Amino Acid and Protein chemistry

What are amino acids

- Amino Acids are the monomeric building units of peptides/proteins

- Amino acids are linked by peptide bonds

- Amino acids present in proteins can be classified as primary and secondary.

Primary vs secondary amino acids

- Primary amino acids are coded by genetic code and they have specific t-RNA.
- Only 20 (21) amino acids are primary amino acids
- Secondary amino acids do no have a code or t-RNA.
- They are formed as a result of post translational modifications.

Classification of amino acids

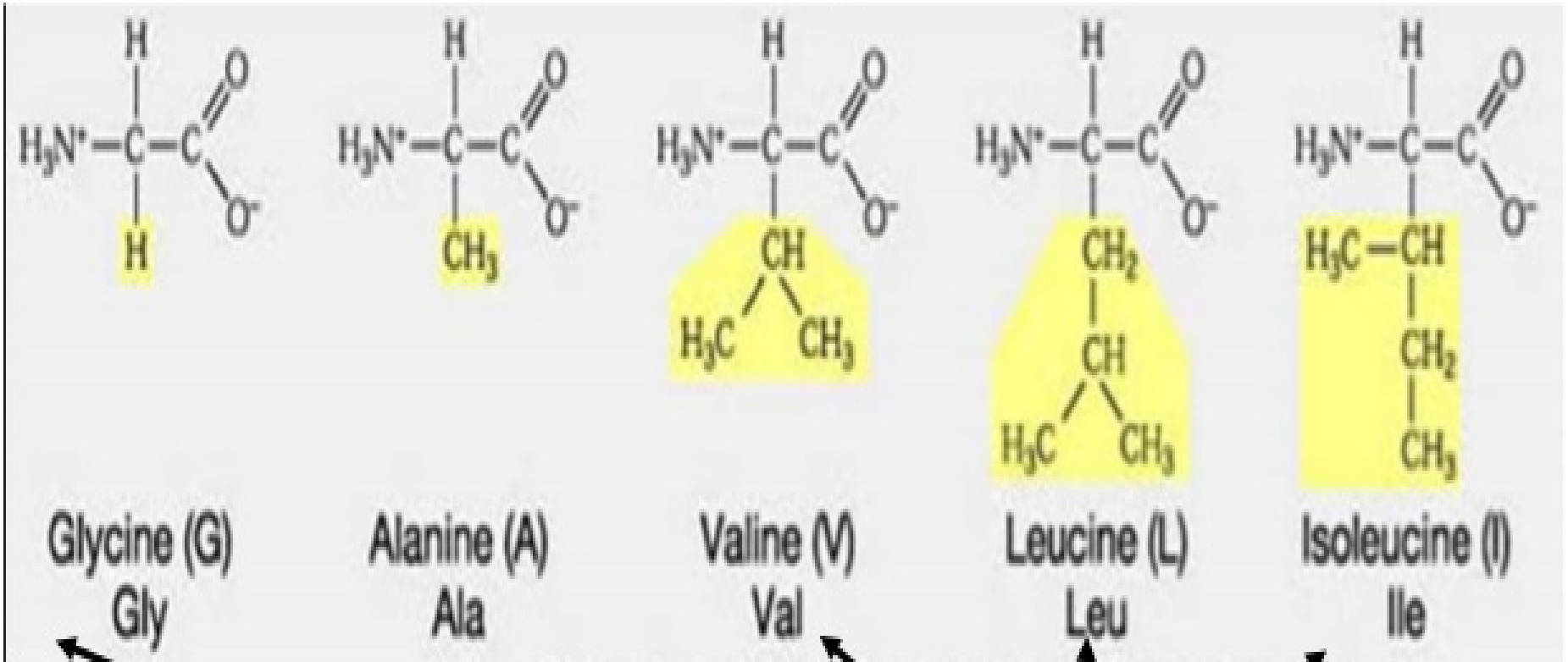
- Amino acids can be classified in 4 ways:
  1. Based on structure
  2. Based on the side chain characters
  3. Based on nutritional requirements
  4. Based on metabolic fate

Classification of primary amino acids

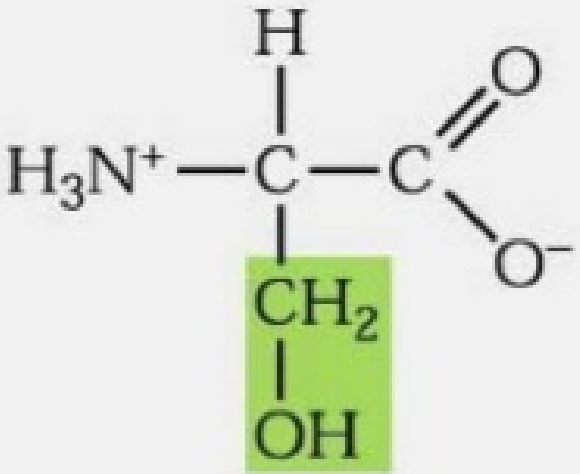
Based on structure:

- A. Aliphatic amino acids:

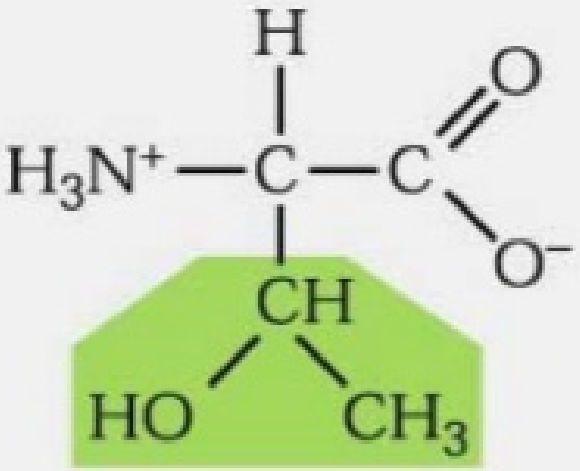
1. Simple amino acids: glycine, alanine.
2. Branch chain amino acids: valine, leucine and isoleucine.
3. Hydroxy amino acids: serine and threonine.
4. Sulphur containing amino acids: cysteine and methionine



Hydroxyl group containing amino acids



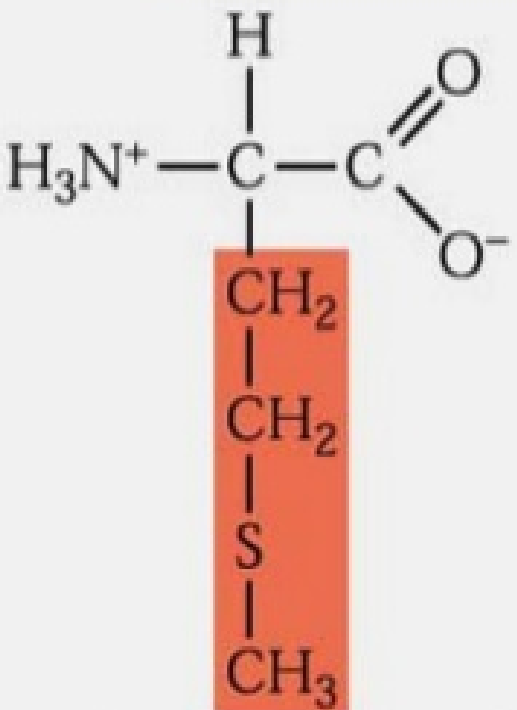
SERINE



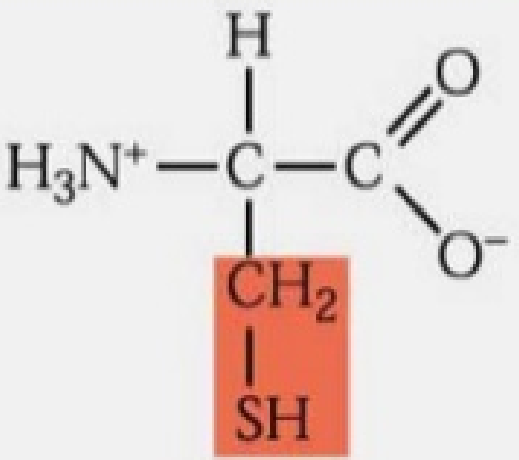
THREONINE

Side chains contain hydroxyl functional groups

Sulfur containing amino acids



METHIONINE



CYSTEINE

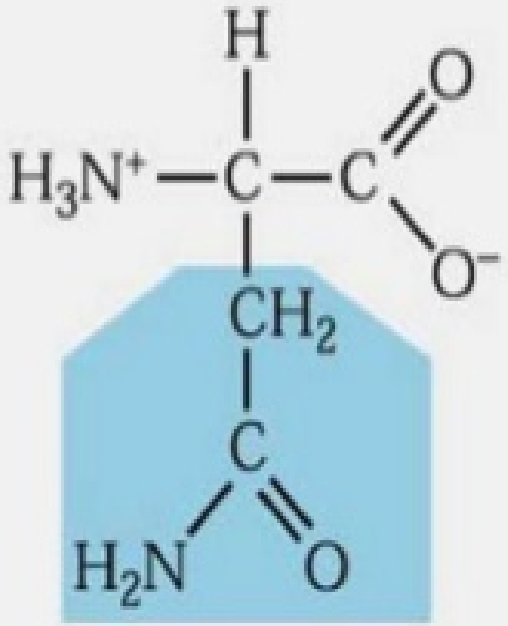
Side chains contain sulfur

- 5. Amide group containing amino acids: asparagine and glutamine.

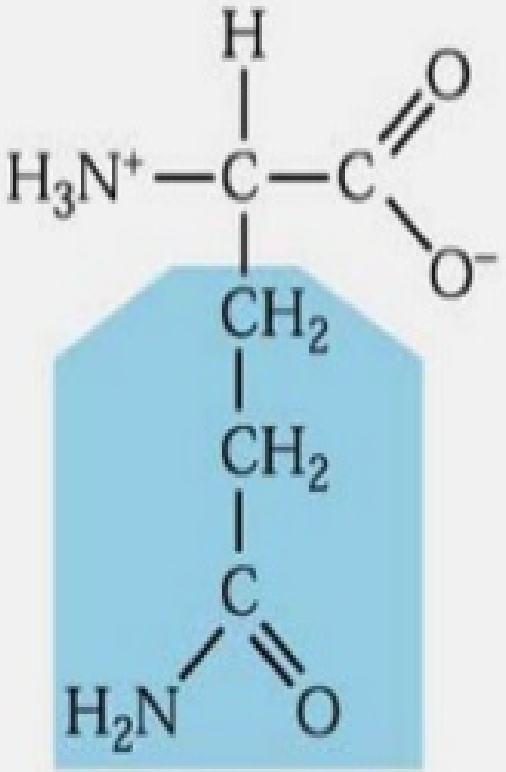
- 6. Dicarboxylic amino acids: glutamic acid and aspartic acid

- 7. Basic amino acids: lysine and arginine, histidine.

Amino acid amides



ASPARAGINE

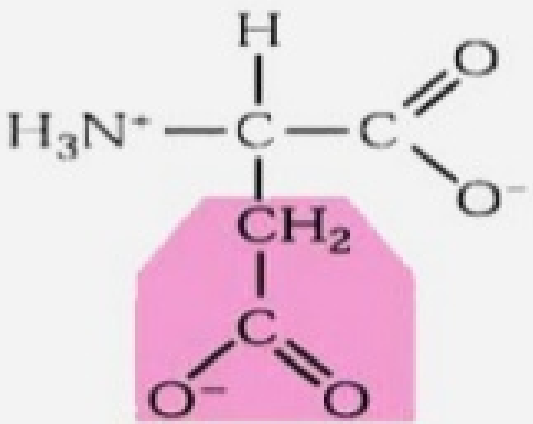


GLUTAMINE

Side chains contain amino functional groups

Monoamino dicarboxylic amino acids

Acidic amino acids

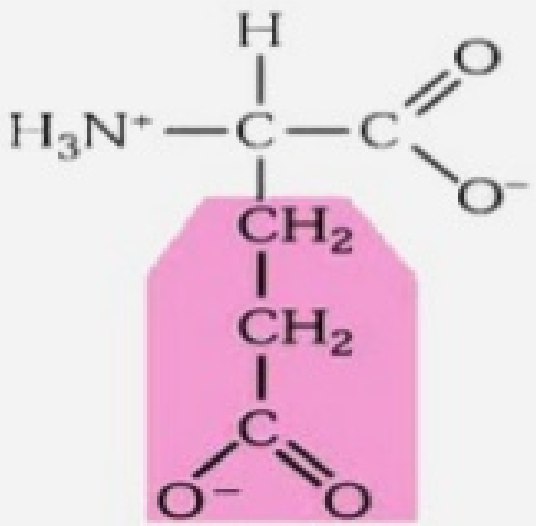


-N-C-C-

amino acid backbone

pH 3 = +1

pH 9 = -2

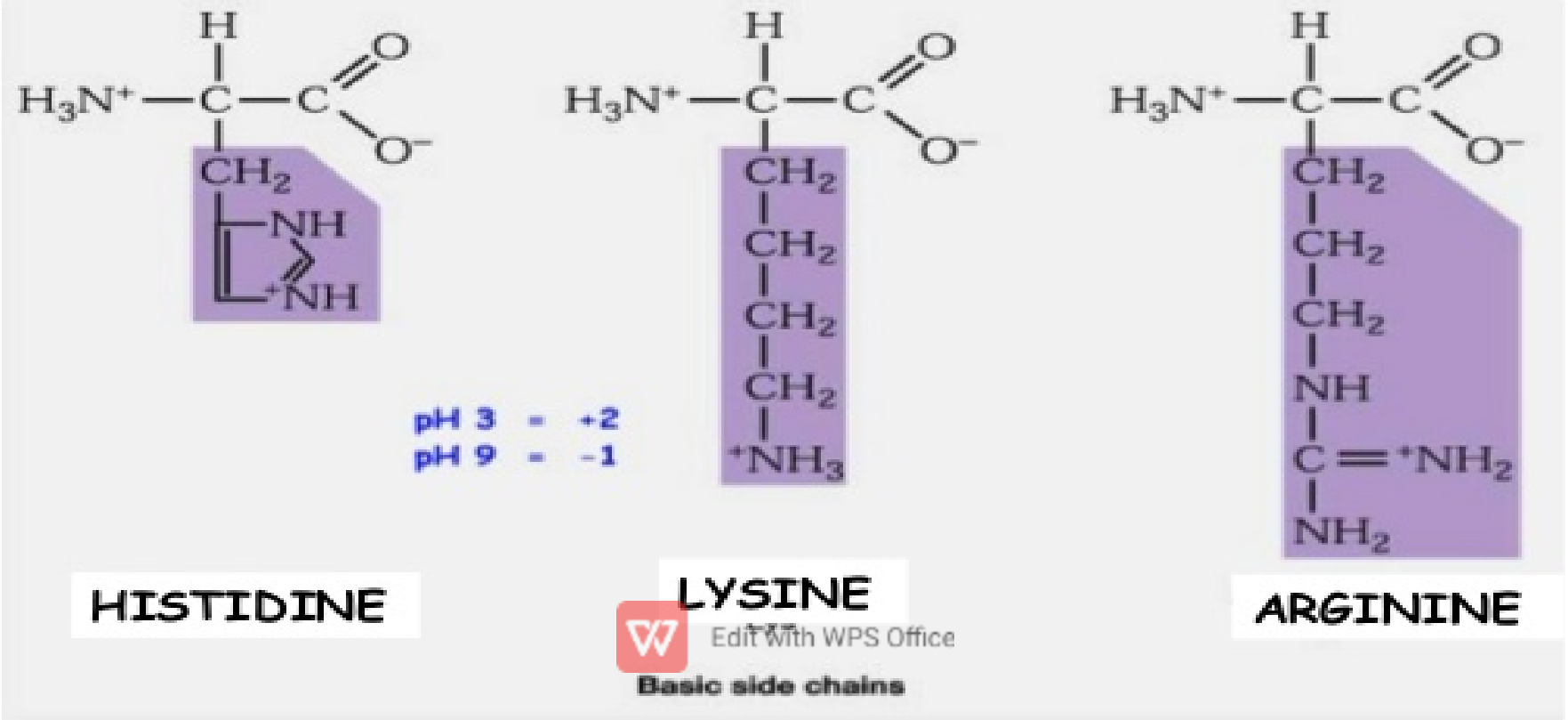


ASPARTATE

GLUTAMATE

Basic amino acids

Diamino monocarboxylic amino acids

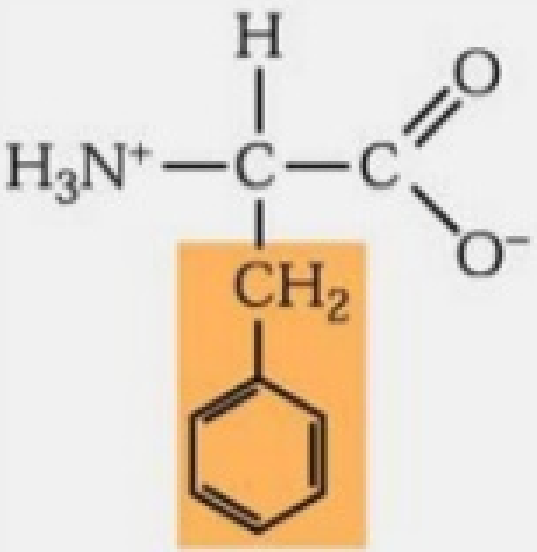


B. Aromatic amino acids:

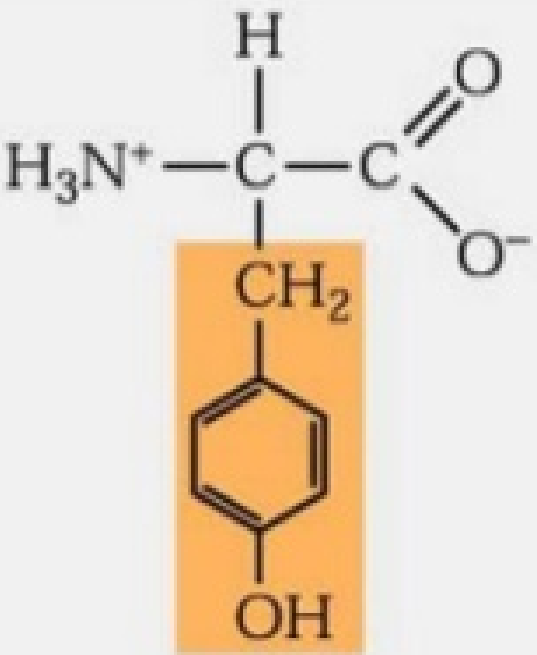
phenylalanine, tyrosine, tryptophan

C. Imino acid: proline

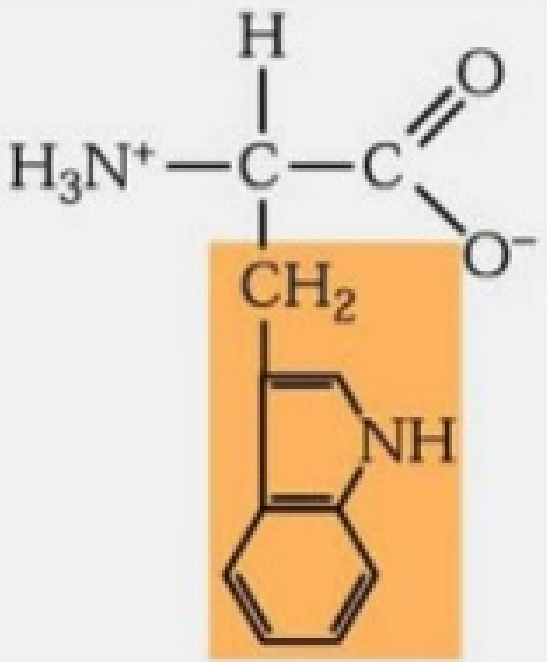
Aromatic amino acids



PHENYLALANINE



TYROSINE



TRYPTOPHAN

Side chains contain ring structures

Based on side chain property

- Non polar amino acids: these are hydrophobic amino acids (lipophilic)

Alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline.

- Polar amino acids:

1. Neutral: glycine, serine, threonine, cysteine, glutamine, asparagine,tyrosine.

2. Basic amino acids: lysine, arginine, histidine.

3. Acidic amino acids: glutamic acid , aspartic acid.

Based on nutritional requirement

- Essential amino acids: cannot be synthesised by the body hence they should be provided by diet:

Isoleucine, leucine, valine, threonine, lysine, methionine, phenylalanine, tryptophan.

- Semi essential amino acids: histidine and arginine: required during growth.

- Non essential amino acids: remaining 10 amino acids

Essential amino acids	• cannot be synthesized in human body • Phenylalanine, Valine,Threonine, Tryptophan, Isoleucine, Methionine, , Leucine, Lysine
Semi-essental amino acid	• synthesized at a rate inadequate to support the growth • Histidine, Arginine
Non-essential amino acids	• which can be synthesized in human body • Glycine, alanine, aspartate, glutamate, asparagine, glutamine, serine, proline, tyrosine, cysteine

Based on metabolic fate

- Purely ketogenic: leucine
- Ketogenic and glucogenic: lysine, isoleucine, phenylalanine, tyrosine, tryptophan.
- Purely glucogenic: remaining 14 amino acids

Secondary amino acids

- Also called derived amino acids.
- Non α amino acids: β alanine, Gamma amino butyric acid.
- Derived and incorporated in proteins: hydroxyproline, hydroxylysine.
- Derived and not incorporated in proteins: ornithine, citrulline, homocysteine, thyroxine.

Non protein amino acids

- Amino acids not found in proteins but serve important functions in metabolic processes :

Examples:

- Ornithine, citrulline (used in urea cycle)

- Homocysteine: formed from methionine, it is an important marker of cardiovascular diseases.

21ST amino acid

- Selenocysteine is the 21st amino acid.
- It resembles serine in structure.
- It is found in some enzymes like: thioredoxin reductase, glutathione peroxidase and deiodinase.

Isoelectric p H(p I)

- Definition : The pH at which aminoacid has no net charge is called isoelectric pH(pI)

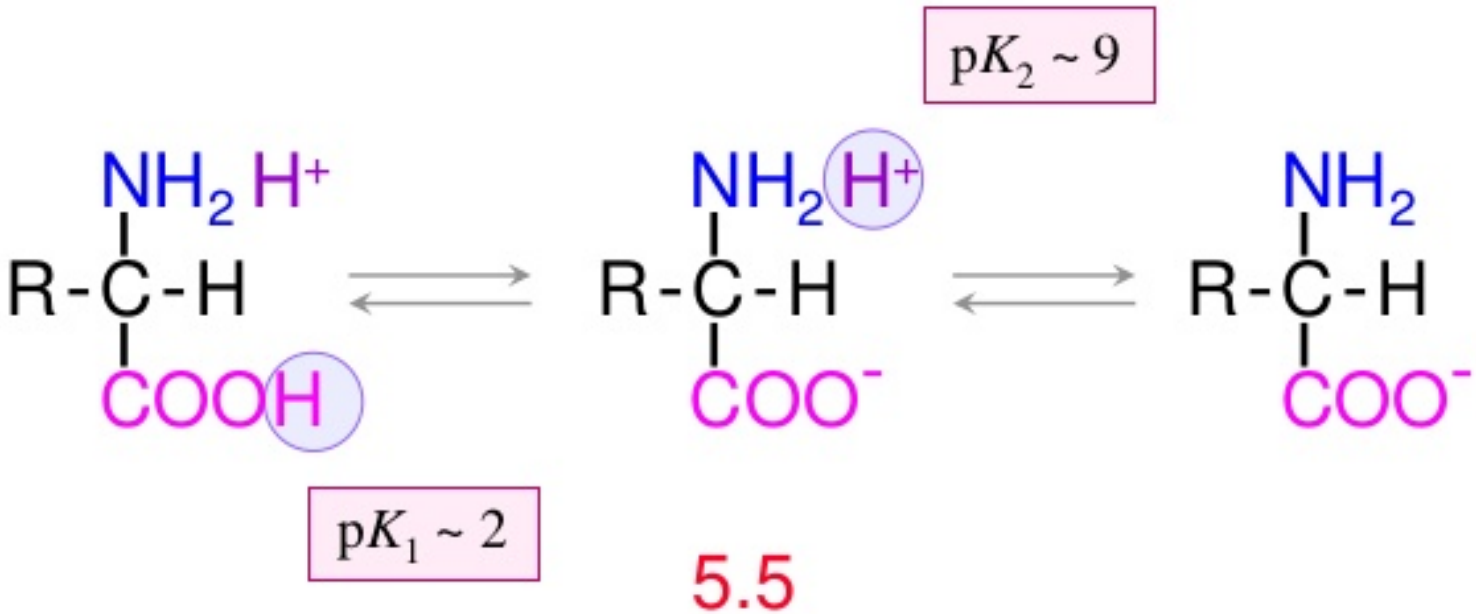
Most Amino acids have two ionisable groups.

- At pI aminoacids exist as zwitterions

- At this pH amino acids are not soluble so they ppt
- No mobility in electric field.

- Buffering capacity is minimum.

Acidic environment **Neutral environment** **Alkaline environment**



calculation

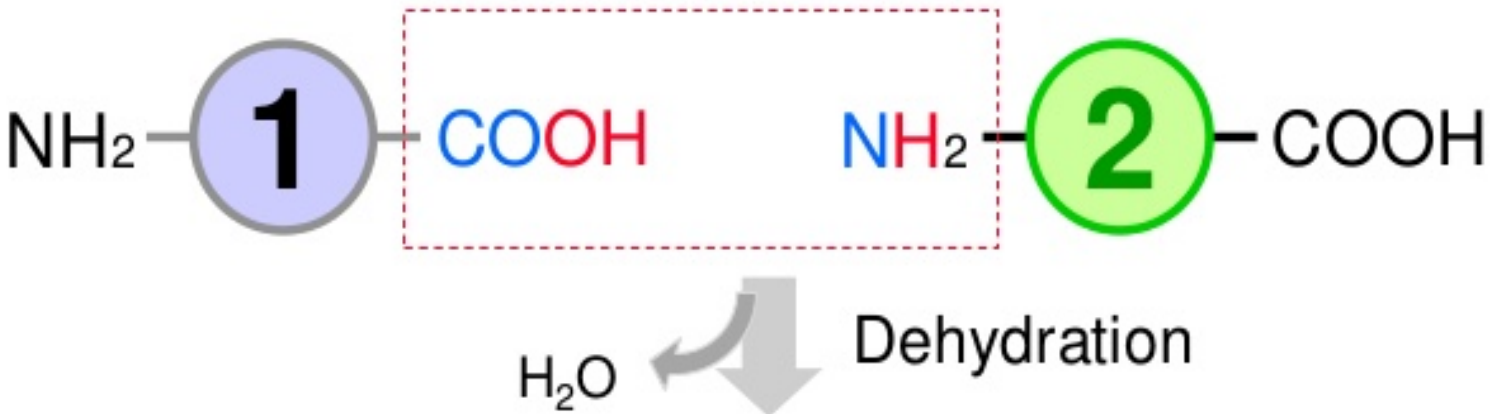
- p I = p K1+ pk2/ 2
- For glycine pk1 is 2.4 and pk2 is 9.8
- pK of imidazoline group of histidine is 6.7 so it is a good buffer at physiological pH (7.4)

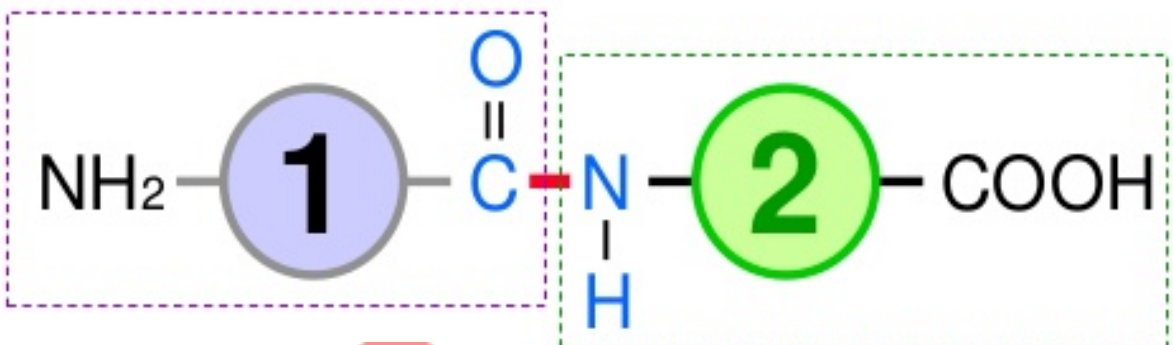
Applications of pI

- For precipitation of proteins
- For separation of amino acids from a mixture by varying the p H.
- Isoelectric focussing: Separation based on mobility in electric field.
- In ion exchange chromatography.

Formation of Peptide Bonds

Amino acids are connected head to tail





Structural organisation of proteins

- Primary
- Secondary
- Tertiary
- Quarternary

Primary structure

- Number and sequence of amino acids in a peptide chain

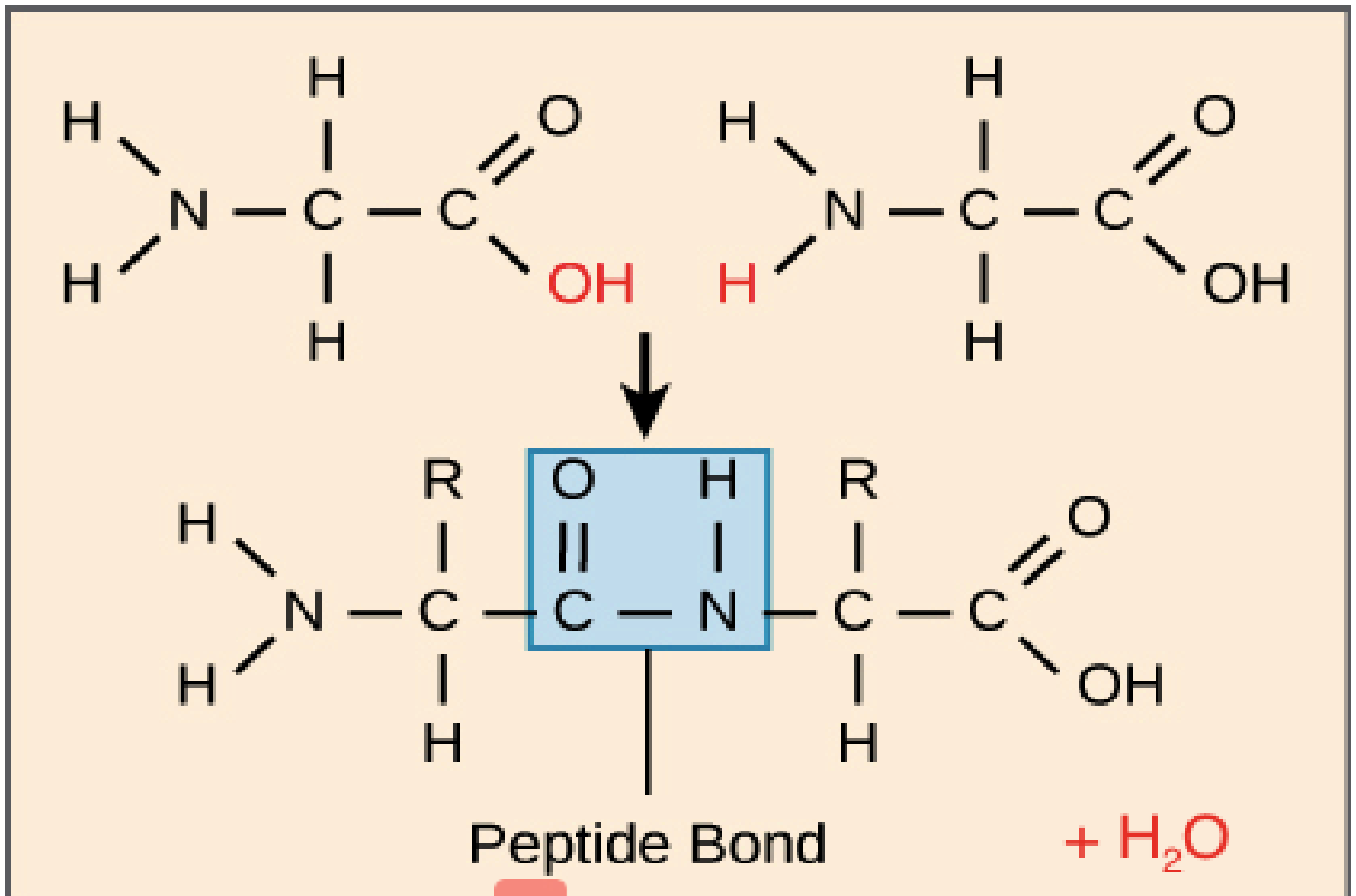
- Bonds stabilising primary structure are:

  Peptide bond and disulphide bond

Characteristics of Peptide bond:

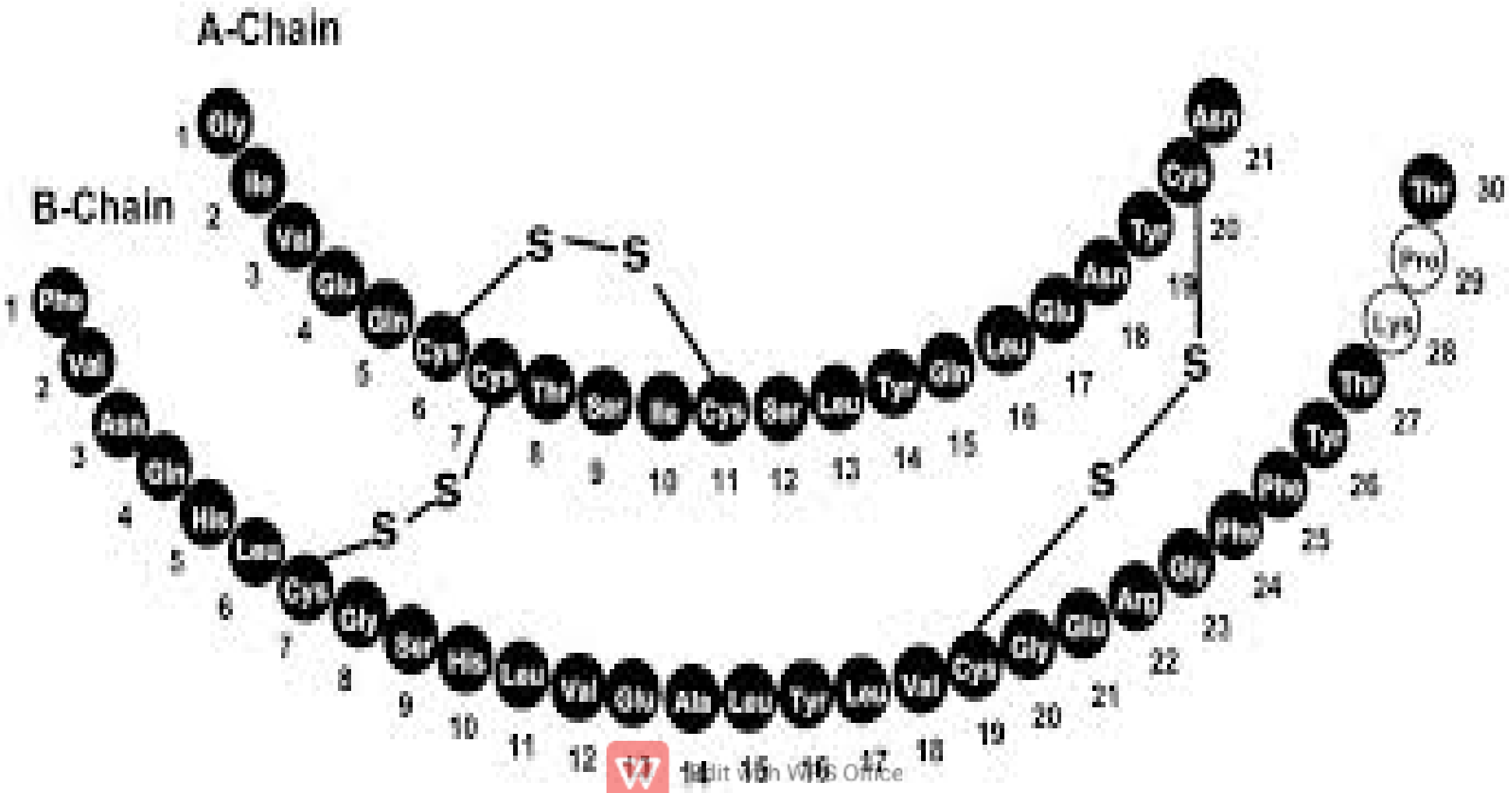
- Polar covalent bond.
- Partial double bond character
- Bond length 1.33 A (between 1.49 and 1.27)
- Transplanar
- Uncharged

- First amino acid is at the N-terminal end, last is at C-terminal end
- Amino acid residues are named by changing the suffix “ine” to “yl” except the last amino acid
- In branched or circular proteins interchain or intrachain disulphide bridges are also part of primary structure



Insulin

- Described by Sanger in 1955
- 2 chains: A (gly) 21 aminoacids and B (Phe) 30 aminoacids
- 2 interchain disulfide bonds and one intrachain S-S bond
- Species variation in 8,9,10 of A chain and C- terminal of B chain
- Proinsulin 86 aminoacids.


A-Chain
B-Chain
Gly
Ile
Val
Glu
Gln
Cys
Cys
Thr
Ser
Ile
Cys
Ser
Leu
Tyr
Gln
Leu
Glu
Asn
Tyr
Cys
Asn
Thr
Lys
Pro
Thr
Tyr
Phe
Phe
Gly
Arg
Glu
Gly
Cys
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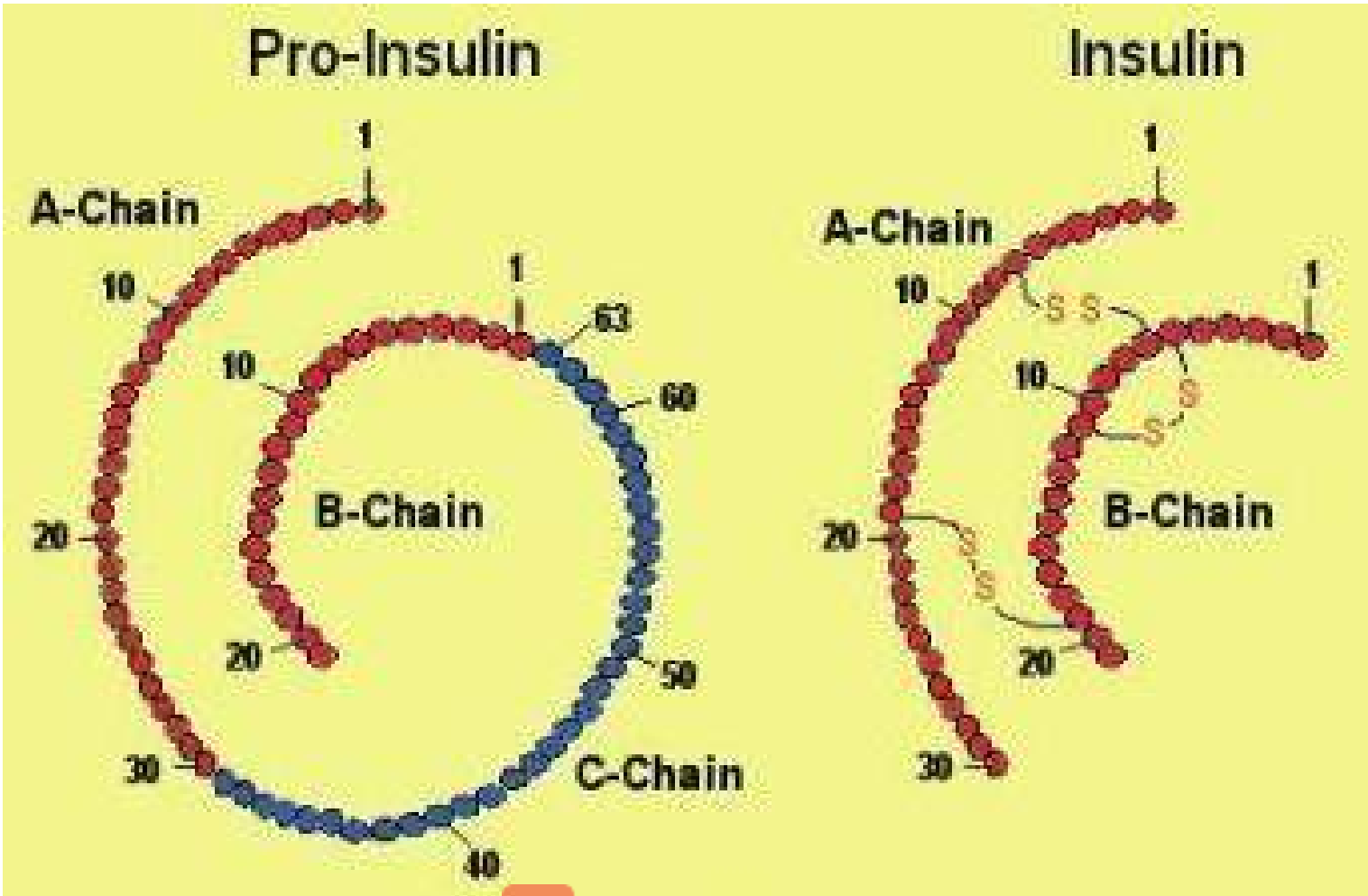


- Primary structure determines biological activity.
- Any mutation in primary structure can lead to disease: eg sickle cell disease

Secondary structure

- Definition: It is the configurational relationship between the residues 3-4 amino acids apart.

- Bonds responsible for secondary structure: Non covalent forces: H-bond, hydrophobic interactions, electrostatic, van der waals forces.

- Hydrogen bond:

H donors are NH (imidazole of histidine), OH(serine and threonine) , NH2 (lysine and arginine).

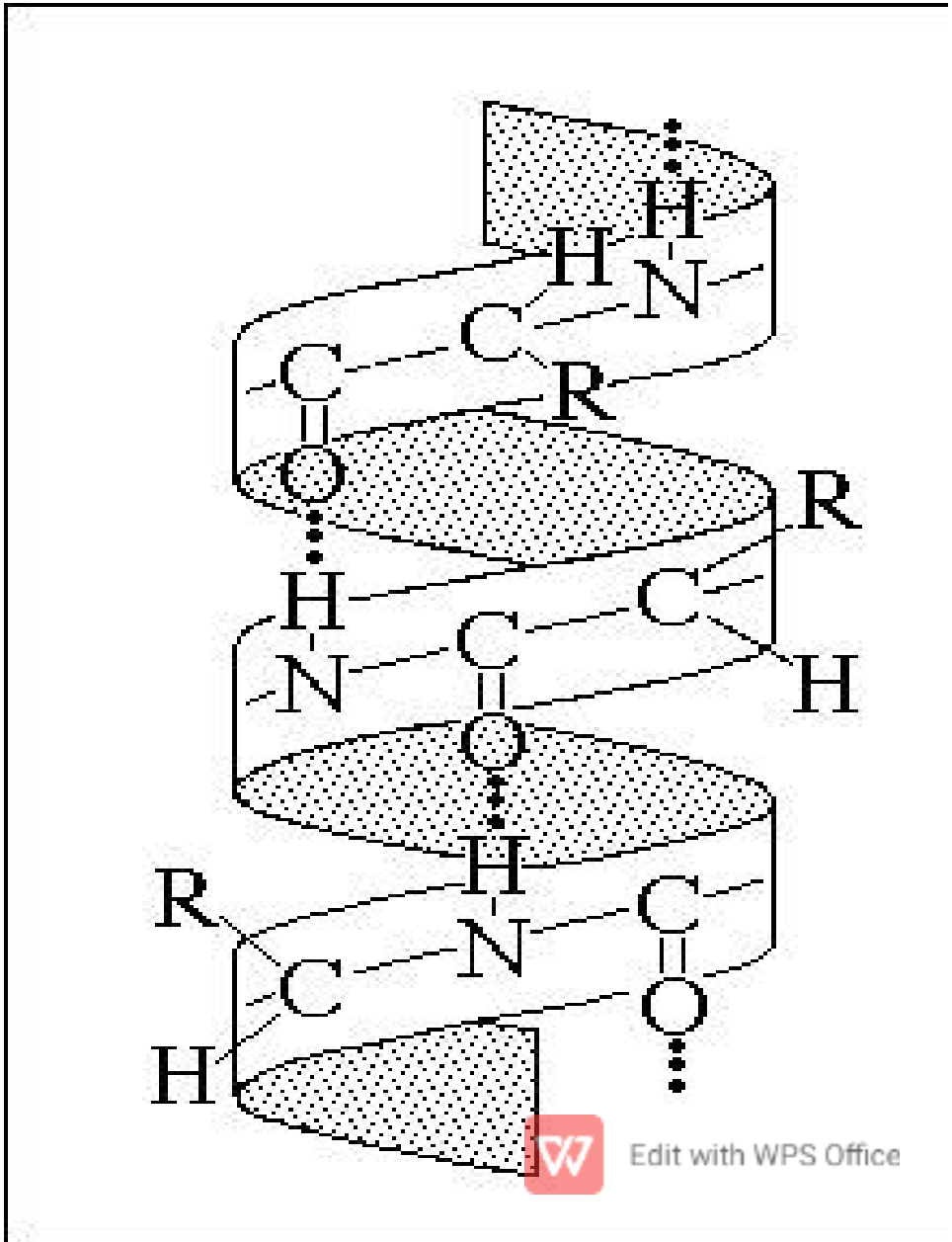
H accepting groups: COO , C=O

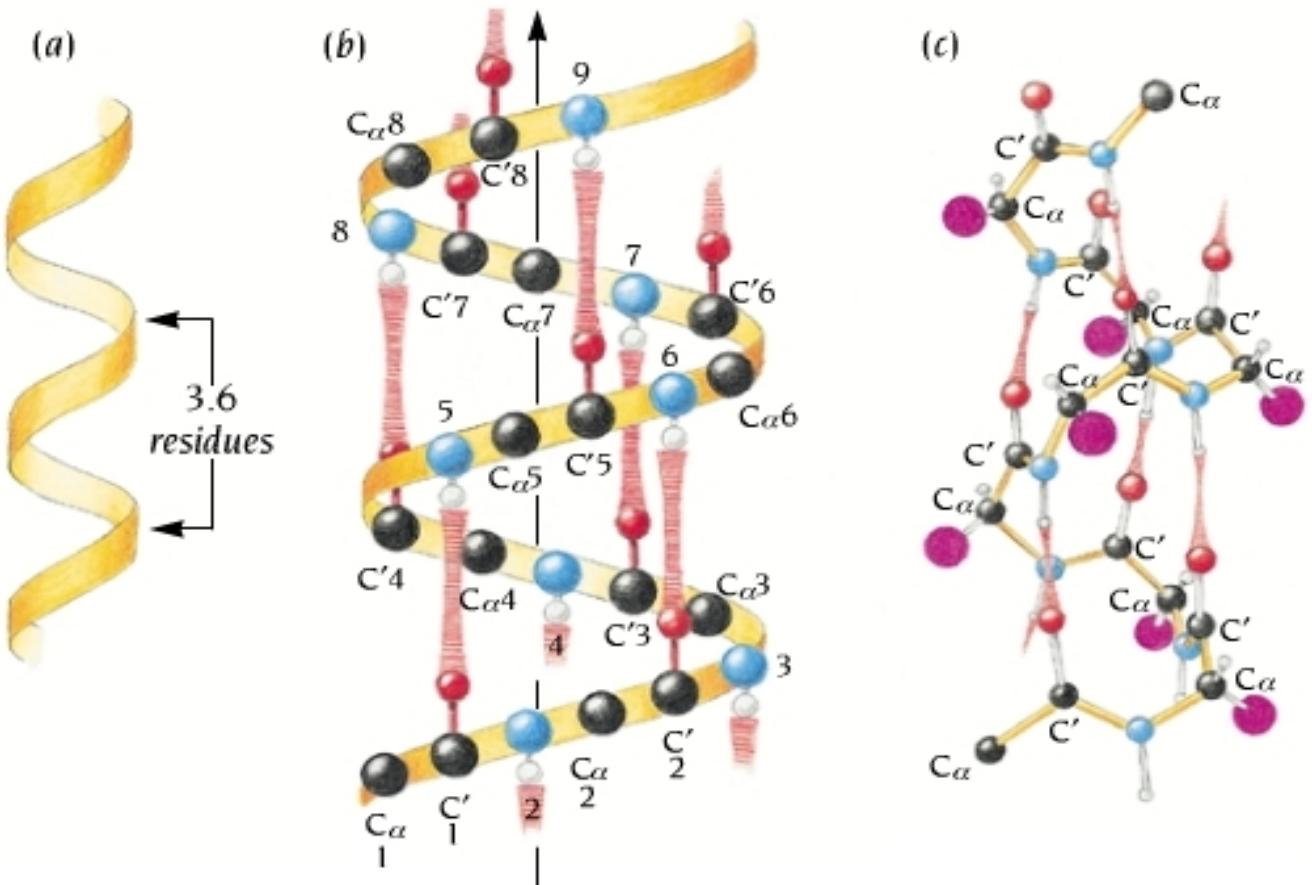
- Electrostatic bond: positive charges by basic amino acid and negative charge from acidic amino acids
- Hydrophobic interactions: between nonpolar aminoacids
- Van der Waals forces: weak but contributes to maximum stability

Alpha Helix

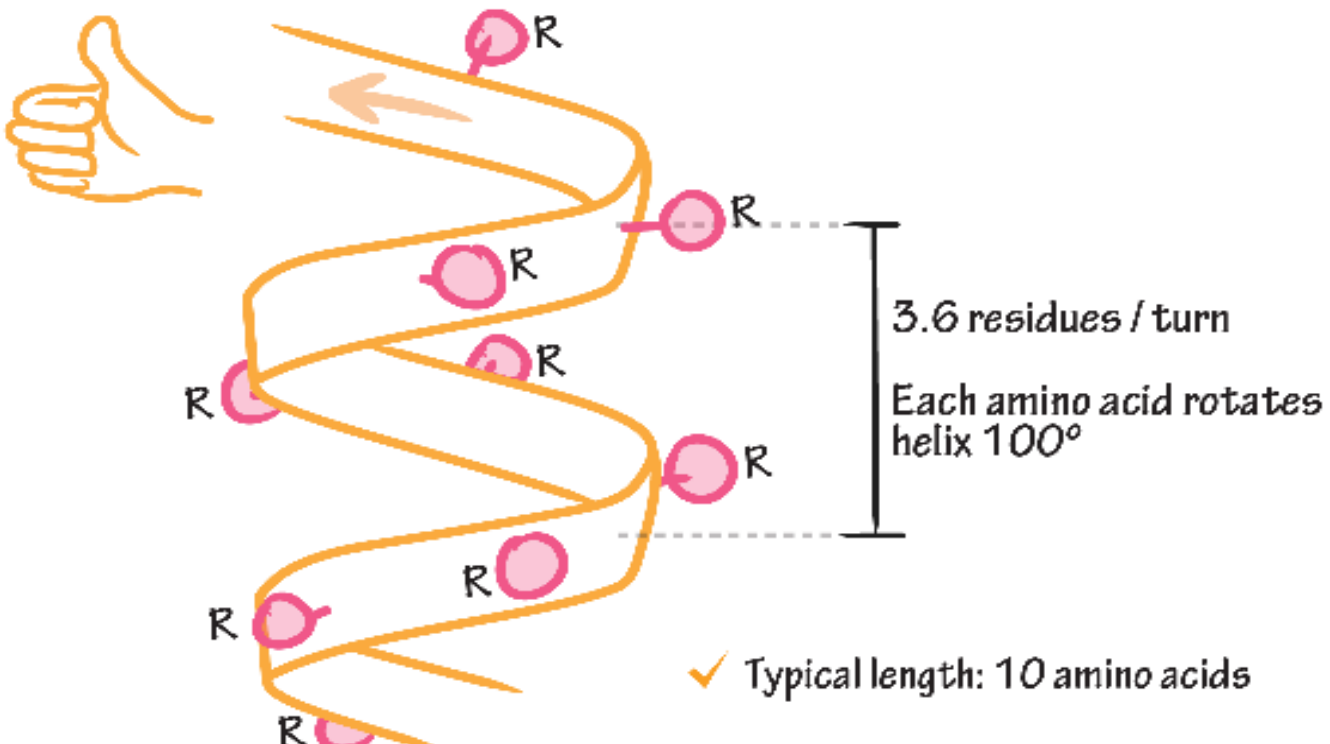
- Described by Pauling and Corey
- Spiral structure consisting of polypeptide chain coiled around a longitudinal axis in helical manner.
- polypeptide bonds form backbone, side chains project out.
- H-bond between NH and C=O of main chain. Between 1^{st} and 4^{th}, 2^{nd} and 5^{th}, 3^{rd} and 6^{th} and so on

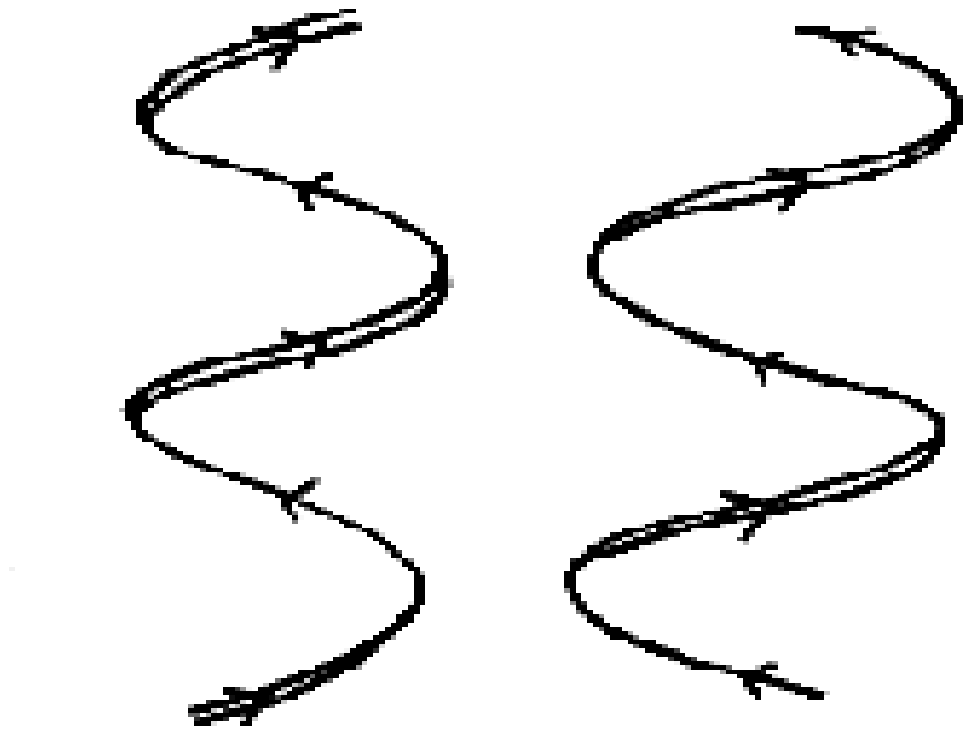
- 3.6 residues in each turn
- Distance between each turn (pitch) : 1.5A
- Right handed helix
- Most stable conformation
- Abundant in Hb and Mb but absent in chymotrypsin





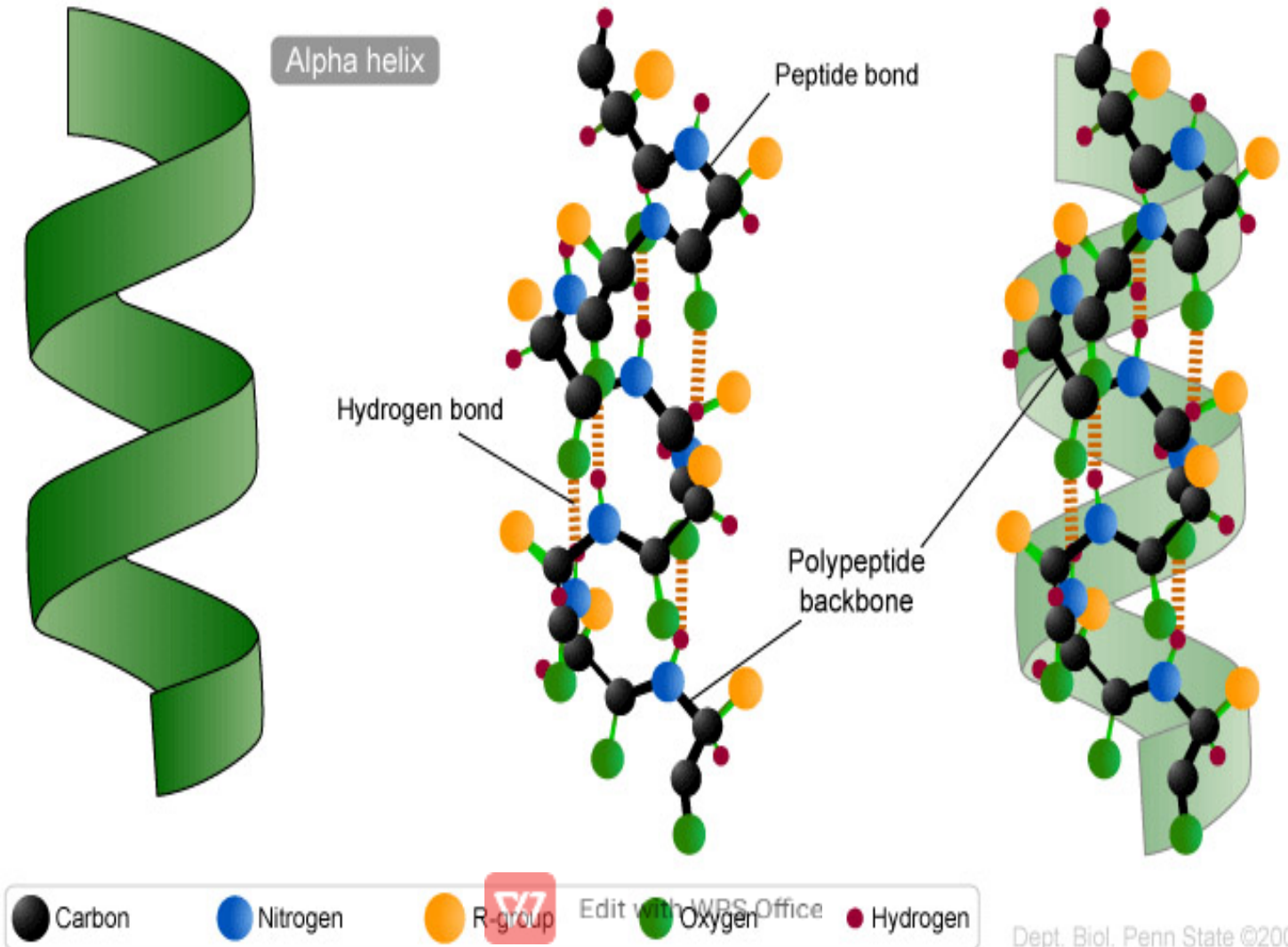
Alpha Helix





Left handed helix

Right handed helix


Alpha helix
Peptide bond
Hydrogen bond
Polypeptide backbone
Carbon
Nitrogen
R-group
Oxygen
Hydrogen
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Dept. Biol. Penn State ©2002

Amino acids not compatible with alpha helix:

1. Proline and hydroxyproline: R group is cyclic so it cannot form helix rather it will bend the helix.

2. Bulky side chains like aromatic amino acids, branched chain, asparagine, aspartic acid.

3. Charged R group, like basic and acidic amino acids.

4. Glycine found only in first turn.

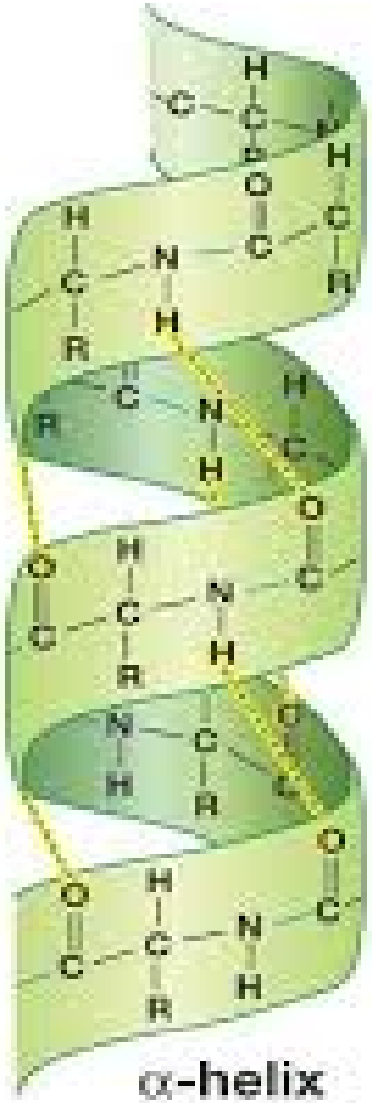
Beta Pleated sheets

- Almost fully extended
- Distance between adjacent aminoacids:3.5 Å
- Stabilised by H bond between neighboring polypeptide segments
- Adjacent strands can be parallel or antiparallel

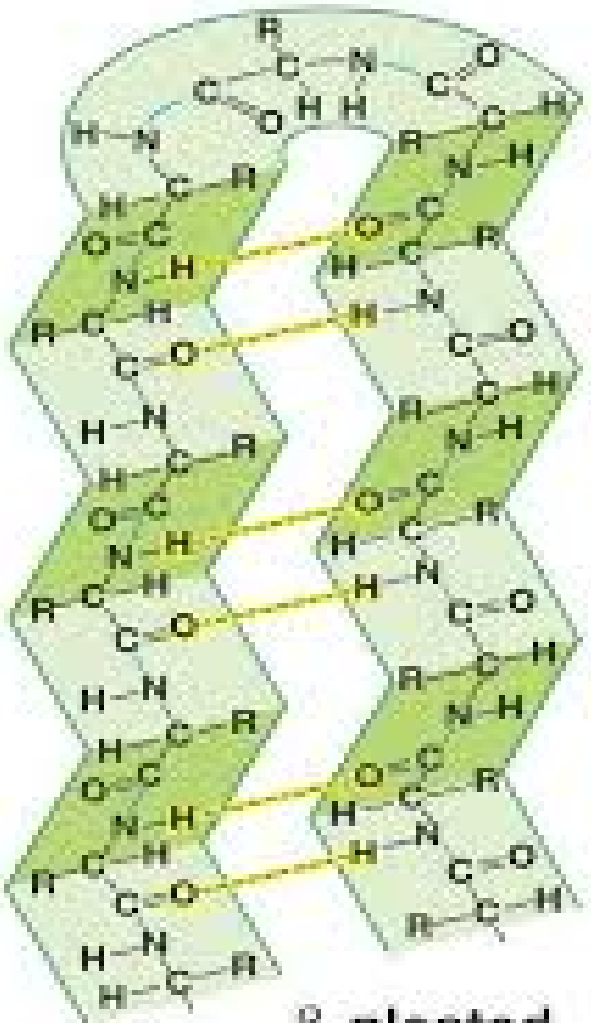
- The side chains of adjacent amino acids orient in opposite directions to avoid steric hindrance.

- Silk fibroin contains plenty of beta pleated sheets.

- Beta bends: abrupt U turns in the proteins stabilized by disulphide bridges.



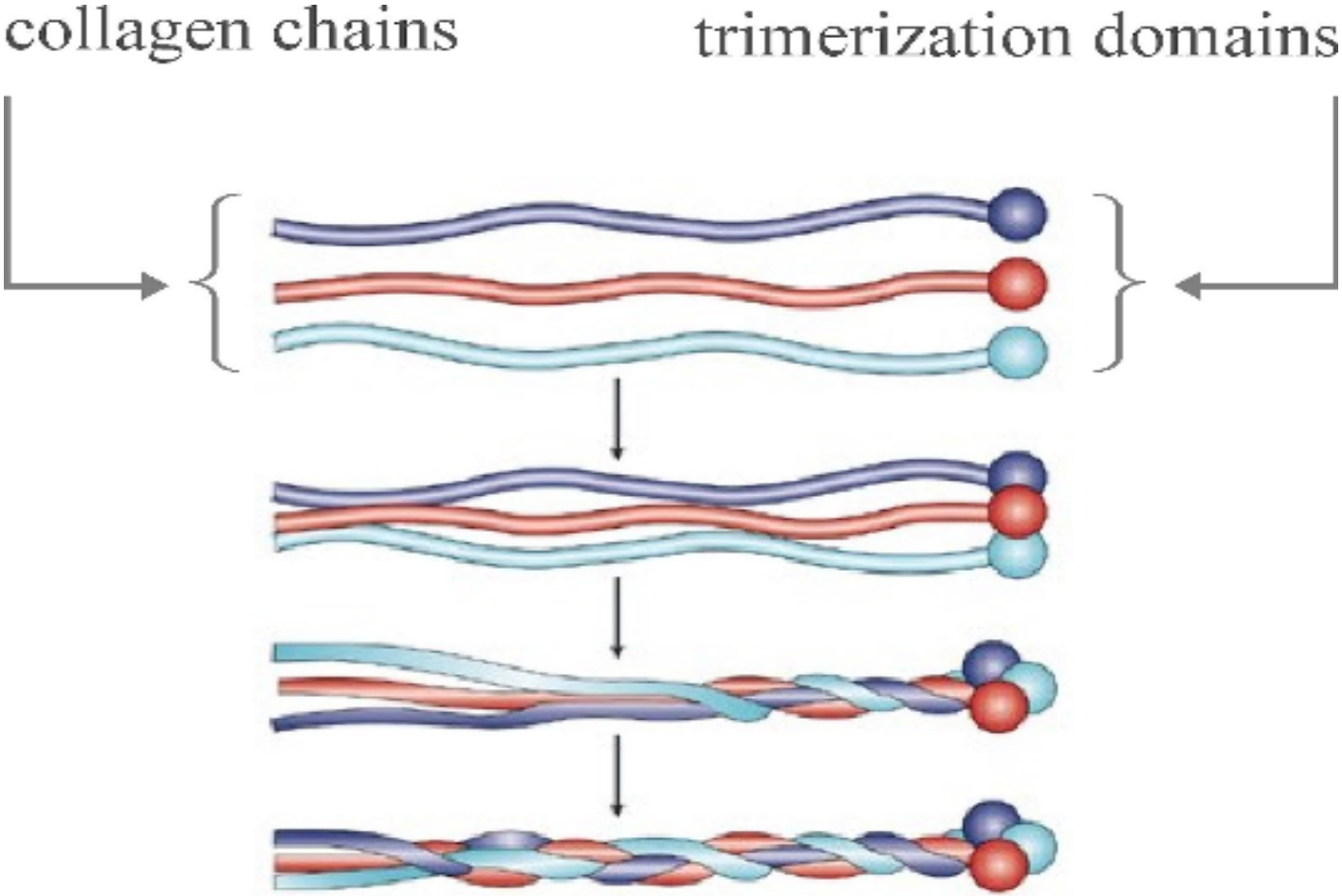
α-helix



β-pleated sheet

Collagen

- Most abundant protein in mammals
- Glycoprotein containing glucose.
- Main fibrous content of skin, bones, tendons, cartilages and teeth.
- Every third residue is glycine
- Superhelical cable: 3 polypeptide chains wound around itself



- 3.3 residues per turn
- Pitch is 2.9Aº
- Three strands are hydrogen bonded to each other

- Hydrogen bonds between hydroxyproline and hydroxylysine stabilises collagen

- Heat denatured collagen is gelatin.

- Functions:

- Collagen provides support to organs
- Helps in anchoring of cells
- Helps in blood clotting

Super secondary motifs

- Beta alpha beta motif
- Beta hairpin motif
- Greek key motif

Motifs: Supersecondary Structures

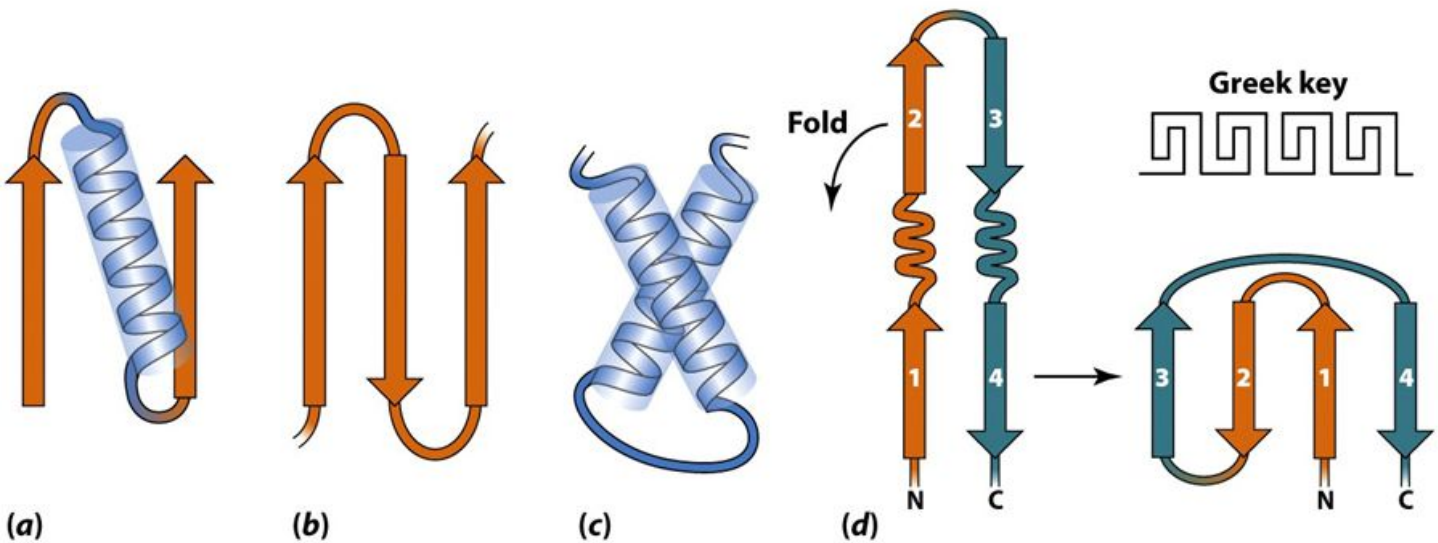
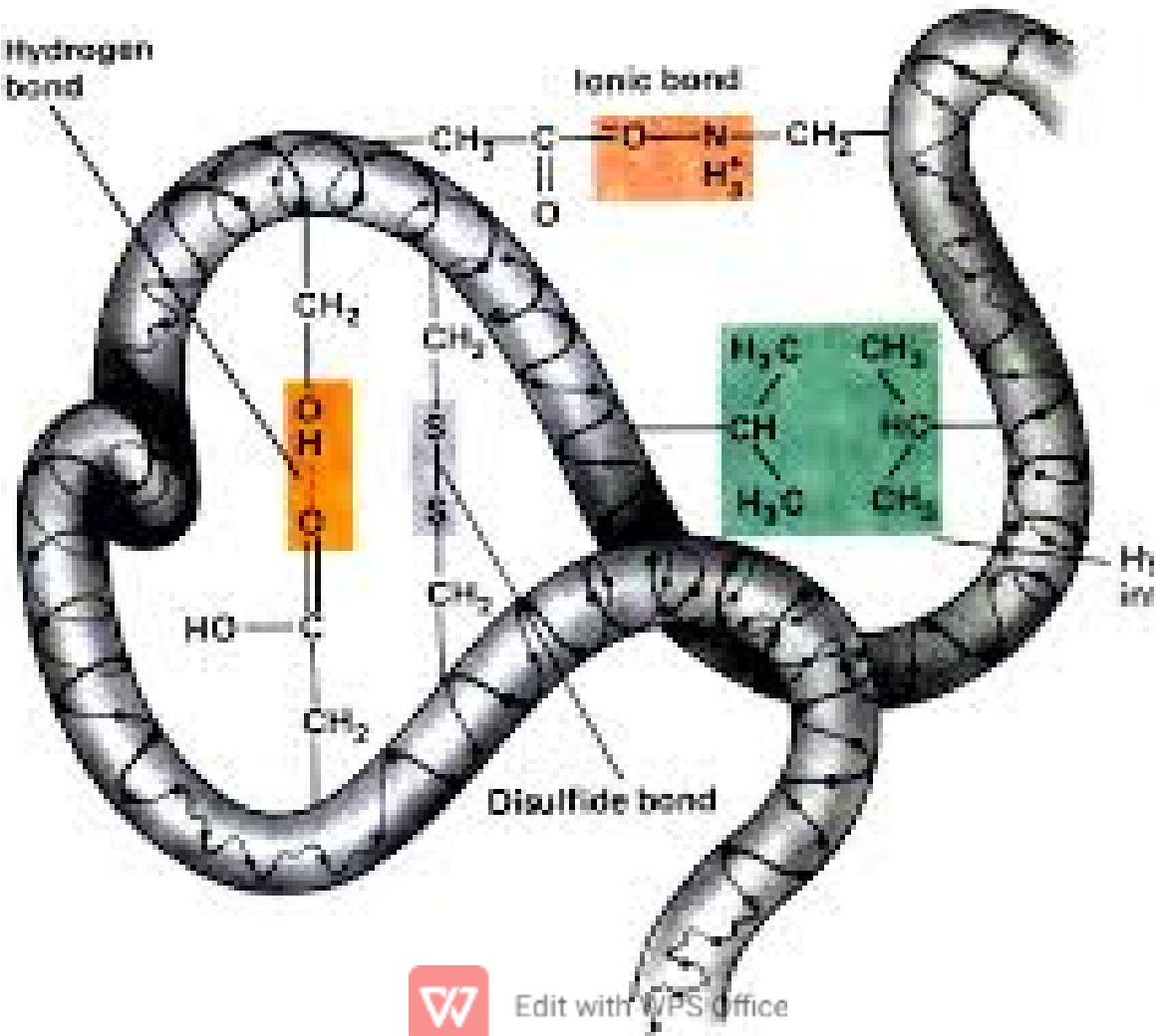
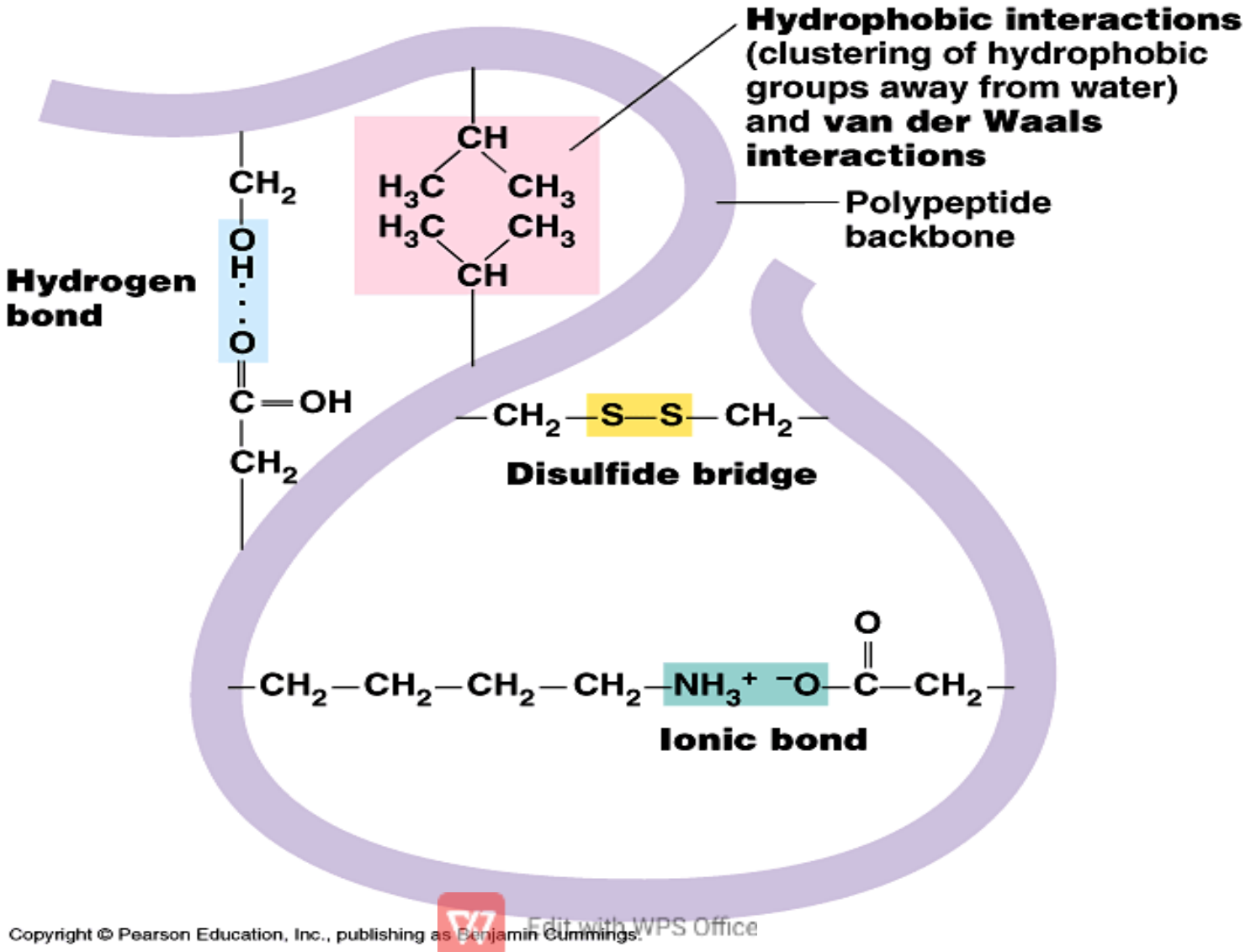


Figure 6-28

Tertiary structure

- 3-D structure of the whole protein
- Steric relationship between amino acids far apart in linear sequence but are close together in three dimensional form
- Hydrophobic, Van der waals and electrostatic forces stabilise it
- Tertiary structure forms domains.
- Domain is the functional unit of a globular protein.





Specific structural motifs in some proteins

Protein	Structural motif present
myoglobin	Alpha helix and beta pleated sheets
collagen	Triple helix
keratin	Coiled coil
elastin	No specific motif
Super oxide dismutase	Anti parallel beta pleated sheets

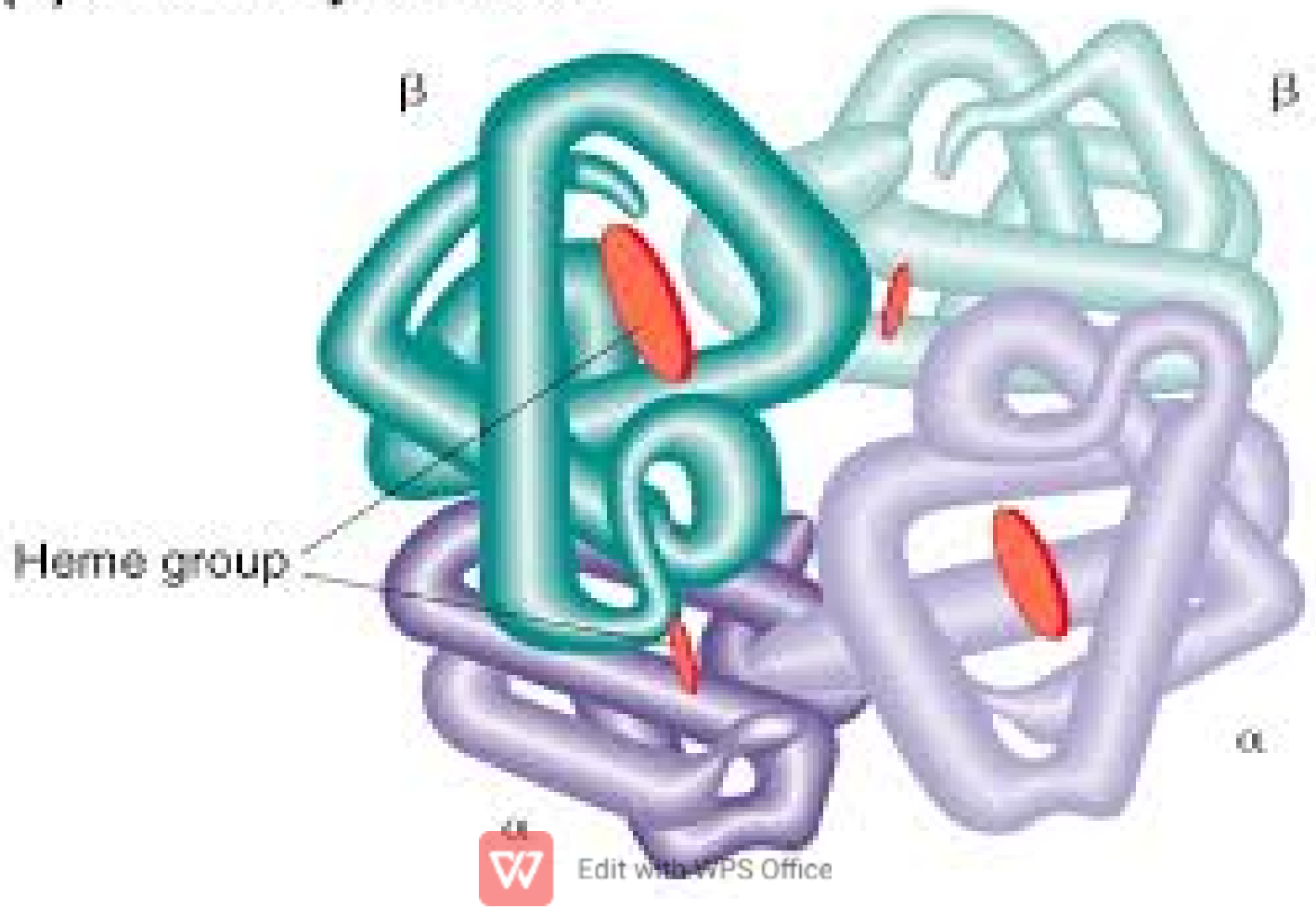
Quarternary structure

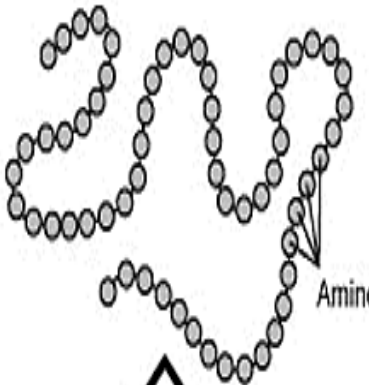
- Polypeptides aggregate to form one protein
- This is called quarternary structure.
- Same forces like secondary structure(H bond, electrostatic, vander waal's, hydrophobic bonds, stabilize it.
- The protein will lose its function if the subunits are dissociated.
- Each polypeptide is called subunit

- Homodimers contain 2 copies of same polypeptide.
- Heterodimers contain different types of polypeptides

- Eg : hemoglobin, immunoglobulin, lactate dehydrogenase enzyme.

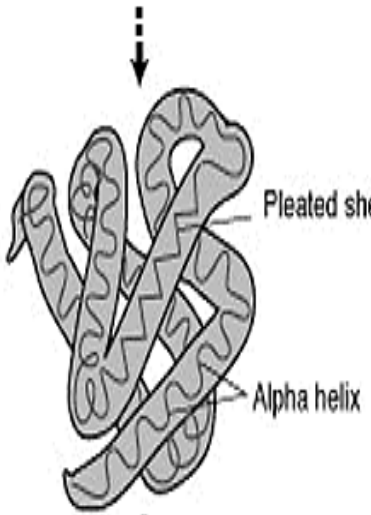
(d) Quaternary structure



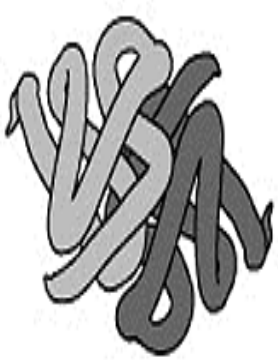


Primary protein structure
is sequence of a chain of amino acids

Secondary protein structure
occurs when the sequence of amino acids are linked by hydrogen bonds

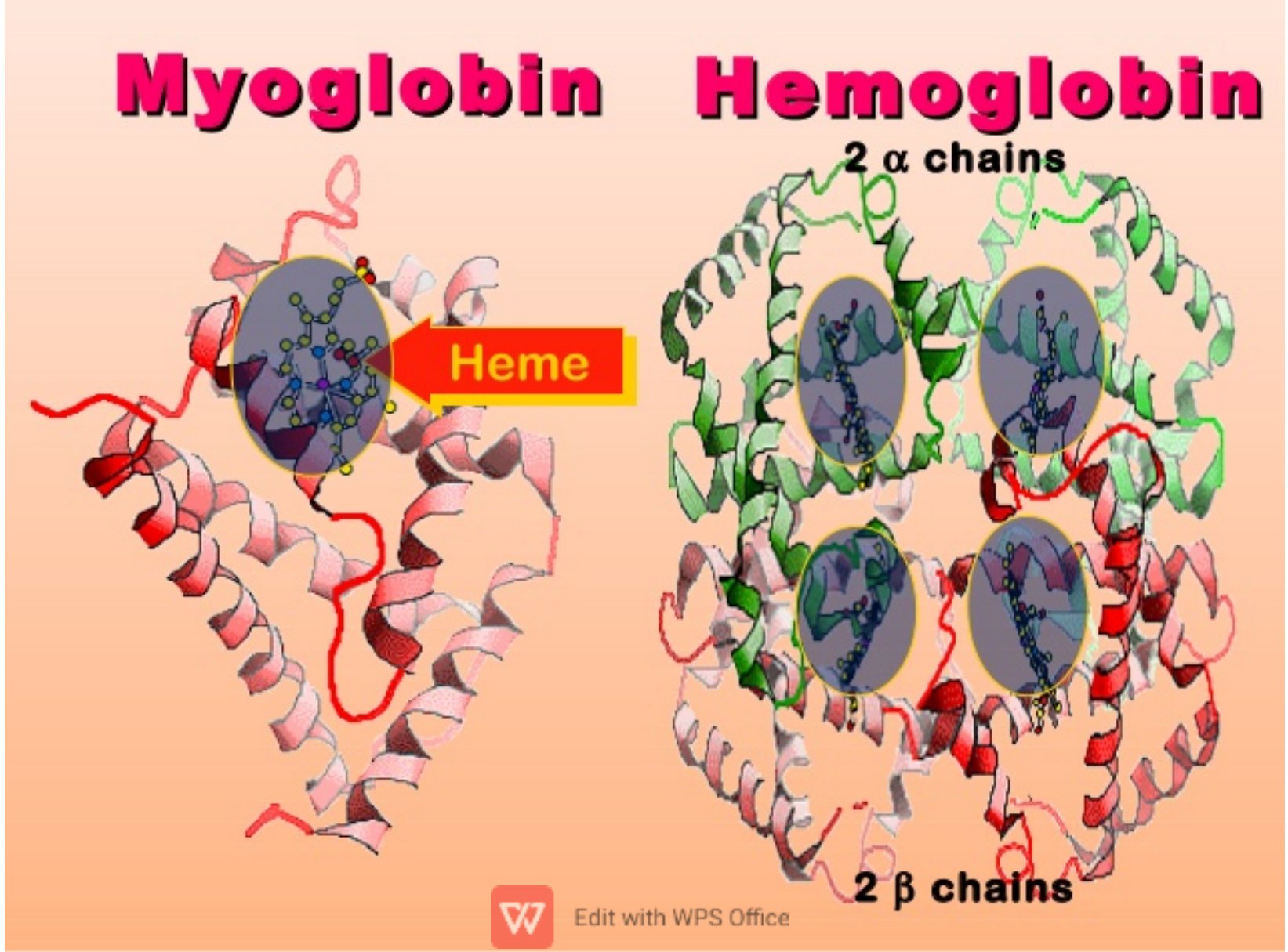


Tertiary protein structure
occurs when certain attractions are present between alpha helices and pleated sheets.



Quaternary protein structure
is a protein consisting of more than one amino acid chain.

	Myoglobin	Hemoglobin
Primary structure	153 amino acids	141 (α-chain) and 146 (β-chain) amino acids
Secondary structure	Eight α-helixes	Eight α-helixes for each α-chain and β-chain
Tertiary structure	Folding of α-helixes	Folding of α-helixes
Quaternary structure	None (monomer)	Tetramer of two identical αβ-dimers
Function	O_2 storage	O_2 delivery


Myoglobin
Hemoglobin
2 α chains
Heme
2 β chains
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Hemoglobin

- It is made of two polypeptide chains alpha and beta.
- The two polypeptide chains in each dimer are held by strong hydrophobic interactions
- The two dimers are held by weak electrostatic and hydrogen bonds
- This helps the dimers to move against each other and form Taut (deoxyhemoglobin) and relaxed states(oxyhemoglobin)

Myoglobin

- Single polypeptide chain
- 8 helices
- Heme is linked to this single chain

- Stores oxygen in skeletal and cardiac muscles

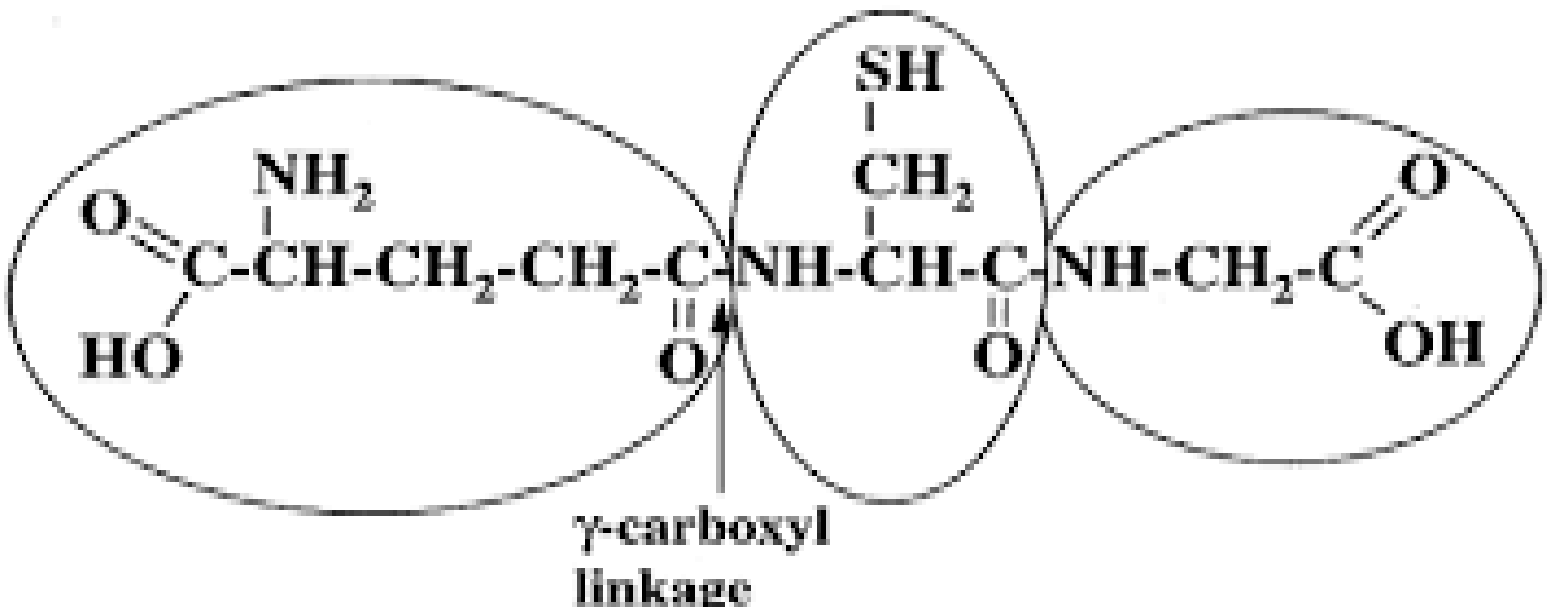
- Do not exhibit cooperativity

Biologically important peptides

Name	Nature of peptide	Biological function
Angiotensin II	Octapeptide	Stimulates release of aldosterone
Gastrin	17 aminoacid	HCl and pepsin secretion in stomach
Vasopressin	Nonapeptide	Increase water reabsorption in DCT
Oxytocin	Nonapeptide	Increase contraction of uterus, milk secretion
Enkephalin	Pentapeptide	Inhibits pathway of pain
Substance P	Pentapeptide	Neurotransmitter

Name	Nature of peptide	functions
Bradykinin	Nonapeptide	vasodilator
Atrial natriuretic peptide	28 amino acids	stimulates excretion of Na in urine
Insulin	2 peptide chains	Regulation of blood glucose

GLUTATHIONE



Glutathione

- Tripeptide having a pseudopeptide bond
- Gamma glutamylcysteinylglycine

- Functions:
  1. Antioxidant: Acts in free radical scavenging reactions.
  2. Detoxification by conjugation.
  3. Activation of some enzymes
  4. Absorption of aminoacids: Meister cycle.
  5. Inactivation of insulin

Physical properties of proteins

- Colloidal properties: scattering of light and exert osmotic pressure.

- Shape: fibrous or globular: length :width ratio >10 it is fibrous protein.

- Isoelectric pH:

1.Net charge on protein will be zero.

2.Aminoacid composition determines pI.

3.No movement in electric field

4. On acidic side proteins are cationic, on basic side they are anionic.

Precipitation reactions

- Polar groups of aminoacids in a protein tend to attract water molecules around them to produce a shell of hydration.
- Neutralising the charge or removing the water of hydration will cause precipitation.
- Salting out: when a salt like ammonium sulphate is added shell of hydration is removed.
- Higher the molecular weight of protein ,lesser the salt required.

- Globulins will precipitate at half saturation of ammonium sulphate but albumin requires full saturation.

- Isoelectric precipitation: casein ppt at pH of 4.6
- Albumin : 4.7

- Organic solvents like alcohol can precipitate proteins: used as disinfectant.

- Heavy metals: in alkaline medium proteins have negative charge, heavy metals can complex with proteins and precipitate them.

- Alkaloidal agents like TCA phosphotungstic acid can also

  precipitate proteins.

Denaturation

- Definition: disruption of the higher order (secondary tertiary and quaternary) structure of a protein is called denaturation.
- Primary structure is not altered because it is covalent bond. (peptide bond)
- Denaturation causes loss of biological activity of proteins

- Generally irreversible

- Example of reversible denaturation:

Ribonuclease denatured by 8 M urea can be renatured after removal of urea by dialysis

Changes in physical properties

- Solubility : water soluble proteins become insoluble.
- Precipitation: protein becomes less symmetric so it precipitates
- Optical rotation changes.
- Sedimentation rate and diffusion rate decreases.

Agents causing denaturation

- Heat: 50-80 degrees
- Detergents and organic solvents
- Strong acids and alkalis
- 8 M urea
- Heavy metal ions
- TCA
- Physical agents: X –Rays, UV Rays, shaking.

Coagulation

- Coagulation is a type of irreversible denaturation.

- Coagulation by heating: heat coagulation is used to detect albumin in urine samples

(heat and acetic acid test).

When heated at isoelectric point some proteins will denature irreversibly to cause thick floating coagulam.

Clinical applications

- Heat and acetic acid test to detect albumin in urine samples: albumin is easily coagulated when a urine sample is heated in a test tube.

- This can be used to detect proteinuria in nephrotic syndrome.

- Detection of Bence Jones protein in multiple myeloma.
- When collagen is heated gelatin is obtained

Classification of Some Proteins and their Functions

Class of Protein	Function in the body	Examples
Structural	Provide structural components	Collagen Keratin
Contractile	Move muscles	Myosin Actin
Transport	Carry essential substances throughout the body	Hemoglobin Lipoprotein
Storage	Store nutrients	Casein Ferritin
Hormone	Regulate body metabolism and nervous system	Insulin Growth hormone
Enzyme	Catalyze biochemical reactions in the cells	Sucrase Trypsin
Protection	Recognize and destroy foreign substances	immunoglobulins

	Fibrous	Globular
Molecules	Long , thin Lie side by side to form fibres.	Fold into spherical 3-D shape.
Examples	- Keratin (in hair) - Collagen (in skin and bone).	- Haemoglobin - Insulin - Enzymes
Solubility in water	Insoluble	Soluble
Roles	Structural: - Collagen in bone and cartilage - Keratin in fingernails and hair.	Metabolic - Enzymes in all organisms, - Plasma proteins, antibodies in mammals.

Based on composition and solubility

- Simple proteins: contain only amino acids: albumin, globulins, protamines, lectins

- Conjugated proteins: they have a prosthetic group: glycoproteins, lipoproteins, chromoproteins, metalloproteins, phosphoproteins

- Derived proteins: degradation products of native proteins: gelatin

Based on Nutritional value

- Complete protein: casein (milk), egg albumin.
- Incomplete proteins: cereals lack lysine, pulses are deficient in methionine.
- Poor proteins: lacks many amino acids: Zein from corn lacks tryptophan and lysine.

DIGESTION AND ABSORPTION OF PROTEINS

- Unlike carbohydrates and fats, proteins are not stored in body.
- Dietary proteins are denatured by cooking.
- Enzymes that help in digestion of proteins are called proteolytic enzymes
- Proteolytic enzymes are secreted as zymogens (inactive form)

- Zymogens are converted to their active forms in the stomach and intestinal lumen
- This prevents autodigestion of the secretory cells.
- Proteins are made up of amino acids linked by peptide bond.
- So digestive enzymes are also called peptidases

Types of proteolytic enzymes

- Endopeptidase :These are the enzymes that act on peptide bond inside the protein molecule

- Exopeptidase: These are the enzymes that act on peptide bonds located at the ends of polypeptide chain. It includes:

Carboxypeptidase: Acts on the carboxyl end of the protein

Aminopeptidase : acts on the amino end of the protein.

- The digestion of protein takes place in:
- Stomach
- Pancreas
- Intestine

Gastric digestion

- In the stomach HCl is secreted which makes the p H optimum for the action of pepsin

- There are two proteolytic enzymes in the stomach:

- Rennin
- pepsin

- Rennin/chymosin: present in infants for curdling of milk.
-

- Milk protein casein is converted to paracasein.
- Paracasein is further digested by pepsin
- Rennin is absent in adults

- Pepsin: It is secreted by the chief cells of stomach as pepsinogen.

- Removal of 44 amino acids from N terminal end converts it to pepsin.

- Optimal pH for activity is 2.
- It is an endopeptidase
-

SITE OF ACTION

- Pepsin breaks the peptide bonds formed by COO group of phenylalanine, tyrosine, tryptophan, methionine.

- By the action of pepsin proteins are converted to smaller peptides

Pancreatic digestion

- The Optimal pH for activity of pancreatic enzymes is 8
- This is provided by alkaline bile and pancreatic juice.
- Secretion of pancreatic juice is stimulated by a hormone cholecystokinin pancreozymin.

- Pancreatic juice contains Trypsin, chymotrypsin, elastase, carboxypeptidase

All these enzymes are serine proteases.

- These enzymes are secreted as zymogens.

Trypsinogen, chymotrypsinogen and proteoelastase.

This is to prevent protect pancreatic cells from auto digestion

Trypsin

- Trypsinogen is activated by enteropeptidase (enterokinase) present in intestinal membrane.

- Once activated it activates other enzyme molecules.

- Site of action:

Trypsin hydrolysis of bonds between COO groups of arginine and lysine.

Clinical significance

- Acute pancreatitis: premature activation of trypsinogen inside the pancreas itself will result in autodigestion of the pancreatic cells.

- This is a life threatening condition.

Chymotrypsin

- Trypsin acts on chymotrypsinogen and forms A,B,C peptides
- Assembly of A,B,C peptides forms the active chymotrypsin
- Site of action: it breaks the bond formed by COO ends of phenylalanine, tyrosine, tryptophan, leucine, valine.

Carboxypeptidase:

- Trypsin and chymotrypsin breaks the proteins into smaller peptides.
- They are further digested by carboxypeptidases
- They are secreted by pancreas as procarboxypeptidase and activated by trypsin.
- These are metalloenzymes requiring zinc

- Site of action: Hydrolyses peptides into dipeptides and tripeptides

- Carboxypeptidase A: acts on Carboxy terminal of acidic aminoacids like glutamic acid and aspartic acid

- Carboxypeptidase B: C terminal of basic aminoacids like histidine, arginine, lysine.

Intestinal digestion

- Intestinal juice is called succus entericus.
- It contains the following enzymes:

- 1. Aminopeptidase: releases N terminal aminoacids

- 2. Dipeptidases and 3. Tripeptidases

Action of proteolytic enzymes

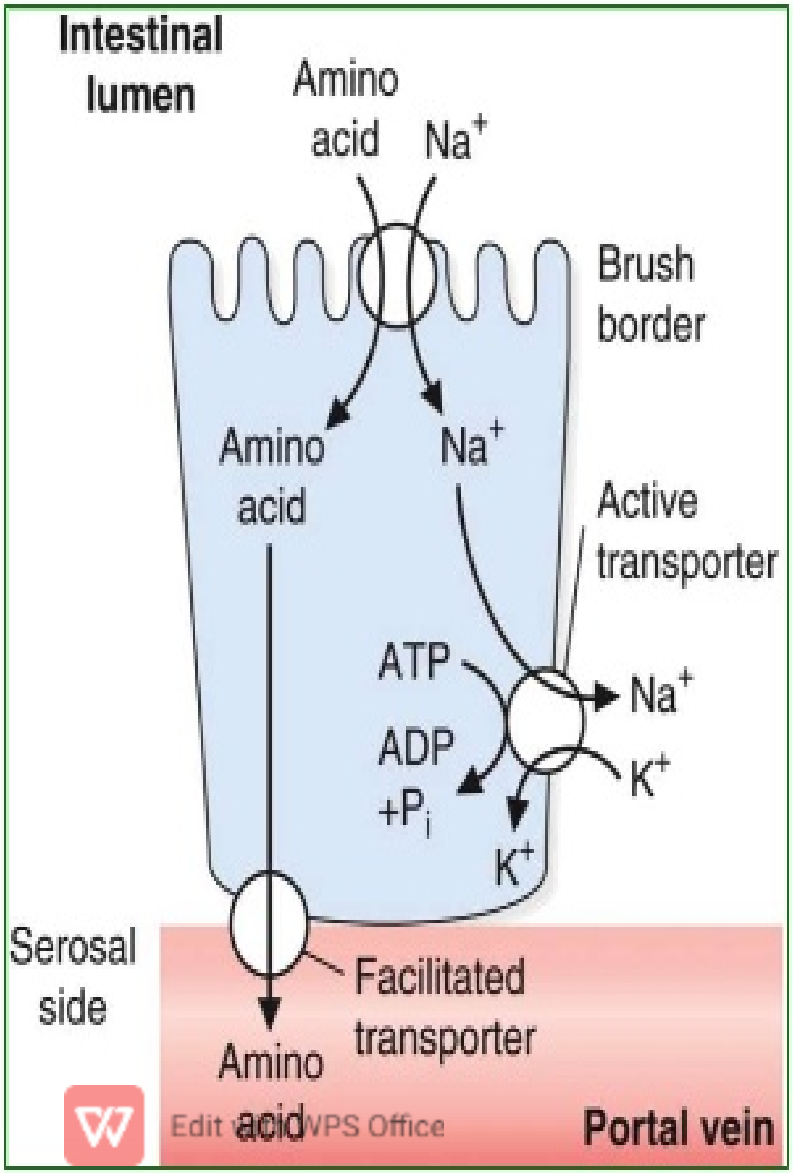
Enzyme	Hydrolysis of bonds formed by carboxyl groups of
Pepsin	Phe, Tyr, Trp, Met
Trypsin	Arg, Lys
Chymotrypsin	Phe, Tyr, Trp, Val, Leu
Elastase	Ala, Gly, Ser
Carboxypeptidase A	C-terminal aromatic amino acid
Carboxypeptidase	C-terminal basic amino acid

Absorption of amino acids

- Occurs mainly in small intestine
- Requires energy from ATP

- The absorption takes place by ATP Na dependent symport

Active transport of Amino acids



- 5 different carriers of amino acids are there:

These are for

1. Neutral aminoacid
2. Basic aminoacid
3. Imino aminoacid
4. Acidic aminoacid
5. Beta aminoacid

MEISTER CYCLE

- Definition: it is a cyclic process for absorption of neutral amino acids. It is also called Gamma glutamyl cycle

- Site: it occurs in intestine, kidney and brain

- Glutathione is required.

Meister cycle process

- Glutathione reacts with aminoacid to form gammaglutamyl aminoacid

- Enzyme required: gammaglutamyl transferase
- Transport of one amino acid requires 3 ATP