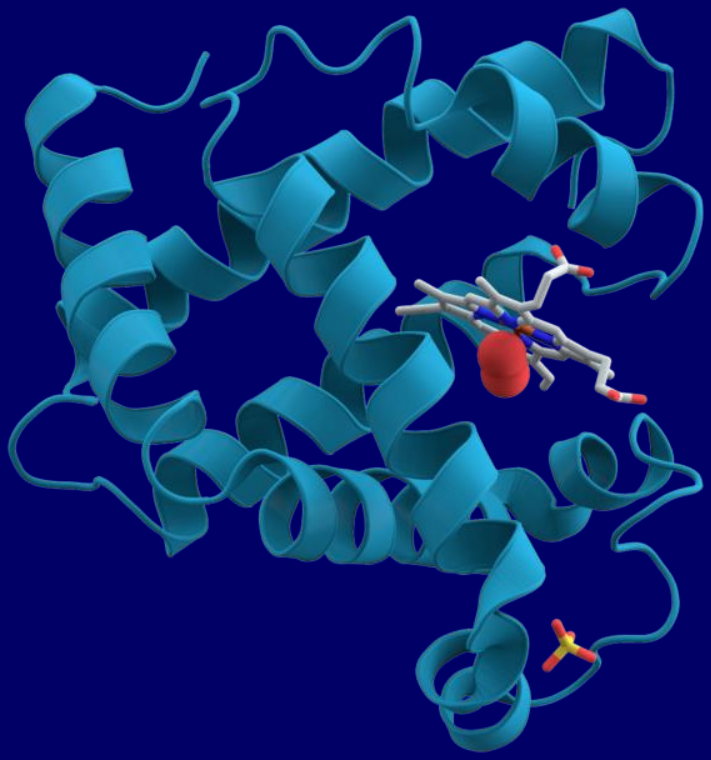
Amino Acids & Proteins

10th lect. of medical chemistry

Dr. Salih Mahdi Salman



Introduction

Proteins are biopolymers built from monomeric units called amino acids.

More than 700 amino acids occur naturally, but 20 of them are commonly found in proteins.

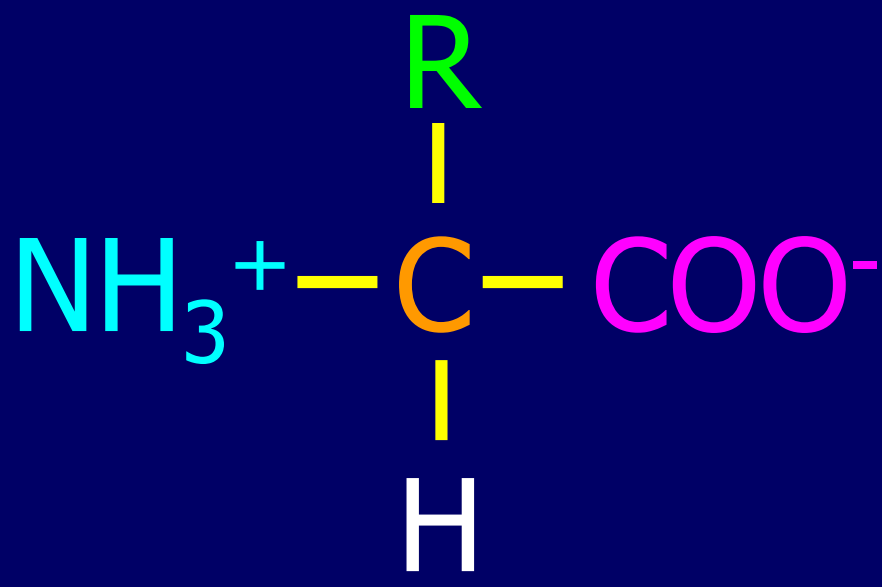
Protein function depends on both:-

1. amino acid content
2. amino acid sequence.

To understand protein function, we must first understand the nature of amino acids.

Amino Acids

Structure



Amino acids contain two functional groups, a protonated amine and carboxylic acid in the form of a carboxylate group.

These functional groups are bonded to a central carbon atom known as the **alpha (α) carbon**, and are referred to as **alpha amino acids**.

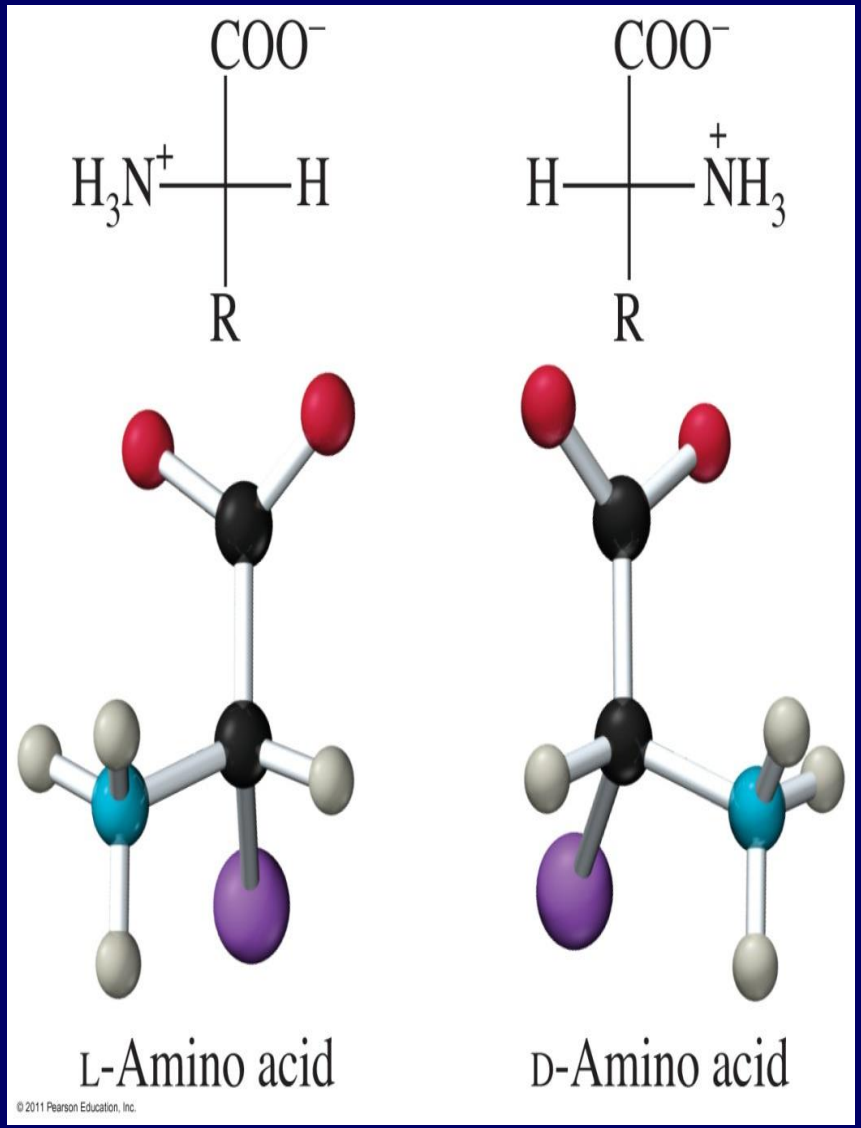
The α carbon is also bonded to a hydrogen atom and a larger side chain. The side chain is unique for each amino acid.

The α carbon on all amino acids, except glycine, is a ***chiral carbon*** because it has four different groups bonded to it.

An amino acid, with a chiral center, has two forms called ***enantiomers***, which are nonsuperimposable mirror images.

When drawing the Fischer projection, the carboxylate group is at the top of the structure and the side chain (R group) is at the bottom.

The protonated amine group can be on the left-hand side (L form) or right-hand side (D form) of the structure.



The L-amino acids are the building blocks for proteins. Some D-amino acids occur in nature, but not in proteins.

There are nine different families of organic compound represented in the structures of different amino acids. They are as follows:

1. Alkanes
2. Aromatics
3. Thioethers
4. Alcohols
5. Phenols
6. Thiols
7. Amides
8. Carboxylic acids
9. Amines

Classification of amino acids

The functional groups divide the amino acids into the following four categories:

Nonpolar, aliphatic (7)

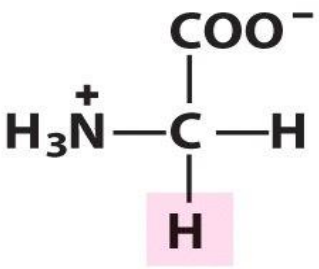
Aromatic (3)

Polar, uncharged (5)

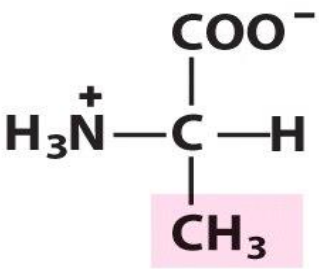
Positively charged (3)

Negatively charged (2)

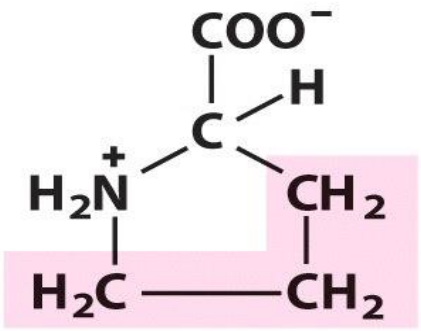
Nonpolar, aliphatic R groups



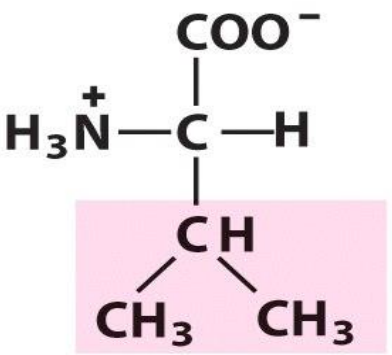
Glycine



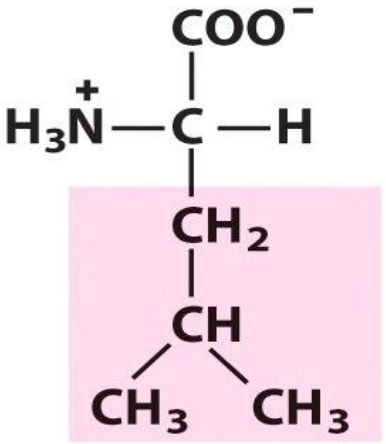
Alanine



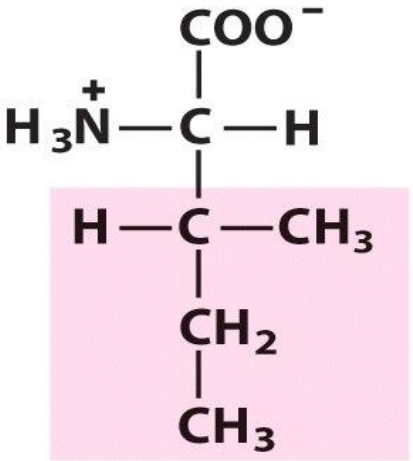
Proline



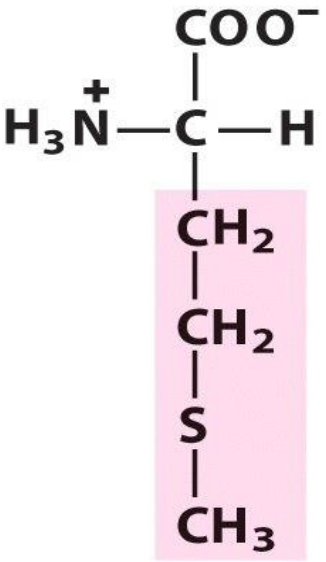
Valine



Leucine

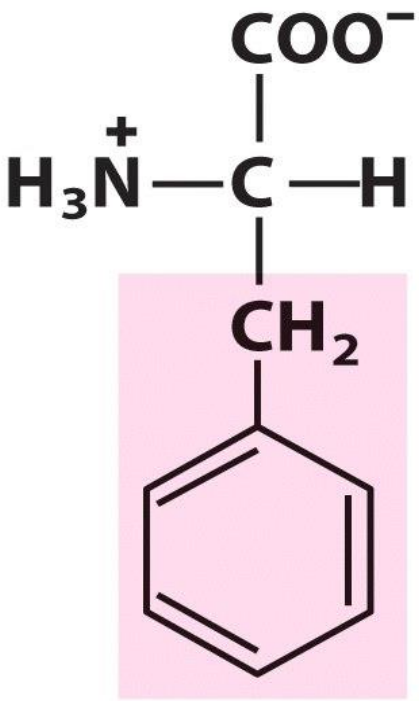


Isoleucine

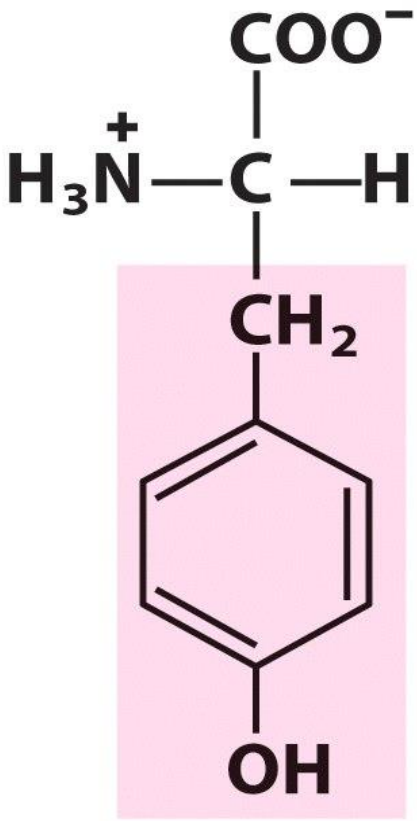


Methionine

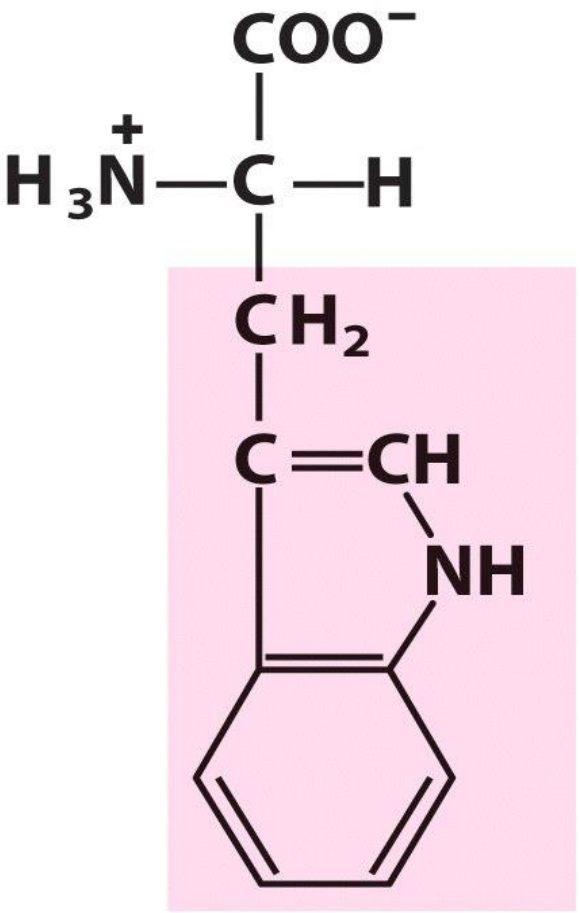
Aromatic R groups



Phenylalanine

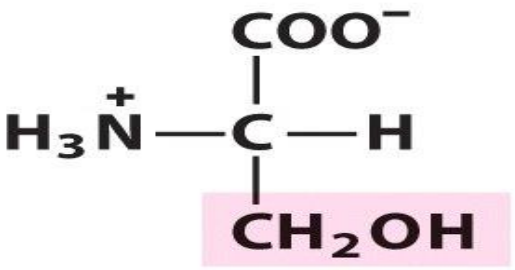


Tyrosine

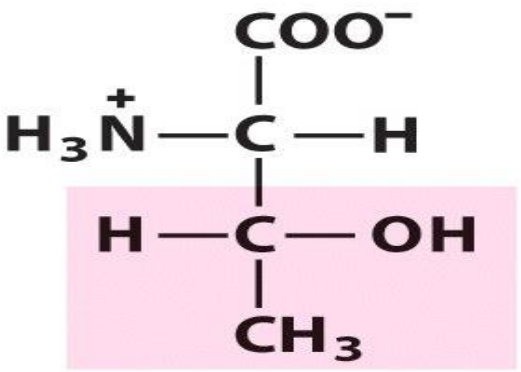


Tryptophan

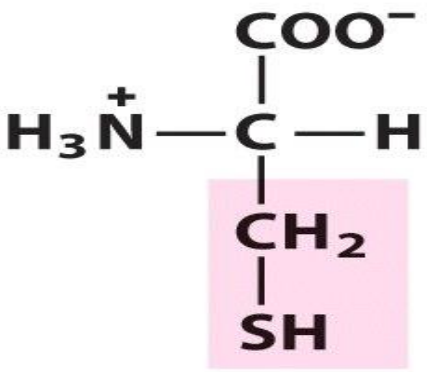
Polar, uncharged R groups



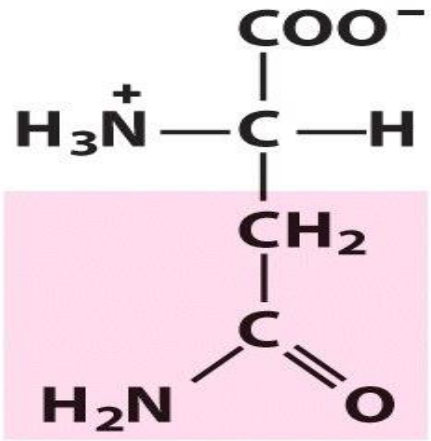
Serine



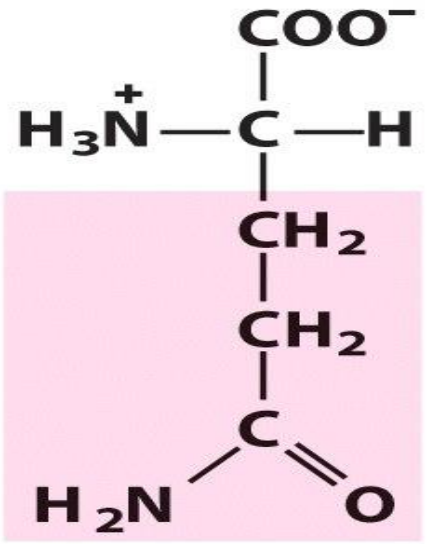
Threonine



Cysteine

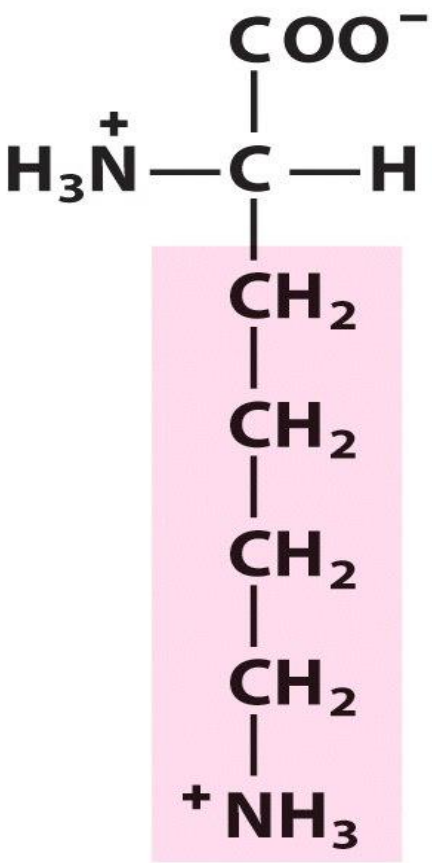


Asparagine

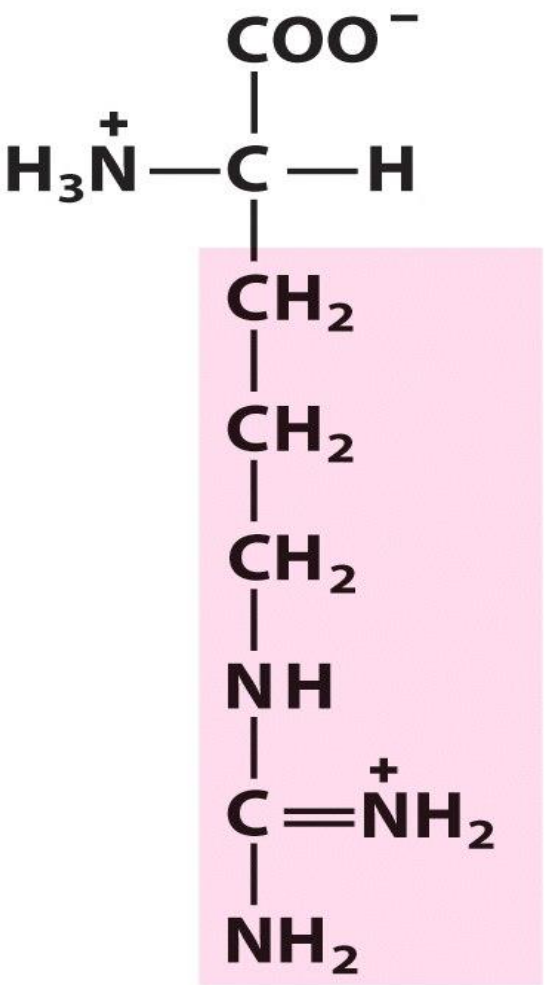


Glutamine

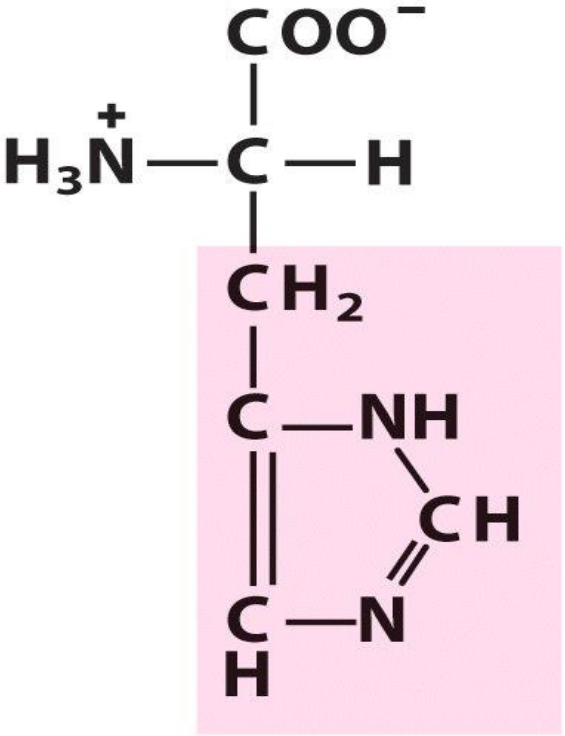
Positively charged R groups



Lysine

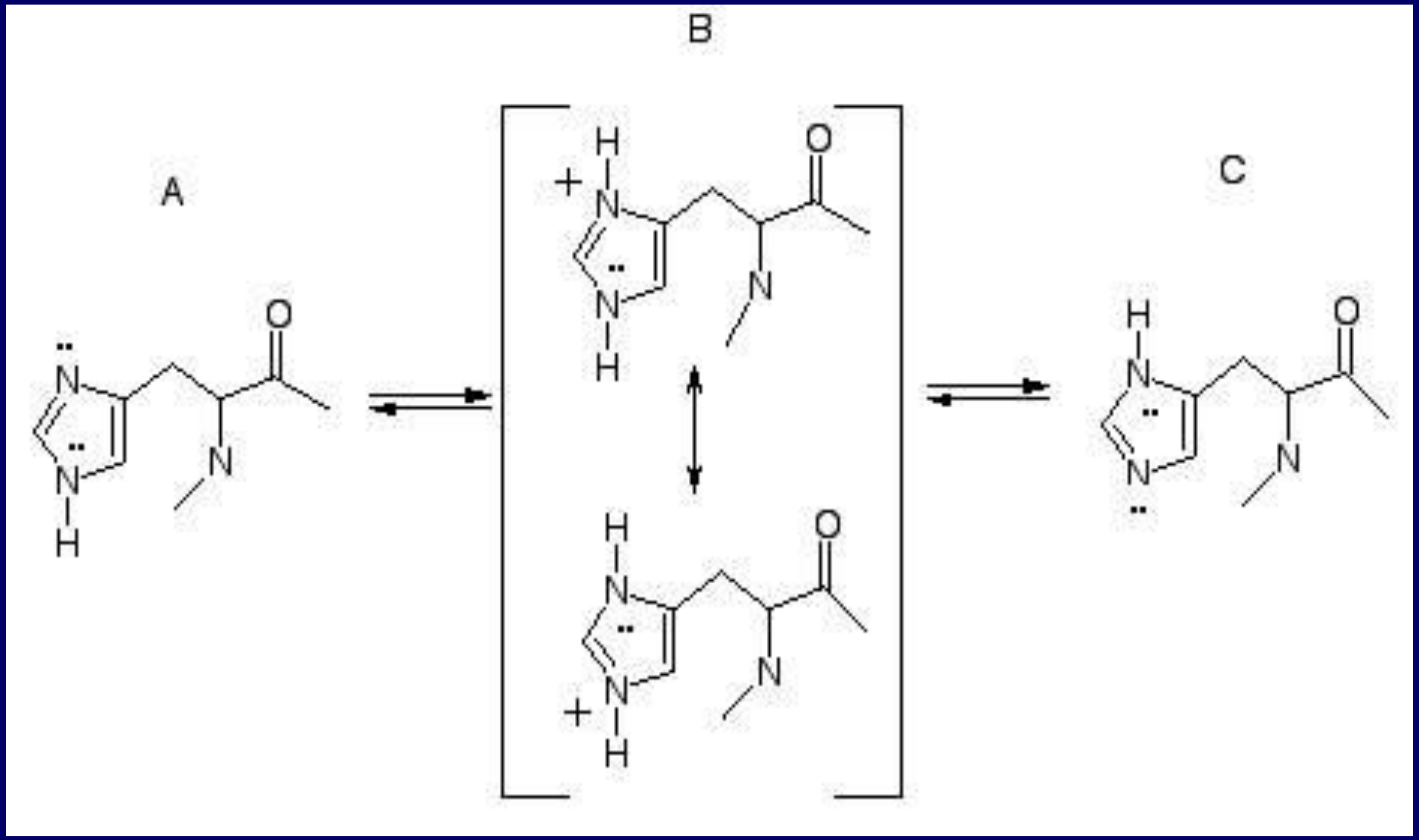


Arginine

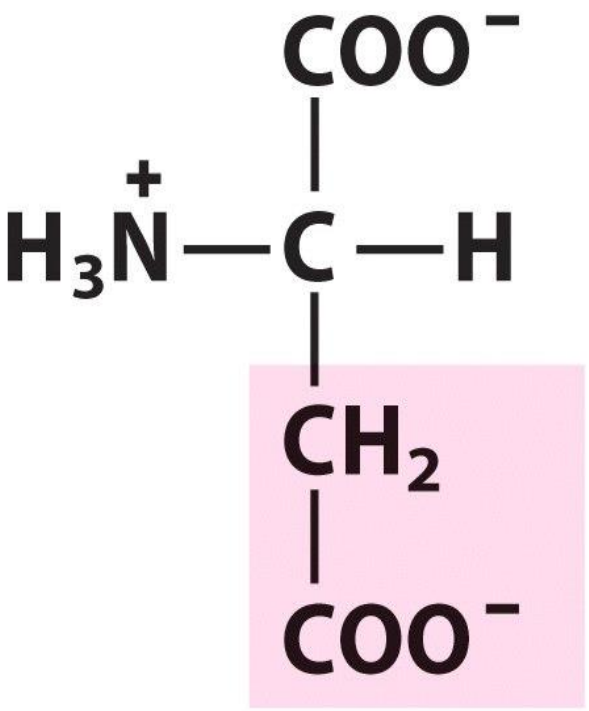


Histidine

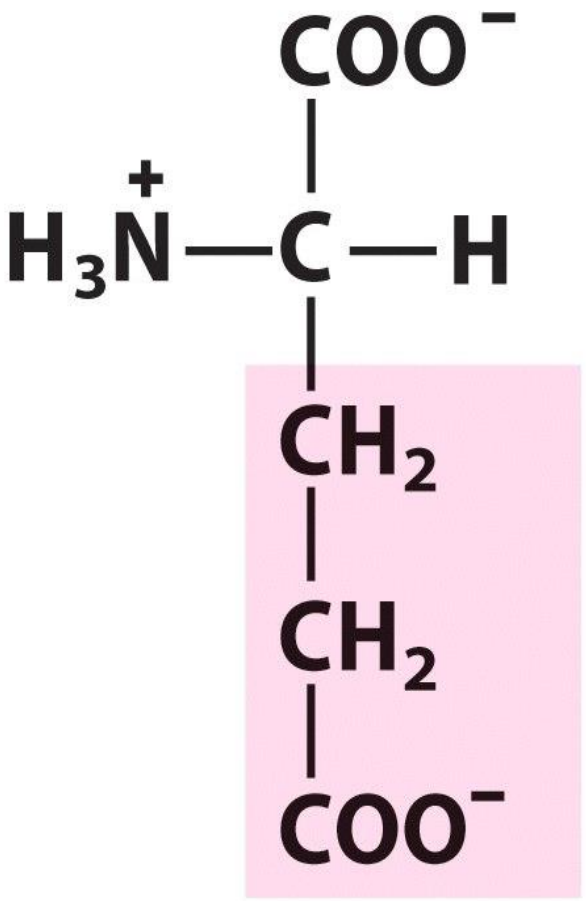
Ionic and Tautomeric States of the Histidine Side Chain



Negatively charged R groups



Aspartate



Glutamate

There are 10 amino acids that are essential amino acids because they cannot be synthesized in the human body and must be obtained in the diet.

The 10 essential amino acids are: valine, leucine, isoleucine, phenylalanine, methionine, tryptophan, threonine, histidine, lysine, and arginine.

Two of these amino acids, arginine and histidine, are essential in children, but not adults.

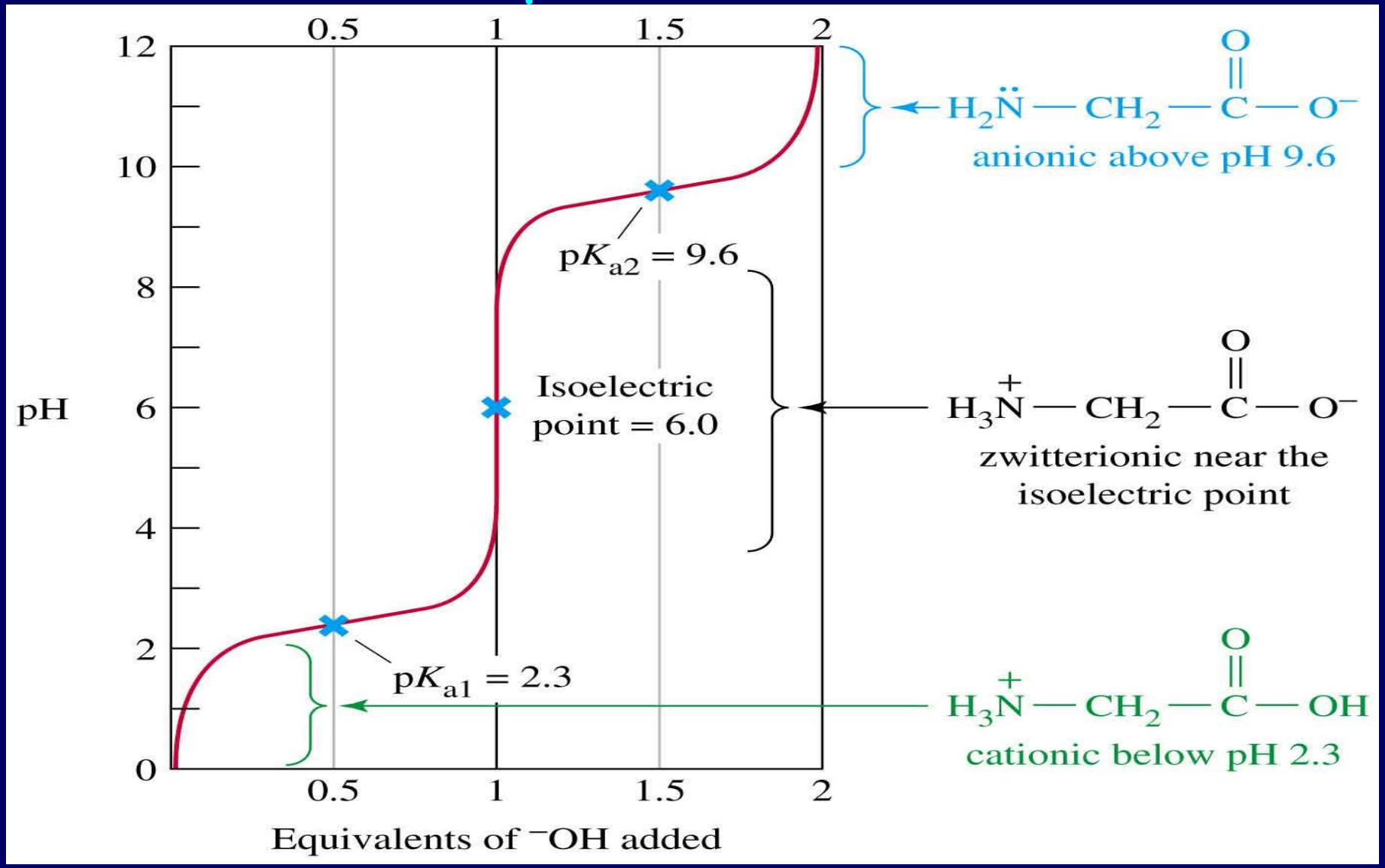
Nonessential amino acids can be synthesized in the body from essential amino acids.
Proteins that contain all the essential amino acids are called ***complete proteins***.

Soybeans and most proteins found in animal products are complete proteins.

Some plant proteins are incomplete proteins because they lack one or more essential amino acid.

Complete proteins can be obtained by combining foods like rice and beans.

Structure and pH

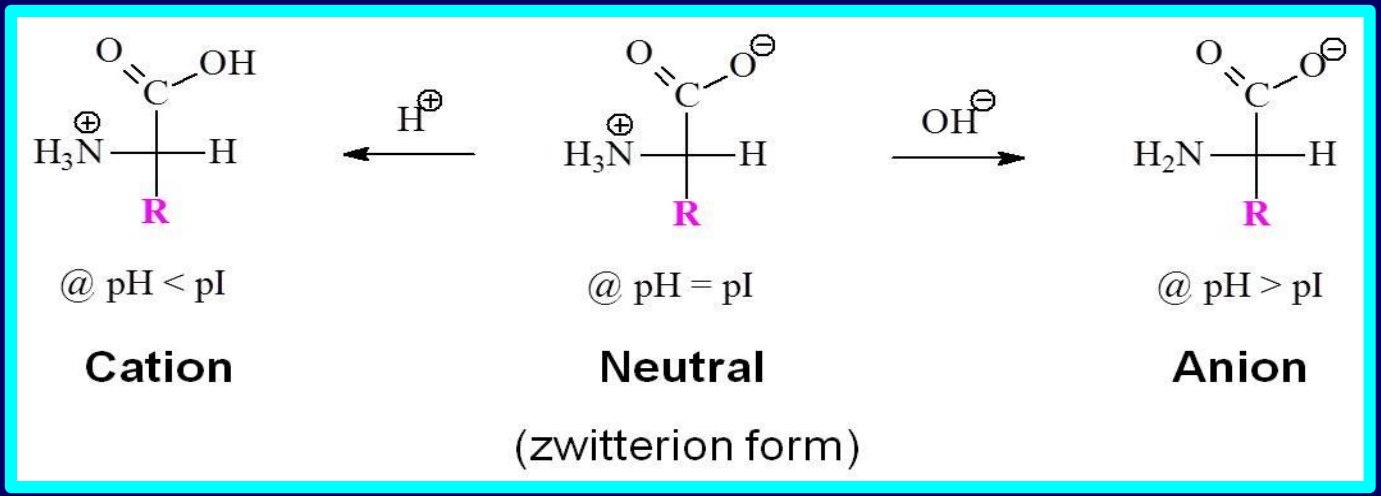


Isoelectric Point

Each amino acid has an **isoelectric point**, (**pI**) numerically equal to the pH at which the **zwitterion** concentration is at a maximum.

The amino acid has no NET charge at its pI; it has one positive and one negative charge.

At a pH less than the value of the isoelectric point, the amino acid is protonated and has a POSITIVE charge; at a pH greater than the pI the amino acid is deprotonated and has a NEGATIVE charge



Peptides and Peptide bonds

The amino group of one molecule condenses with the acid group of another to form peptide bond.

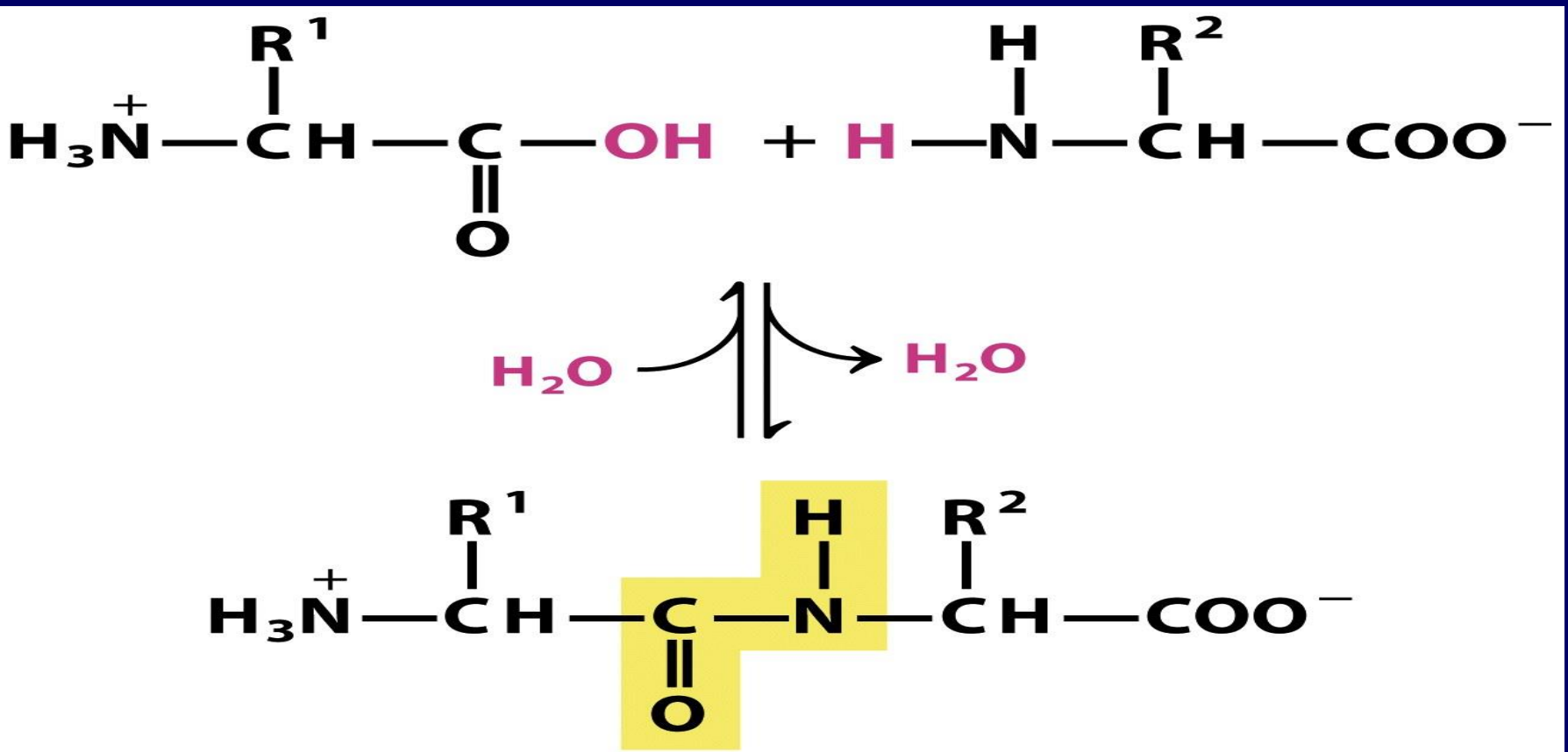


Figure 3-13
Lehninger Principles of Biochemistry, Fifth Edition

Peptides are compounds in which an amide bond links the amino group of one a-amino acid and the carboxyl group of another.

An amide bond of this type is often referred to as a peptide bond

Polymerization of amino acids – form peptides and proteins

A condensation reaction between the carboxyl of one amino acid and the amino group of another forms a Dipeptides (dimerization reaction) .

Peptides are di, tri, tetra and oligopeptides.

Oligopeptides – condensation of 2 – 10 AA units.

Polypeptides – condensation of 11 – 100 AA units.

Proteins – more than 100 AA units

Protein Formation

The product formed during the condensation of alanine and valine is known as a dipeptide, which is represented as Ala—Val or AV.

In this dipeptide, alanine is called the *N-terminus* because it has an unreacted α-amino group.

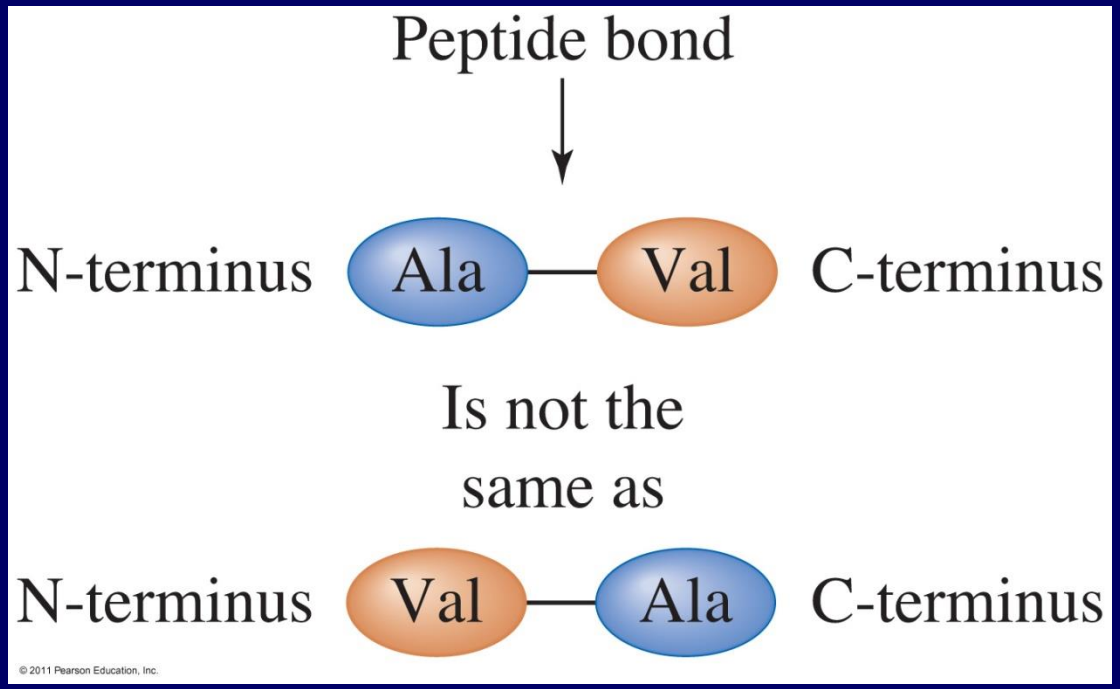
Valine is called the *C-terminus* because it has an unreacted α-carboxylate group.

Structures are always written from N-terminus to C-terminus.

Two amino acids can combine in two ways forming two different dipeptides.

The two dipeptides formed from condensation of Ala and Val are Ala—Val and Val—Ala.

They are structural isomers, different compounds, and have different properties.


Peptide bond
N-terminus
Ala
Val
C-terminus
Is not the
same as
N-terminus
Val
Ala
C-terminus
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The Three-Dimensional Structure of Proteins

Peptides and proteins have three-dimensional shapes or structures.

Proteins have four levels of structure:

1. Primary (1°)
2. Secondary (2°)
3. Tertiary (3°)
4. Quaternary (4°)

Each level of protein structure is a result of interactions between the amino acids of the protein.

1. Primary structure

The amino acid sequence (also called primary structure) of a protein is the order of the amino acids in the protein chain.

The sequence is always read from the N-terminus to the C-terminus of the protein.

For example:

$^{+}H_3N$-Lys-Val-Phe-Ala-Met-Cys-Leu-Leu-Arg-Val-COO^-

Or (in one-lettercode): KLVFAMCLLRV

2. Secondary Structure

The ***secondary structure*** of a protein describes repeating patterns of structure within the three-dimensional structure of a protein.

The two most common secondary structures are:

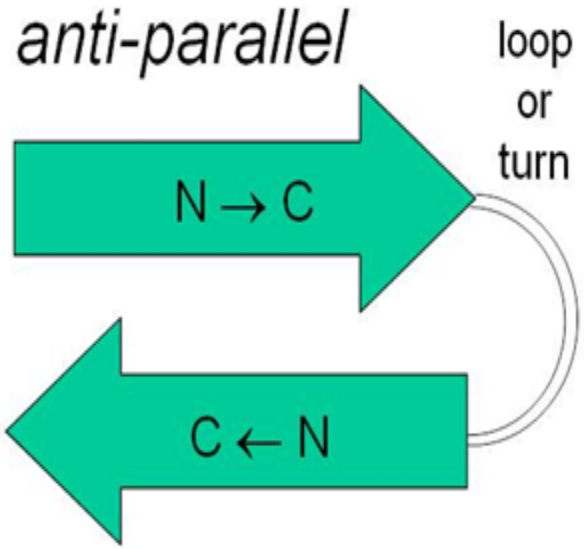
1. Alpha helix (α helix)
2. Beta-pleated sheet (β-pleated sheet)

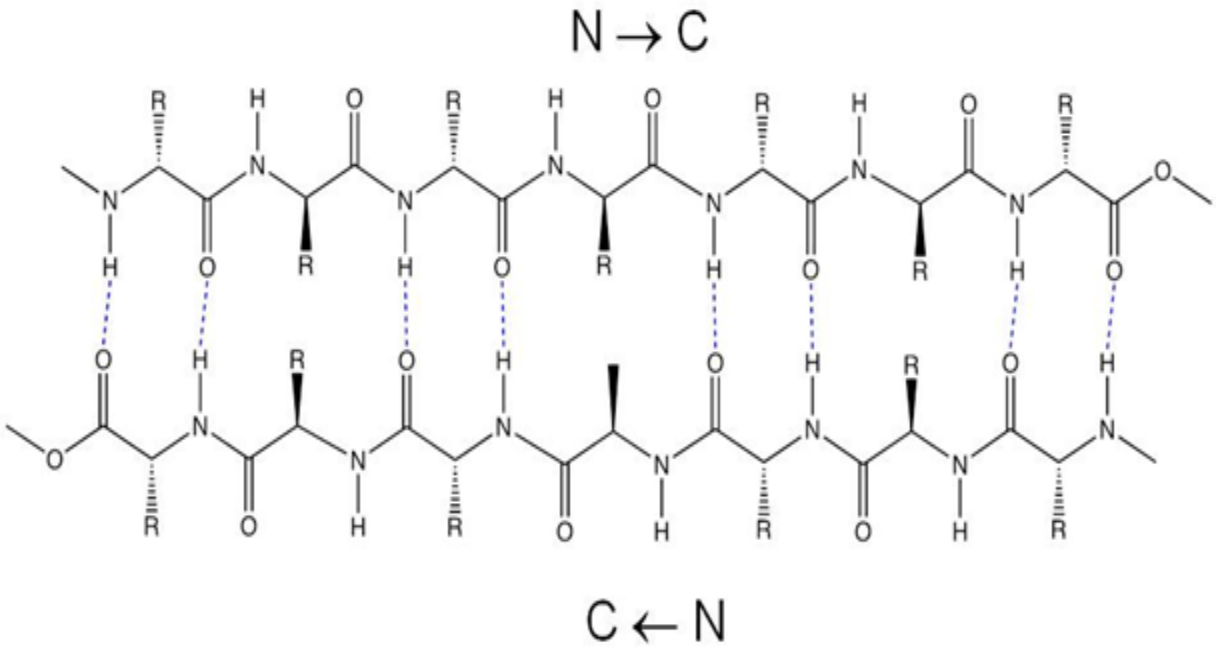
The α helix is a coiled structure, and much like the coil of a telephone cord, it is a right-handed coil.

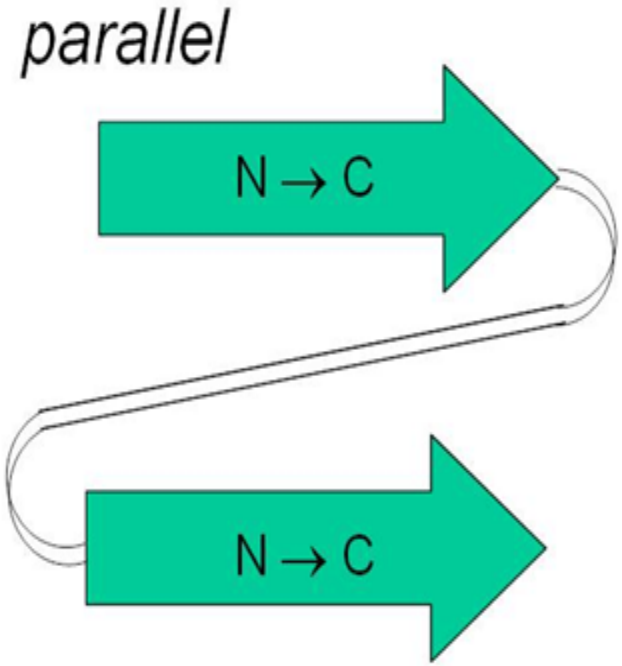
This coil is stabilized by hydrogen bonds between the carbonyl oxygen of one amino acid and the N—H hydrogen atom of another amino acid located four amino acids from it in the primary structure.

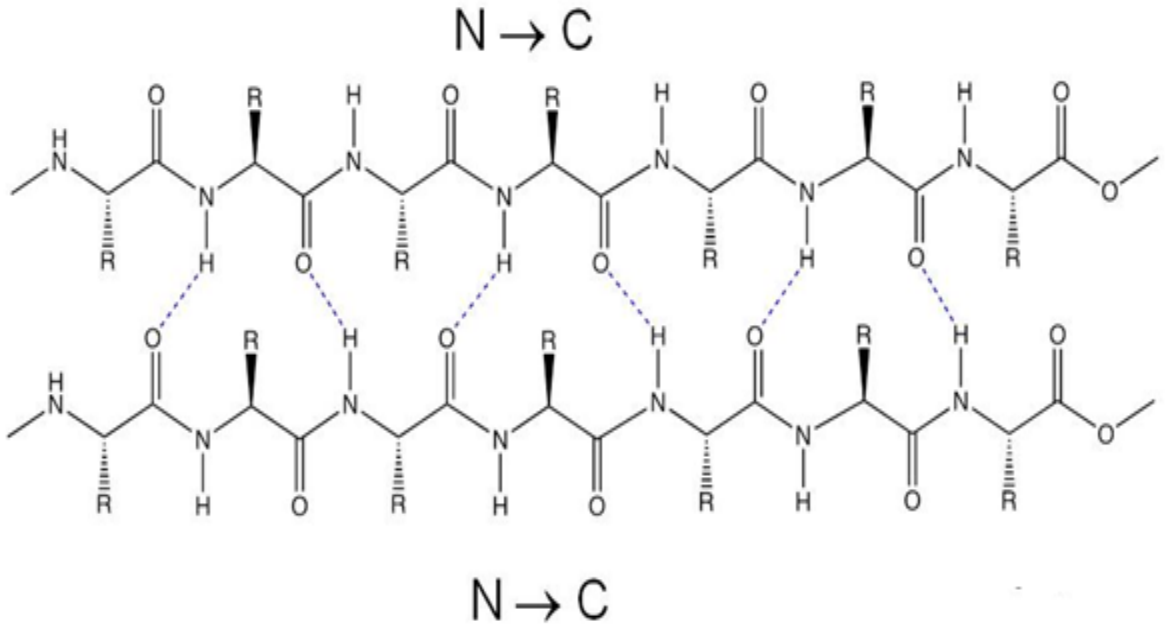
The coil is able to stretch and recoil, and is a strong structure. The side chains project outward from the axis of the helix.

A secondary structure involves hydrogen bonding along the backbone.


anti-parallel
loop
or
turn
N → C
C ← N


N → C
C ← N


parallel
N → C
N → C

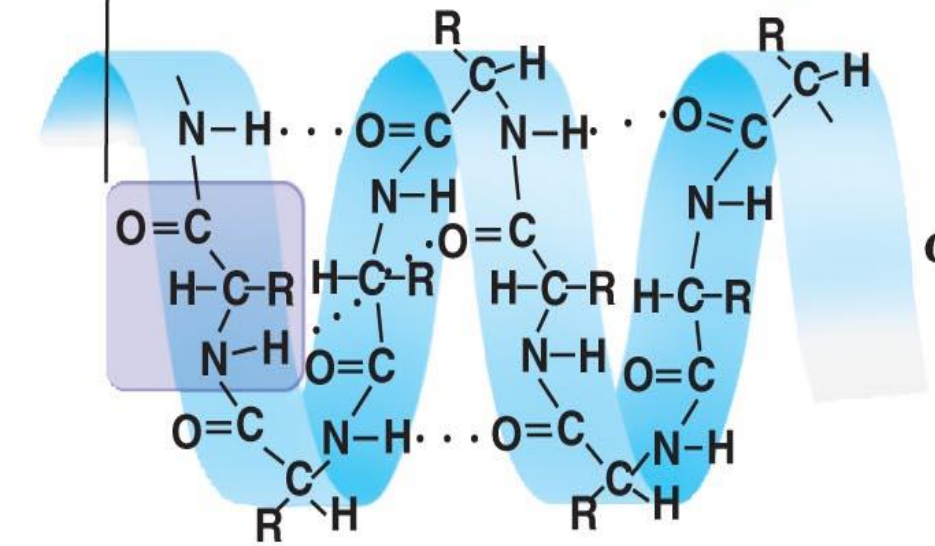

N → C
N → C

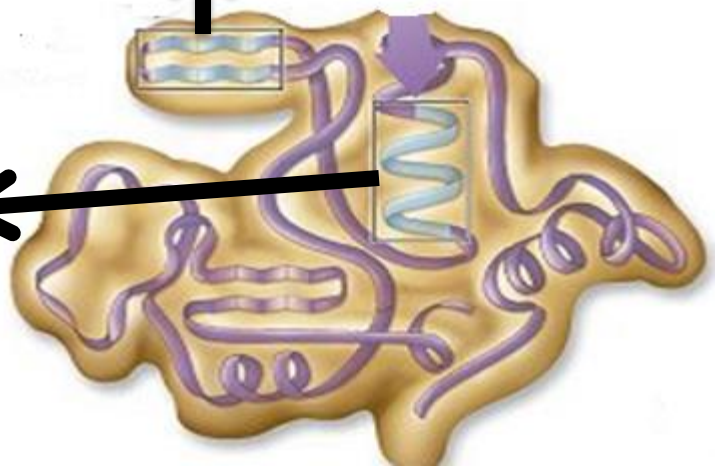
Secondary Structure

β pleated sheet

Examples of amino acid subunits

α helix





3. Tertiary Structure

The *tertiary structure* is the three-dimensional structure of the protein.

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.

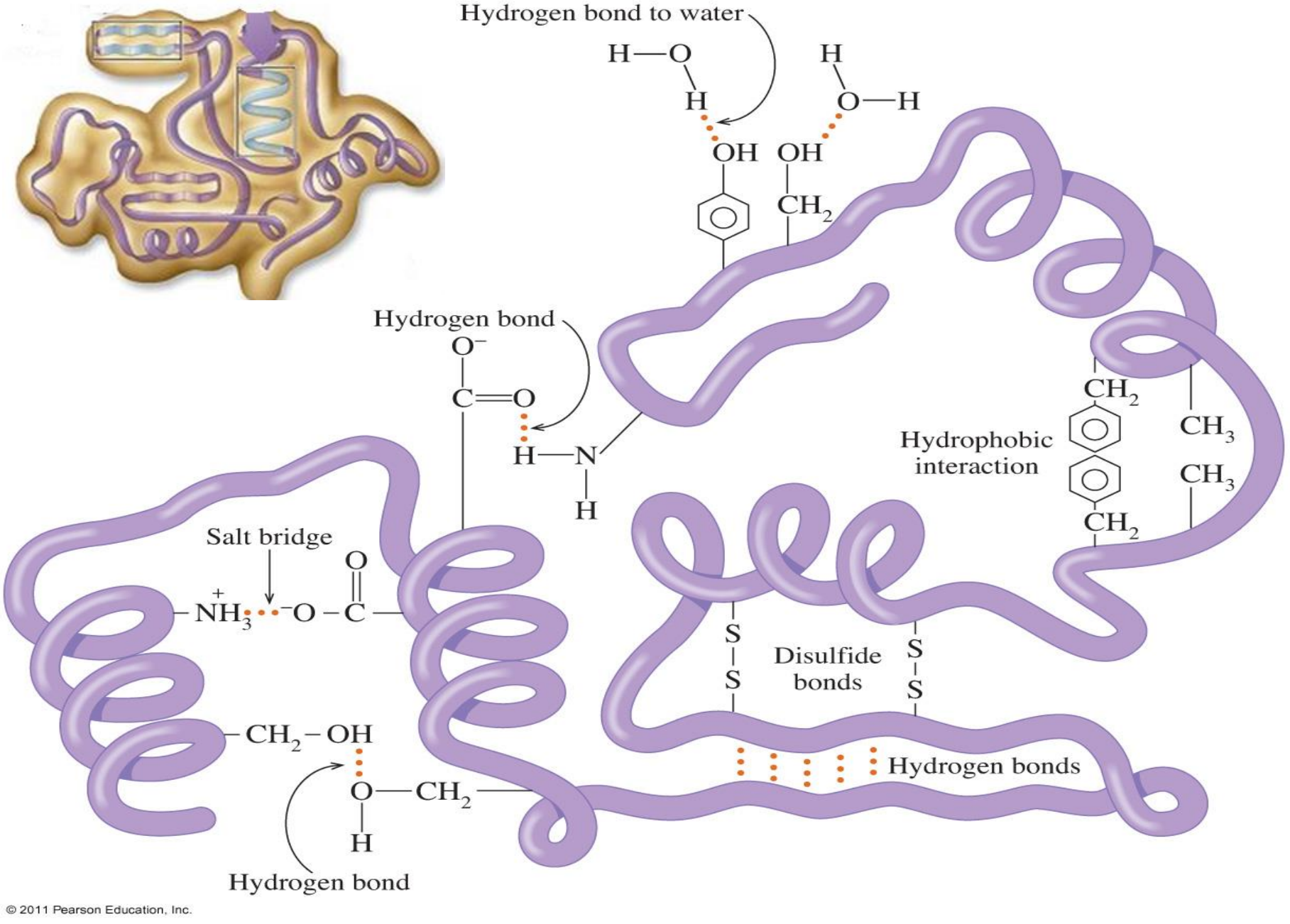
Stabilization of the tertiary structure is by:

1. Attractive forces between the side chains and aqueous environment
2. Attractive forces between side chains themselves

These attractive forces cause the protein to fold into a specific three-dimensional shape.

Interactions in the tertiary structure involves:

1. Nonpolar or hydrophobic interactions
2. Polar or hydrophilic interactions
3. Salt bridges (ionic interactionsz)
4. *Disulfide bonds*, which are covalent bonds formed between –SH groups of two cysteine molecules.


Hydrogen bond to water
Hydrogen bond
Hydrophobic interaction
Salt bridge
Disulfide bonds
Hydrogen bonds
Hydrogen bond
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4. Quaternary Structure

The *quaternary structure* is two or more polypeptide chains interacting to form a biologically active protein.

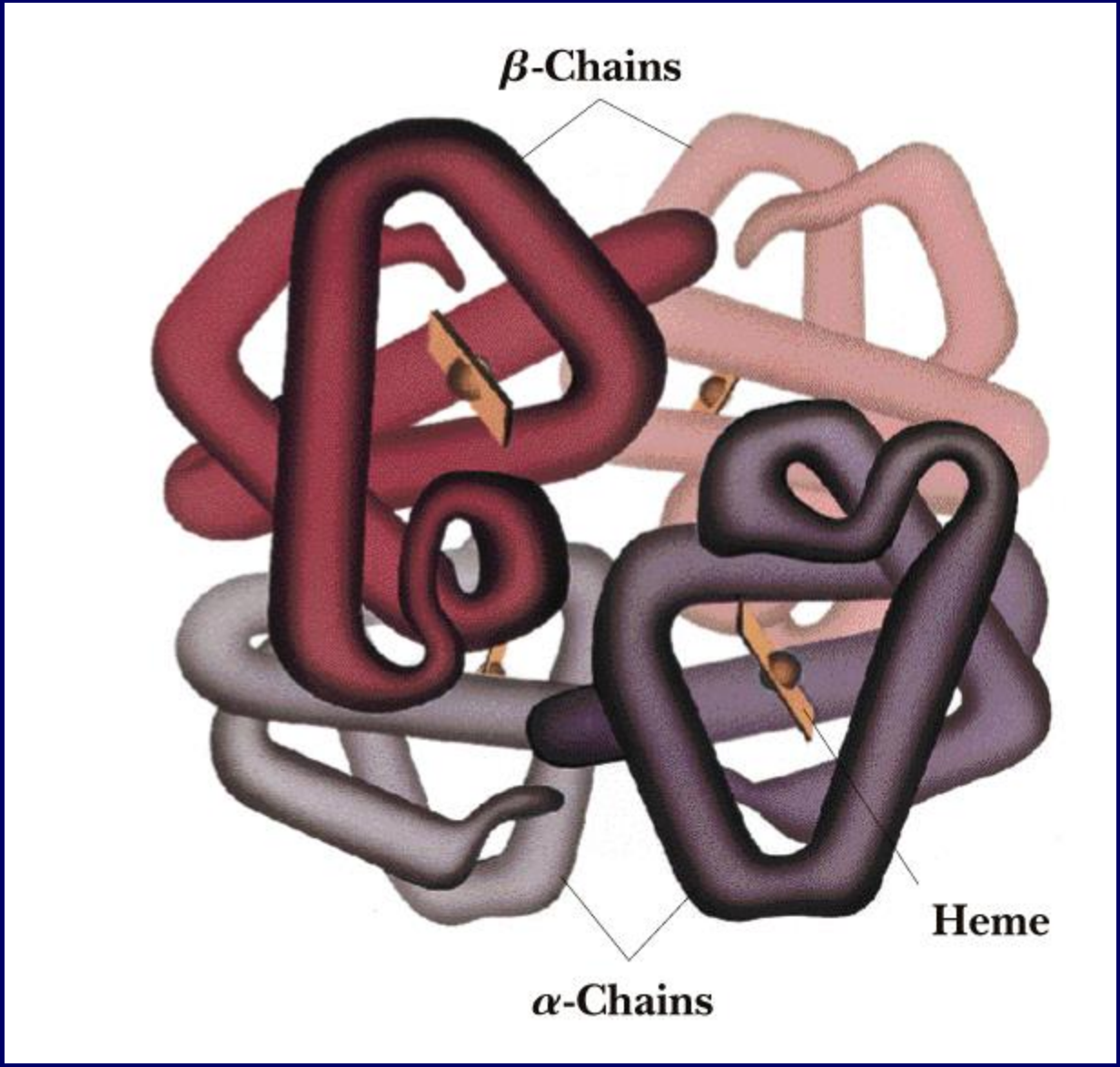
Hemoglobin, an oxygen transport protein, is an example of a protein with a quaternary structure.

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.

Not all proteins have a quaternary structure.


β-Chains
Heme
α-Chains

SUMMARY OF LEVELS OF STRUCTURE AND STABILIZING FORCES IN PROTEINS

Level of Structure	Forces Stabilizing Structure
Primary (1°)	Peptide bonds
Secondary (2°)	Hydrogen bonding along the protein backbone between amino acids close together in sequence
Tertiary (3°)	London forces, hydrogen bonding, dipole–dipole and ion–dipole interactions, salt bridges, and disulfide bonds between amino acids far away from each other in sequence.
Quaternary (4°)	Same as tertiary structure but between subunits

Determining the Sequence

An Eight Step Strategy.

1. If more than one polypeptide chain, separate (8 M urea ,6 M HCl , ammonium sulfate)
2. Cleave (reduce) any disulfide bridges (Performic acid CH_2O_3 oxidation, sulfhydryl reducing agents)
3. Determine amino acid composition of each chain (Complete hydrolysis in 6 N HCl , followed by quantitative analysis e.g HPLC , GC-MS.
4. Determine N- and C-terminal residues.

N-terminal analysis:

- Dansyl chloride method
- Edman's reagent (phenylisothiocyanate)
- Leucine aminopeptidase ,
- Sanger's Reagent

C-terminal analysis

Enzymatic analysis (carboxypeptidase) is common

1. Carboxypeptidase A cleaves any residue except Pro, Arg, and Lys
2. Carboxypeptidase B only works on Arg and Lys

5. Cleave each chain into smaller fragments and determine the sequence of each chain

Enzymatic fragmentation using :- trypsin, chymotrypsin, staphylococcal protease

Chemical fragmentation using cyanogen bromide (CNBr acts only on methionine residues)

6. Repeat step 5, using a different cleavage procedure to generate a different set of fragments

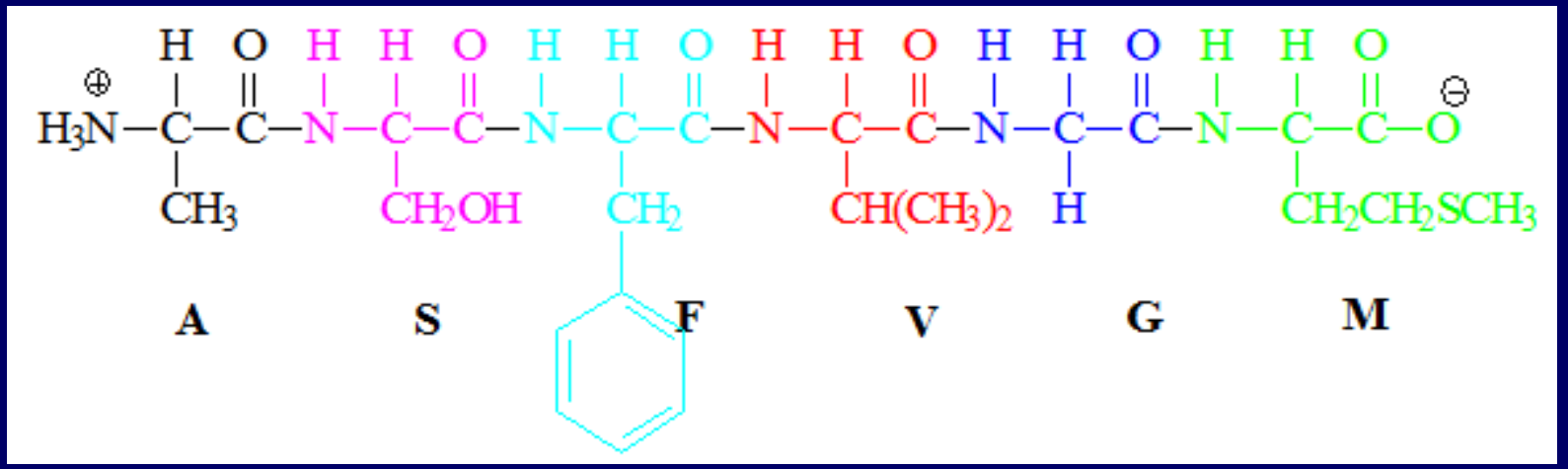
The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

Treatment*	Cleavage points†
Trypsin	Lys, Arg (C)
Submaxillarus protease	Arg (C)
Chymotrypsin	Phe, Trp, Tyr (C)
Staphylococcus aureus V8 protease	Asp, Glu (C)
Asp-*N*-protease	Asp, Glu (N)
Pepsin	Phe, Trp, Tyr (N)
Endoproteinase Lys C	Lys (C)
Cyanogen bromide	Met (C)

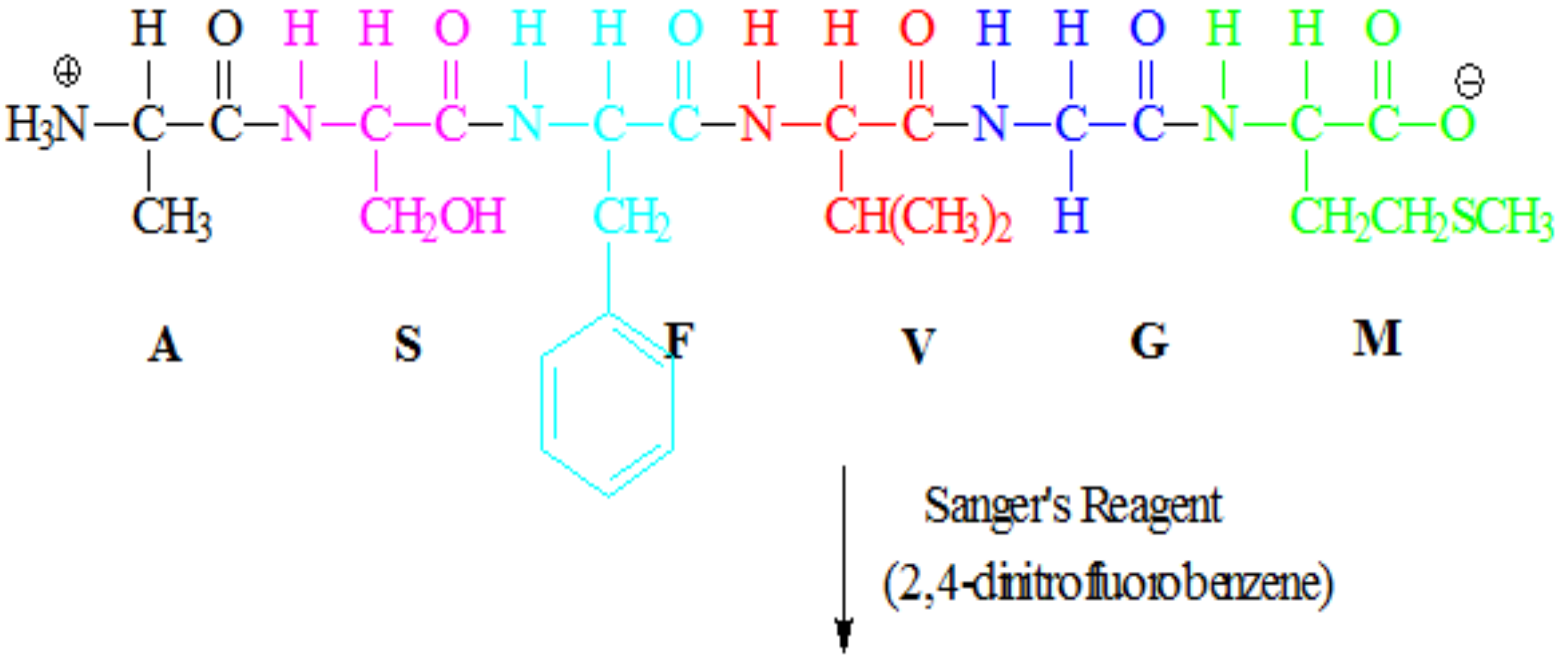
peptide bond cleavage occurs on either the carbonyl (C) or the amino (N) side of the indicated amino acid residues.

7. Reconstruct the sequence of the protein from the sequences of overlapping fragments
8. Determine the positions of the *disulfide cross-links.*

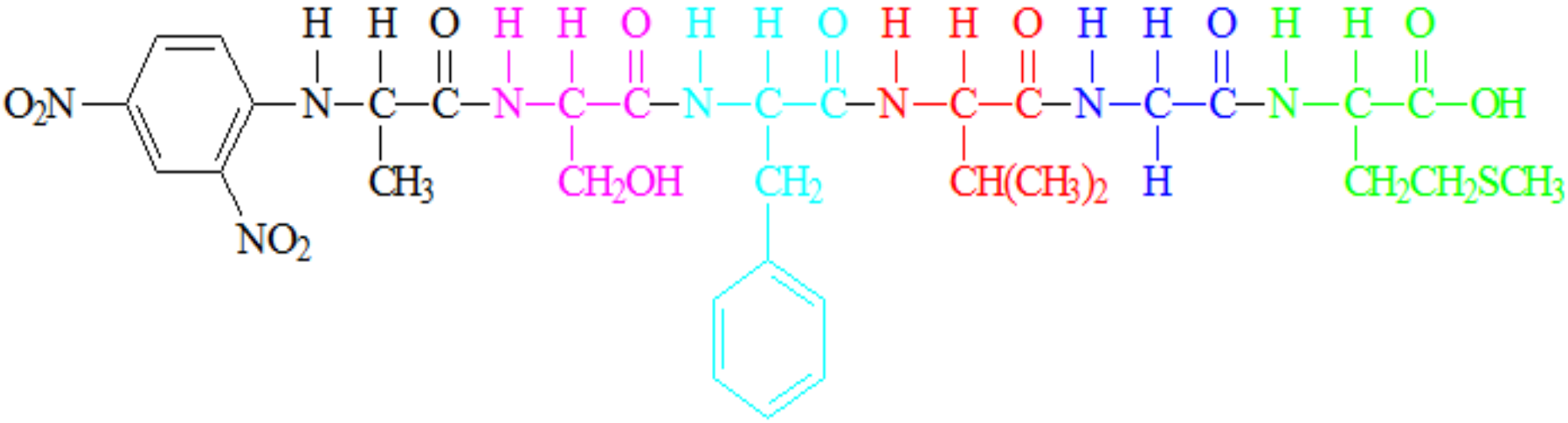
Ex.

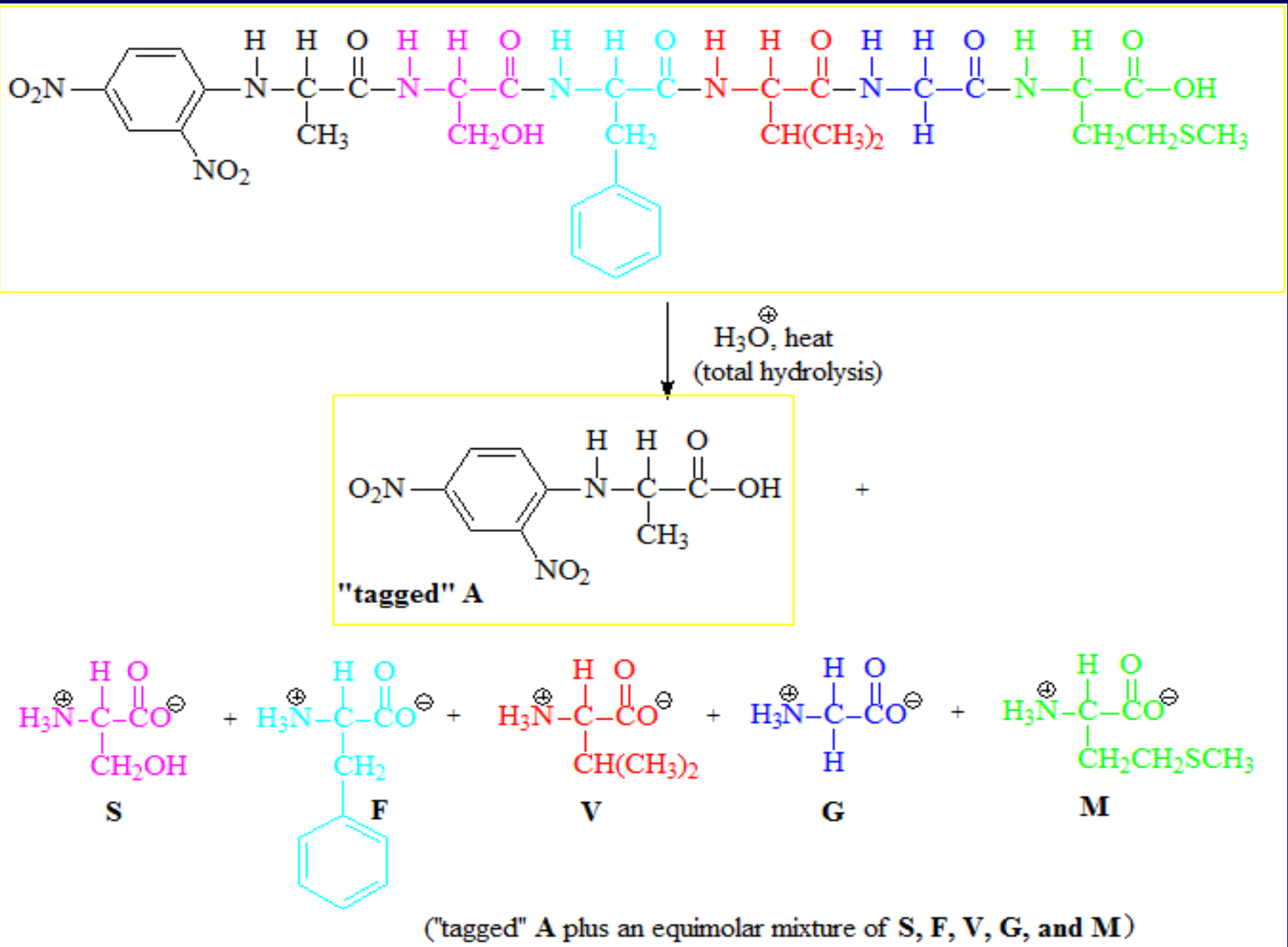


Sanger's Reagent: N-terminal Amino Acid Analysis

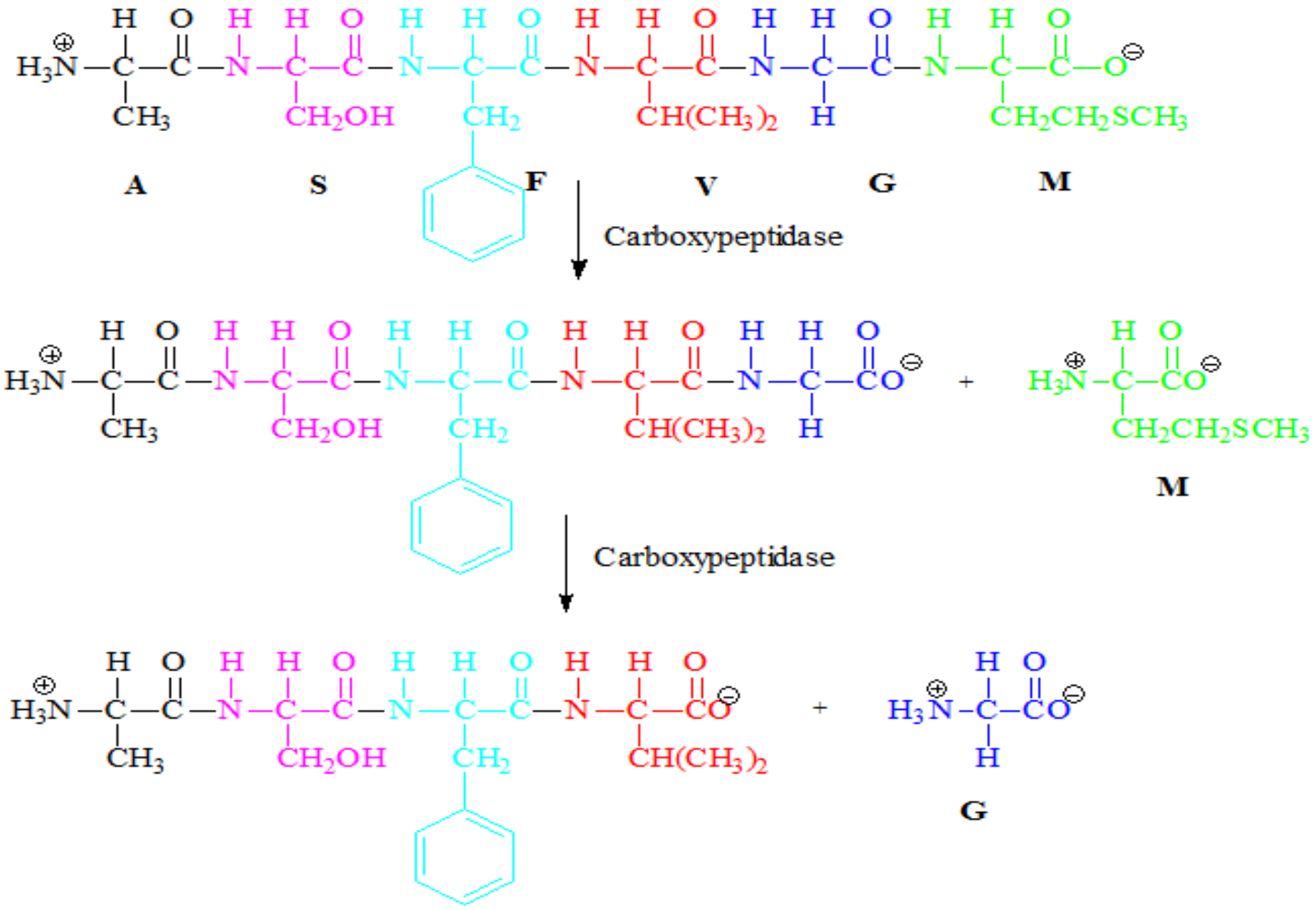


Sanger's Reagent
(2,4-dinitrofluorobenzene)

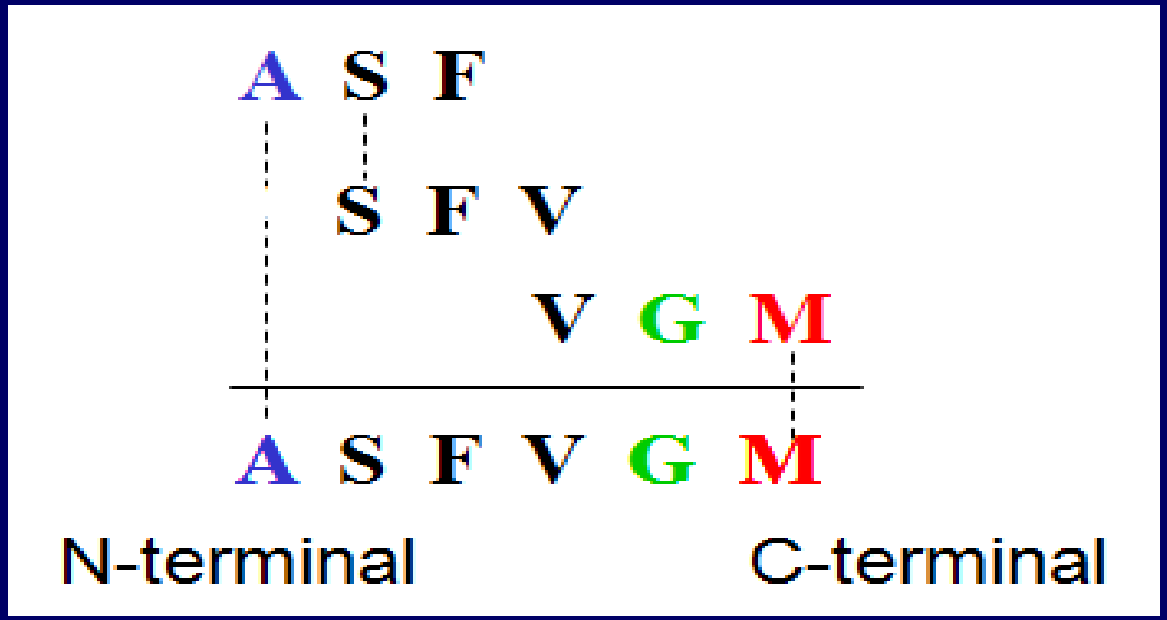



H3O⊕, heat
(total hydrolysis)
"tagged" A
+
S
+
F
+
V
+
G
+
M
("tagged" A plus an equimolar mixture of S, F, V, G, and M)

Carboxypeptidase: C-terminal AA Analysis



Partial hydrolysis give the following identifiable tripeptides: V-G-M, A-S-F, and S-F-V. What is the 1° structure of the hexapeptide (written as usual, with the N-terminal aa on the left and the C-terminal aa on the right)?



Proteins are classified into groups based on their three-dimensional shape.

1. ***Globular proteins*** are compact, spherical structures that are soluble in an aqueous environment. Myoglobin, which stores oxygen in muscle, is an example.
2. ***Fibrous proteins*** are long, threadlike structures that have high helical content. Keratins, found in hair, nails, the scales of reptiles, and collagen, are examples.

Denaturation of Proteins

Denaturation : is a process that disrupts secondary, tertiary, and quaternary structures.

The primary structure is not destroyed during denaturation.

A protein will lose its biological activity if it loses its three-dimensional shape.

Denaturing agents including:-

Denaturing Agent	Disrupted Forces	Examples
Heat above 50 °C	Hydrogen bonds and hydrophobic interactions	Cooking food
Acids and bases	Salt bridges and hydrogen bonds	Lactic acid from bacteria, which denatures milk proteins in the preparation of yogurt and cheese
Organic compounds	Disulfide bonds	Thiols, which are used in hairstyling for hair straightening or permanent waves
Heavy metal ions Ag^{+}, Pb^{2+}, Hg^{2+}	Disulfide bonds and salt bridges	Mercury and lead poisoning
Mechanical agitation	Hydrogen bonds and London forces	Whipped cream and meringue made from egg whites

Heat, acid, base, heavy metal salts, agitation

Active protein

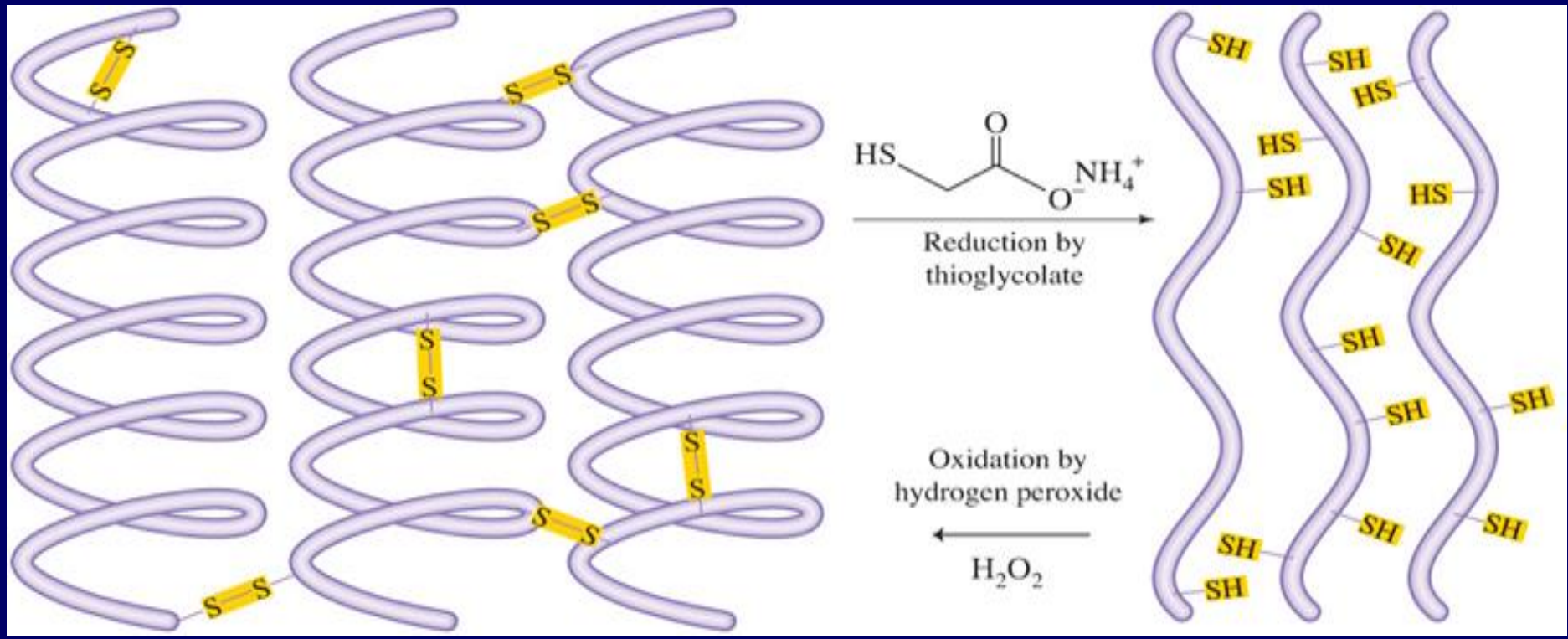
Denatured protein

Some Application for denaturation

Curling straight hair or straightening curly hair requires protein denaturation.

Both processes require disruption of disulfide bonds found in the hair protein keratin.

The disruption of disulfide bonds reshapes the hair and forces the reformation of disulfide bonds in new places.



Ammonium thioglycolate, which reduces (breaks) disulfide bonds, and hydrogen peroxide (reforms disulfide bonds) are two chemical agents used in hairstyling.

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.

Proteins function

Proteins function as follows:

1. They transport oxygen in the blood.
2. They are the primary components of skin and muscle.
3. They work as defense mechanisms against infection.
4. They serve as biological catalysts called ***enzymes***.
5. They also control the metabolism of hormones.

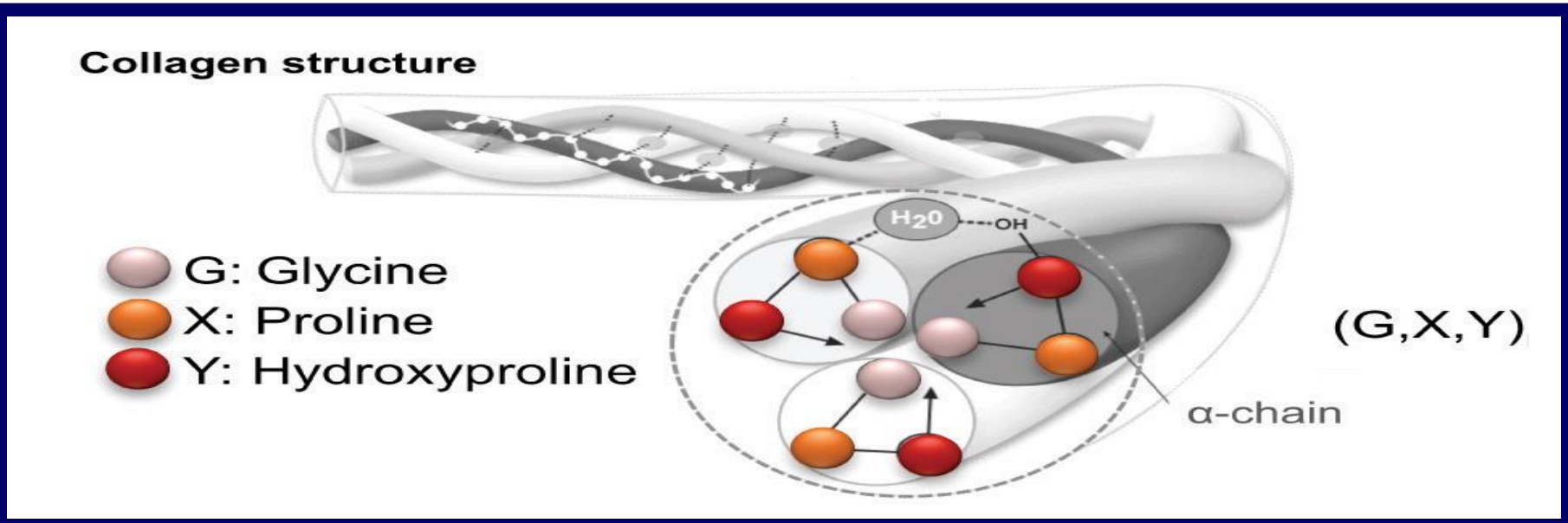
Examples of Biologically Important Proteins

Collagen

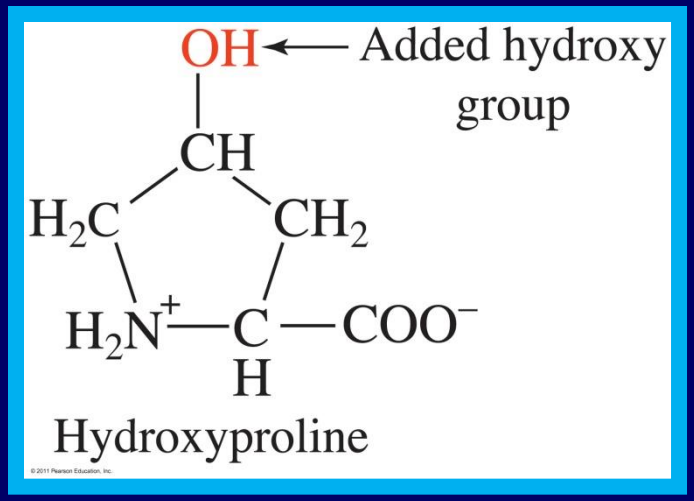
- ***Collagen*** is the most abundant protein in the body. One-third of the bodies protein is collagen.

- It is found in connective tissue like cartilage, skin, blood vessels, and tendons.

- A special quaternary structure called a ***triple helix*** forms the fibrous structure of collagen.



- Each polypeptide chain of a triple helix is a left-handed helix.
- Collagen primarily contains the amino acids glycine, proline, alanine, and hydroxyproline.
- Hydrogen bonds between the polypeptide chains in collagen are formed through the hydroxyl group on hydroxyproline.



- Proline is converted to hydroxyproline with the aid of Vitamin C.
- A deficiency of Vitamin C causes scurvy, which is a collagen malformation disease. Scurvy can be reversed with a vitamin C diet.

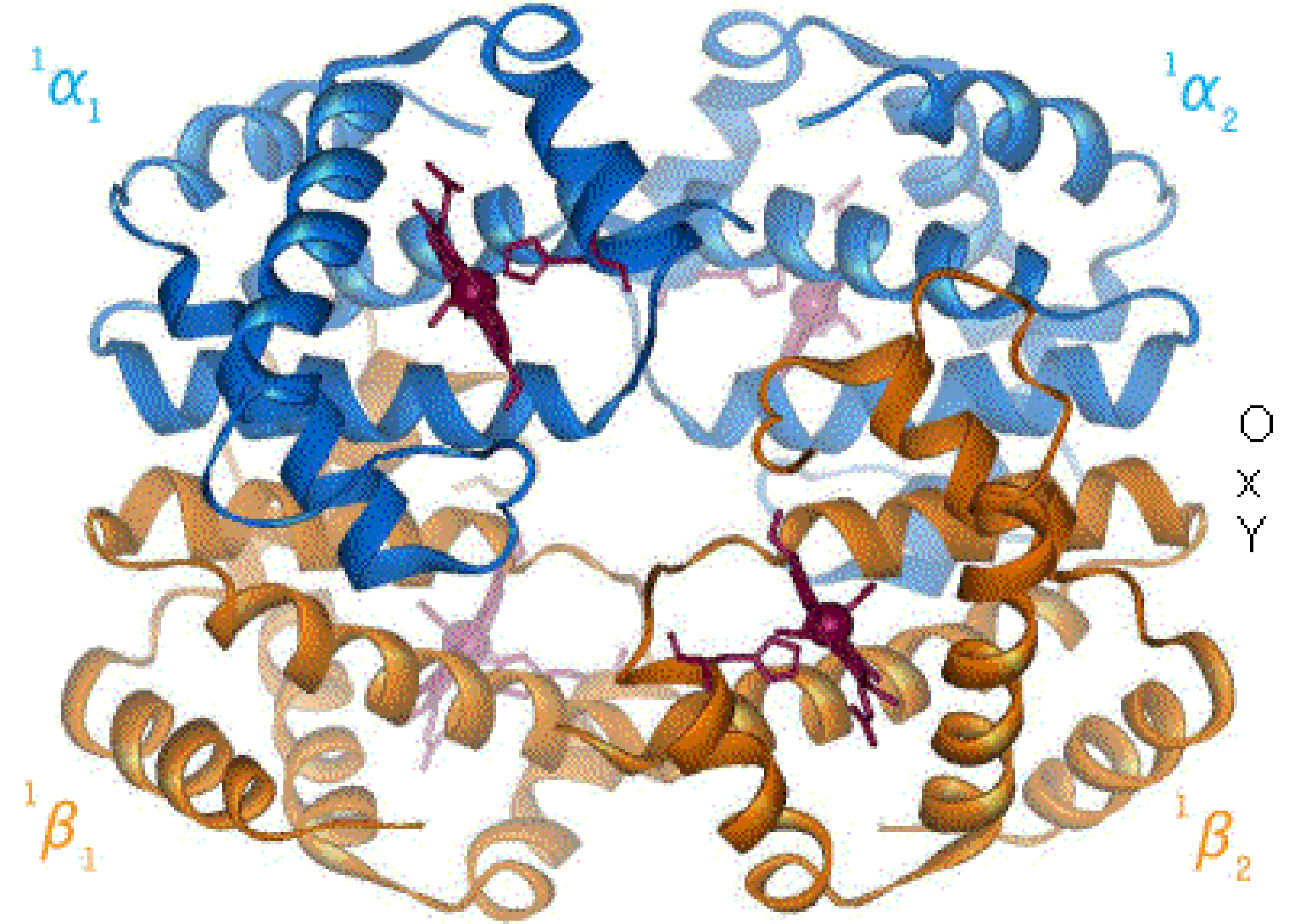
Hemoglobin

- ***Hemoglobin*** transports oxygen in blood.

- It is composed of two alpha subunits and two beta subunits held together by hydrogen bonds, London forces, and salt bridges.

- Each subunit contains a nonprotein part called a ***prosthetic group***, which is called a ***heme***.

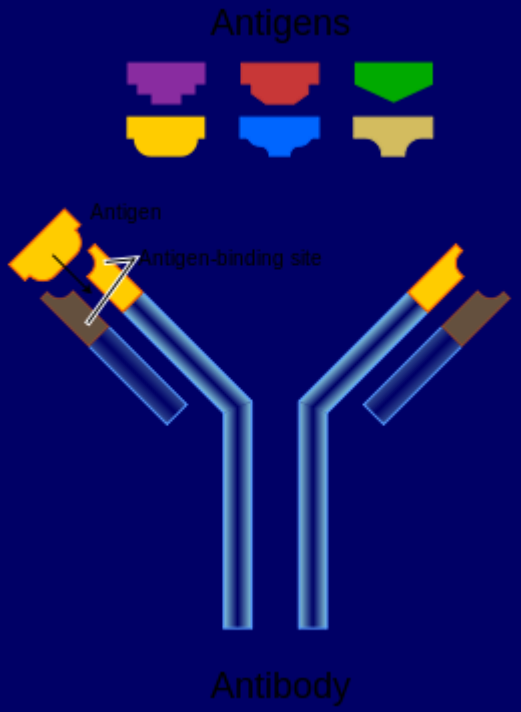
- Each heme group binds an Fe^{2+}, which binds O_2, so a molecule of hemoglobin can bind up to four molecules of O_2.
- When O_2 binds to the Fe^{2+} of hemoglobin, the shape of hemoglobin changes, which allows the hemoglobin to hold on to the oxygen until it is delivered to tissues. This change in shape is known as a ***conformational change***.
- After the oxygen is delivered to the tissues, the shape of the hemoglobin changes back to its pre-oxygenated form.

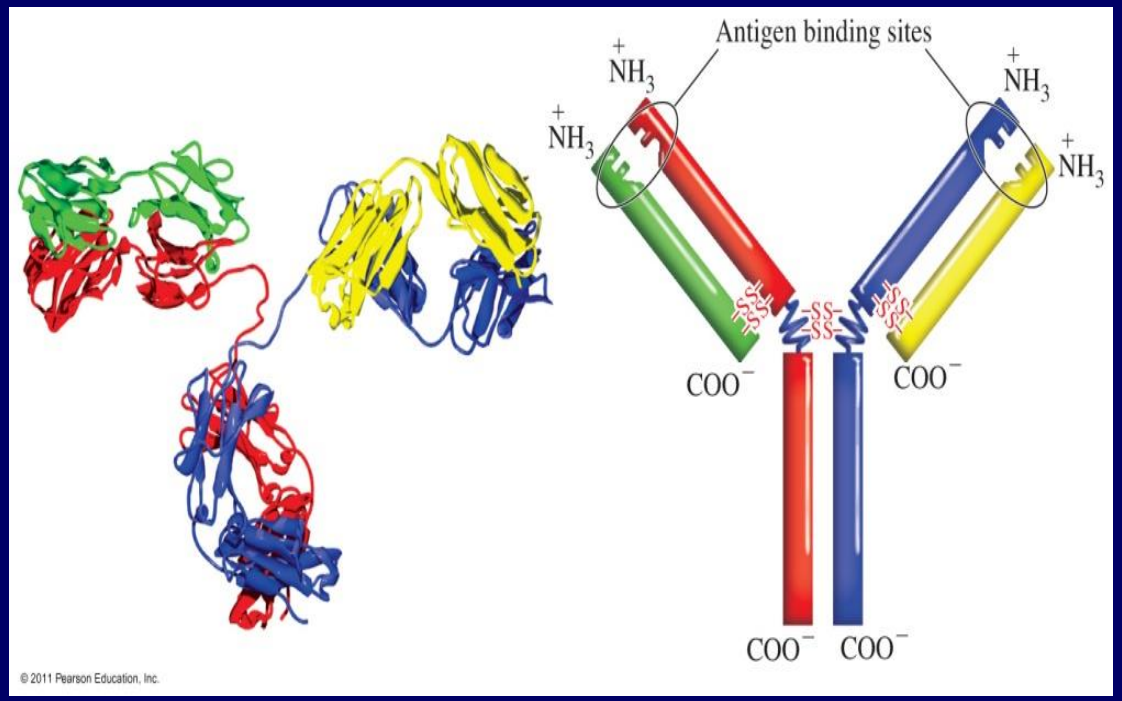

$^1\alpha_1$
$^1\alpha_2$
O
X
Y
$^1\beta_1$
$^1\beta_2$

Antibodies (Defense System)

- ✓Antibodies, also known as immunoglobulins, are produced in our bodies when a foreign agent like bacteria enters.
- ✓The foreign agent recognized by antibodies is known as an antigen.
- ✓Antibodies consists of four polypeptide chains held together by disulfide bonds and intermolecular forces.
- ✓Antibodies are "Y" shaped. Antigens bind at the top of each arm of the Y.

The top of each Y has a unique primary structure for a particular antigen and binds only one antigen, which is then destroyed by the immune system.





Integral Membrane Proteins

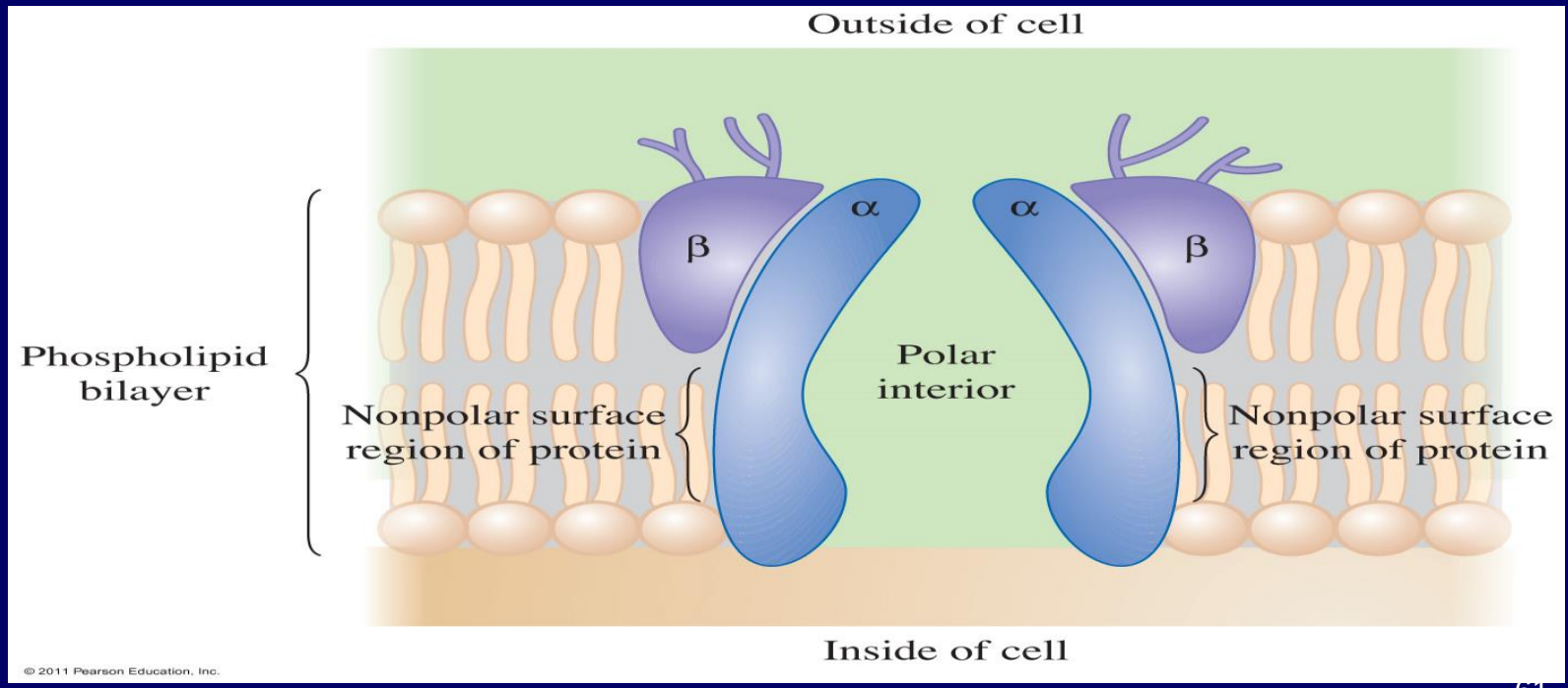
Integral membrane proteins span the nonpolar region of a cell membrane and facilitate the movement of polar substances across the membrane.

An important integral membrane protein involved in electrolyte balance is the sodium potassium pump (Na^+/K^+ ATPase).

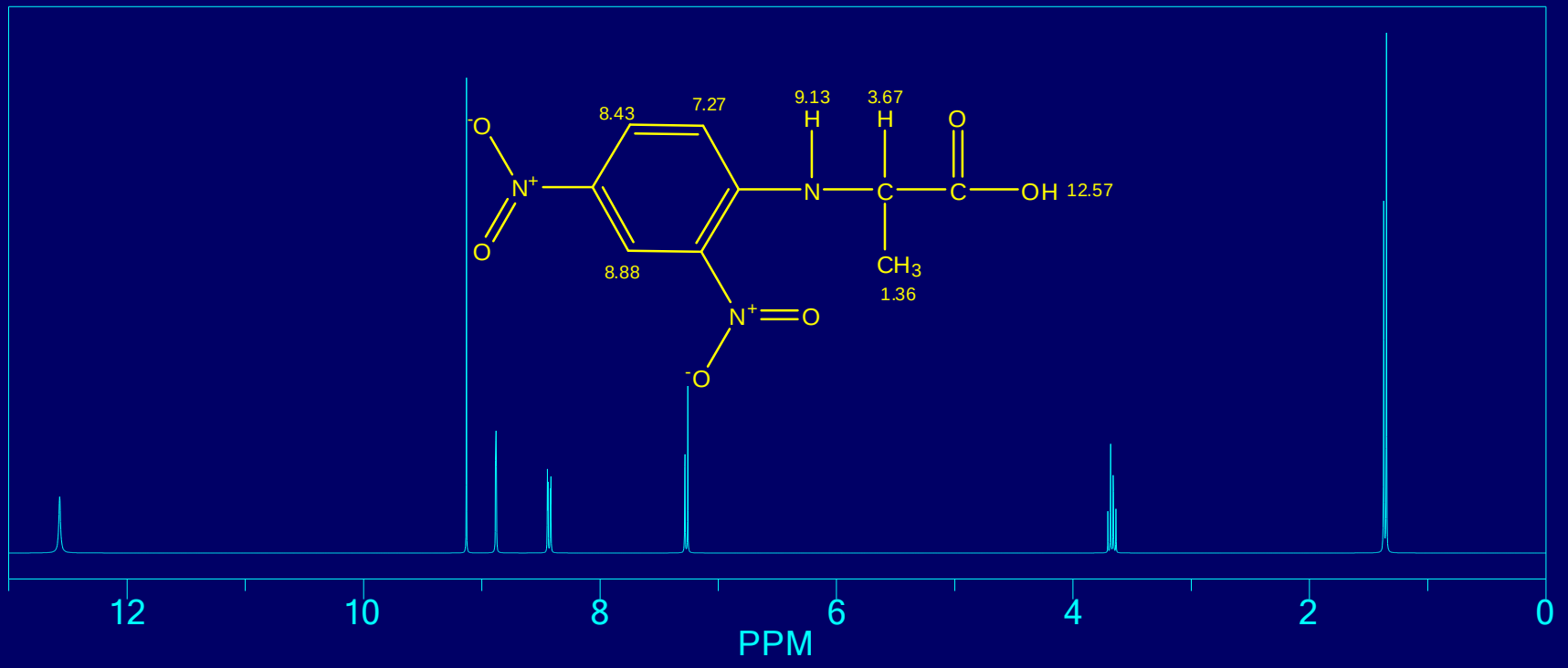
The sodium potassium pump protein is composed of four polypeptide chains held together by intermolecular forces.

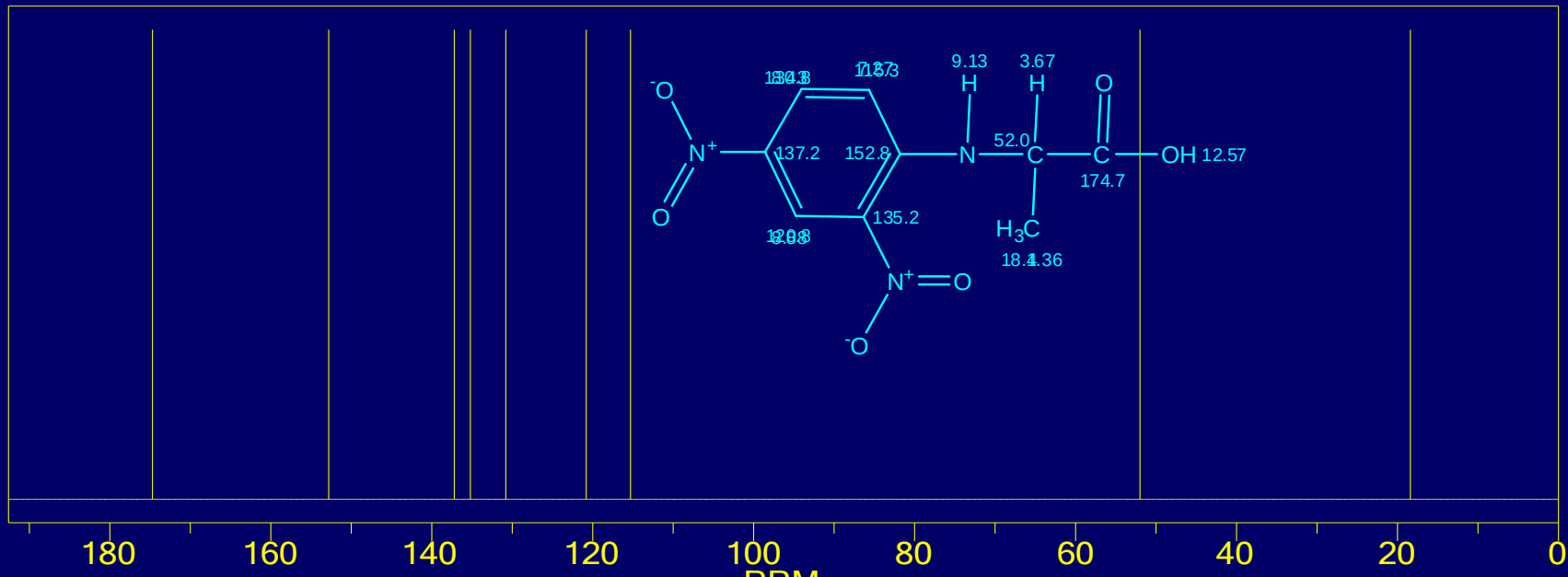
The side chains embedded in the nonpolar region of the cell membrane are nonpolar, allowing them to interact with the nonpolar region of the membrane.

The central cavity of the protein is lined with polar amino acids, allowing polar molecules to pass.




Thank you for your attention


8.43
7.27
9.13
H
3.67
H
O
-O
N+
O
N
C
C
OH
12.57
8.88
CH3
1.36
N+
O
-O
12
10
8
6
4
2
0
PPM


9.13
3.67
H
H
O
-O
N+
137.2
152.8
N
52.0
C
C
OH
12.57
174.7
O
135.2
H3C
1.36
N+
O
-O
180
160
140
120
100
80
60
40
20
0