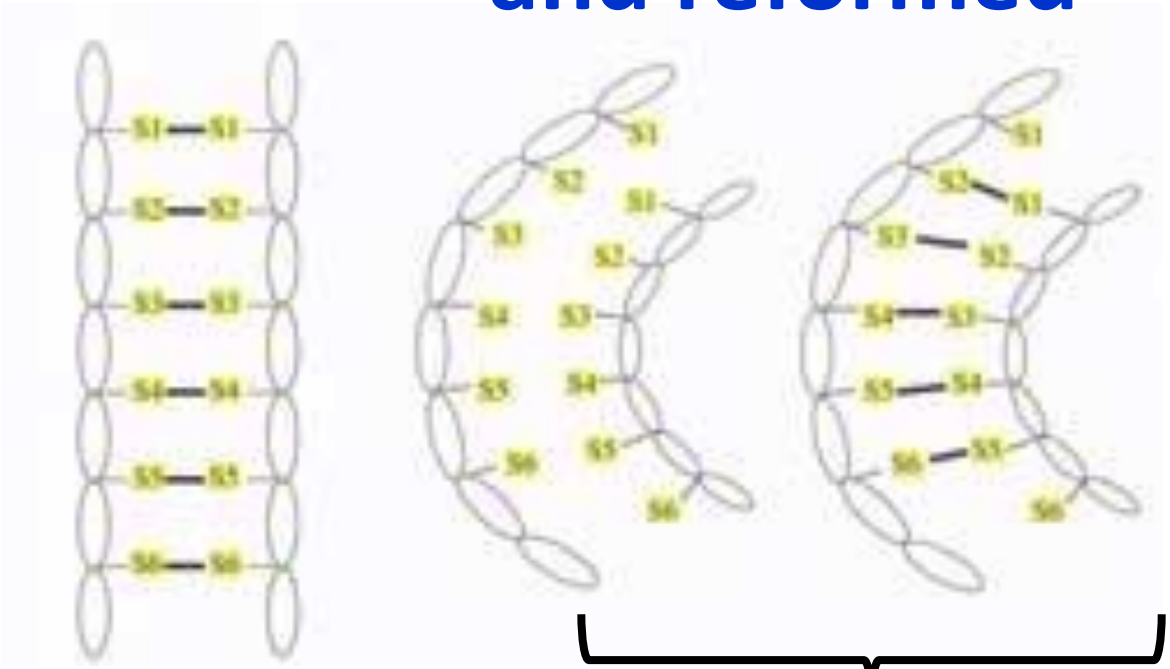


Sulfhydrl group

A Disulfide Bridge



When hair is permed – disulfide bridges in keratin are broken and reformed

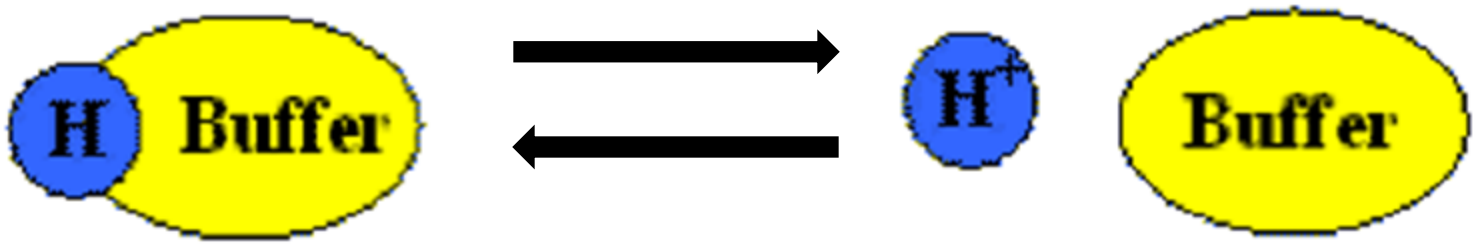


Disulfide bridges in straight hair

Disulfide bridges broken & reformed

Buffering capacity of proteins

A buffer can donate or accept H^+ to stabilize the pH.



Why are buffers needed?

To keep solution at a constant pH.

- Amino acids are usually ionized at physiologic pH
- Therefore, amino acids have amphoteric properties:
  - In acidic medium ; the amino acid is positively charged, so it behaves as a base (proton acceptor).
  - In alkaline medium ; the amino acid is negatively charged, so it behaves as an acid (proton donor).

Amino Group

$NH_3^+ \underset{\text{lower pH}}{\overset{\text{higher pH}}{\rightleftharpoons}} NH_2$

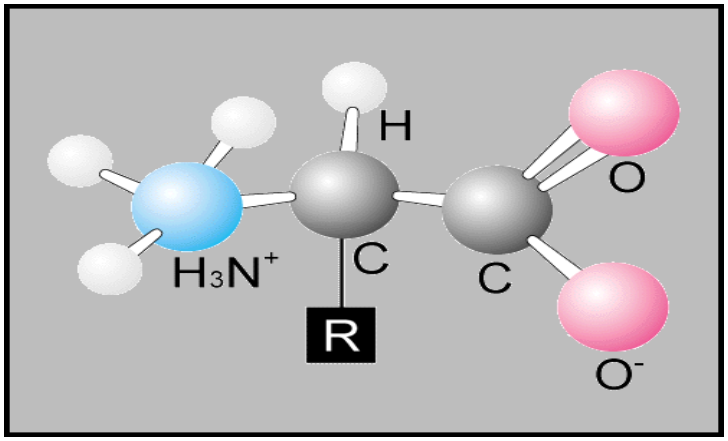
Carboxyl Group

$COOH \underset{\text{lower pH}}{\overset{\text{higher pH}}{\rightleftharpoons}} COO^- + H^+$

Isoelectric point

جديد

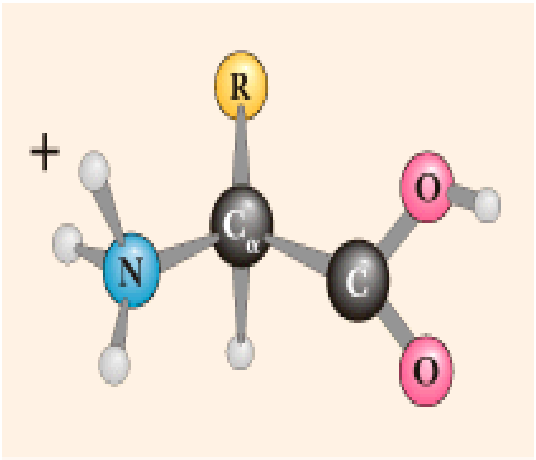
- Amino acids can exist as **ampholytes** or **zwitterions** in solution, depending upon pH of the medium.
- The pH at which the amino acids exist as zwitterions, with no net charge on them is called Isoelectric pH or Isoelectric point.
- In acidic medium, the amino acids exist as cations
- In alkaline medium , they exist as anions.



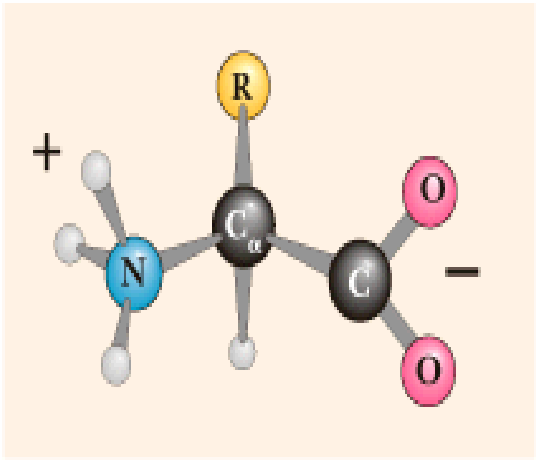
Due to no net charge, there is no electrophoretic mobility at Isoelectric pH.
Solubility and buffering capacity are also minimum at Isoelectric pH

Isoelectric point

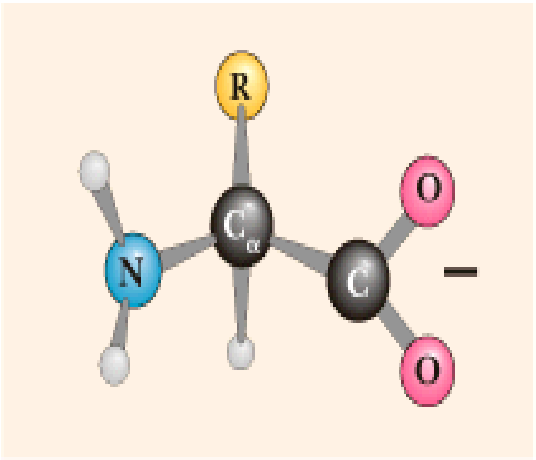
pH 1 Net charge +1

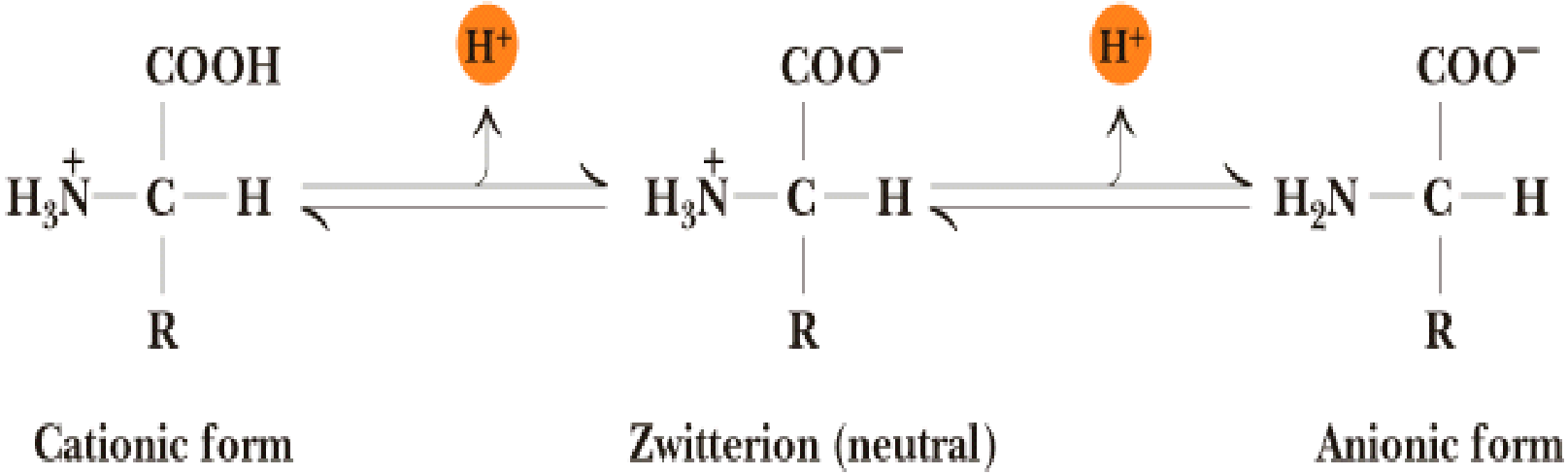


pH 7 Net charge 0



pH 13 Net charge −1





Cationic form

Zwitterion (neutral)

Anionic form

Titration of amino acids

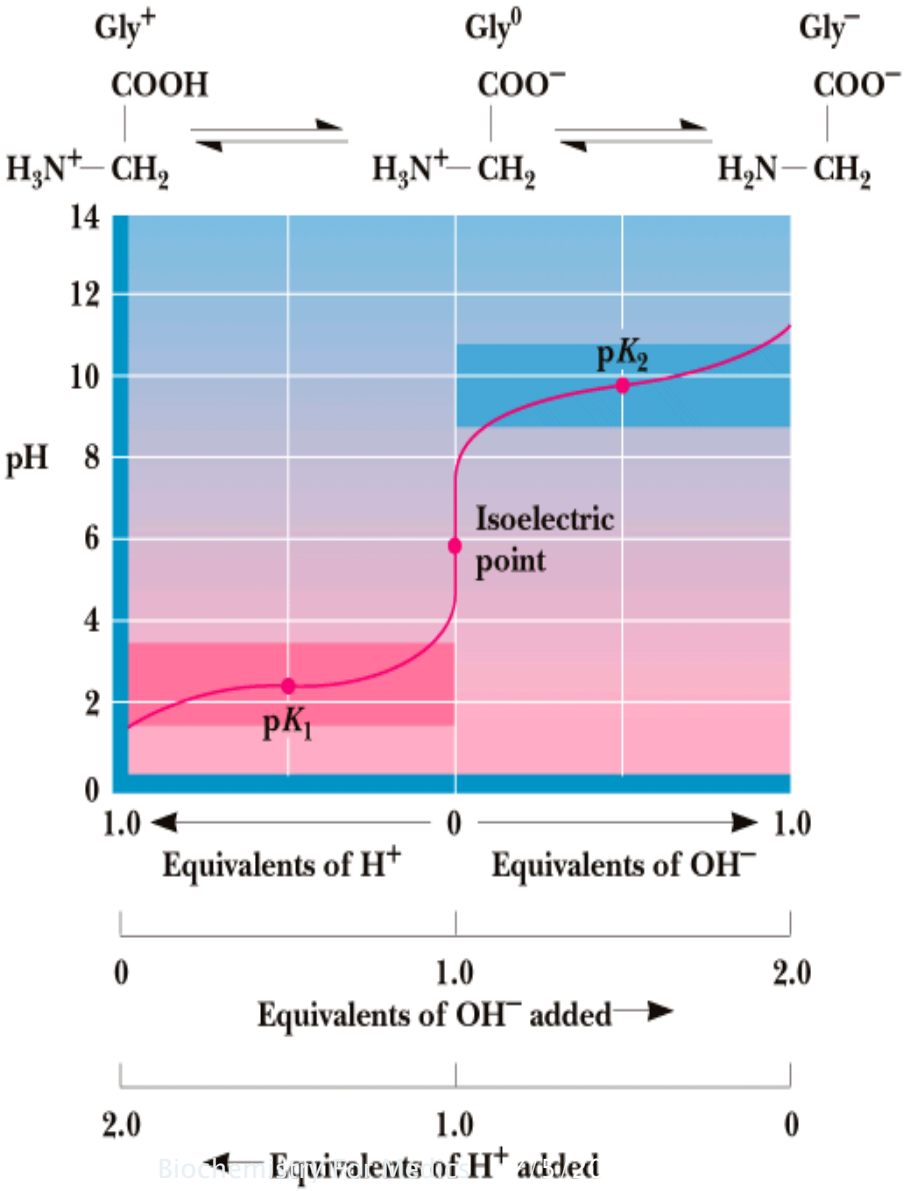
- If HCl is added drop wise to am amino acid solution, at a particular pH, 50 % of the molecules are in the cationic form and 50% are in the zwitterion form. This pH is pK1(with regard to COOH).
- If the titration is done from the Isoelectric point with NaOH, molecules acquire the anionic form. When 50 % of the molecules are in the anionic form and 50% are in the zwitterion form. This pH is pK2(with regard to NH_2)

Titration of Glycine

For mono amino mono carboxylic amino acids-

$$pI = \frac{pK1 + pK2}{2}$$

The buffering action is maximum in and around pK1or at pK2 but is minimum at pI

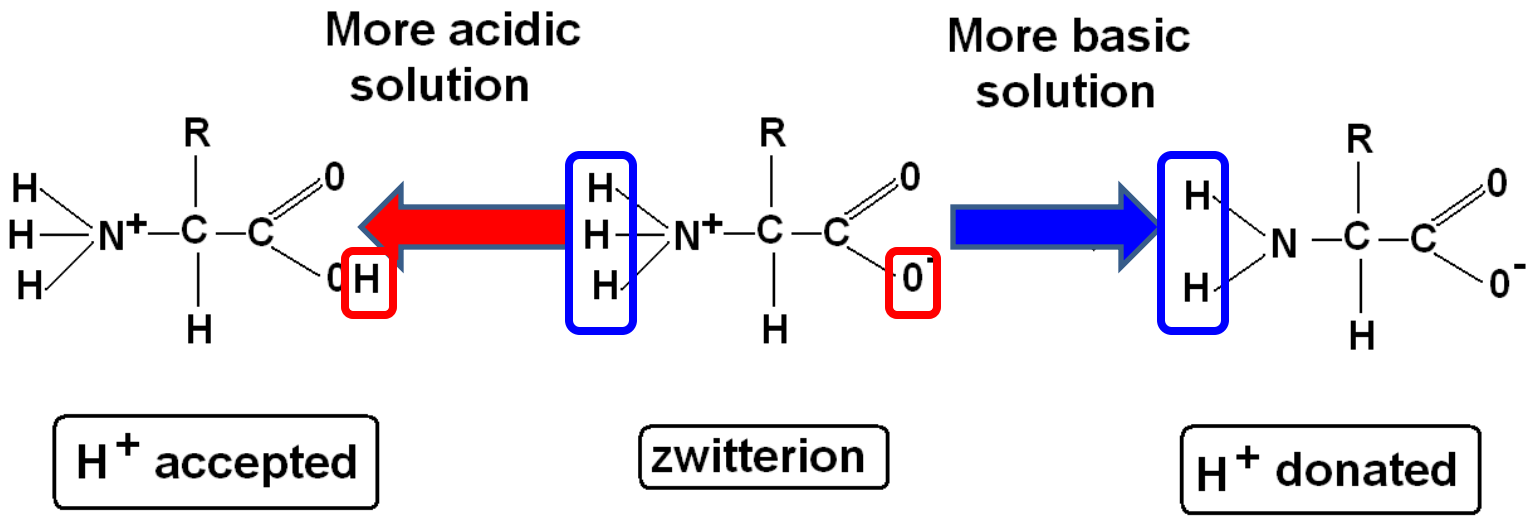


pK_a of some amino acids

TABLE **pK_a values of some amino acids**

Amino acid	pK_a values (25°C) α-COOH group	α-NH_3^+ group	Side chain
Alanine	2.3	9.9	
Glycine	2.4	9.8	
Phenylalanine	1.8	9.1	
Serine	2.1	9.2	
Valine	2.3	9.6	
Aspartic acid	2.0	10.0	3.9
Glutamic acid	2.2	9.7	4.3
Histidine	1.8	9.2	6.0
Cysteine	1.8	10.8	8.3
Tyrosine	2.2	9.1	10.9
Lysine	2.2	9.2	10.8
Arginine	1.8	9.0	12.5

Buffering capacity of amino acids



Zwitterion: a compound with both acidic and basic groups

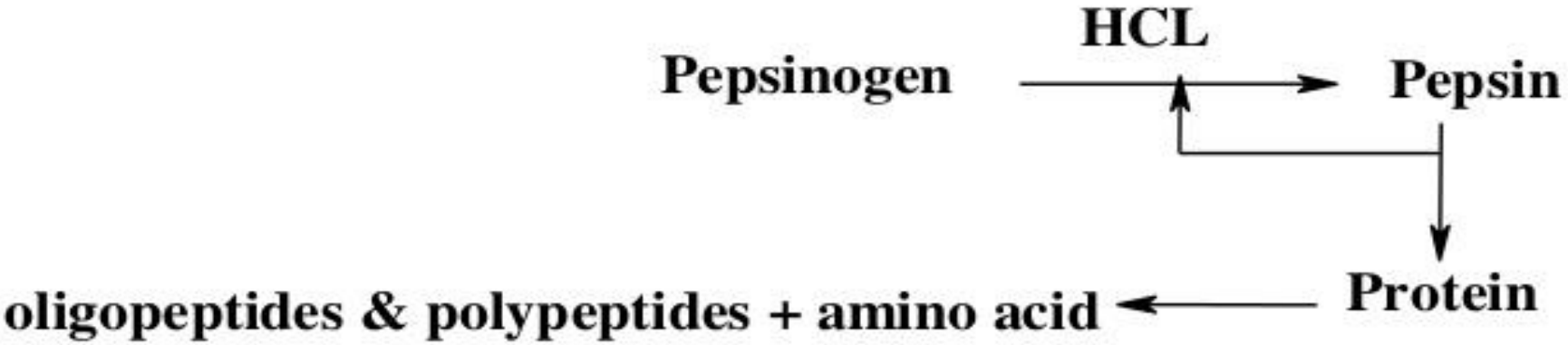
Isoelectric point is that pH at which a zwitter-ion carries no net electrostatic charge

DIGESTION OF PROTEIN

- Proteins are broken down by hydrolyases (peptidases or proteases):
- **Endopeptidases** attack internal bonds and liberate large peptide fragments (pepsin, trypsin, Chymotrypsin & Elastase)
- **Exopeptidases** remove one amino acid at a time from – COOH or $-NH_2$ terminus (aminopeptidase & carboxypeptidase)
- **Endopeptidases are important for initial breakdown of long polypeptides into smaller ones which then attacked by exopeptidases.**
- Digestion of protein can be divided into: a gastric, pancreatic and intestinal phases.

I. Gastric Phase of Protein Digestion: (represents 15% of protein digestion)

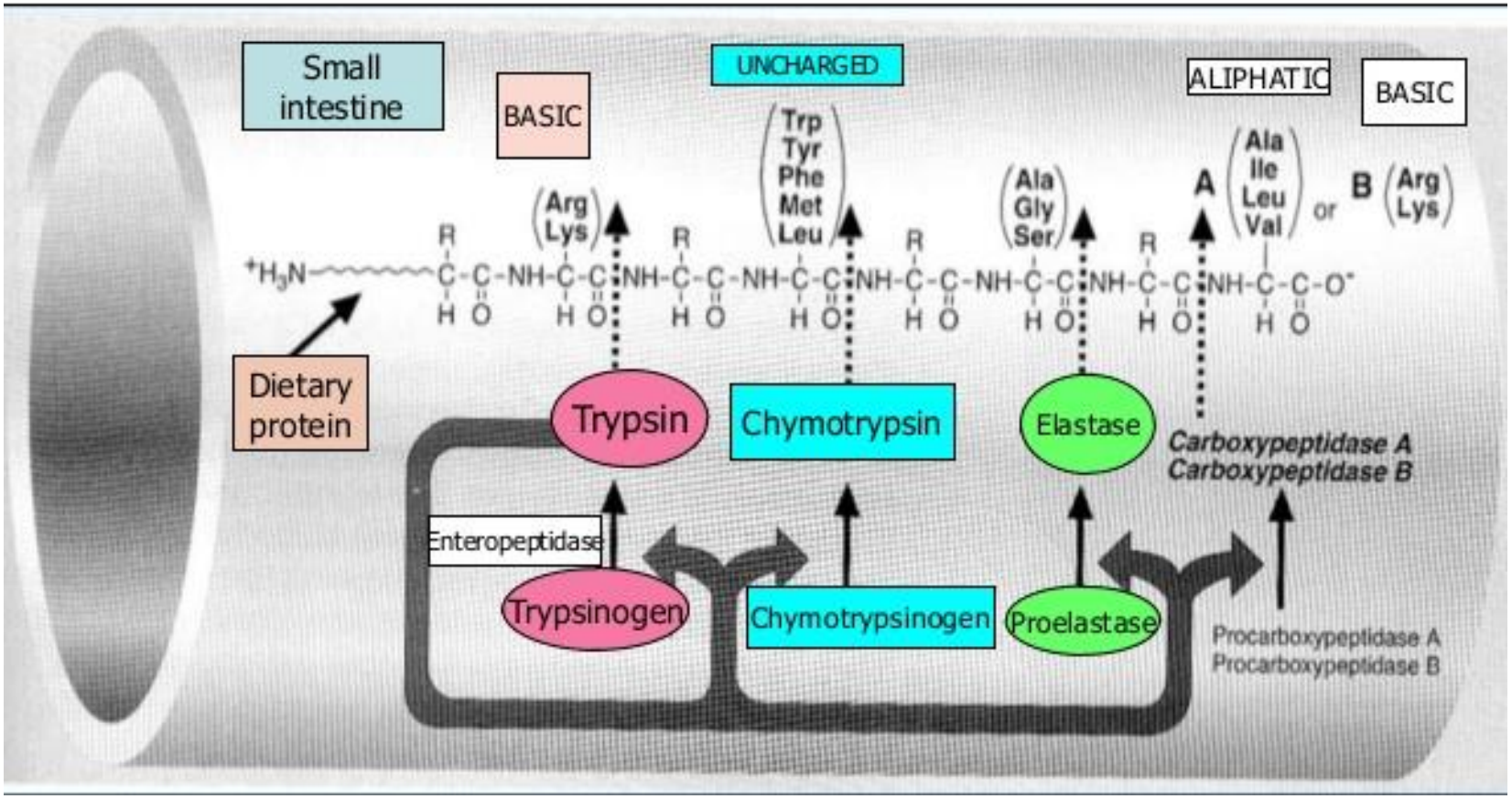
1) **Pepsin:** in adult stomach , secreted as pepsinogen.It is specific for peptide bond formed by aromatic or acidic amino acids



2) **Rennin:** in infants for digestion of milk protein (casein).

II. Pancreatic Phase of Protein Digestion

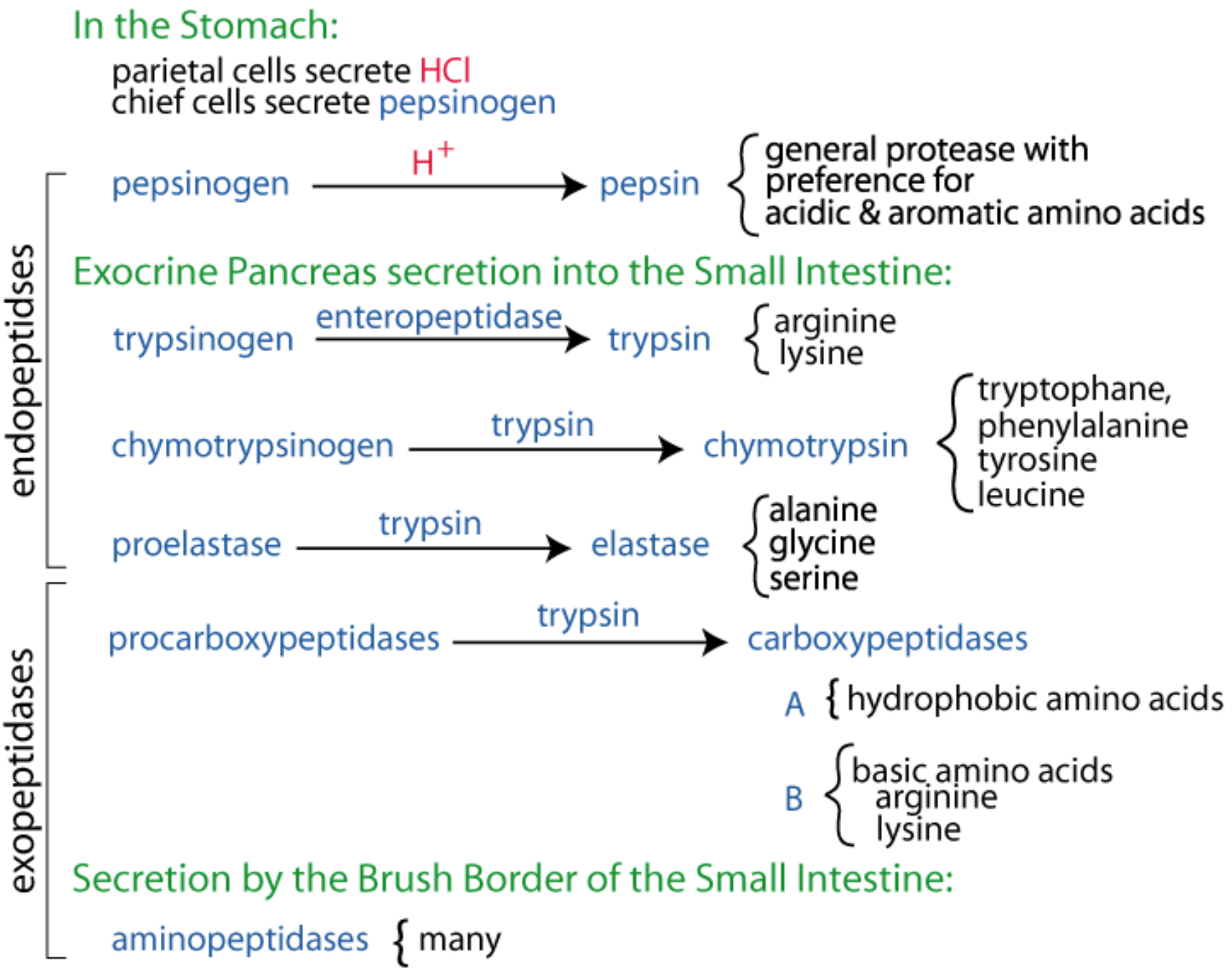
- This phase ends with **free amino acids** and **small peptides** of 2-8 amino acid residues which account for 60% of protein digestion



III. Intestinal Phase of protein digestion:

- Intestinal enzymes are:
  aminopeptidases (attack peptide bond next to amino terminal of polypeptide) & **dipeptidases**
- The end product is **free amino acids**

  dipeptides & tripeptides.


In the Stomach:
parietal cells secrete HCl
chief cells secrete pepsinogen
endopeptidases
pepsinogen
H+
pepsin
general protease with preference for acidic & aromatic amino acids
Exocrine Pancreas secretion into the Small Intestine:
trypsinogen
enteropeptidase
trypsin
arginine
lysine
chymotrypsinogen
trypsin
chymotrypsin
tryptophane,
phenylalanine
tyrosine
leucine
proelastase
trypsin
elastase
alanine
glycine
serine
exopeptidases
procarboxypeptidases
trypsin
carboxypeptidases
A
hydrophobic amino acids
B
basic amino acids
arginine
lysine
Secretion by the Brush Border of the Small Intestine:
aminopeptidases
many

Amino Acids Absorption

- Amino acids are absorbed in the small intestine
- Amino acids are transported to the liver from the intestines via the portal vein
- In the liver, amino acids are
  - Used to synthesize new proteins
  - Converted to energy, glucose, or fat
  - Released to the bloodstream and transported to cells throughout the body

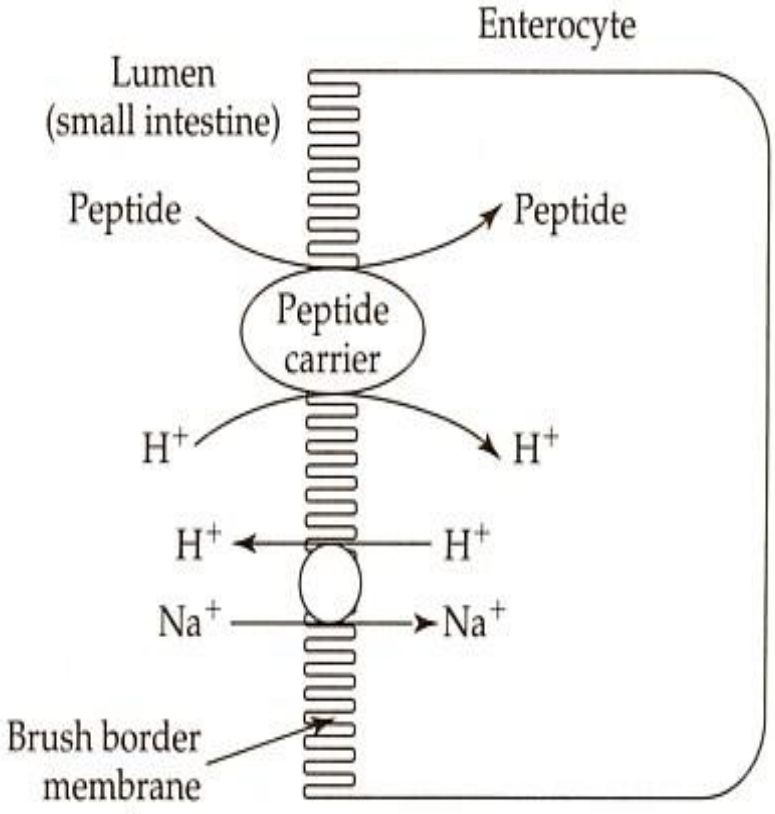
Peptide Absorption

Free amino acids are taken into the enterocytes by a Na+-linked secondary transport system of the apical membrane. Di- and tri -

peptides, however, are taken up by a H+-linked transport system.

The peptides are hydrolyzed in the cytosol to amino acids that are

released into the portal system by facilitated diffusion. Thus, only free amino acids are found in the portal vein after a meal containing protein. These amino acids are either metabolized by the liver or released into the general circulation.



Structure of a Protein

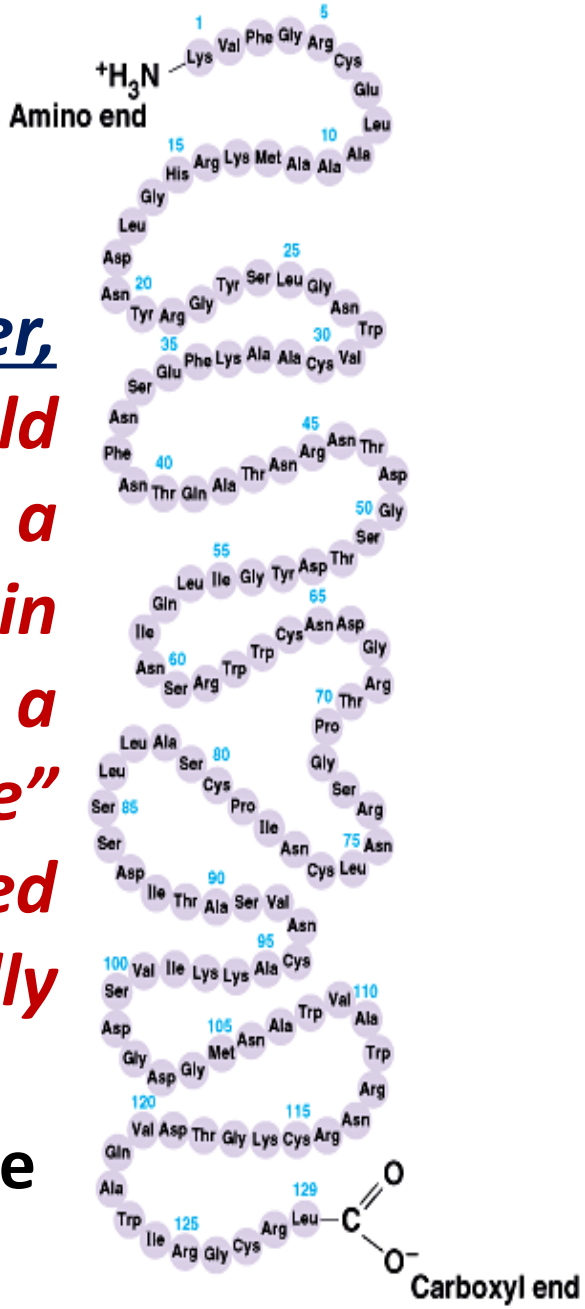
- each protein has a characteristic three dimensional shapes called its conformation

- *four* levels of organization exist:-
  1) Primary structure
  2) Secondary structure
  3) Tertiary structure
  4) Quaternary structure

Primary structure of a protein:

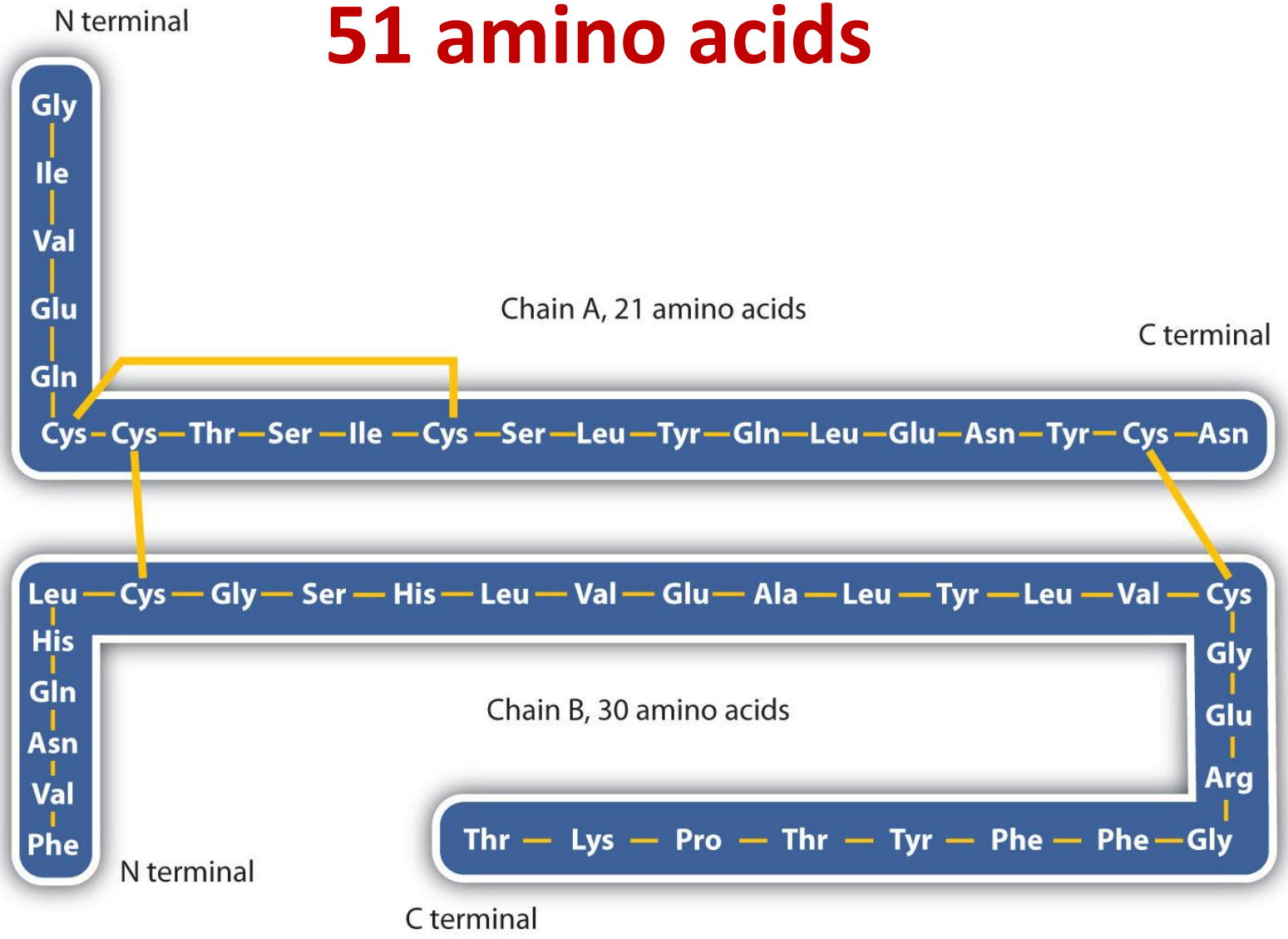
the one-dimensional structure of a protein

- ***Includes the sequence, number, arrangement of amino acids held together by peptide bonds in a polypeptide chain***
  Each component AA in a polypeptide is known as a "residue"
  -Proteins have a precisely defined AA sequence which is genetically determined
- **the primary structure of each type of protein is unique**



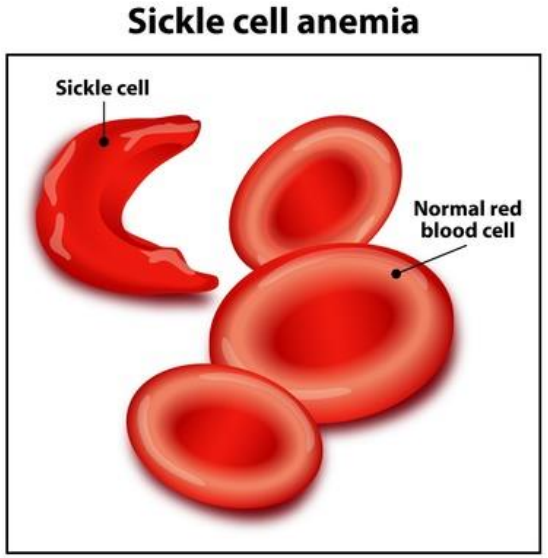
Primary structure of insulin:

51 amino acids



- **A change in just one amino acid can change the *structure and function* of a protein.**
- **For example, sickle cell anemia is a disease that results from an altered structure of the protein hemoglobin, resulting from a change of the sixth amino acid from glutamic acid to valine. (This is the result of a single base pair change at the DNA level)**

This single AA change is enough to change the *conformation* of hemoglobin



Polypeptides that have similar amino acid sequences and have arisen from the same ancestral gene are said to be **homologous**. Sequence comparisons among homologous polypeptides have been used to trace the genetic relationships of different species

Secondary structure: the arrangement in space

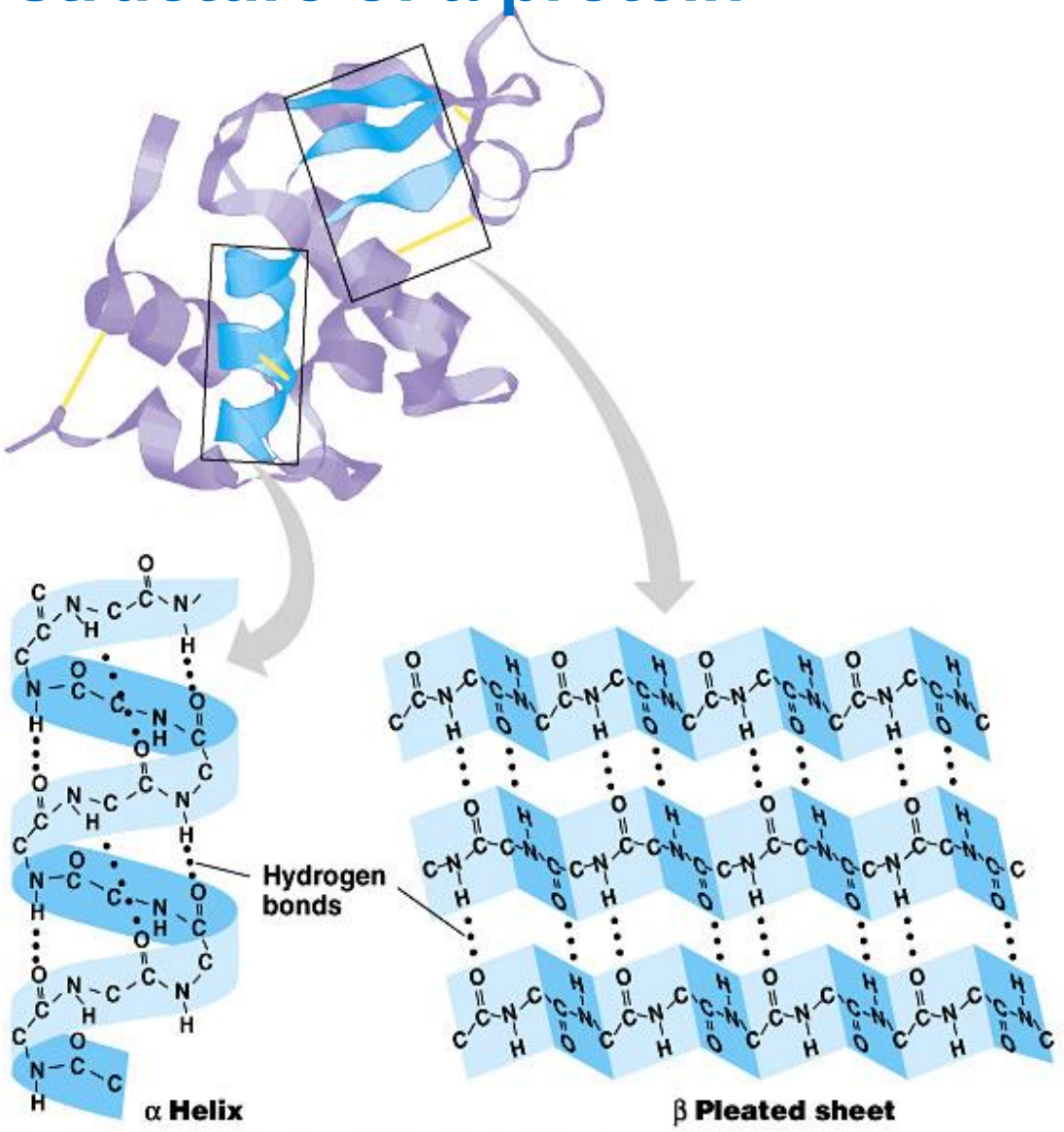
- ***The way in which the polypeptide is arranged in space= folding of the polypeptide chain into ordered structure***

- **secondary structure of many different proteins may be the same**

- **bonds present:**
    1. **Peptide**
    2. **Hydrogen bonds: between carbonyl oxygens and amide hydrogens**

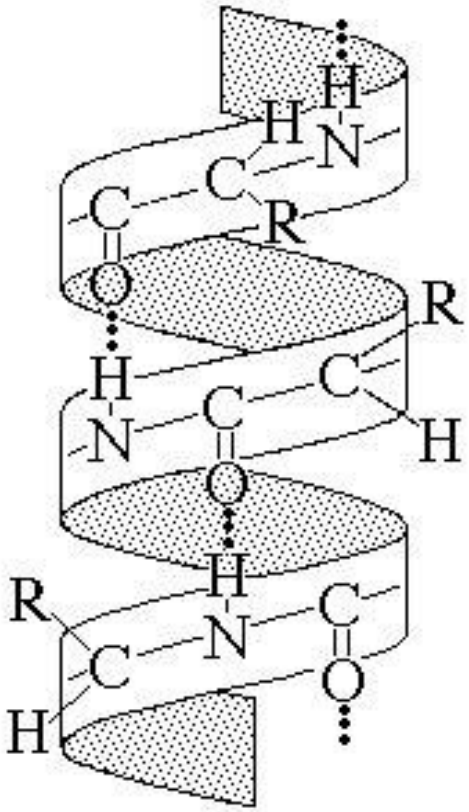
Hydrogen bonds between amino acids lead to the secondary structure of a protein

Two common secondary structures are the α-helix and β-pleated sheet



Secondary Structure Types

Secondary Structure – α helix

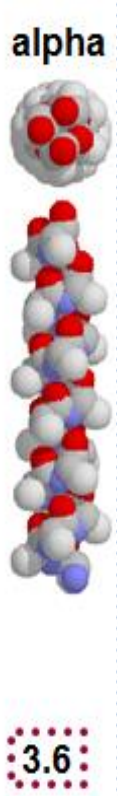


α-helix

The telephone cord shape of the α-helix is held in place by Hydrogen bonds between every N-H group and the oxygen of a C=O group in the next turn of the helix, four amino acids down the chain. The typical α-helix is about 11 amino acids long.

+ve

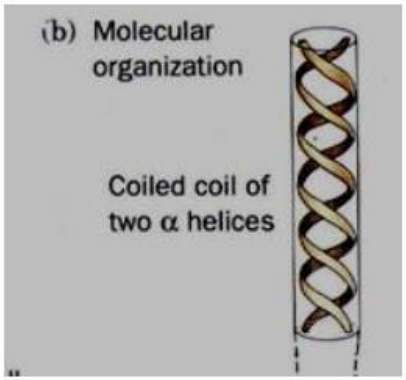
- ve



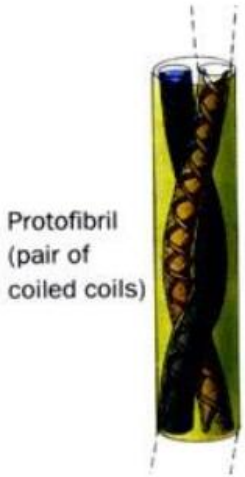
Keratins:(hair, skin)

- is entirely helical and thus fibrous

- hardness & rigidity of keratin is determined by the number of disulfide bonds between the constituent polypeptide chains



Keratin (角蛋白)

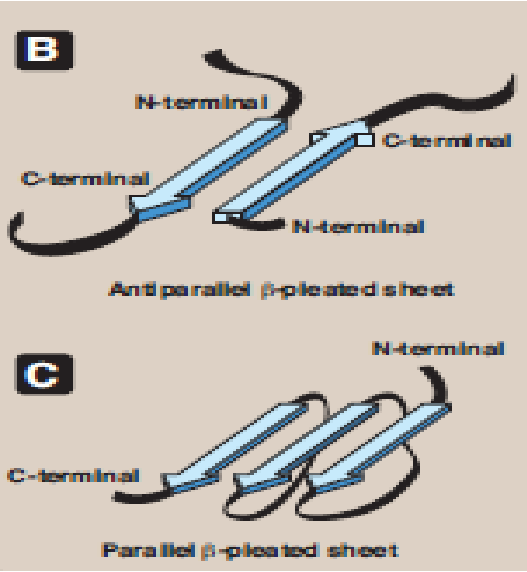


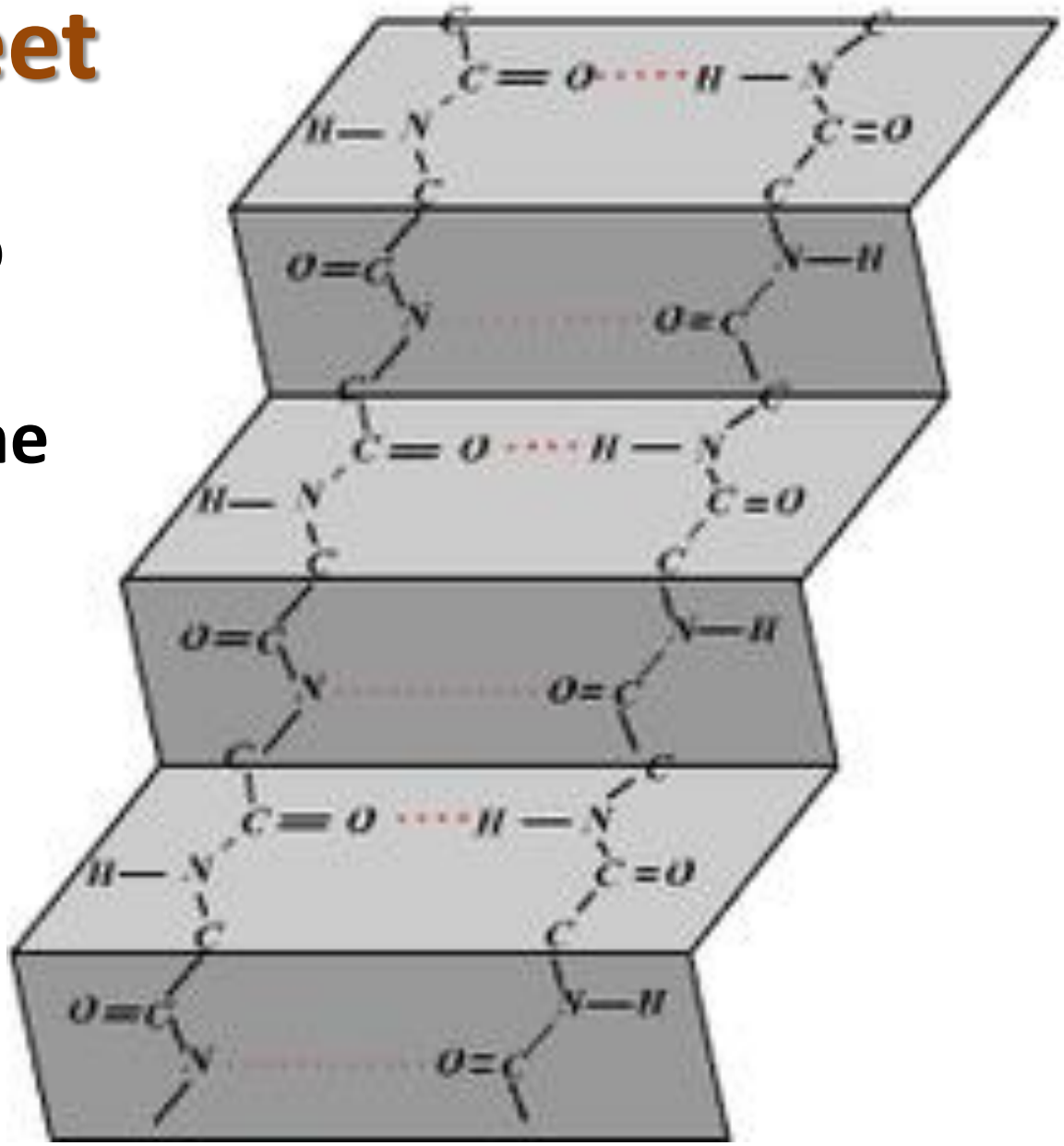
Protofibril (初原纤维)

microfibril (微管)

β-pleated sheet

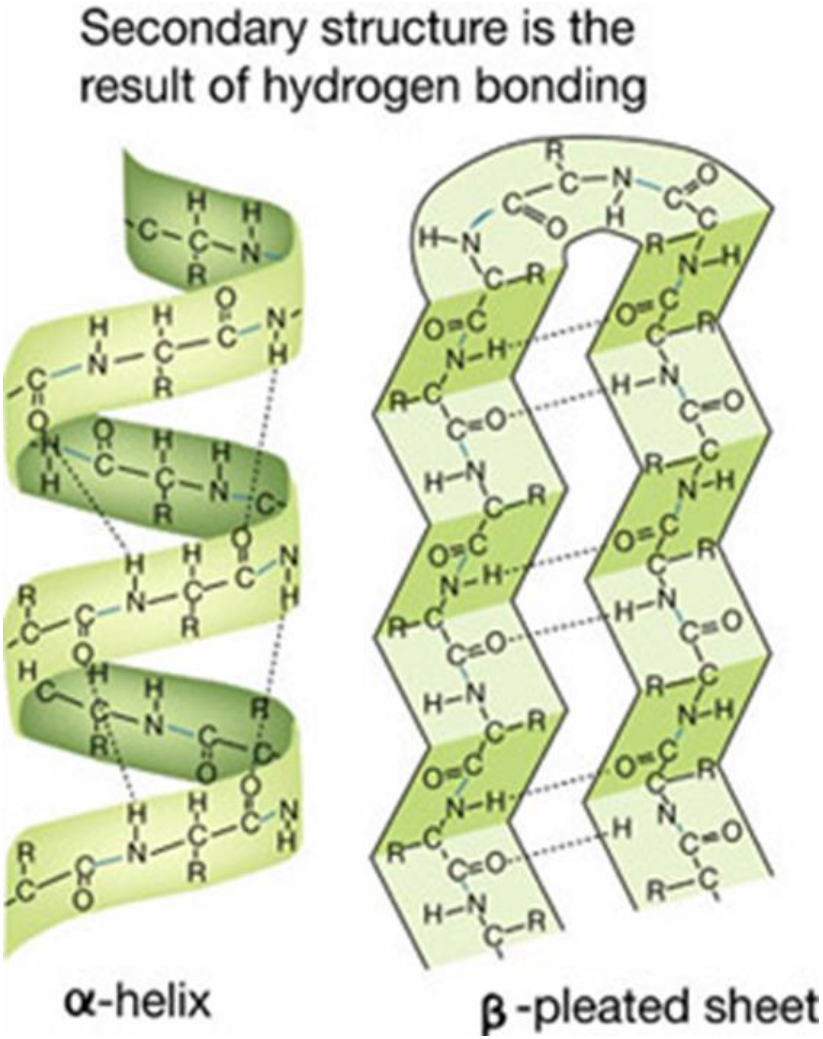
- occurs when two adjacent peptide chains bind to one another



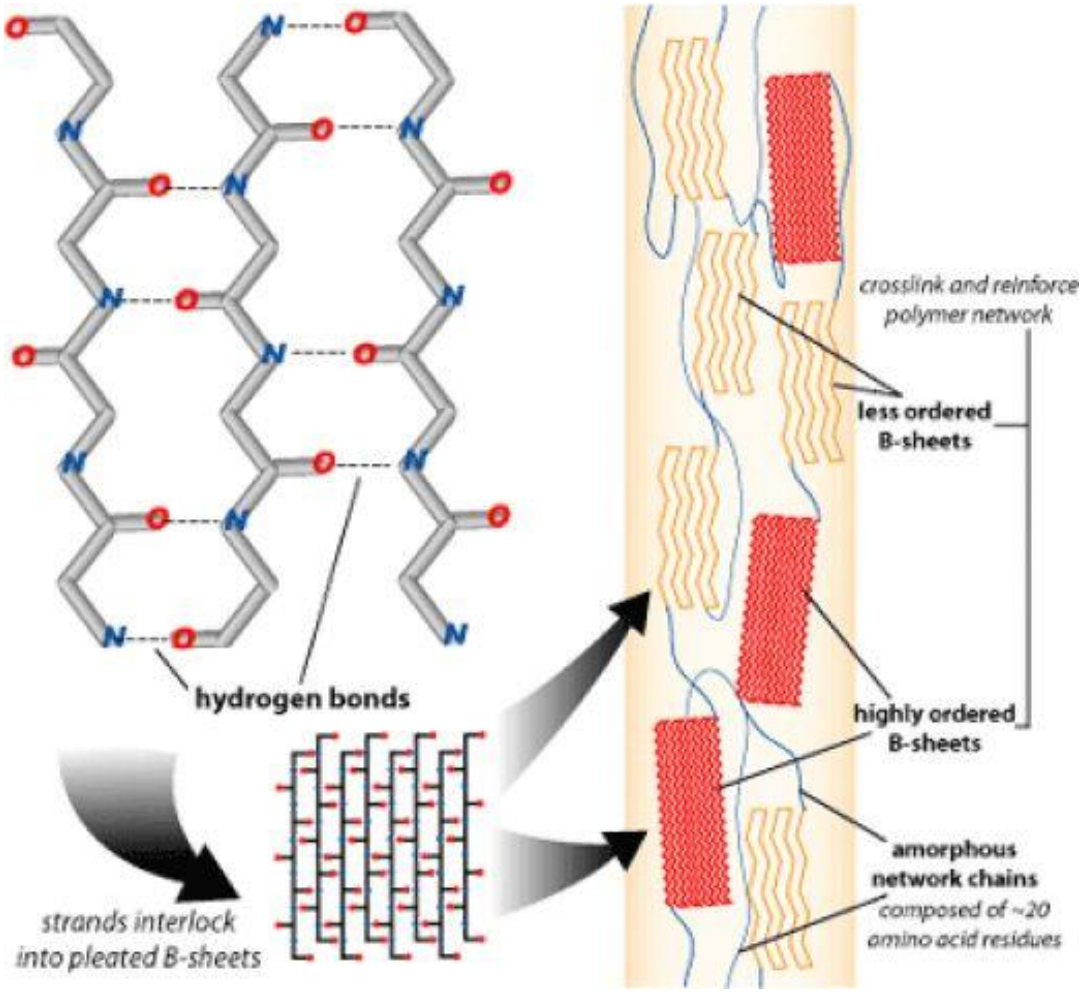


β-Pleated Sheet

- Polypeptide chains lie **adjacent** to one another; may be parallel or antiparallel
- R groups alternate, first above and then below plane
- Each peptide bond is s-trans and planar



Silk is an example of a β-pleated sheet



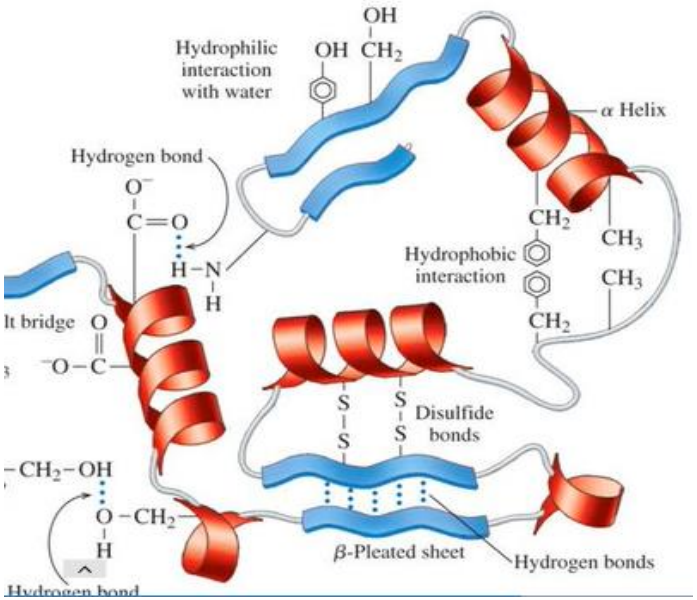
Silk Protein Structure

Tertiary Structure

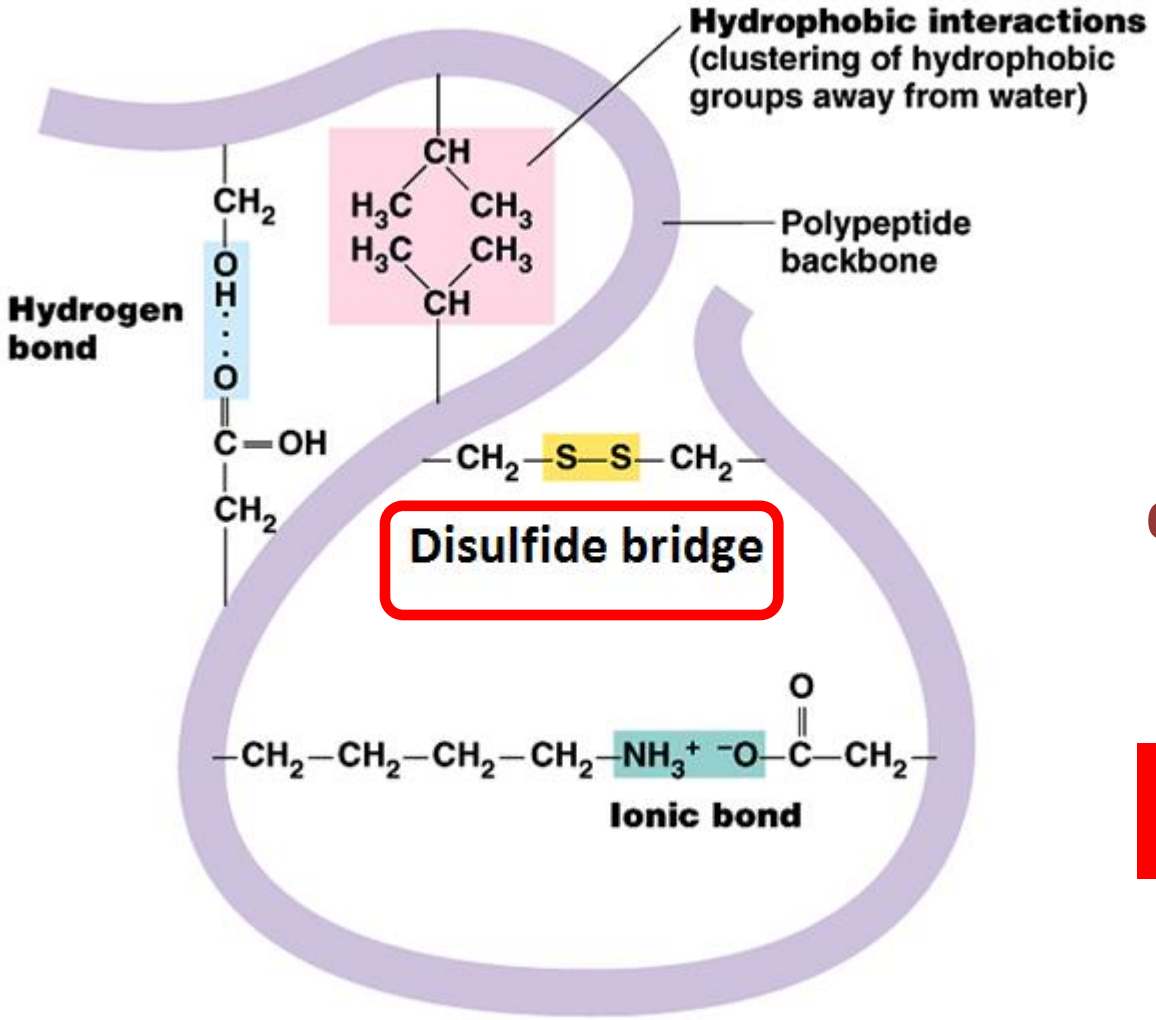
The tertiary structure of a protein:

- **Gives a specific 3-dimesional shape to the polypeptide chain=the overall conformation of a polypeptide chain: 3D arrangement of AA residues**
- **Involves the attractions and repulsions of the R groups of distant AA of the polypeptide chain**
- **Is stabilized by:**

1. Hydrophobic and hydrophilic interactions, salt bridges
1. Hydrogen bonds
2. Disulfide bonds



Further folding of the polypeptide chain contributes to the tertiary structure of a protein



Which amino acid forms disulfide bridges?

Cysteine

Components of Tertiary Structure

- **Fold** – used differently in different contexts – most broadly a reproducible and recognizable 3 dimensional arrangement
- **Domain** – a compact and self folding component of the protein that usually represents a discreet structural and functional unit
- **Motif** (aka supersecondary structure) a recognizable subcomponent of the fold – several motifs usually comprise a domain

Like all fields these terms are not used strictly making capturing data that conforms to these terms all the more difficult

Quaternary structure:

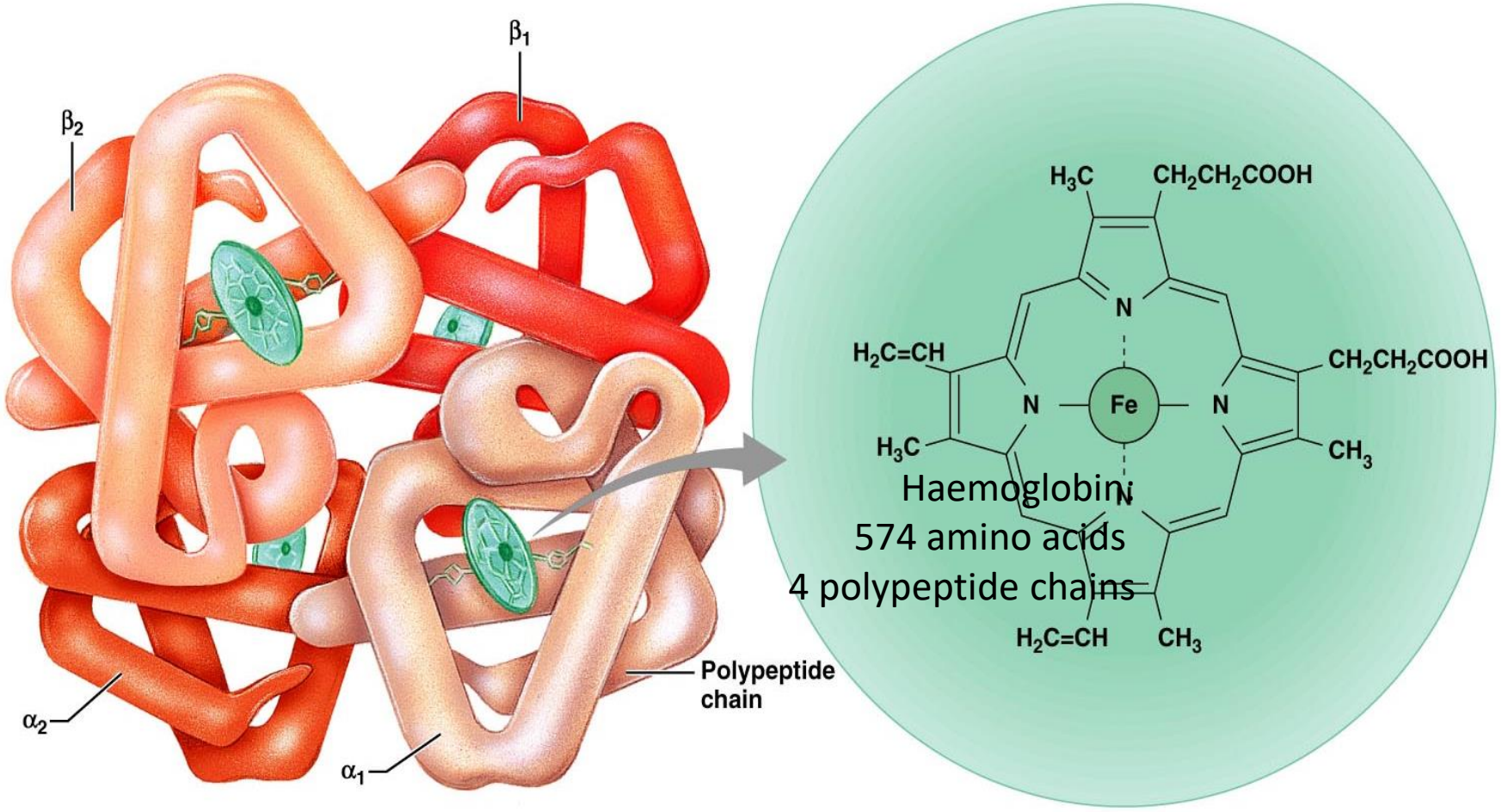
occurs in many highly complex proteins

- ***The precise arrangement of the aggregation of polypeptide chains held together by hydrophobic interactions, H-bonds and ionic bonds***

- ***Quaternary structure exists in proteins consisting of 2 or more separate polypeptide, each polypeptide unit is called: protomer or subunit***

- ***Proteins with 2 subunits are called: dimers: CPK***

  4 subunits: tetramers: Hb



(a) Haemoglobin

(b) Iron-containing haem group

Haemoglobin:

574 amino acids

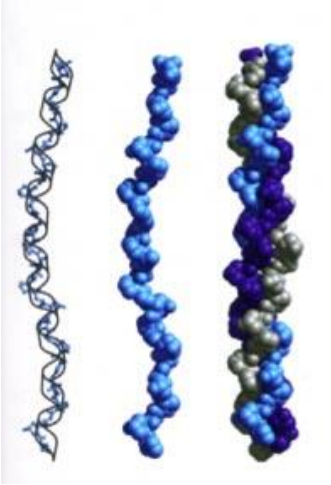
4 polypeptide chains

The final three-dimensional shape of a protein can be classified as:

Fibrous

- ✓ Tough
- ✓ Insoluble in water

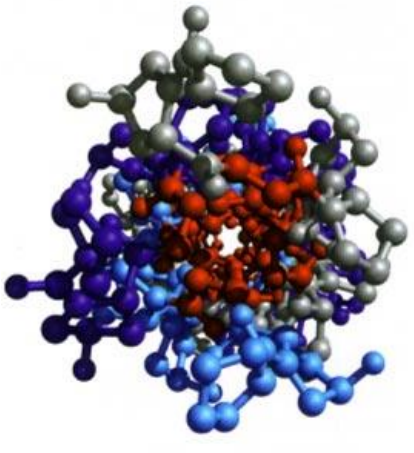
Keratin
Silk
Collagen



Globular

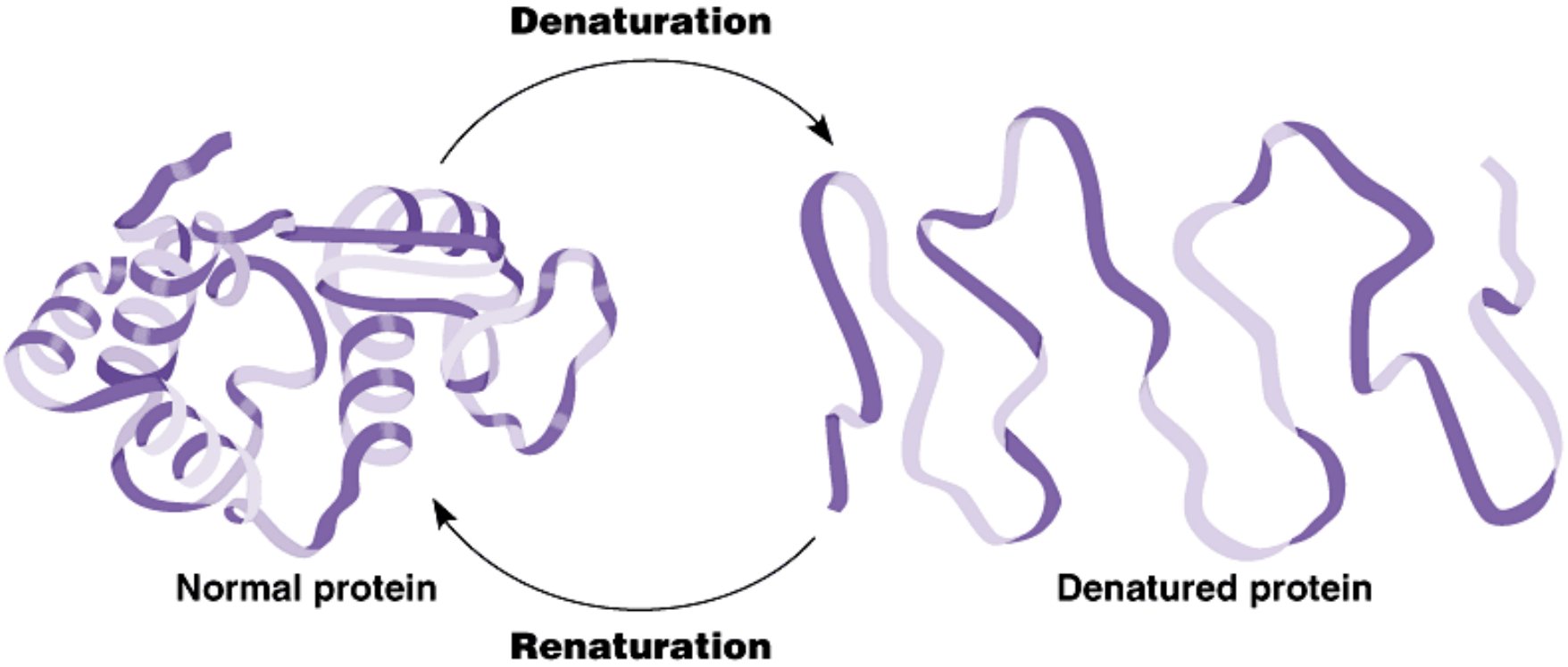
- ✓ Soluble

Enzymes
Antibodies



FIBROUS PROTEINS	GLOBULAR PROTEINS
Do not have a tertiary structure.	Have tertiary structure. Quaternary may or may not be present
Long fibres or sheets in shape.	Spherical in shape
Insoluble in water	Dissolve in water to form colloidal solution
The length of polypeptide chain may vary in two samples of the same fibrous protein	The length of polypeptide chain is always identical in two samples of the same globular protein.
Perform structural functions	Perform metabolic functions.
eg. Keratins, collagen, elastin and fibroin.	Egg albumin, serum globulin etc.

The loss of the specific three-dimensional conformation (secondary structure) of a protein



A protein spontaneously refolds into its original structure under suitable conditions

Denaturation involves

- the disruption of bonds in the secondary, tertiary, and quaternary protein structures.
- *heat* and *organic compounds* that break apart H bonds and disrupt hydrophobic interactions.
- *acids* and *bases* that break H bonds between polar R groups and disrupt ionic bonds.
- *heavy metal ions* that react with S—S bonds to form solids.
- *agitation*, such as whipping, that stretches peptide chains until bonds break.

Denaturation agents can be:

i) Heat :weak hydrogen bonds and non polar hydrophobic interactions are disrupted

ii) Strong acids & alkalis and high concentrations of salts

iii) Heavy metals (e.g. mercury)

iv) Organic solvents and detergents

Applications of Denaturation

Denaturing Agent	Bonds Disrupted	Examples
Heat above 50 °C	Hydrogen bonds; hydrophobic interactions between nonpolar R groups	Cooking food and autoclaving surgical items
Acids and bases	Hydrogen bonds between polar R groups; salt bridges	Lactic acid from bacteria, which denatures milk protein in the preparation of yogurt and cheese
Organic compounds	Hydrophobic interactions	Ethanol and isopropyl alcohol, which disinfect wounds and prepare the skin for injections
Heavy metal ions Ag^+, Pb^{2+}, and Hg^{2+}	Disulfide bonds in proteins by forming ionic bonds	Mercury and lead poisoning
Agitation	Hydrogen bonds and hydrophobic interactions by stretching polypeptide chains and disrupting stabilizing interactions	Whipped cream, meringue made from egg whites