The University of Zambia

CHE 2112
Fundamentals of Biochemistry

Protein Biochemistry

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Definition

- any of a class of nitrogenous organic compounds which have large molecules composed of one or more long chains of amino acids
- Contain a an array of amino acids- polymers of amino acids .

are very important biological molecules that play crucial roles in virtually all biological processes

BIOLOGICAL FUNCTIONS OF PROTEINS

1. **Catalytic function:**

   Nearly all chemical reactions in biological systems are catalyzed by specific enzymes.

2. **Transport and storage:**

   For example;

   - **Hemoglobin transports oxygen in erythrocytes**
   - **Myoglobin carries & stores oxygen in muscle.**
   - **Albumin transports free fatty acids in blood.**
   - **Transferrin transports iron in blood.**

3. **Coordinated motion:** **Actin and myosin are contractile proteins in muscle.**

BIOLOGICAL FUNCTIONS OF PROTEINS (cont.)

4. Structural and Mechanical support:

For Example; collagen, a fibrous protein in skin and bone.

5. Defense function:

For Example Clotting factors prevent loss of blood.

Immunoglobulins protects against infections.

6. Generation and transmission of nerve impulses:

For example, rhodopsin is the photoreceptor protein in retinal rod cells.

7. Control of growth and differentiation:

For Example

- **growth factor proteins.**
- **hormones such as insulin and thyroid-stimulating hormone.**

General structure of protein

- All biologically known protein are polymers of a set of twenty known amino acids.

- All biologically known amino acids are α-L amino acids.

AMINO ACIDS

are the basic building blocks of

PROTEINS

Each AMINO ACID

has

An amino group,

A carboxyl group,

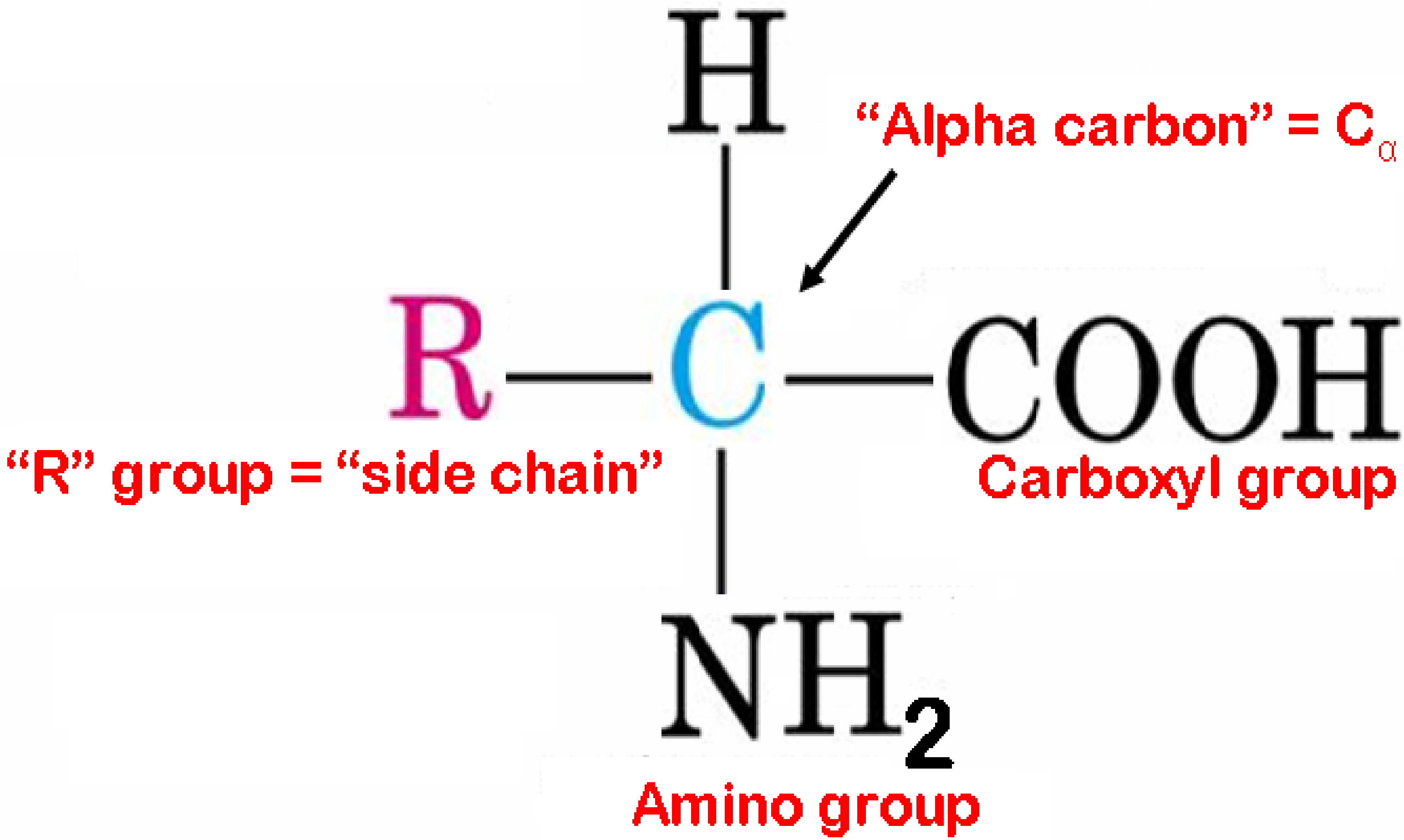
A hydrogen atom and

a specific side chain (R group)

Bonded to

the α-carbon atom

- **This side chain is unique for every amino acid.**


H
"Alpha carbon" = Cα
R
C
COOH
"R" group = "side chain"
Carboxyl group
NH2
Amino group

Classification of amino acids

- Side chain reaction classification
- Biological classification
- Metabolic classification

Side chain classification

1- Hydrophobic (non-polar) R-group)	2- Hydrophilic (polar) R-group		
Glycine (Gly-G) Alanine (Ala-A) Valine (Val-V) Leucine (Leu-L) Isoleucine (Ile– I) Methionine (Met– M) Proline (Pro– P) Phenylalanine (Phe– F) Tryptophan (Trp–W)	**Uncharged** Aspargine (Asn – N) Glutamine (Gln – Q) Serine (Ser – S) Threonine (Thr – T) Tyrosine (Tyr – Y) Cysteine (Cys – C)	**Positively charged** Lysine (Lys – K) Arginine (Arg – R) Histidine (His – H)	**Negatively charged** Aspartic acid (Asp – D) Glutamic acid (Glu – E)

Non polar (hydrophobic) amino acids

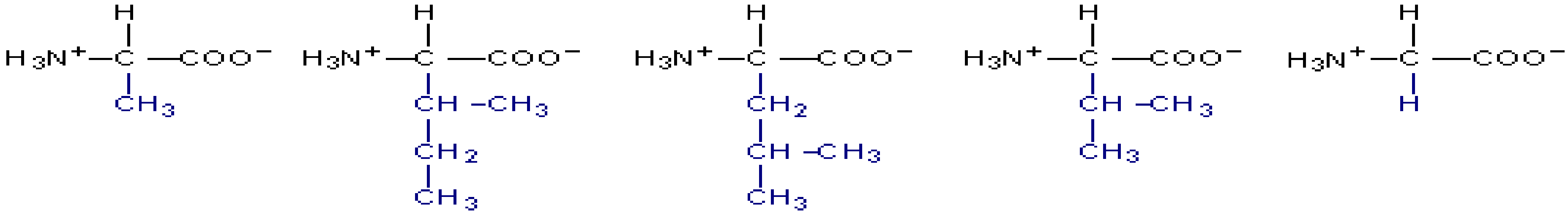
- Side chains of non polar (hydrophobic) a.a. can **not** participate in hydrogen or ionic bonds, but they form hydrophobic interactions.
- In aqueous environment, non polar a.a. tend to be present in the interior of proteins.
- They include:
- Amino acids with aliphatic R group (glycine, alanine,
- Amino acids with aliphatic branched R group (valine, leucine and isoleucine).
- Amino acids with aromatic R group (phenylalanine, tryptophan)
- Amino acids with sulfur group (methionine) and
- Imino acid (proline).

Polar (hydrophilic) amino acids

- Side chains of polar (hydrophilic) a.a. can participate in hydrogen or ionic bonds.
- Therefore, in aqueous environment polar a.a. tend to be present on the surface of proteins.
- **Polar (hydrophilic) amino acids** are classified into:

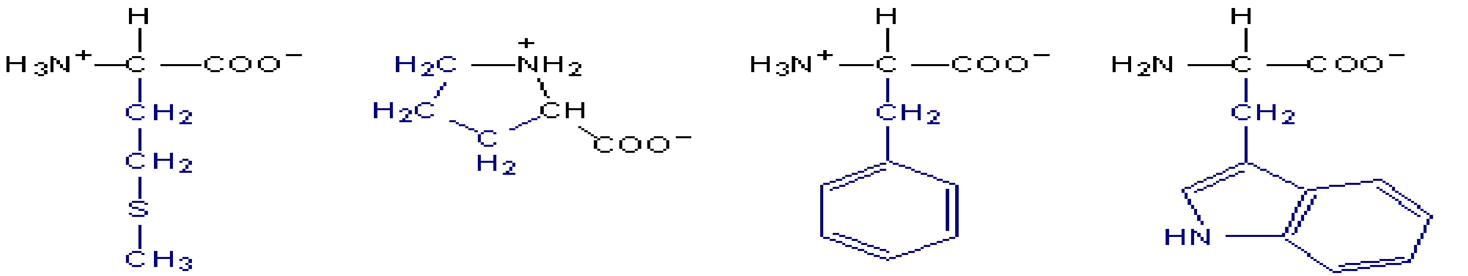
  - Polar charged amino acids include
    - acidic (**Negatively charged):** (aspartic and glutamic a.) and
    - basic (**Positively charged group):** (arginine, lysine, histidine) amino acids.
  - Polar non charged amino acids include:
    - Amino acids with OH group (serine, threonine, tyrosine)
    - Amino acids with SH group (cysteine)
    - Amino acids with amide group (glutamine, asparagines)

alanine (Ala, A) isoleucine (Ile, I) leucine (Leu, L) valine (Val, V) glycine (Gly, G)



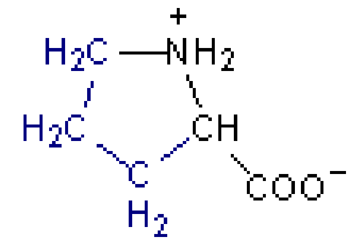
amino acids: non-polar aliphatic R-groups; plus glycine

methionine (Met, M) proline (Pro, P) phenylalanine (Phe, F) tryptophan (Trp, W)

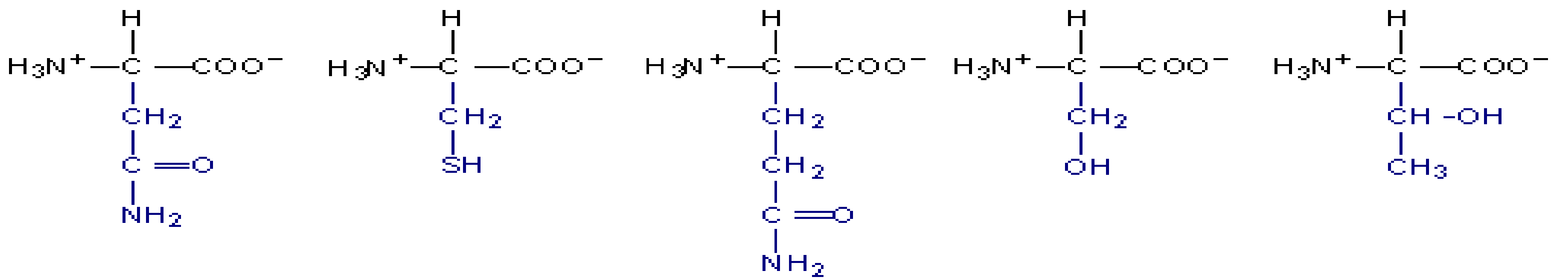


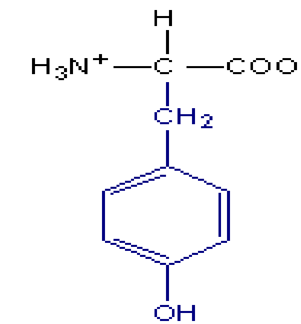
amino acids: non-polar R-groups (cont.)

proline (Pro, P)



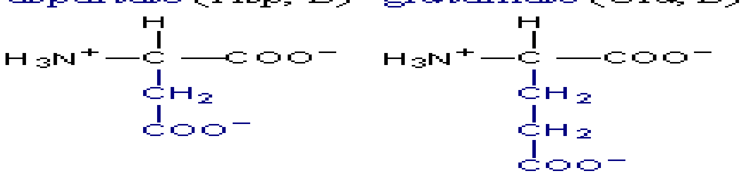
asparagine (Asn, N) cysteine (Cys, C) glutamine (Gln, Q) serine (Ser, S) threonine (Thr, T) tyrosine (Tyr, Y)

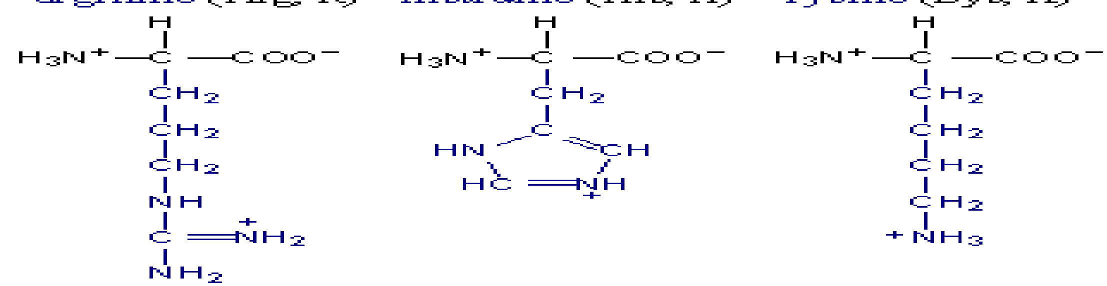




amino acids: polar but uncharged R-groups

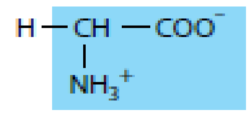
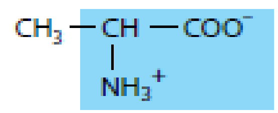
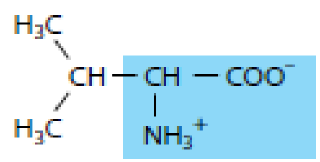
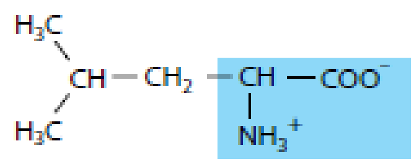
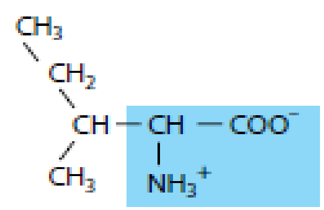
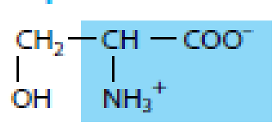
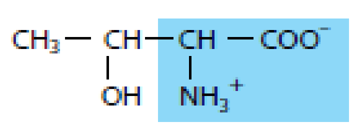
aspartate (Asp, D) glutamate (Glu, E) arginine (Arg, R) histidine (His, H) lysine (Lys, K)





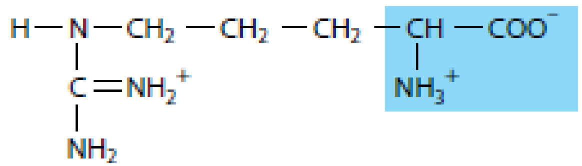
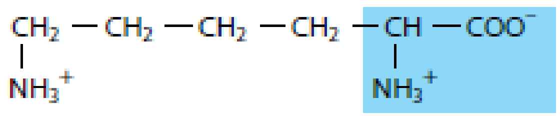
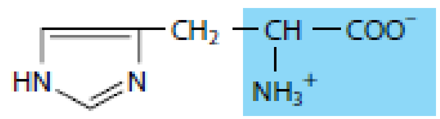
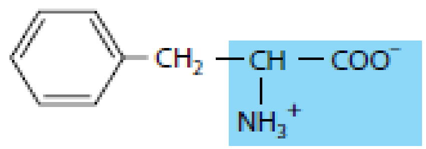
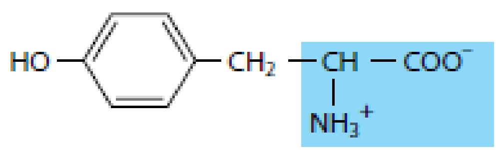
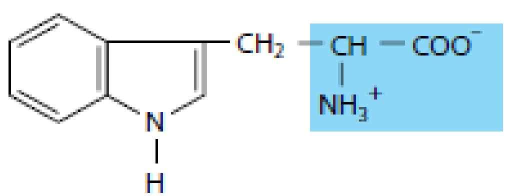
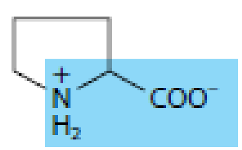
amino acids: charged R-groups

Table 3–1. L- α-Amino acids present in proteins.

Name	Symbol	Structural Formula	pK_1	pK_2	pK_3
With Aliphatic Side Chains			α-COOH	α-NH_3^+	R Group
Glycine	Gly [G]		2.4	9.8	
Alanine	Ala [A]		2.4	9.9	
Valine	Val [V]		2.2	9.7	
Leucine	Leu [L]		2.3	9.7	
Isoleucine	Ile [I]		2.3	9.8	
With Side Chains Containing Hydroxylic (OH) Groups					
Serine	Ser [S]		2.2	9.2	about 13
Threonine	Thr [T]		2.1	9.1	about 13
Tyrosine	Tyr [Y]	See below.			

With Side Chains Containing Hydroxylic (OH) Groups					
Serine	Ser [S]	$CH_2(OH){-}CH(NH_3^+){-}COO^-$	2.2	9.2	about 13
Threonine	Thr [T]	$CH_3{-}CH(OH){-}CH(NH_3^+){-}COO^-$	2.1	9.1	about 13
Tyrosine	Tyr [Y]	See below.			
With Side Chains Containing Sulfur Atoms					
Cysteine	Cys [C]	$CH_2(SH){-}CH(NH_3^+){-}COO^-$	1.9	10.8	8.3
Methionine	Met [M]	$CH_3{-}S{-}CH_2{-}CH_2{-}CH(NH_3^+){-}COO^-$	2.1	9.3	
With Side Chains Containing Acidic Groups or Their Amides					
Aspartic acid	Asp [D]	$^-OOC{-}CH_2{-}CH(NH_3^+){-}COO^-$	2.0	9.9	3.9
Asparagine	Asn [N]	$H_2N{-}C(=O){-}CH_2{-}CH(NH_3^+){-}COO^-$	2.1	8.8	
Glutamic acid	Glu [E]	$^-OOC{-}CH_2{-}CH_2{-}CH(NH_3^+){-}COO^-$	2.1	9.5	4.1
Glutamine	Gln [Q]	$H_2N{-}C(=O){-}CH_2{-}CH_2{-}CH(NH_3^+){-}COO^-$	2.2	9.1	

(continued)

Name	Symbol	Structural Formula	pK_1	pK_2	pK_3
With Side Chains Containing Basic Groups			α-COOH	α-NH_3^+	R Group
Arginine	Arg [R]		1.8	9.0	12.5
Lysine	Lys [K]		2.2	9.2	10.8
Histidine	His [H]		1.8	9.3	6.0
Containing Aromatic Rings					
Histidine	His [H]	See above.			
Phenylalanine	Phe [F]		2.2	9.2	
Tyrosine	Tyr [Y]		2.2	9.1	10.1
Tryptophan	Trp [W]		2.4	9.4	
Imino Acid					
Proline	Pro [P]		2.0	10.6	

Biological classification

1- Non essential amino acids: These are Glycine, Alanine, Serine, Tyrosine, Cysteine, Arginine, Asparagine, Aspartic, Glutamic acid , Glutamine and Proline.

2- Essential amino acids:

They include Valine, Leucine, Isoleucine, Threonine, Methionine,, Lysine, Histidine, Phenylalanine and Tryptophan.

Metabolic classification

- ***Glucogenic amino acids:*** These amino acids could give intermediates which finally can give glucose.
- ***Purely ketogenic amino acids***: They include Leucine & Lysine. They give ketone bodies after its degradation in the body, but no glucose.
- ***Mixed amino acids:*** These are amino acids that can give both ketone bodies and glucose intermediates. These are Phenylalanine, Tyrosine, Tryptophan, Isoleucine and Lysine.

* The rest of amino acids are all purely glucogenic.

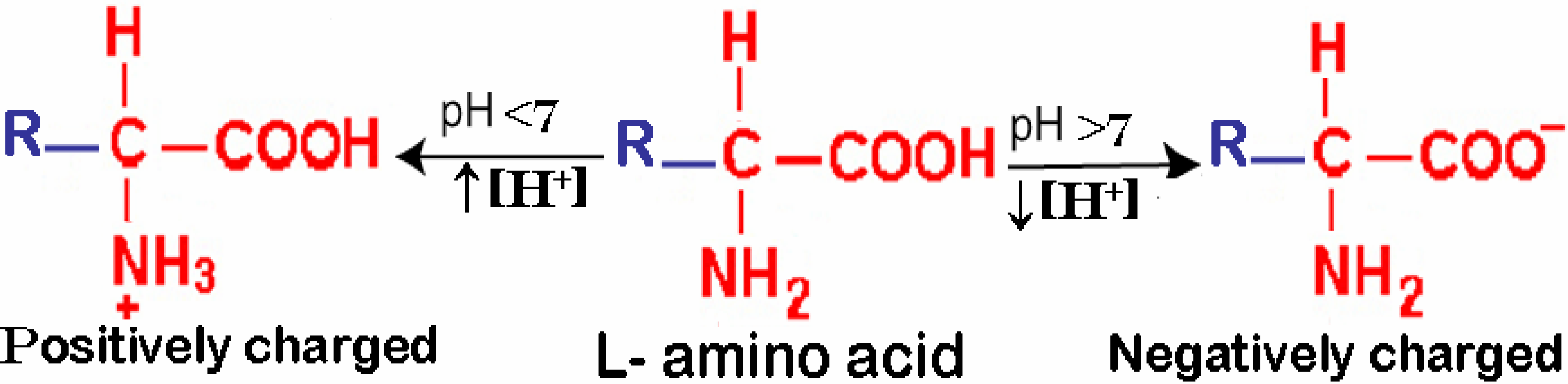
	Glucogenic	Glucogenic and Ketogenic	Ketogenic
Nonessential	Alanine Arginine* Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Histidine* Proline Serine	Tyrosine	
Essential	Methionine Threonine Valine	Isoleucine Phenyl-alanine Tryptophan	Leucine Lysine

Mirror image forms of amino acids

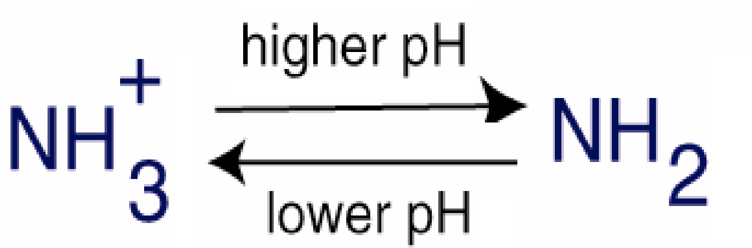
- Tetrahedral alpha-carbon atom,
- alpha amino acids acids are **chiral** exist in one or two **mirror image forms** called the **L isomer** and **D isomer** (orientation of the NH_3 group) determines the isomer, L isomer-left side, D isomer right hand side)
- Only L isomers are constituents of proteins

Ionic properties of amino acids

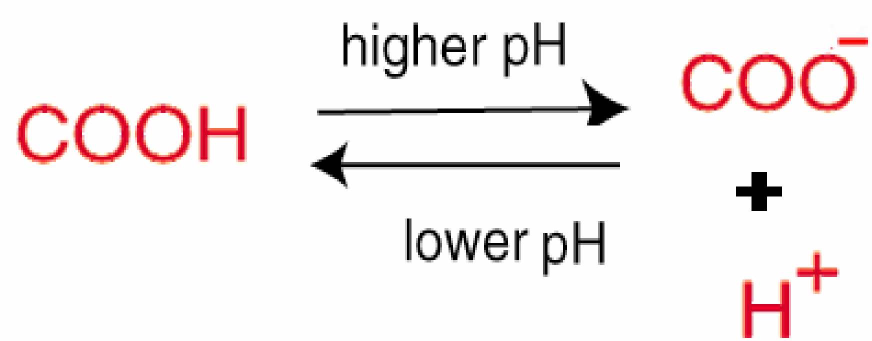
- Amino acids have amphoteric properties .
- They contain acidic (COOH) and Basic (NH2) groups.
- The amino acids are usually ionized at physiological pH .
- In acidic medium ; amino acid is positively charged (behave as a base : proton acceptor)
- In alkaline medium ; the carboxylic acid is negatively charged (behave as an acid: Proton donor)



Amino Group



Carboxyl Group



Isoelectric point or “pI”

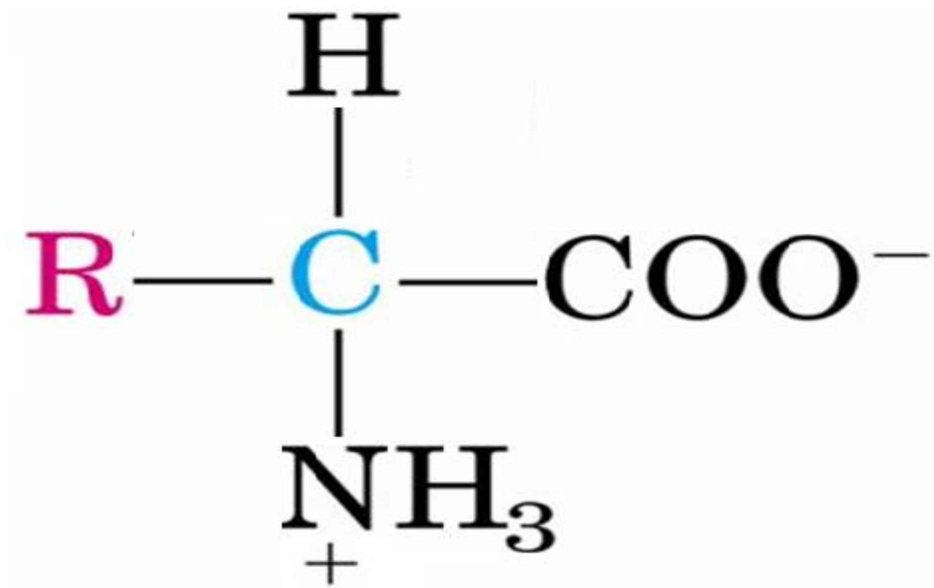
- At certain pH “ specific for each amino acid “ the amino acid can exist in the dipolar from : fully ionized but with no net electric charge .
- The characteristic pH at which the net electric charge is zero is called the Isoelectric point or “pI”.
- The amino acid at the isoelectric pI is called

“ Zwitter Ion “ and is electrically neutral not migrating in an electric field

- “Zwitter in German means hybrid or hermaphrodite”.

Isoelectric point or "pI"

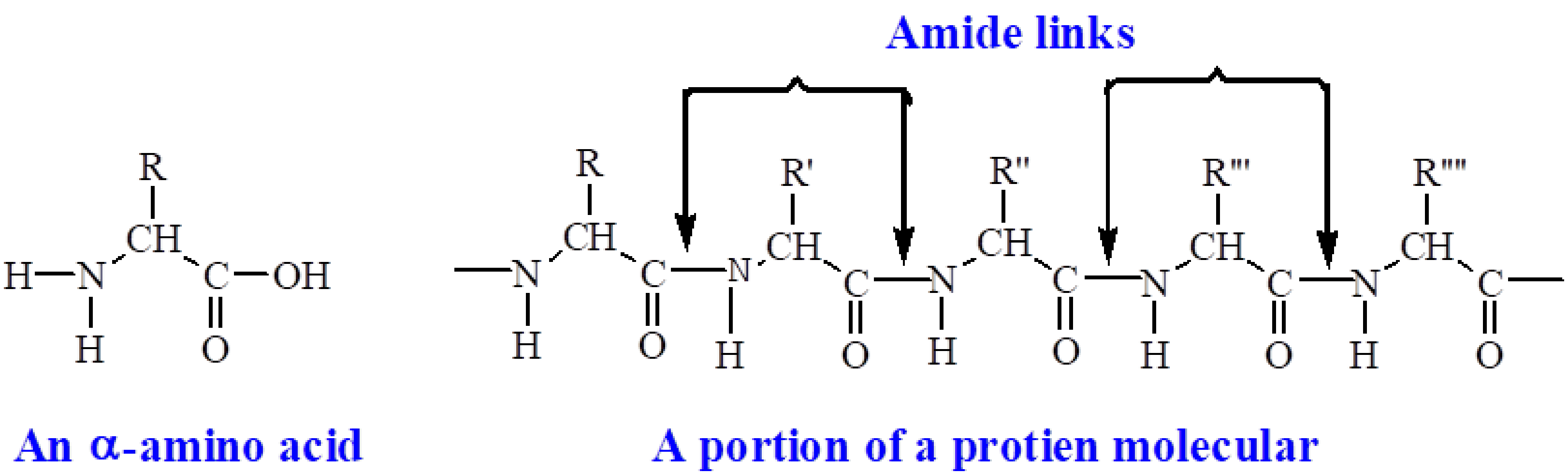
- At certain pH "specific for each amino acid" the amino acid can exist in the dipolar from : fully ionized but with no net electric charge.

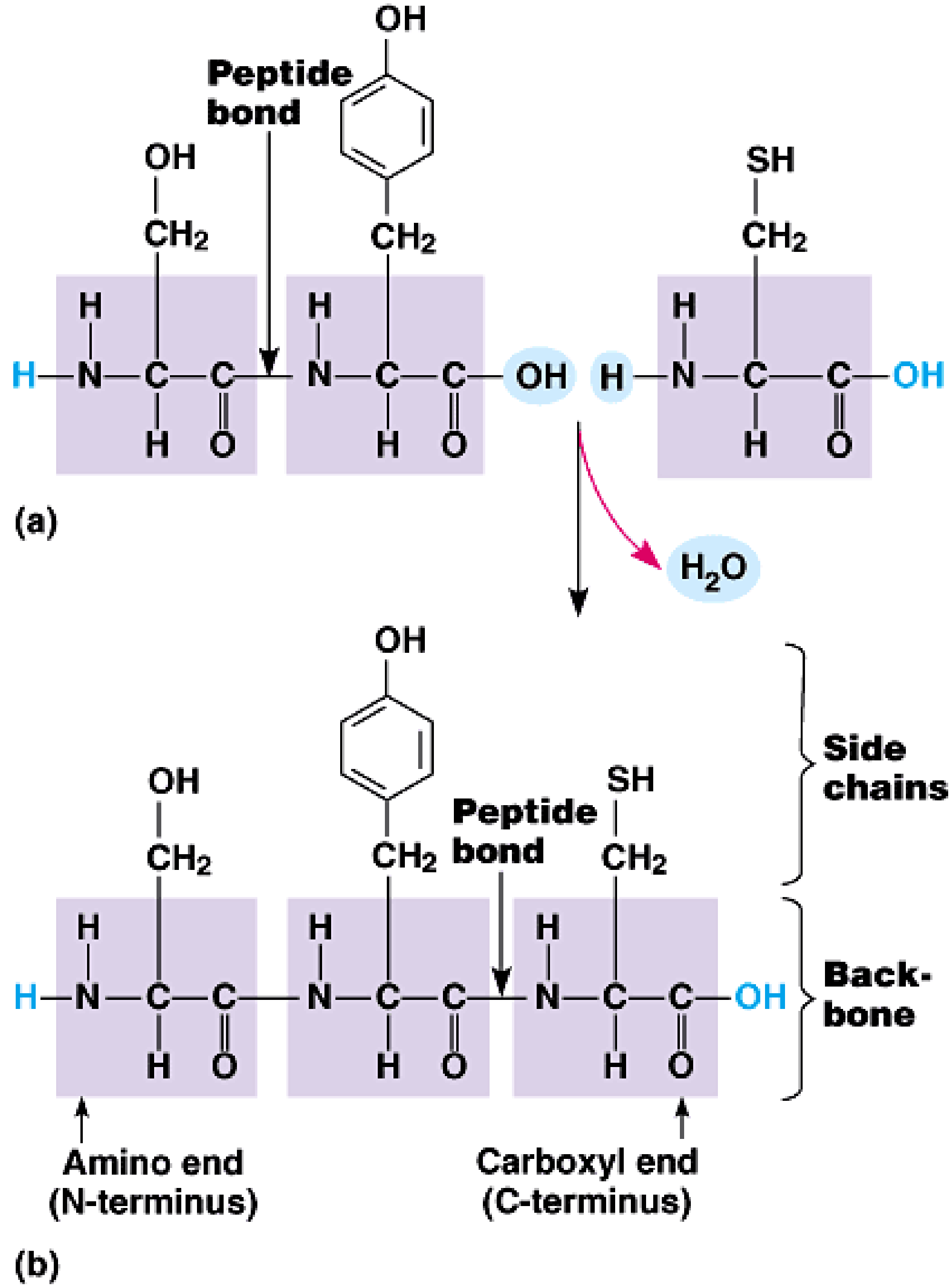


Zwitter ion

Polymerization of amino acids

- Amino acids polymerize by forming peptide bonds joining amino acid monomer units in a **dehydration synthesis reaction.**
- The macromolecule formed is called a polypeptide/amino acid residues/protein




Peptide bond
OH
SH
CH2
H
N
C
O
H2O
(a)
Side chains
Back-bone
Amino end (N-terminus)
Carboxyl end (C-terminus)
(b)

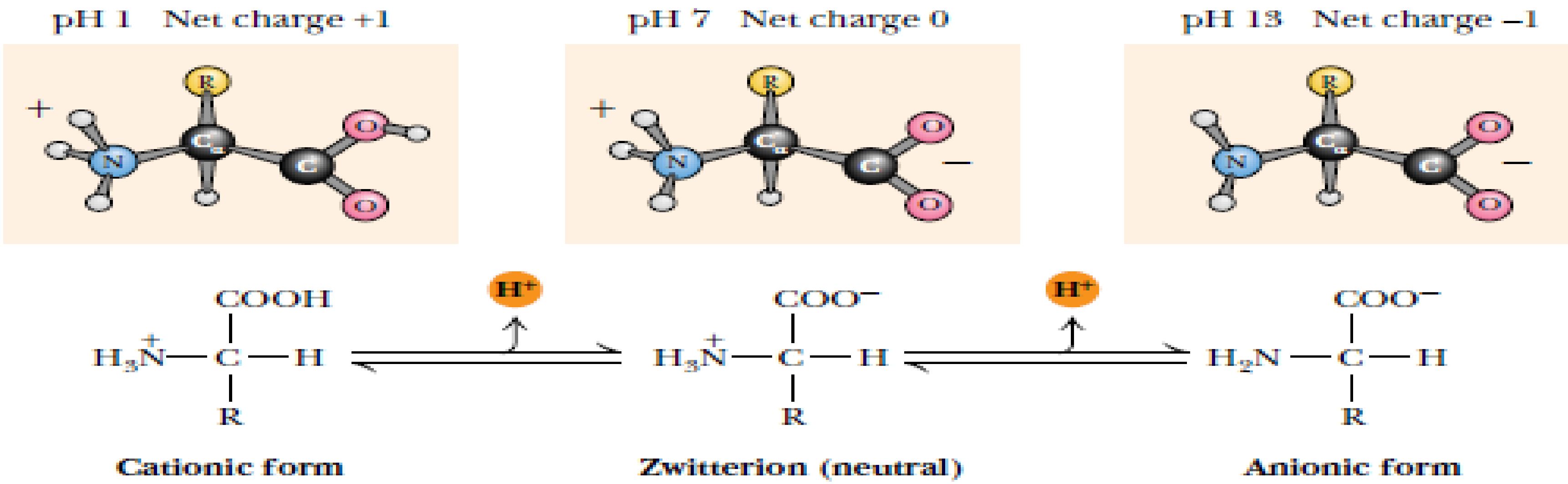
Polypeptide

- A series of a.a joined by peptide bonds forms a polypeptide chain
- Peptide bond is resistant to corrosion
- Each a.a in a polypeptide chain is called a residue
- Has polarity-two ends that are different; amino group at one end, carboxyl at other end
- Amino end-**beginning of a polypeptide**
- **Sequence of a.a is written starting with the amino-terminal residue**
- **Tyr-Gly-Phe-Leu,** tyrosine is the amino terminal, Leucine carboxyl terminal

Polypeptide contains a regularly repeating part called
main chain or backbone
Rich in hydrogen bonding potential

Titration of amino acids

- One important piece of information derived from the titration curve of an amino acid is the relationship between its net electric charge and the pH of the solution.
- At pH 5.97, the point of inflection between the two stages in its titration curve, glycine is present predominantly as its dipolar form, fully ionized but with no *net* electric charge
- The characteristic pH at which the net electric charge is zero is called the **isoelectric point** or **isoelectric pH,** designated **pI.**
- For glycine, which has no ionizable group in its side chain, the isoelectric point is simply the arithmetic mean of the two p*K*a values:



three forms as Gly^+, Gly^0, and Gly^-, we can write the first dissociation of Gly as

$$Gly^+ + H_2O \rightleftharpoons Gly^0 + H_3O^+$$

and the dissociation constant K_1 as

$$K_1 = \frac{[Gly^0][H_3O^+]}{[Gly^+]}$$

Values for K_1 for the common amino acids are typically 0.4 to 1.0×10^{-2} *M*, so that typical values of pK_1 center on values of 2.0 to 2.4 (see Table 4.1). In a similar manner, we can write the second dissociation reaction as

$$Gly^0 + H_2O \rightleftharpoons Gly^- + H_3O^+$$

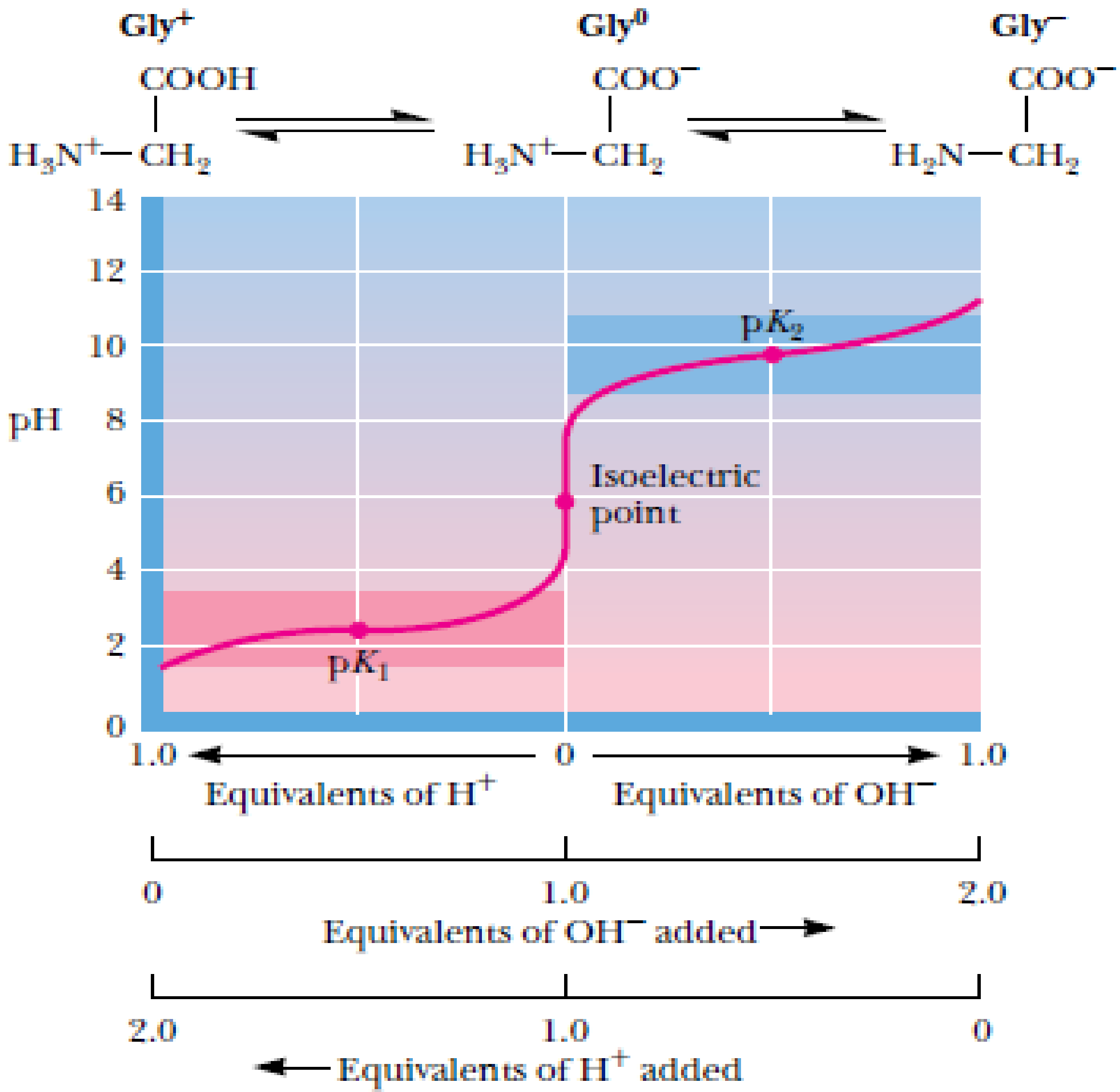


FIGURE 4.7 • Titration of glycine, a simple amino acid. The isoelectric point, pI, the pH where the molecule has a net charge of 0, is defined as $(pK_1 + pK_2)/2$.

Amino Acids Differ in Their Acid-Base Properties

- The shared properties of many amino acids permit some simplifying generalizations about their acid-base behaviors.
- First, all amino acids with a single -amino group, a single -carboxyl group, and an R group that does not ionize have titration curves resembling that of glycine.
- Second, amino acids with an ionizable R group have more complex titration curves, with *three* stages corresponding to the three possible ionization steps; thus they have three p*K*a values.
- The additional stage for the titration of the ionizable R group merges to some extent with the other two

Titration of an amino acids with ionisable side chain

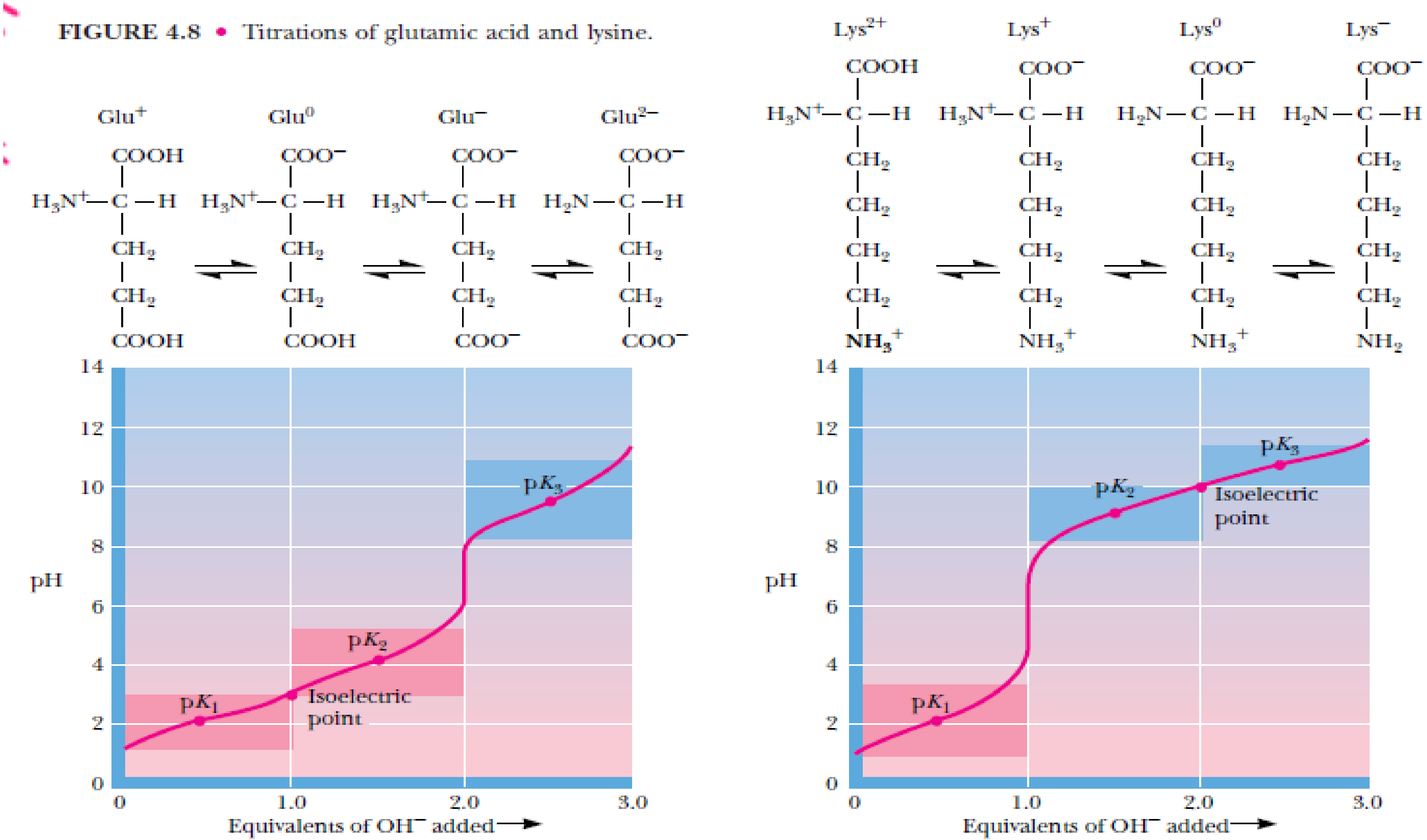


FIGURE 4.8 • Titrations of glutamic acid and lysine.

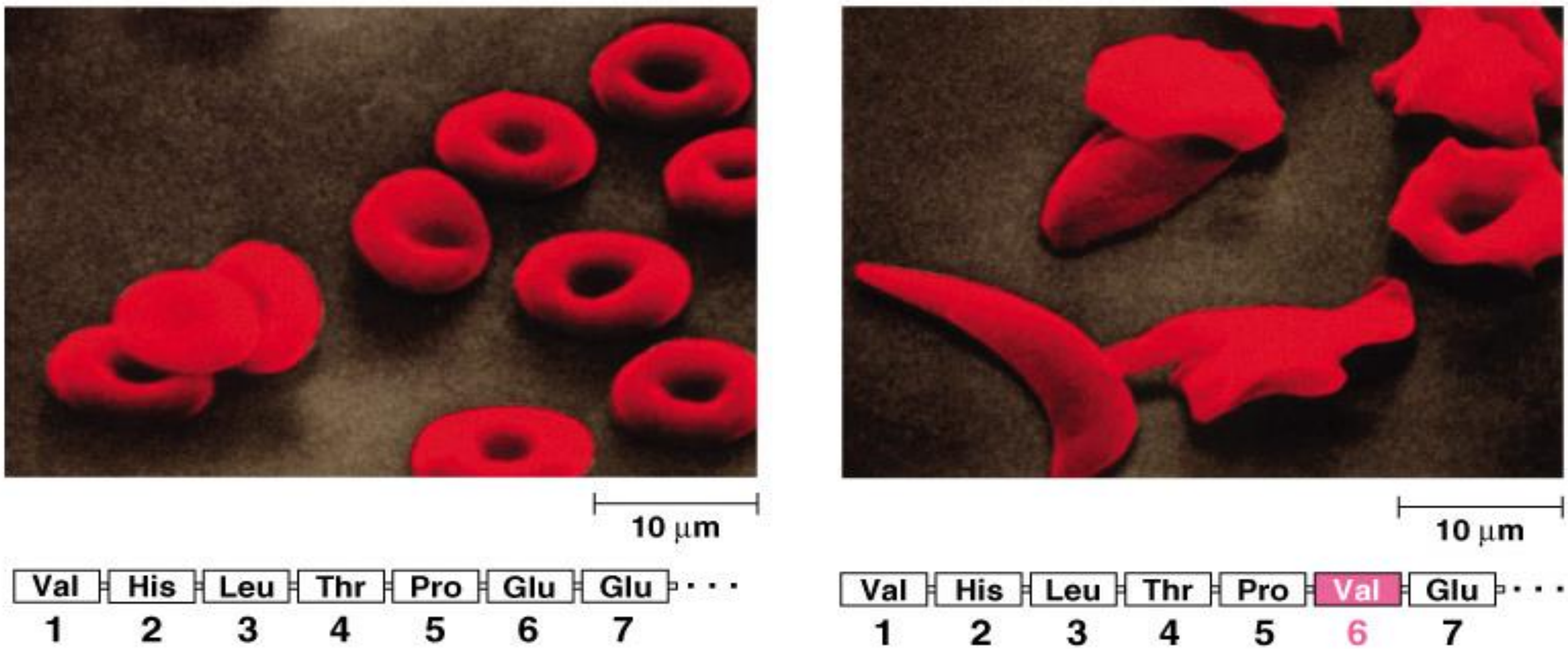
Protein Structure

- **Proteins** are the most abundant and important organic molecules
- Basic elements:
  - carbon (C), hydrogen (H), oxygen (O), and nitrogen (N)
- Basic building blocks:
  - 20 amino acids

Protein Structure – 4 levels

- **Primary structure: the exact sequence of the different α-amino acids along the protein chain**
- amino acid sequence determined by gene (DNA)
- ***Primary structure determines the 3-D structure of a protein, and the 3D structure determines the protein's function***
- slight change in amino acid sequence can affect protein's structure & it's function
  - even just one amino acid change can make all the difference!

Sickle cell anaemia

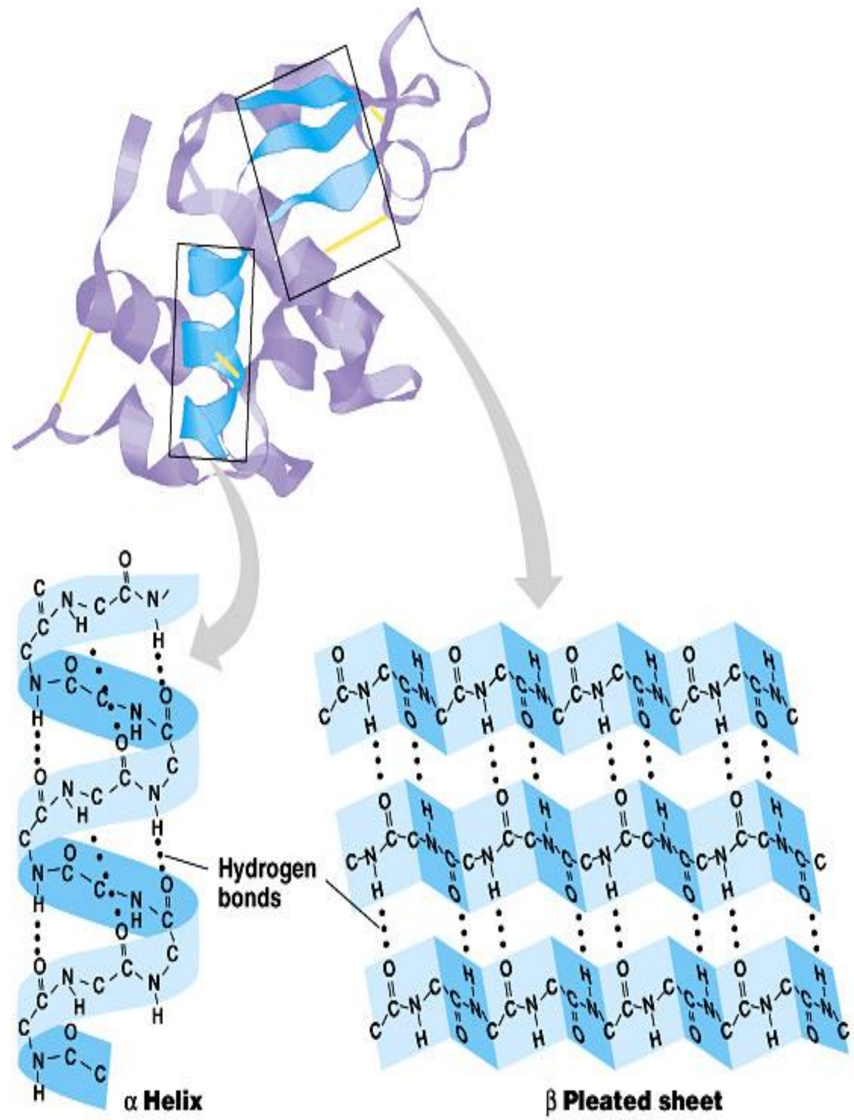


(a) Normal red blood cells and the primary structure of normal hemoglobin

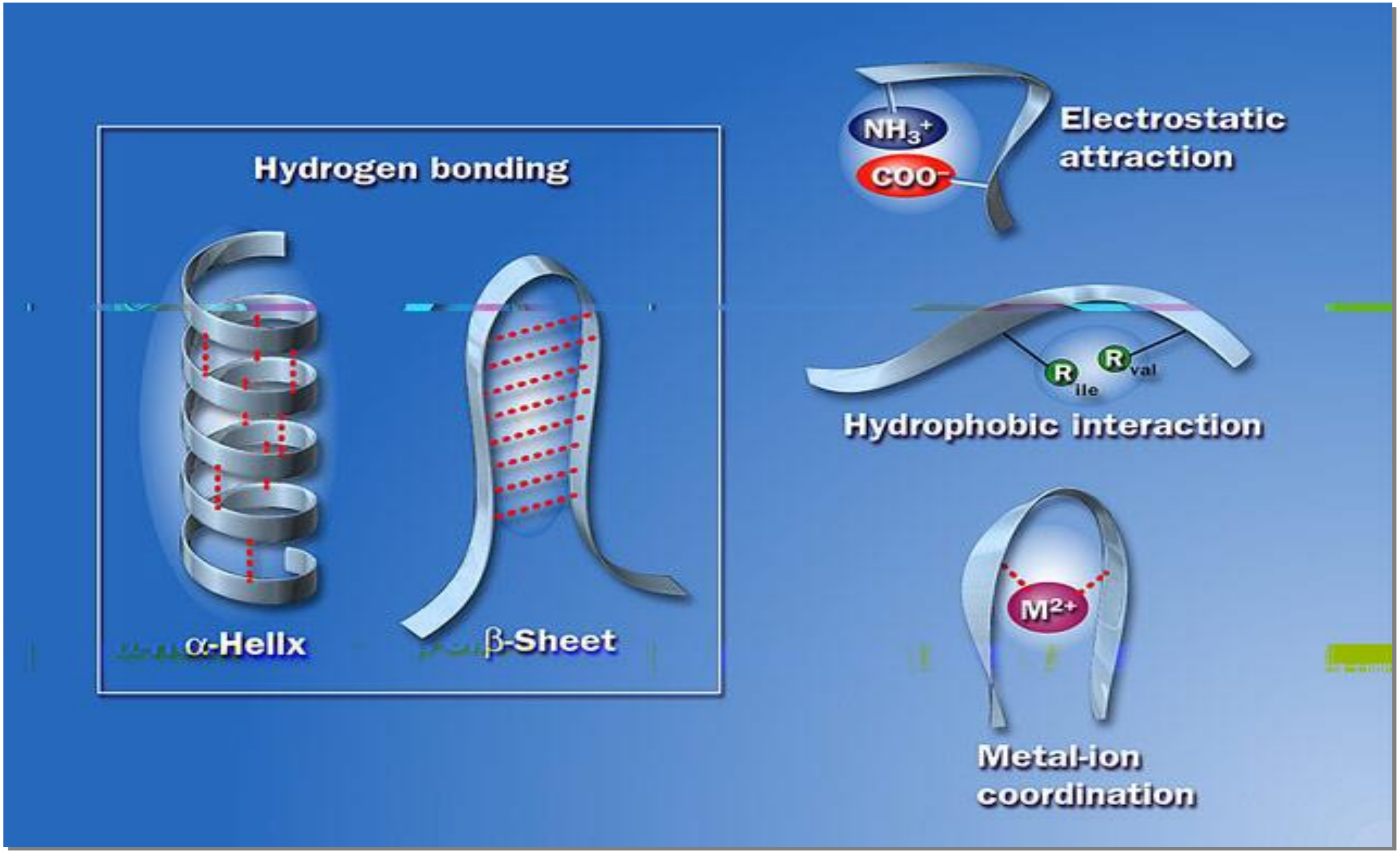
(b) Sickled red blood cells and the primary structure of sickle-cell hemoglobin

Secondary structure

- "Local folding"
  - folding along short sections of polypeptide
    - interaction between adjacent amino acids
    - H bonds between backbones (O:H)
    - □ α-helix
    - □ β-pleated sheet
    - Fibrous proteins – only have secondary structure
      - Keratin
      - Silk



Secondary (2°) structure



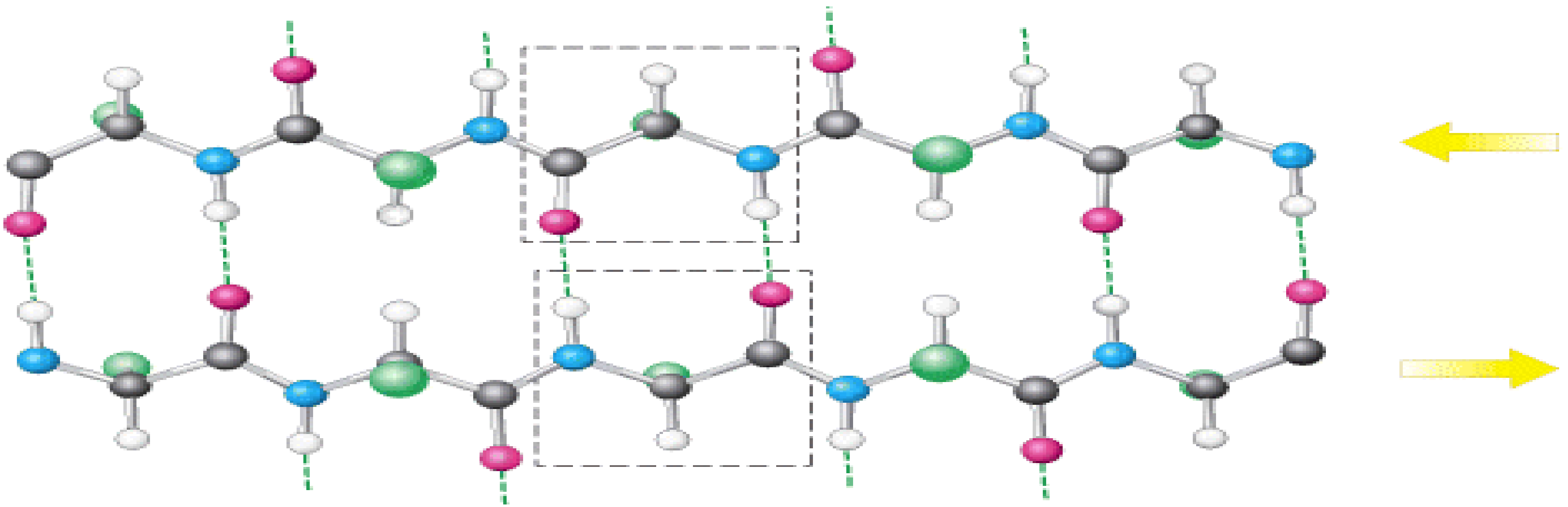
Alpha helix

- Rod-like structure with a tightly coiled backbone
- Stabilised by H-bonds between NH and CO group of the main chain
- CO group forms H bonds with NH group of a.a situated four residues ahead in the sequence
- Has a rise of 1.5A, also called translation
- There are 3.6 a.a. residues per turn of the helix
- Left or right handed helix, right handed helices are energetically more favorable due to fewer steric clashes btwn side chains & backbone
- All helices in proteins are right handed

Beta sheet

- A polypeptide chain, called a β- *strand, has* almost fully extended rather than being tightly coiled as in the α-helix. A range of extended structures are sterically allowed .
- The distance between adjacent amino acids along a β strand is approximately 3.5 Å, in contrast with α distance of 1.5 Å along an α helix.
- The side chains of adjacent amino acids point in opposite directions .

Parallel / Anti-parallel stands



Tertiary (3°) structure

- "Whole molecule folding"
  - **created when the secondary structure fold and form bonds to stabilize the structure into a unique shape**
  - determined by interactions between R groups
    - Hydrophobic interactions
    - anchored by disulfide bridges
    - Ionic Bonds between R groups
    - Hydrogen bonds between backbones
    - Van der Waals Force
      - Globular (spherical) proteins – have tertiary structure
      - enzymes

Tertiary structure

- Refers to the 3 D structure formed from the polypeptide chains.
- Tertiary structures of water-soluble proteins have common features

1. An interior formed by a.a with hydrophobic side chains
2. A surface formed largely by hydrophilic a.a that interact with the aqueous environment

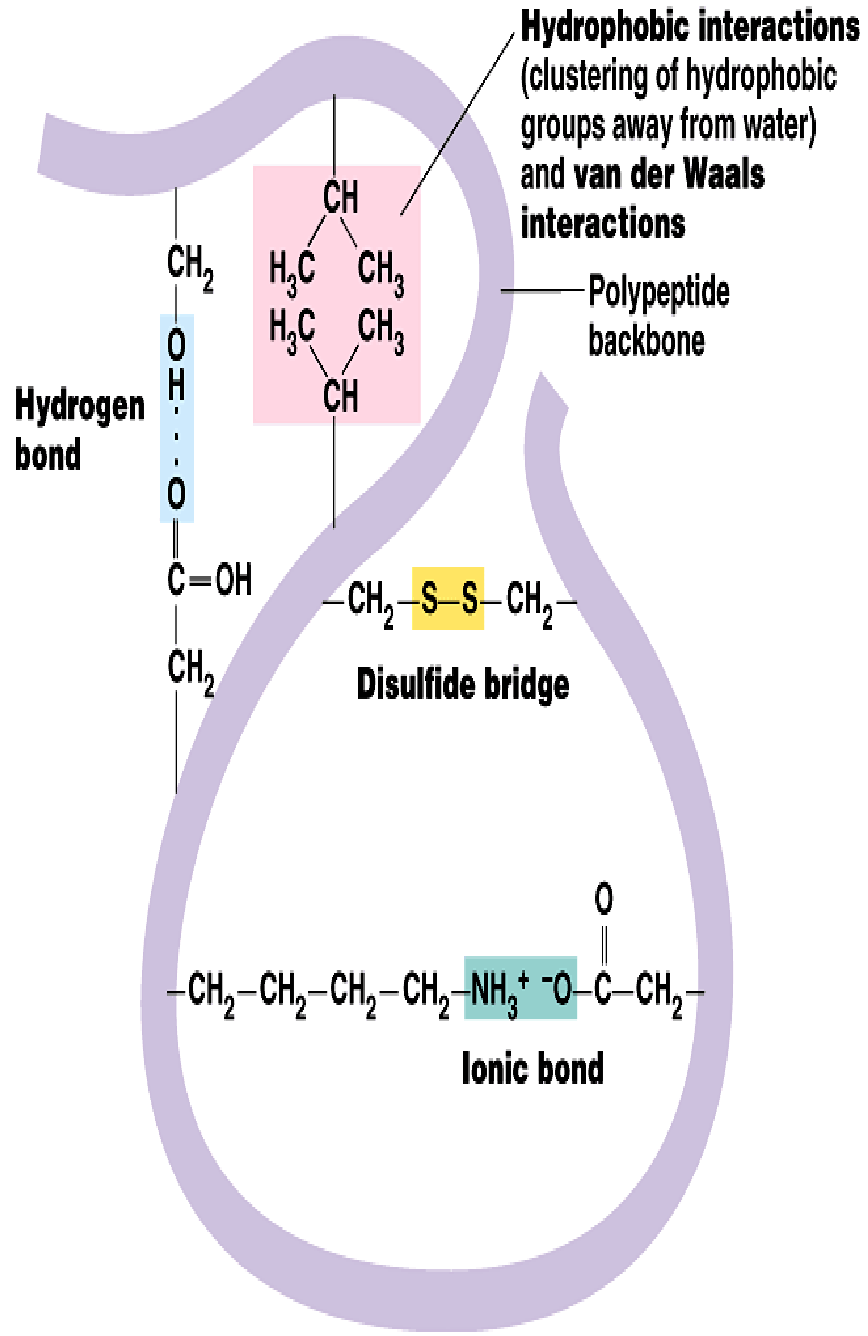
Such interactions are important for the formation of cell membranes

- Many a helices and b strands are **amphipathic**; that is, the a
- helix or b strand has a hydrophobic face, which points into the protein interior, and a more polar face, which points into solution.
- Each polypeptide is called a subunit

- It involves twisting and folding of the polypeptide chain caused by hydrophobic and hydrophilic interactions between the side chains of the amino acids.

- The nonpolar amino side chains end up in the interior of the protein away from the aqueous environment.

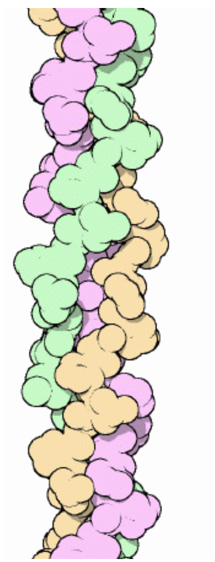
- The polar side chains appear on the surface of the protein since they are attracted to the aqueous surroundings.

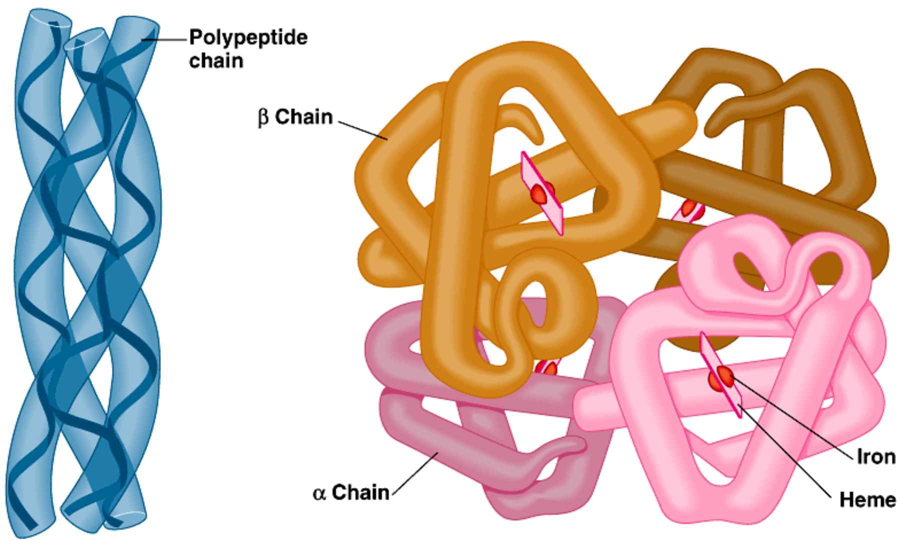


Quaternary structure

- The ***quaternary structure*** is the interaction of two or more polypeptide chains to form a biologically active protein.
- Subunits are held by noncovalent bonds
  - Hemoglobin – 4 polypeptides
  - Collagen – 3 polypeptides

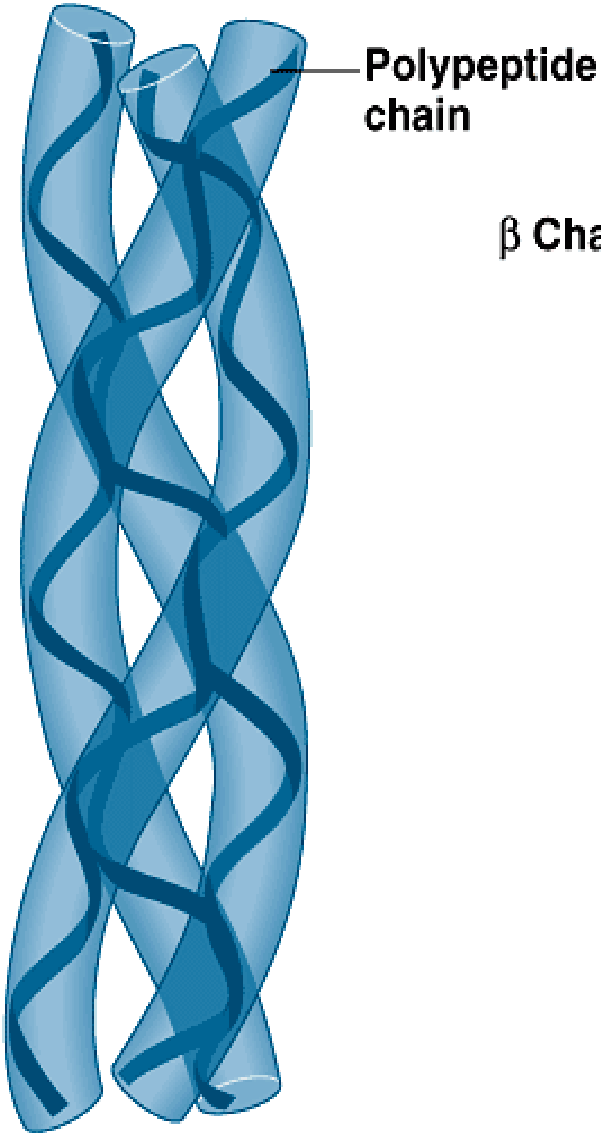
collagen = skin & tendons



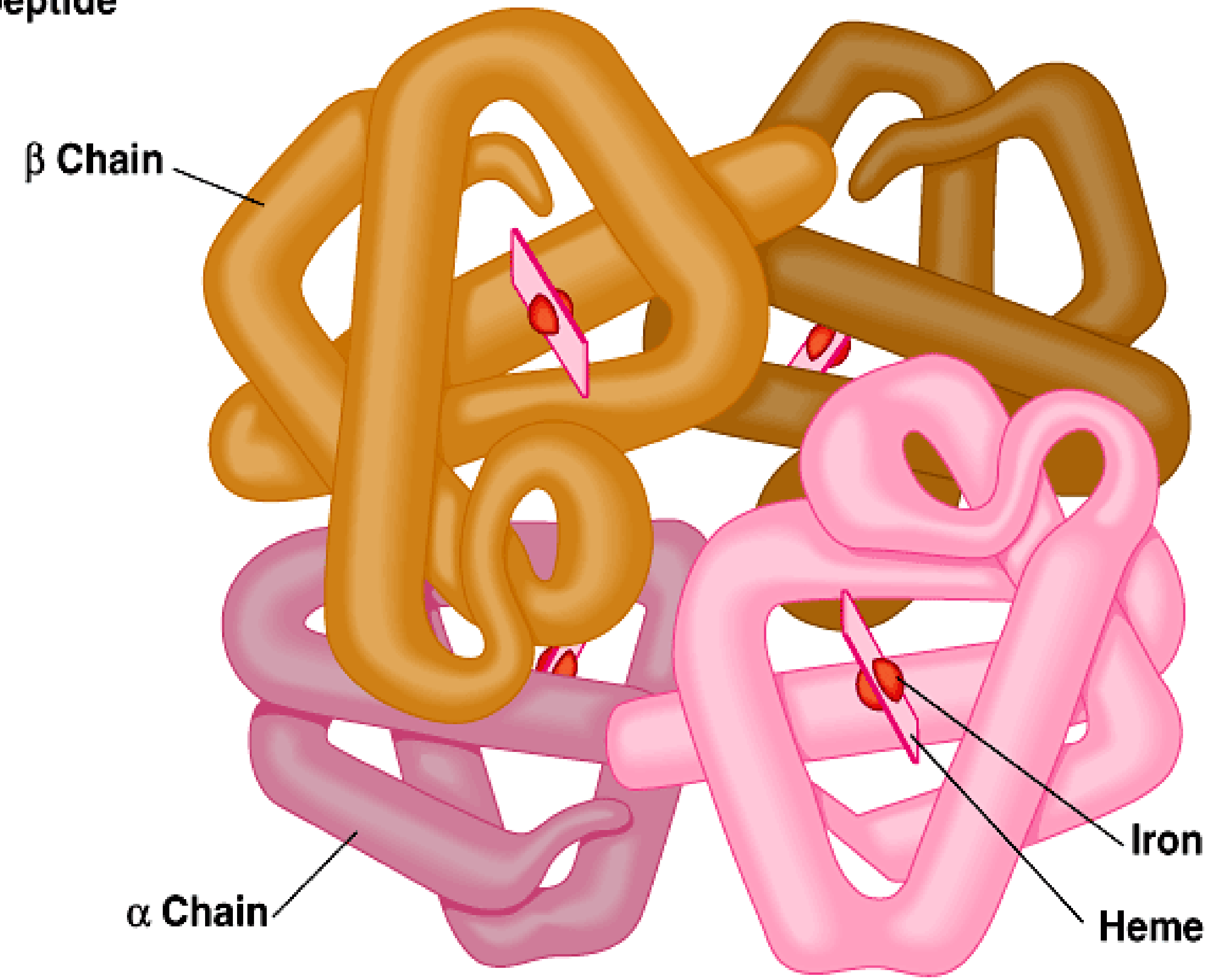


(a) Collagen (b) Hemoglobin

- Hemoglobin, an oxygen transport protein, is an example of a protein with a quaternary structure.
  It consists of four polypeptide chains or subunits.
  It has two identical alpha subunits and two identical beta subunits.
  All four subunits must be present for the protein to function as an oxygen carrier.

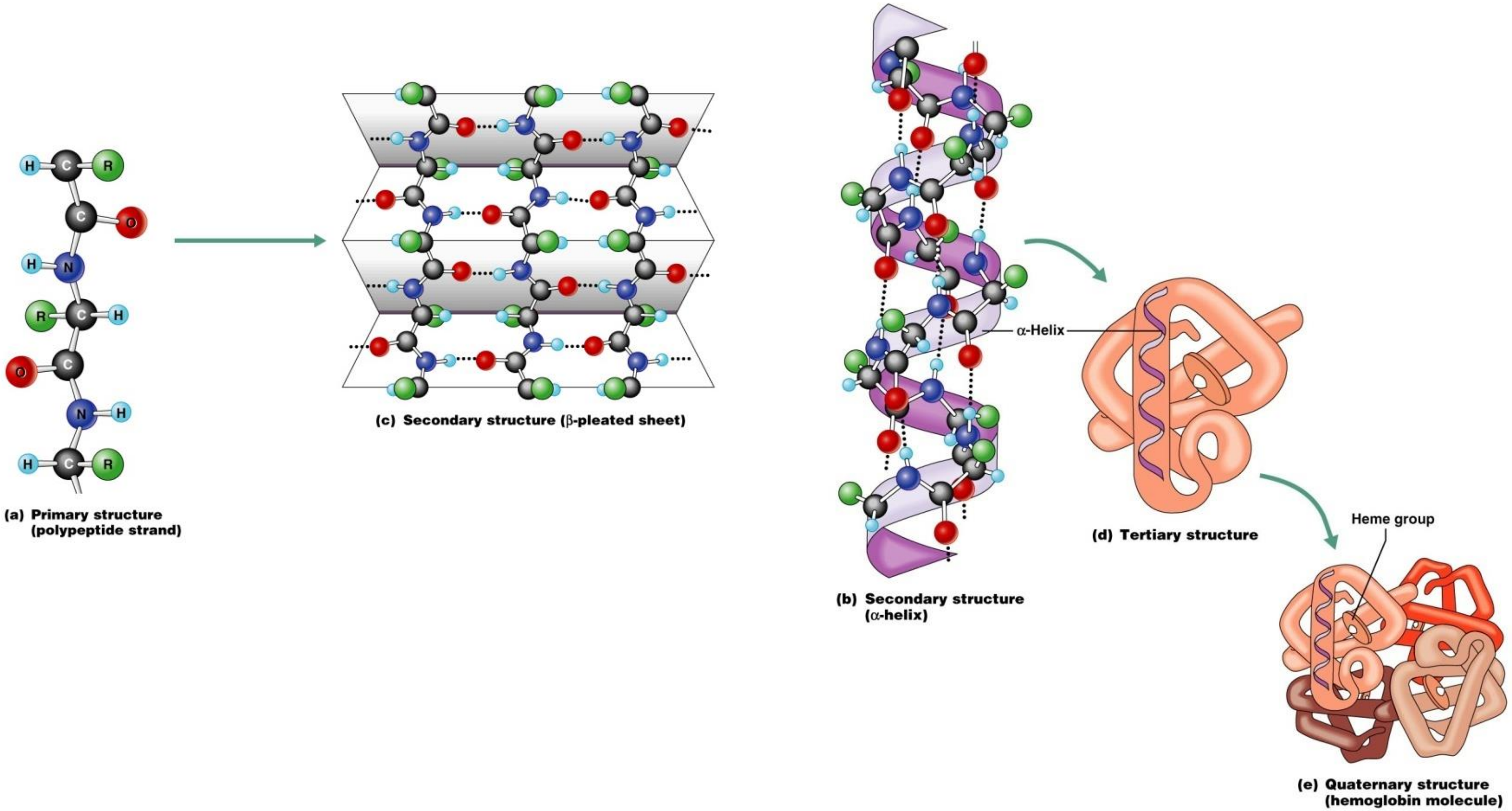


(a) Collagen



(b) Hemoglobin

Levels of Protein structure



Shape and Function

- Protein function is based on **shape**
- Shape is based on **sequence** of amino acids
- **Denaturation**:
  - loss of shape and function (due to heat, pH change or other factors)

Protein denaturation

- Is a process that disrupts secondary, tertiary, and quaternary structures.
- The primary structure is not destroyed during denaturation.
- Protein becomes insoluble and looses biological activity
- Denaturation can be caused by

-strong acids or bases

-concentrated inorganic salts

-an organic solvent (alcohol or chloroform)

-radiation or

heat

- The process of denaturation is used as an antidote for lead or mercury poisoning.
- Egg whites can be given to an individual who has ingested a heavy metal. Egg whites are denaturated by the heavy metals and a precipitate is formed.
- Vomiting is induced to eliminate the metal-protein precipitate.

END