PROTEINS

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Introduction

- most abundant biological macromolecules, occurring in all cells and its parts.
- word is derived from Greek "**protos**" meaning first or foremost; indicating the importance of proteins.
- The word "protein" was first used by a Dutch Scientist **G. J, Mulder** in1839.
- Chemically proteins are high molecular weight complex, nitrogenous compounds which are optically active and are made up of amino acid linked in peptide linkage (-CONH).
- Proteins are the molecular instruments through which genetic information is expressed. Enzymes, hormones, antibodies, transporters, muscles, lens protein of eye, feathers, nails, horn, milk proteins etc. are examples of proteins. Among these, enzymes and hormones are the most varied and specialized.

Functions

Proteins are a diverse group of large and complex polymer molecules, made up of long chains of **amino acids**.

They have a wide range of biological roles, including:

- **structural:** proteins are the main component of body tissues, such as muscle, skin, ligaments and hair
- **catalytic:** all enzymes are proteins, catalyzing many biochemical reactions

- **signalling:** many hormones and receptors are proteins
- **immunological:** all antibodies are proteins.

Proteins Come In Many Varieties!

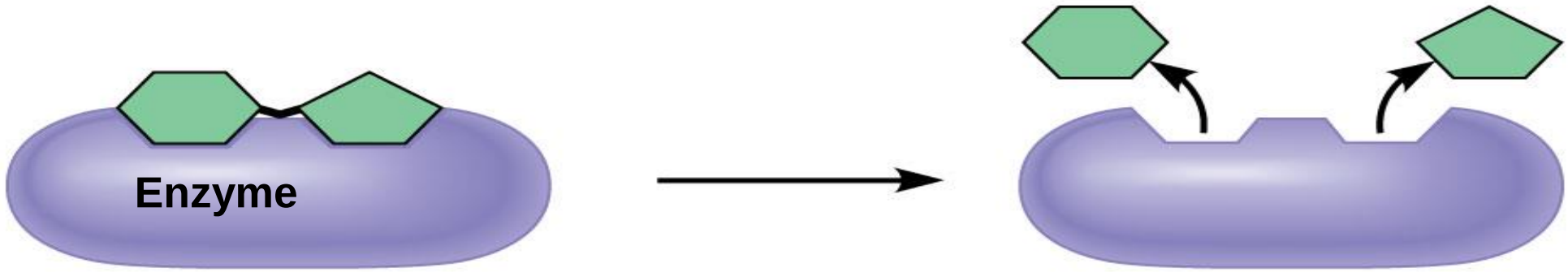
- Proteins include a diversity of structures, resulting in a wide range of functions
- Proteins account for more than 50% of the *dry* mass of most cells
- Protein functions include structural support, storage, transport, cellular communications, movement, and defense against foreign substances

Enzymatic

Enzymatic proteins

Function: Selective acceleration of chemical reactions

Example: Digestive enzymes catalyze the hydrolysis of bonds in food molecules.

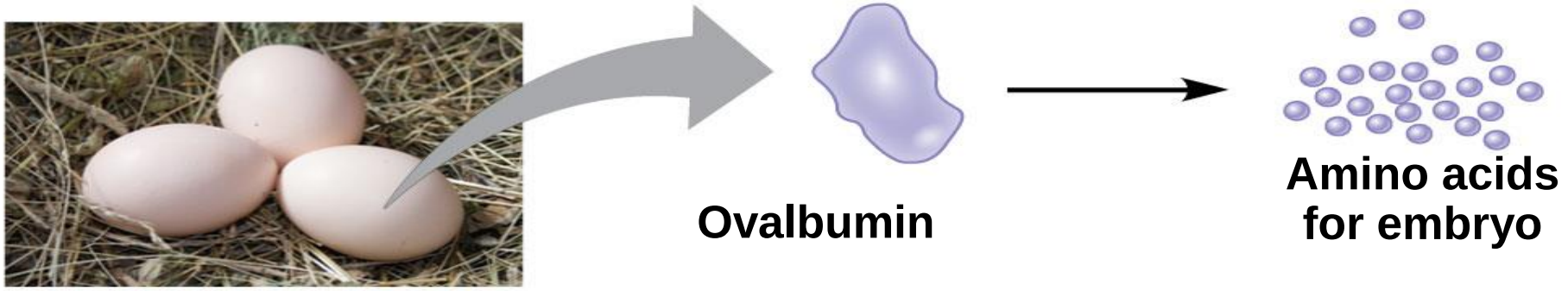


Storage

Storage proteins

Function: Storage of amino acids

Examples: Casein, the protein of milk, is the major source of amino acids for baby mammals. Plants have storage proteins in their seeds. Ovalbumin is the protein of egg white, used as an amino acid source for the developing embryo.

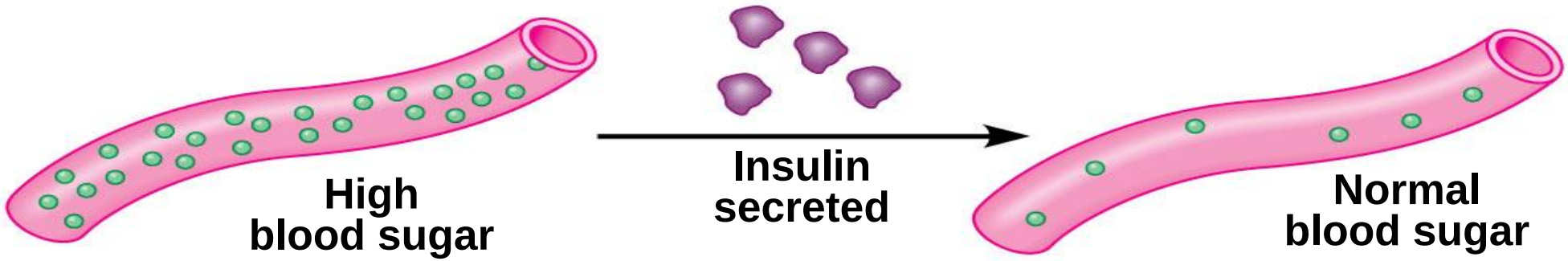


Hormonal

Hormonal proteins

Function: Coordination of an organism's activities

Example: Insulin, a hormone secreted by the pancreas, causes other tissues to take up glucose, thus regulating blood sugar concentration

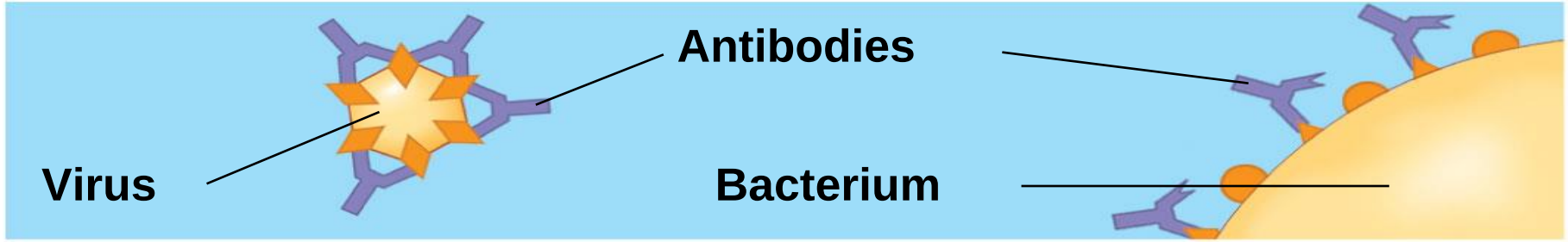


Defensive

Defensive proteins

Function: Protection against disease

Example: Antibodies inactivate and help destroy viruses and bacteria.

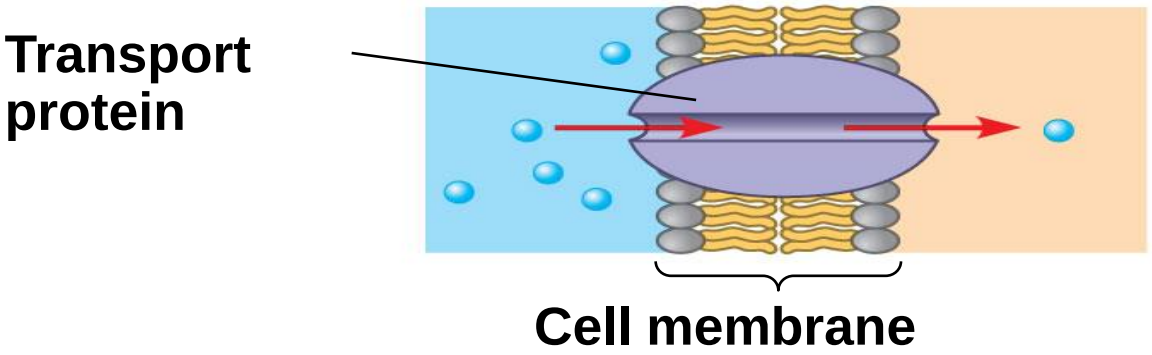


Transport

Transport proteins

Function: Transport of substances

Examples: Hemoglobin, the iron-containing protein of vertebrate blood, transports oxygen from the lungs to other parts of the body. Other proteins transport molecules across cell membranes.

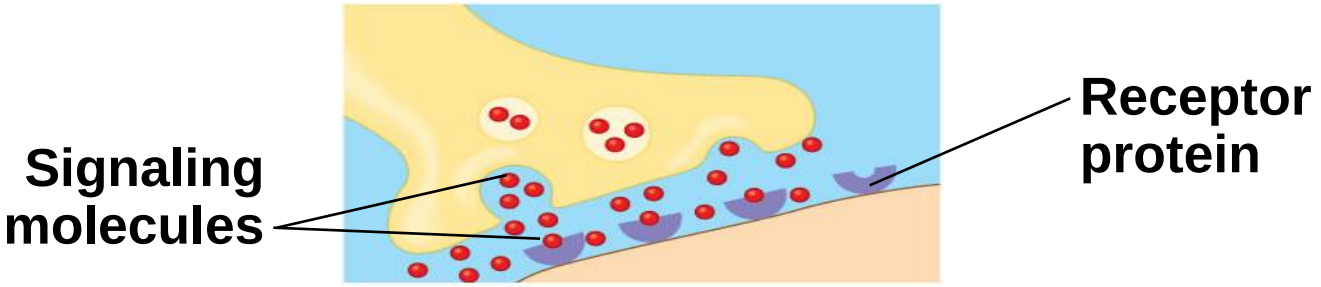


Receptor

Receptor proteins

Function: Response of cell to chemical stimuli

Example: Receptors built into the membrane of a nerve cell detect signaling molecules released by other nerve cells.

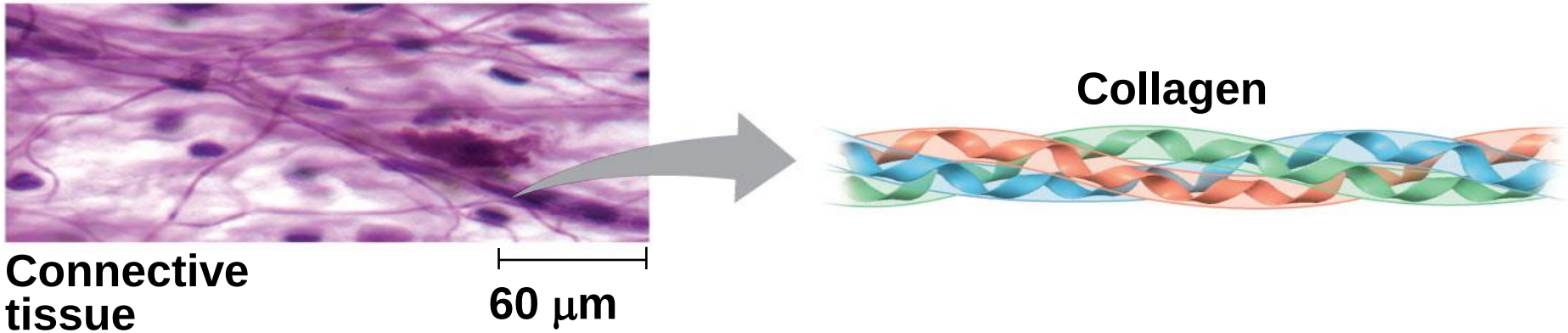


Structural

Structural proteins

Function: Support

Examples: Keratin is the protein of hair, horns, feathers, and other skin appendages. Insects and spiders use silk fibers to make their cocoons and webs, respectively. Collagen and elastin proteins provide a fibrous framework in animal connective tissues.



GENERAL PROPERTIES OF PROTEINS

- polymer of amino acids united in peptide linkage.
- contain C, H, O, N and sometimes P & S. Iron, Cu, I & Zn are occasionally present.
- MW high. Due to their higher MW, they do not pass from membranes like cellophanes.
- generally soluble in water, weak salt solutions, dilute acids and alkalies.
- Owing to their large size, they form colloidal solution and exhibit its properties.
- possess free ionic groups, so they migrate in an electric field.
- They can be precipitated from solution
- They are amphoteric substances as they have -COOH and $–NH_2$ groups. Thus they can react with both acids and alkalies.
- Certain proteins are heat coagulable like albumin and globulin.

CLASSIFICATION OF PROTEINS

Classification of Proteins

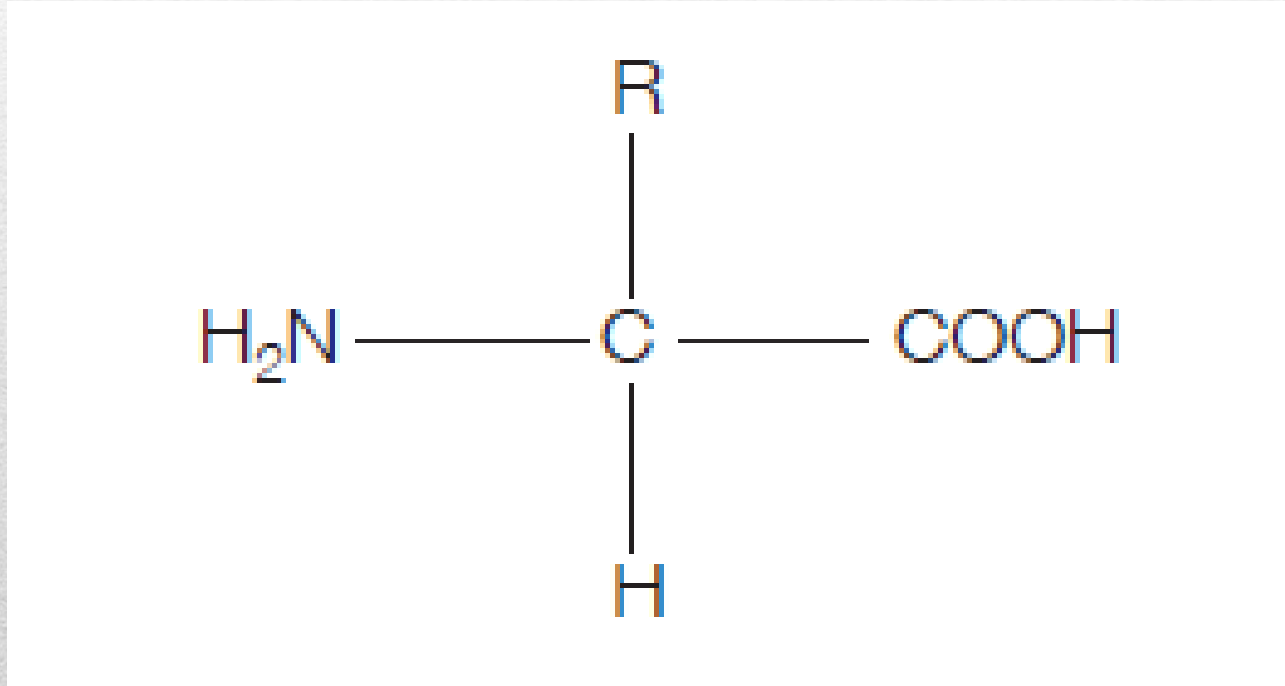
Based on hydrolysis

- **Simple**: Hydrolyzed to amino acids only
- **Conjugated**: Bonded to a nonprotein group, such as sugar, nucleic acid, or lipid
- **Derived:** derived from above 2 by the action of acids, alkalies or enzymes. Generally they are product of partial to complete hydrolysis.

Classification of Proteins

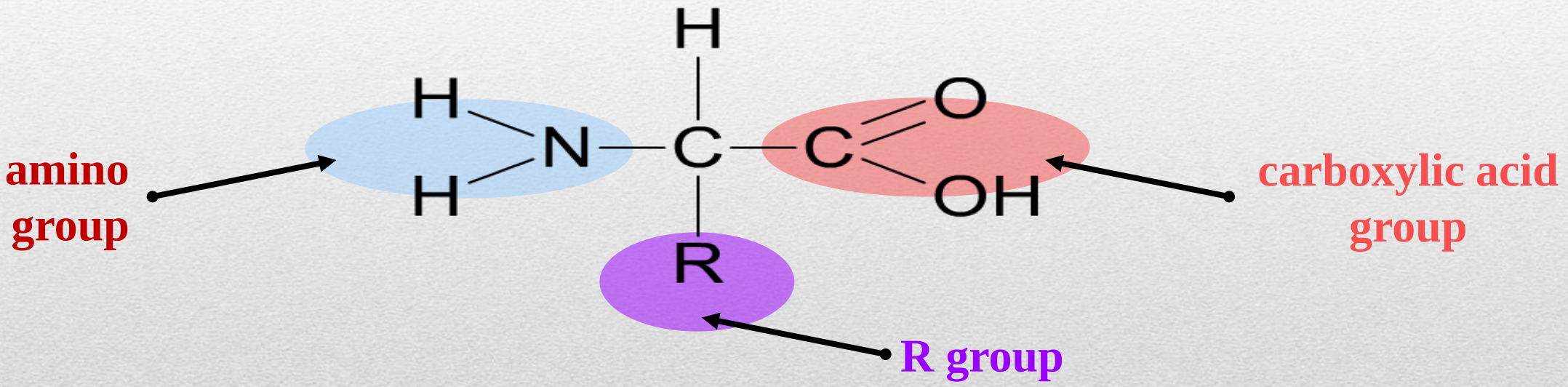
- **Based on hydrolysis (Axial Ratio)**
- **Fibrous**: Long, stringy filaments, insoluble in water; function as structure. Axial ratio >10
- **Globular**: Folded into spherical shape; function as enzymes, hormones, or transport proteins. Axial ratio < /=10

Amino Acids



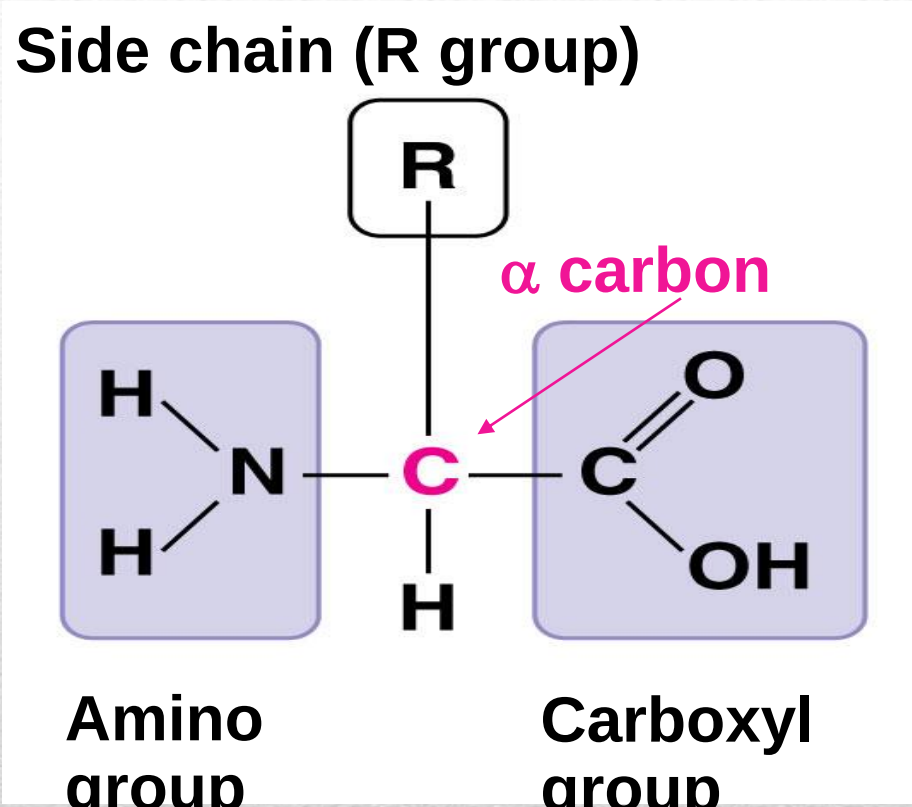
The general structure of amino acids

All amino acids have the same general structure: the only difference between each one is the nature of the **R group**. The R group therefore defines an amino acid.



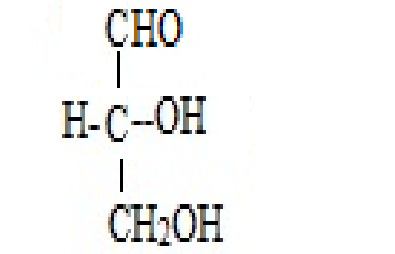
The R group represents a side chain from the central ‘alpha’ carbon atom, and can be anything from a simple hydrogen atom to a more complex ring structure.

- **Amino acids** are organic molecules with carboxyl and amino groups
- Amino acids differ in their properties due to differing side chains, called R groups

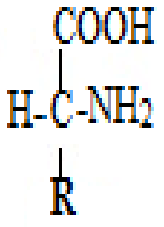


Amino Acids

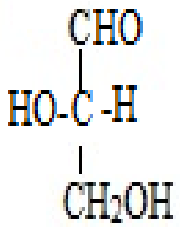
- the α-carbon is bonded to four different group: a carboxylic acid (**-COOH**), an amino (**-NH_2**) group, a hydrogen atom and an R group (except glycine)
- So optically active & exist in two forms: D and L amino acids.
- This D and L forms can be compared with relation to D-Glyceraldehyde as in the case of carbohydrates and as per Fischer convention.
- Most natural amino acids are of "L" series. D occurs in few



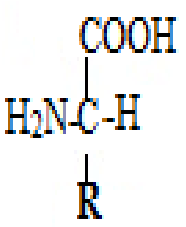
D- GLYCERALDEHYDE



D- AMINO ACID



L- GLYCERALDEHYDE



L- AMINO ACID

Standard Amino Acids

- Twenty standard alpha-amino acids
- Differ in side-chain characteristics:
  - —H or alkyl
  - Contains an —OH
  - Contains sulfur
  - Contains a nonbasic nitrogen
  - Has —COOH
  - Has a basic nitrogen

- alanine - ala - A
- arginine - arg - R ***
- asparagine - asn - N
- aspartic acid - asp - D
- cysteine - cys - C
- glutamine - gln - Q
- glutamic acid - glu - E
- glycine - gly - G
- histidine - his - H ***
- isoleucine - ile - I
- leucine - leu - L
- lysine - lys - K
- methionine - met - M
- phenylalanine - phe - F
- proline - pro - P
- serine - ser - S
- threonine - thr - T
- tryptophan - trp - W
- tyrosine - tyr - Y
- valine - val - V

"Essential Amino Acids"
are those that must be ingested in the diet
(our body can't make them)

Essential Amino Acids

- Arginine (Arg)
- Threonine (Thr)
- Lysine (Lys)
- Valine (Val)
- Phenylalanine (Phe)
- Tryptophan (Trp)
- Methionine (Met)
- Histidine (His)
- Leucine (Leu)
- Isoleucine (Ile)

Nomenclature

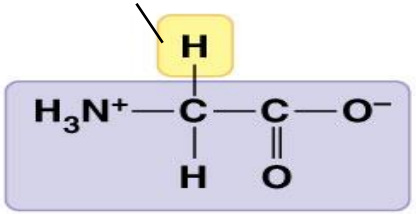
- Common names: names by which we commonly know them. Like: Glycine, asparagine, tyrosine etc
- Trivial names: 3 letter symbol given to AA. Derived from common names. Like: Glycine = Gly
- Single alphabet system: single alphabet allotted to AA, written in caps. Mostly restricted to long sequences. Like: Glycine = G
- IUPAC/ Systemic Names: names written as per their chemical structure as per IUPAC. **Not used in biological system**

Classification of amino acids

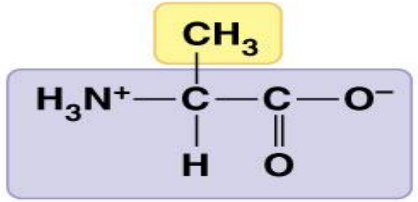
- Based on “R’ group
- 5 groups depending upon their “R” groups—
  - **Amino acids with non polar Aliphatic side chains**
  - **Amino acids with polar uncharged side chains**
  - **Amino acids with aromatic side chains**
  - **Amino acids with Positively charged side chains**
  - **Amino acids with negatively charged side chains**

Non Polar AA (Nonpolar side chains; hydrophobic)

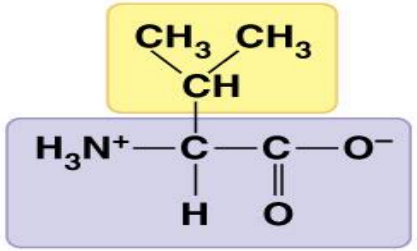
Side chain



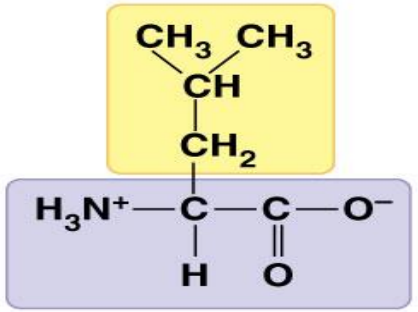
Glycine
(Gly or G)



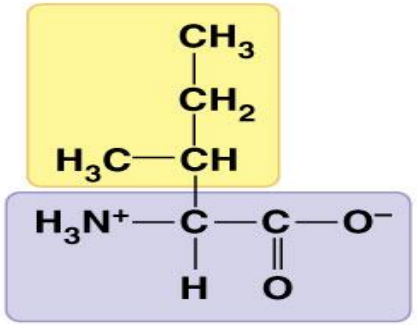
Alanine
(Ala or A)



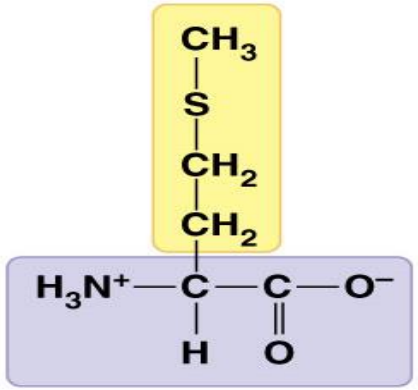
Valine
(Val or V)



Leucine
(Leu or L)

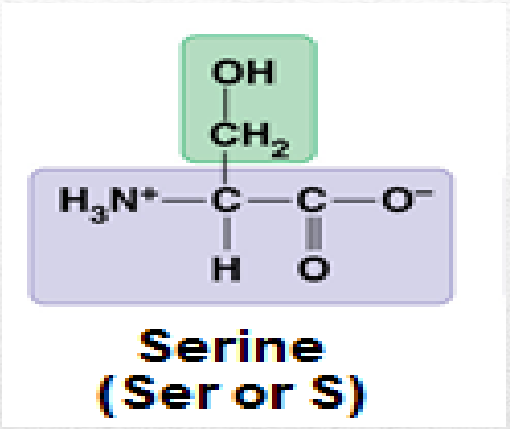


Isoleucine
(Ile or I)

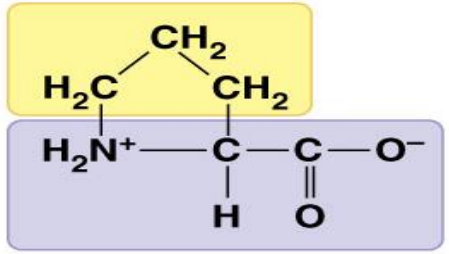


Methionine
(Met or M)

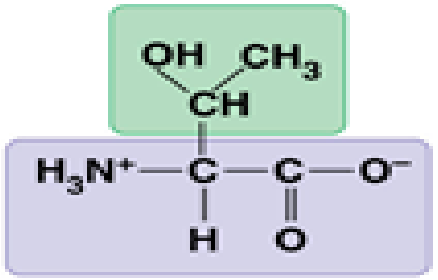
Polar AA (Polar side chain: Hydrophilic)



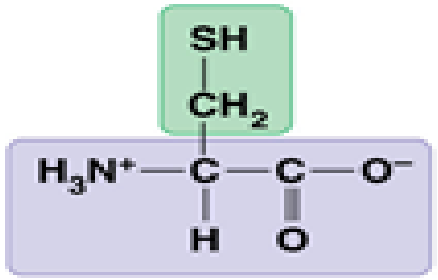
Serine
(Ser or S)



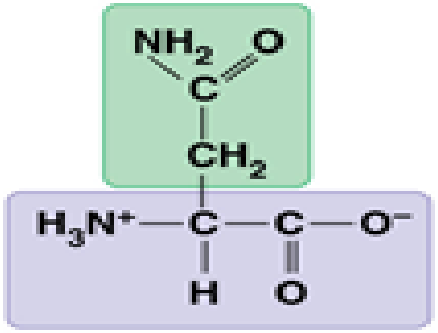
Proline
(Pro or P)



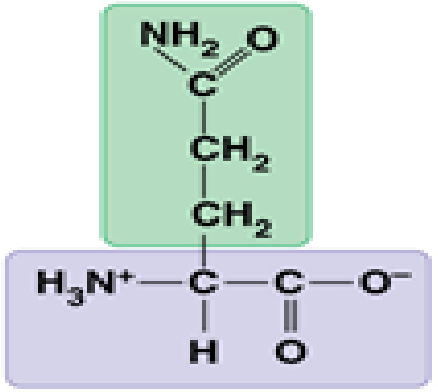
Threonine
(Thr or T)



Cysteine
(Cys or C)

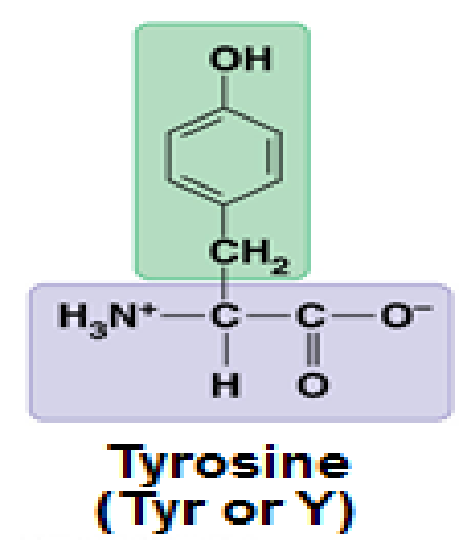


Asparagine
(Asn or N)

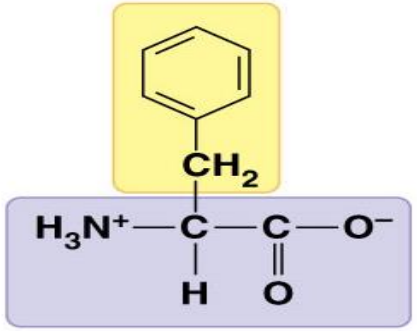


Glutamine
(Gln or Q)

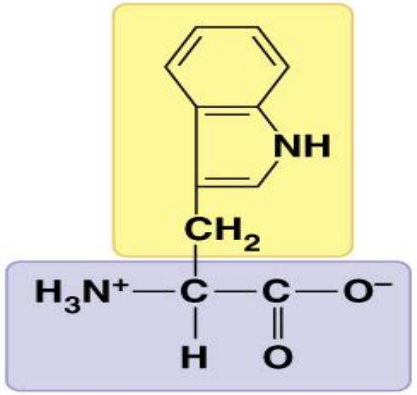
Aromatic Amino Acid: AA with aromatic side chain



Tyrosine
(Tyr or Y)

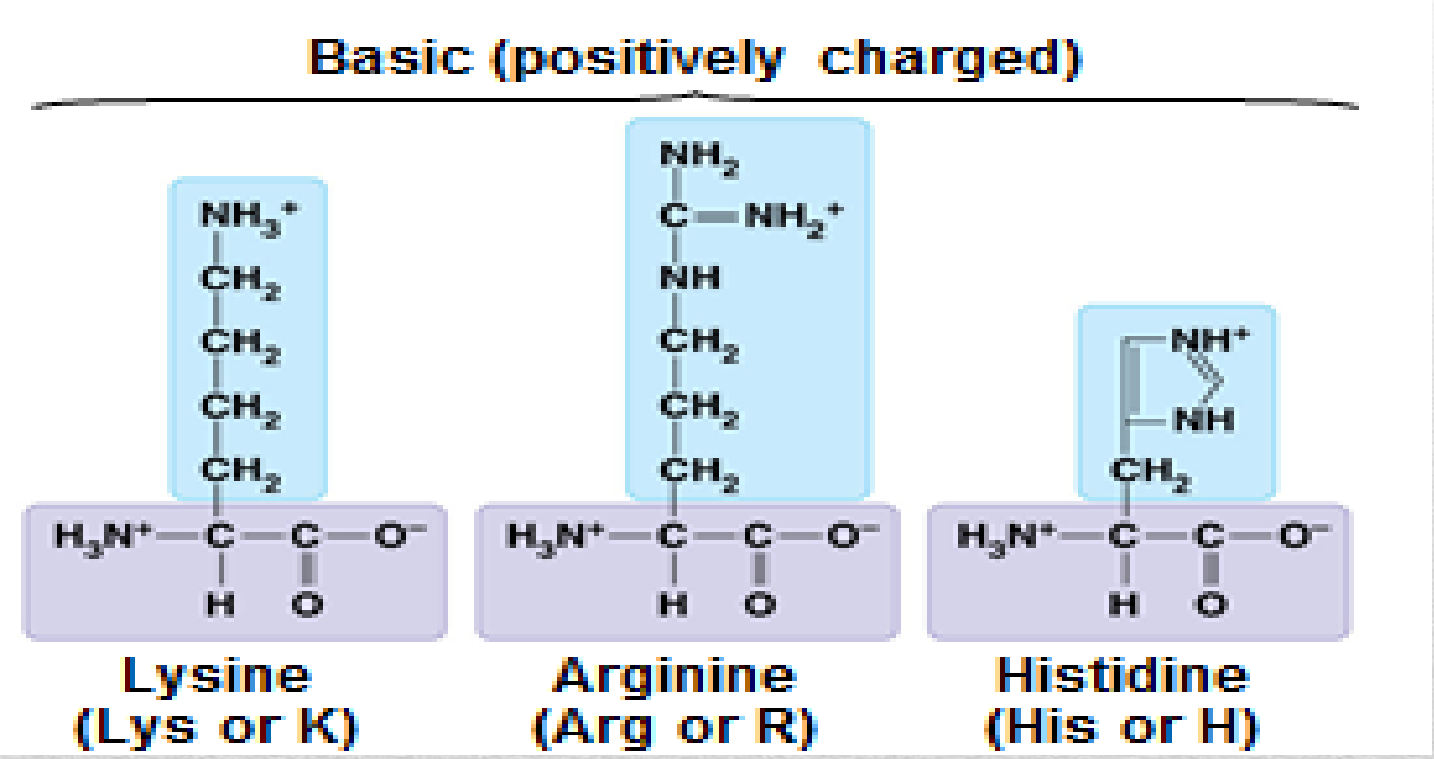


Phenylalanine
(Phe or F)

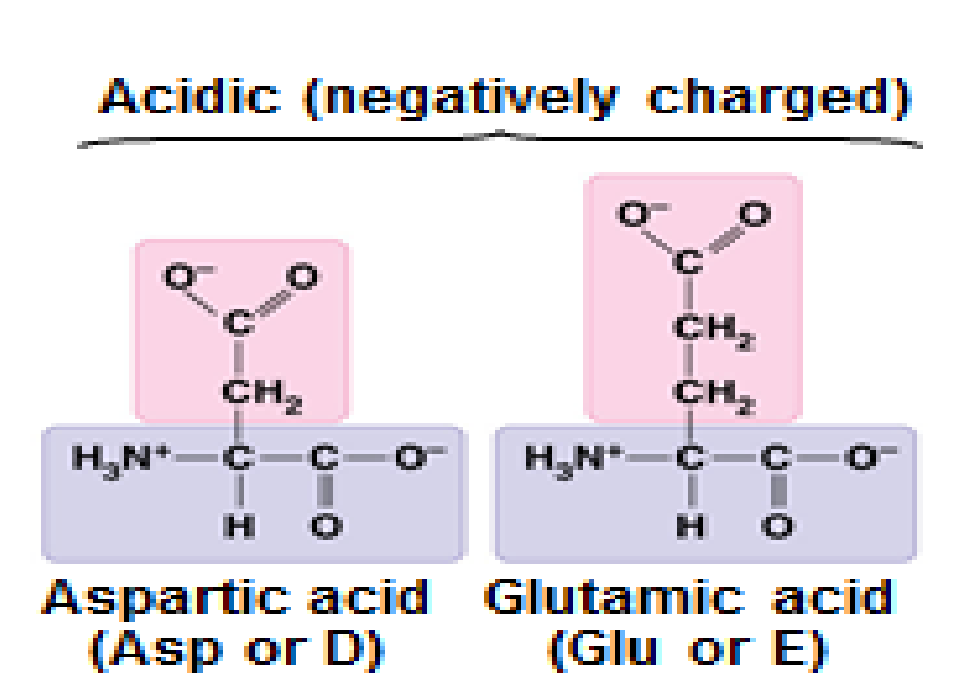


Tryptophan
(Trp or W)

Positively charged AA: Hydrophilic



Negatively charged AA: Hydrophilic



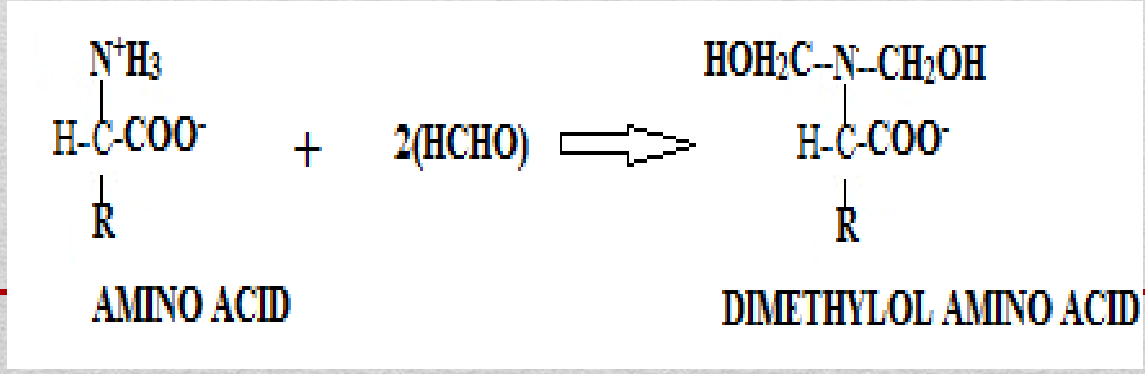
Properties of AA: PHYSICAL

- Generally soluble in water and insoluble in organic solvents.
- They may be tasteless, sweet or bitter. Sodium glutamate is used as a flavouring agent.
- Except glycine, all are optically active.
- are amphoteric substances, as they may have both charges. When an acid is added to amino acids, they assume a positive charge and moves towards cathode in an electric field. Similarly, when an alkali is added, they assume a negative charge and move towards anode. That's why, they have two pKa values. The pH where amino acids exist as "Zwitter ions" is known as **isoelectric point** (pI) and is calculated as the average of both pKa values.

Chemical reactions of AA

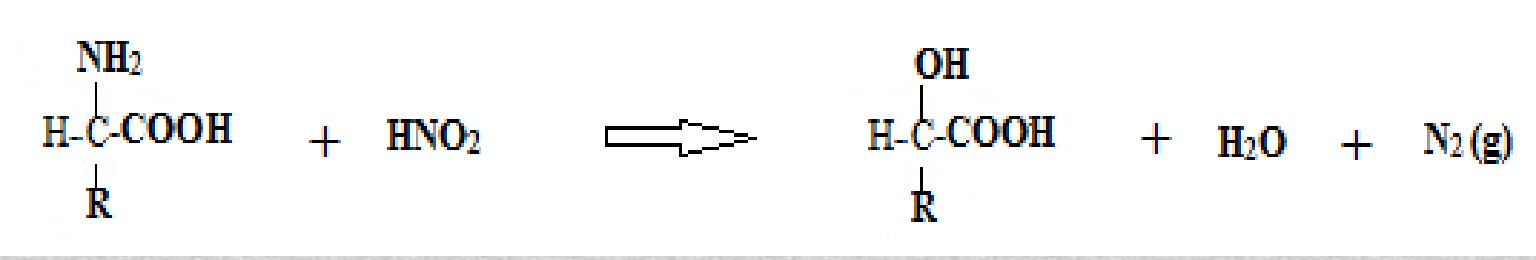
Reaction with formaldehyde

- Amino acids react with formaldehyde & form **Dimethylol amino acid**.
- Due to this the solution of AA becomes acidic due to involvement of amino group.
- amino acids cannot be titrated against alkalies using standard methods as they form neutral solution due to zwitter ion formation.
- After treatment with formaldehyde, it can be titrated with standard alkalies. This was first observed by **Sorenson**, so this method is also known as **Sorenson Formal Titration**.



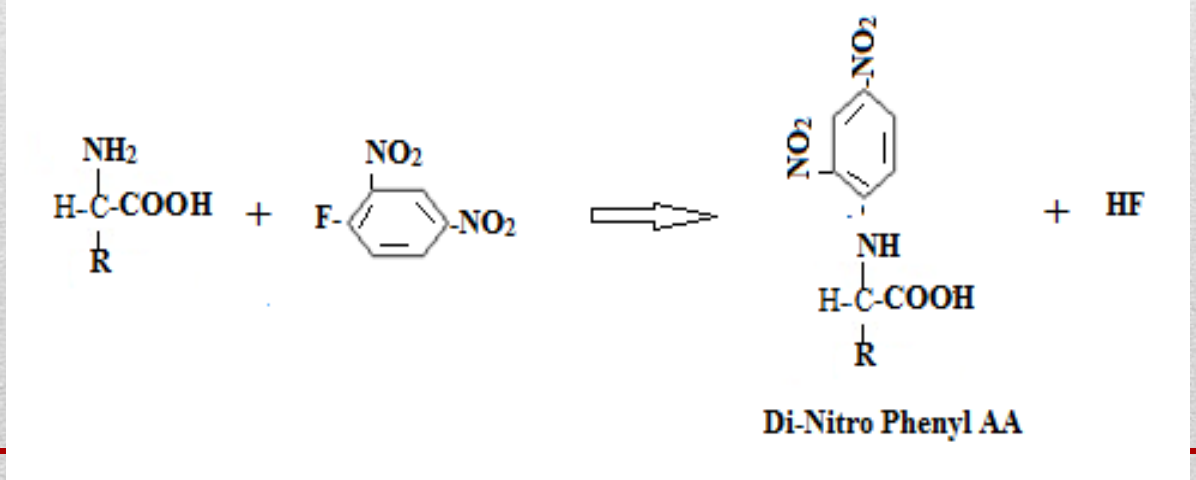
Reaction with Nitrous acid

- Nitrous acid (HNO_2) reacts with amino group of amino acids to form corresponding hydroxyl acid and liberate nitrogen gas.
- This nitrogen gas can be measured volumetrically and one molecule of nitrogen gas is produced by one molecule of amino group. This forms the basis of **Van Slyke's method** of estimation of amino groups in amino acids and proteins.



Reaction with 1-Fluoro-2,4 Dinitrobenzene (FDNB)

- FDNB condenses with a free NH_2 group in cold mild alkaline solution (e.g. sodium bicarbonate) to give Dinitrophenyl amino acid (DNP-AA).



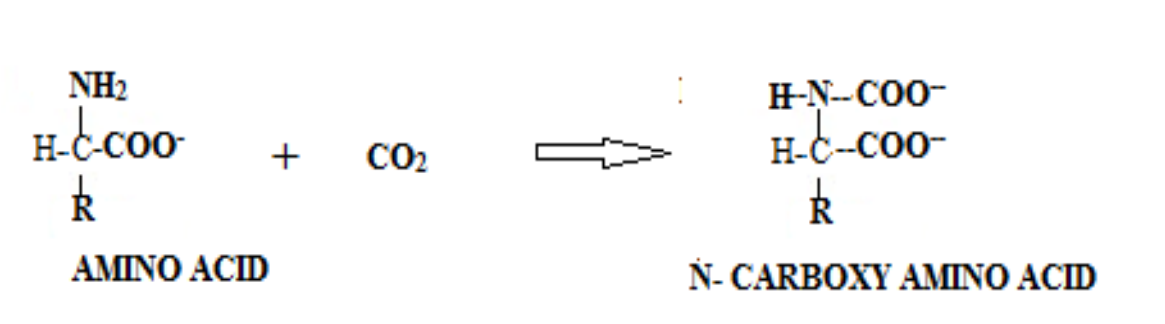
Reaction with 1-Fluoro-2,4 Dinitrobenzene (FDNB)

- DNP-AA are bright yellow in colour and are now soluble in ether.
- Amino acids can be separated from the DNP bond by acid hydrolysis and identified by chromatography.
- **Sanger** used this reagent to identify and sequence Insulin that contain 51 amino acid; so FDNB is also called as Sanger's reagent.

Reaction with Ninhydrin

- When amino acids are boiled with Ninhydrin (Triketo hydrindene hydrate), they are quantitatively deaminated to NH_3 and keto acid by oxidative deamination.
- Carboxylic group of keto acid is spontaneously converted to CO_2 and can be measured. This reaction is specific for release of CO_2.
- In the reaction, ninhydrin is specifically reduced to a compound which condenses with ammonia and more ninhydrin to form a blue coloured compound known as "Ruhemann's Purple."

Reaction with carbon di oxide

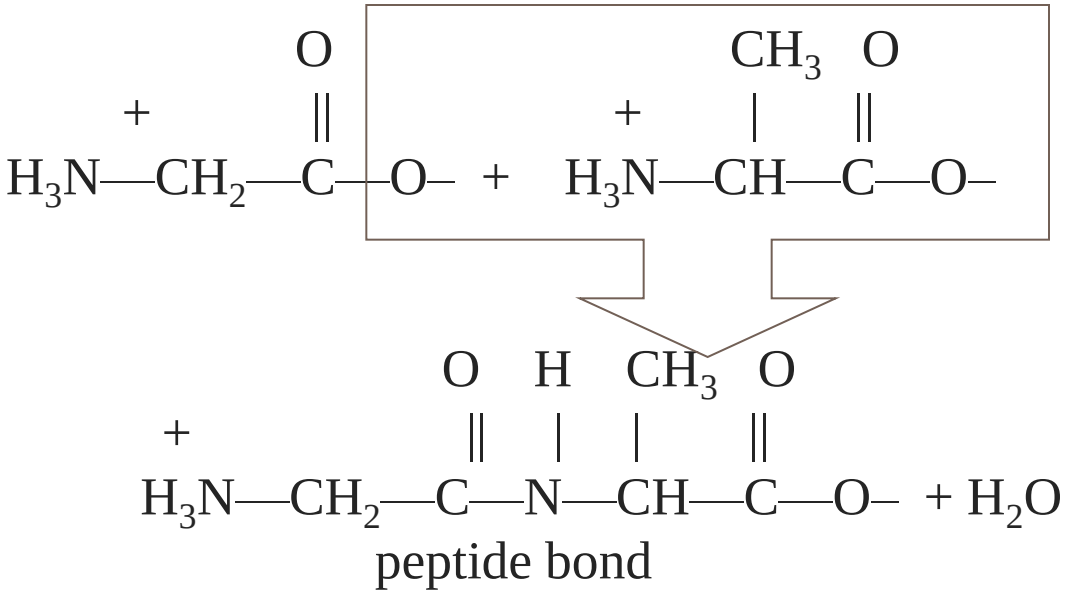


- This reaction is also known as "Siegfried's carbamino reaction.
- When CO_2 is passed through the alkaline solution of an amino acid, the CO_2 is added to the amino group and forms a **carbamino** compound.

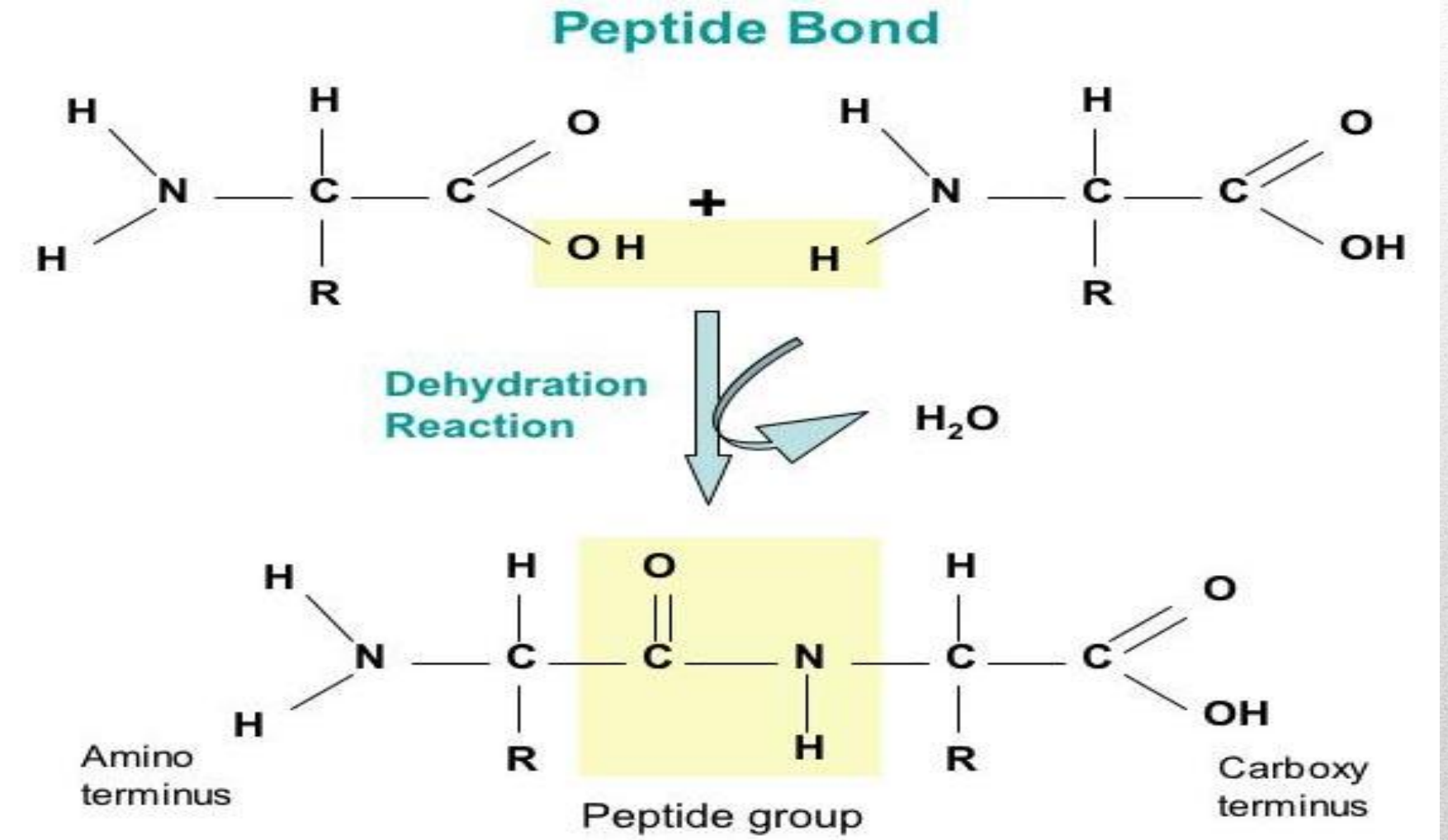
FORMATION OF PEPTIDE BOND

A peptide bond

- Is an amide bond.
- Forms between the carboxyl group of one amino acid and the amino group of the next amino acid.

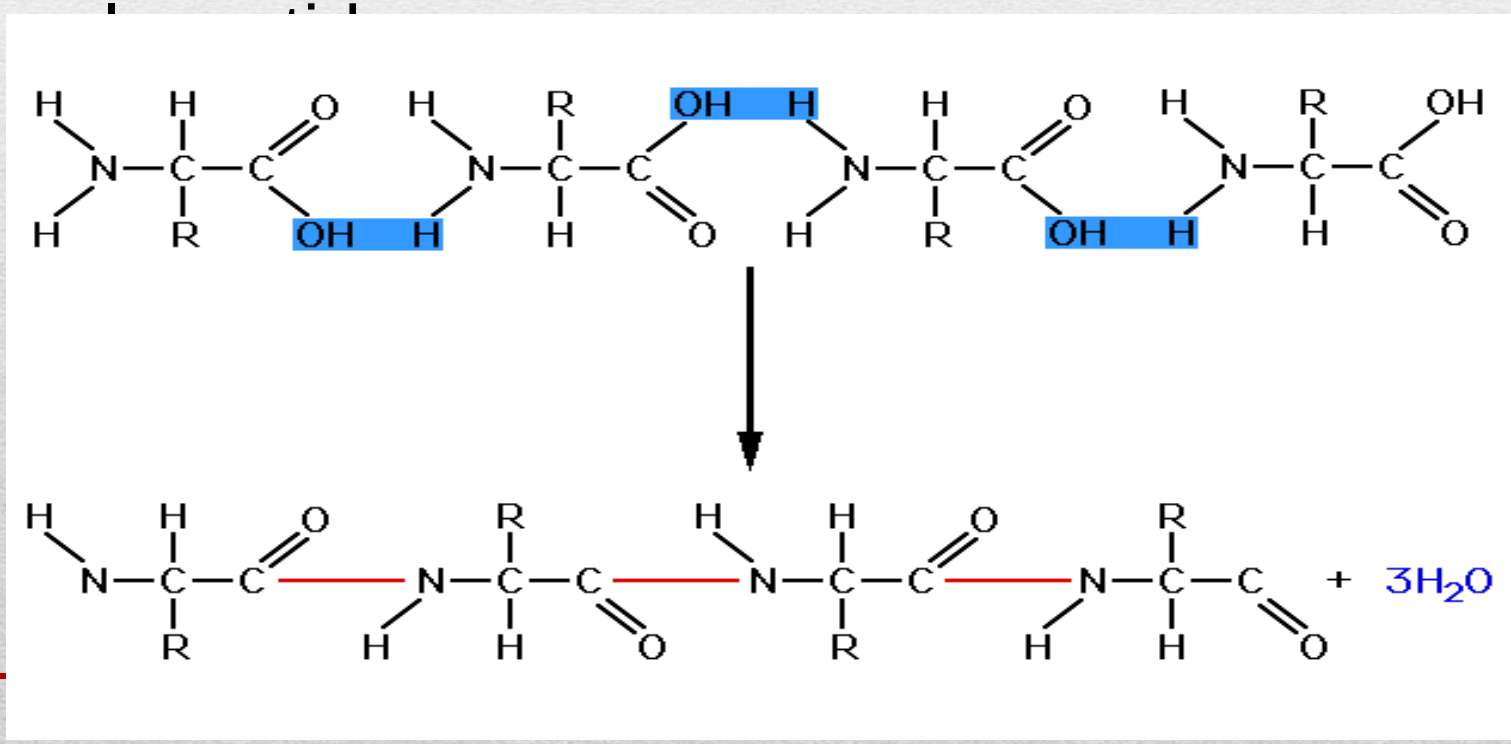


Peptide Bonds



Polypeptides

- **Polypeptides** are unbranched polymers built from the same set of 20 amino acids
- A **protein** is a biologically functional molecule that consists of one or more



Peptide Bonds

- Amino acids are linked by **peptide bonds**
- A polypeptide is a polymer of amino acids
- Polypeptides range in length from a few to more than a thousand monomers (Yikes!)
- Each polypeptide has a unique linear sequence of amino acids, with a carboxyl end (C-terminus) and an amino end (N-terminus)

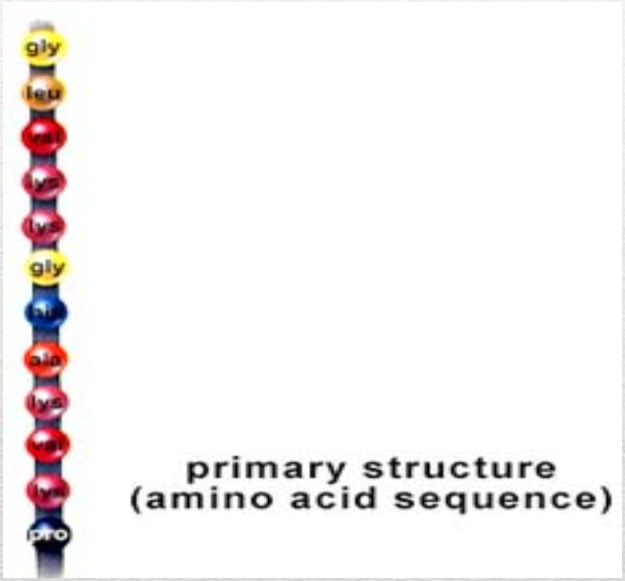
Optical Properties of the Amino Acids

- All AA except glycine have **chiral** carbon; so optical active
- Accordingly they are either **dextrorotatory** or **levorotatory**.
- All of the amino acids in proteins exhibit the same absolute steric configuration as L-glyceraldehyde and are all L-α-amino acids.
- D-amino acids are never found in proteins, although they exist in nature. D-amino acids are often found in polypetide antibiotics.
- The aromatic R-groups in amino acids absorb ultraviolet light with an absorbance maximum in the range of 280nm. The ability of proteins to absorb ultraviolet light is predominantly due to the presence of the tryptophan which strongly absorbs ultraviolet light.

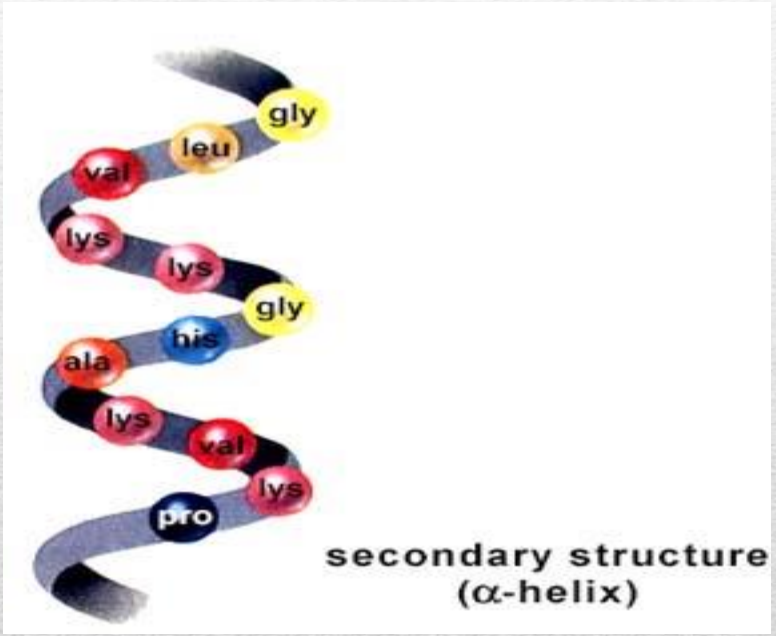
STRUCTURAL ORGANIZATION OF PROTEINS (Protein Structure)

- protein structure is by its shape: **Globular** and **Fibrous**, as we have seen in protein classification.

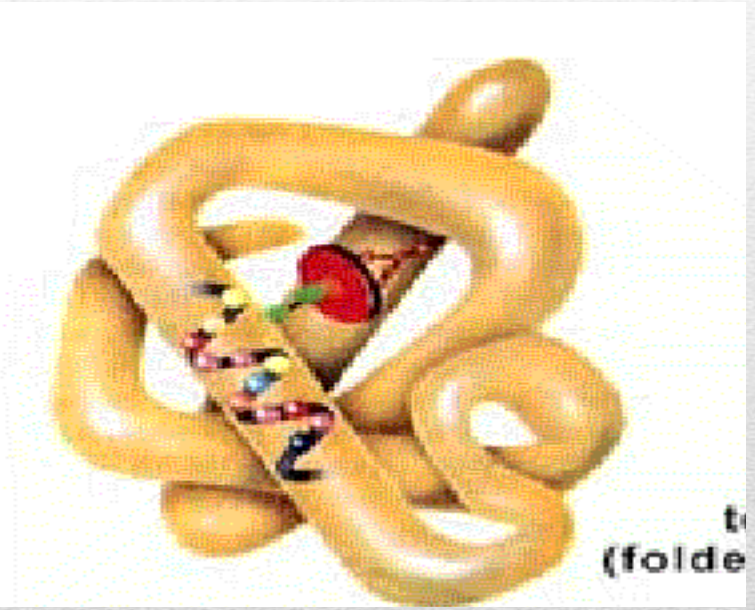
Protein Structure



primary structure
(amino acid sequence)



secondary structure
(α-helix)



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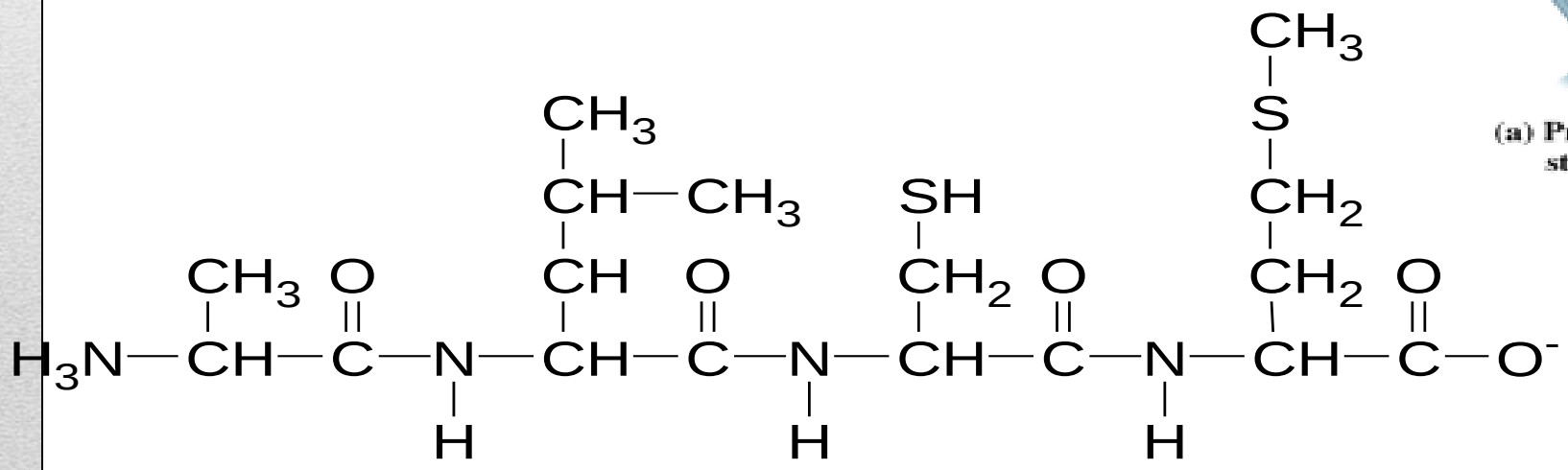
Levels of Organization

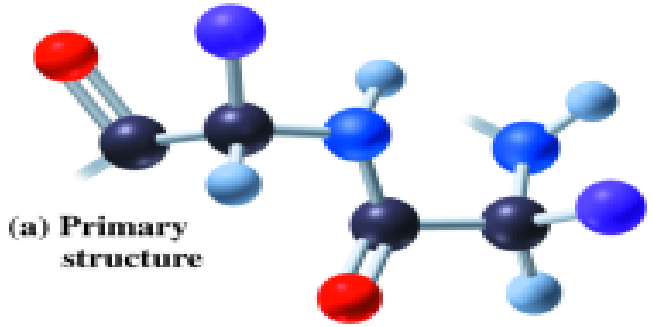
- Primary structure
  - Amino acid sequence of the protein
- Secondary structure
  - H bonds in the peptide chain backbone
    - α-helix and β-sheets
- Tertiary structure
  - Non-covalent interactions between the R groups within the protein
- Quaternary structure
  - Interaction between 2 polypeptide chains

Primary Structure of Proteins

The primary structure of a protein is

- The number & particular sequence of amino acids.
- The backbone of a peptide chain or protein.



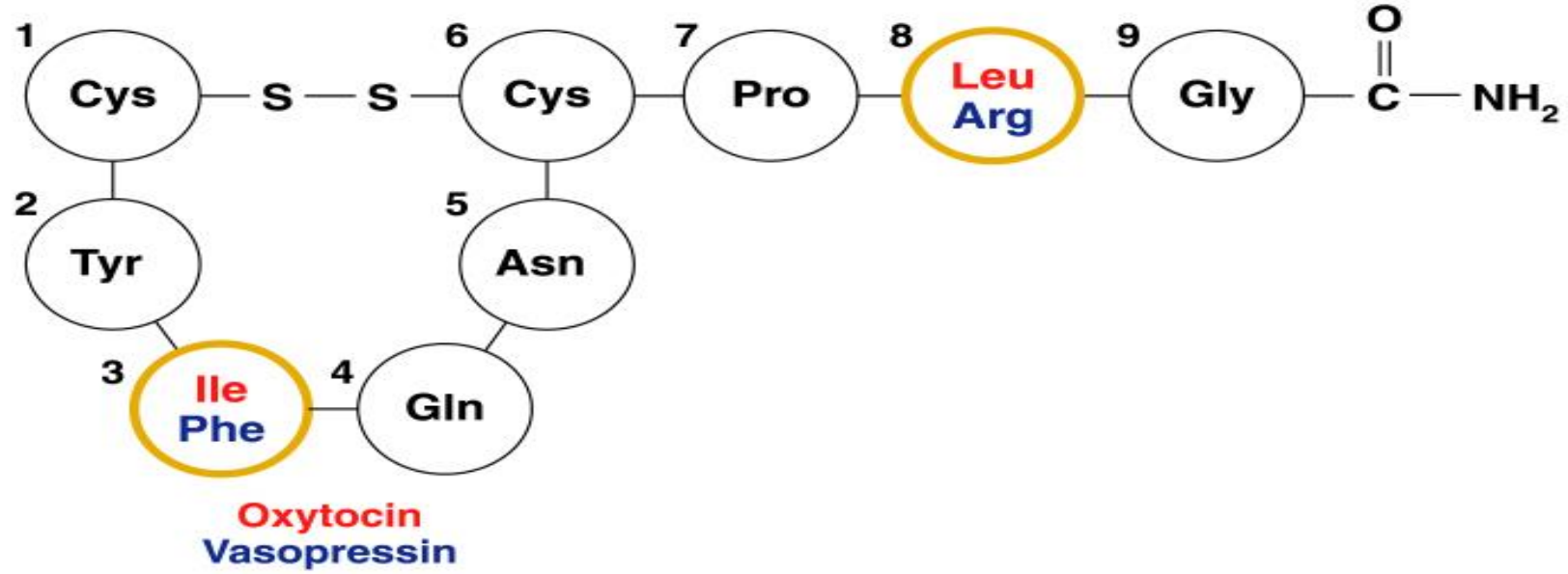


Ala–Leu–Cys–Met

Primary Structures

The nonapeptides oxytocin and vasopressin

- Have similar primary structures.
- Differ only in the amino acids at positions 3 and 8.



Secondary Structure – Alpha Helix

The secondary structures of proteins indicate the

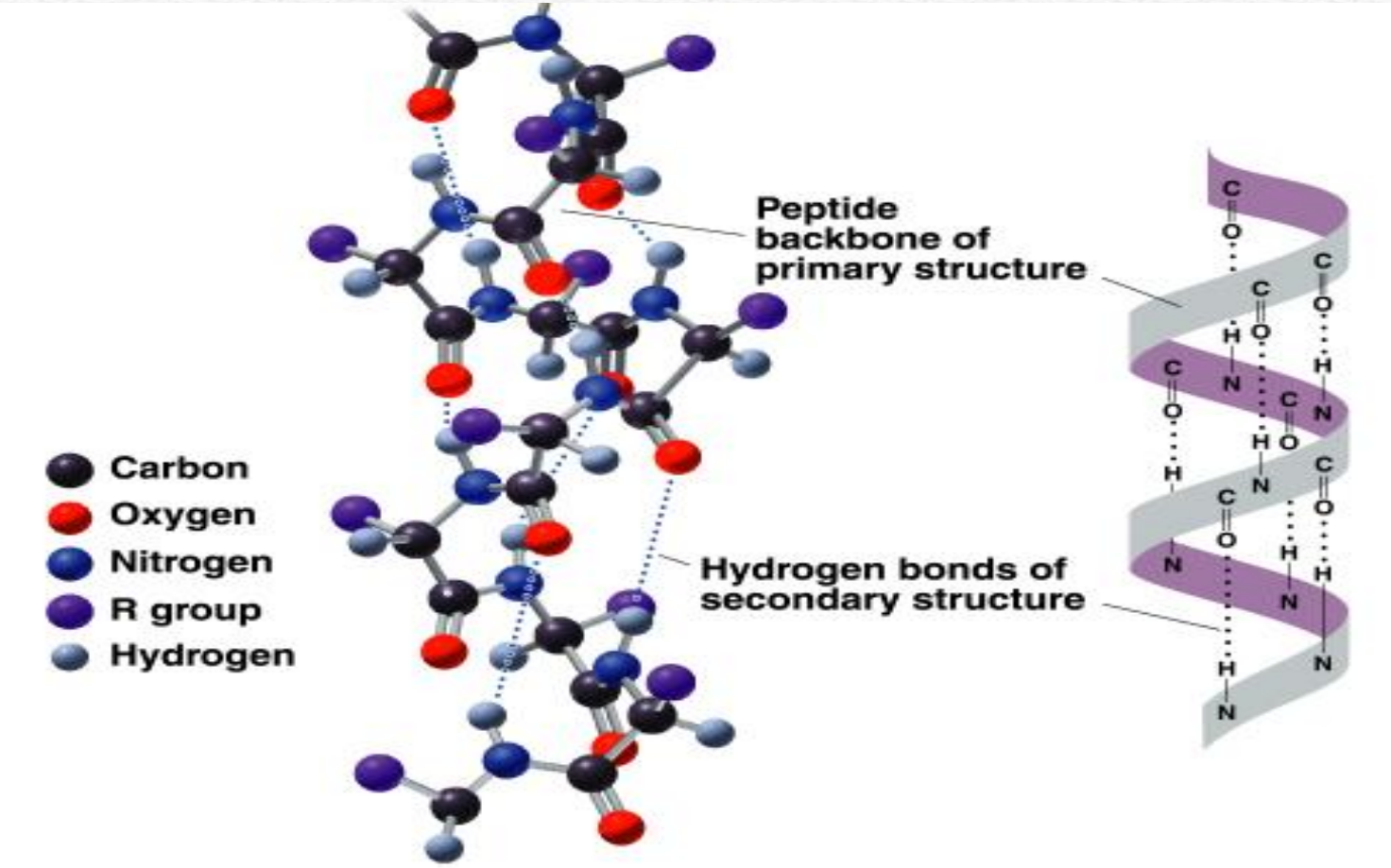
three-dimensional spatial arrangements of the

polypeptide chains.

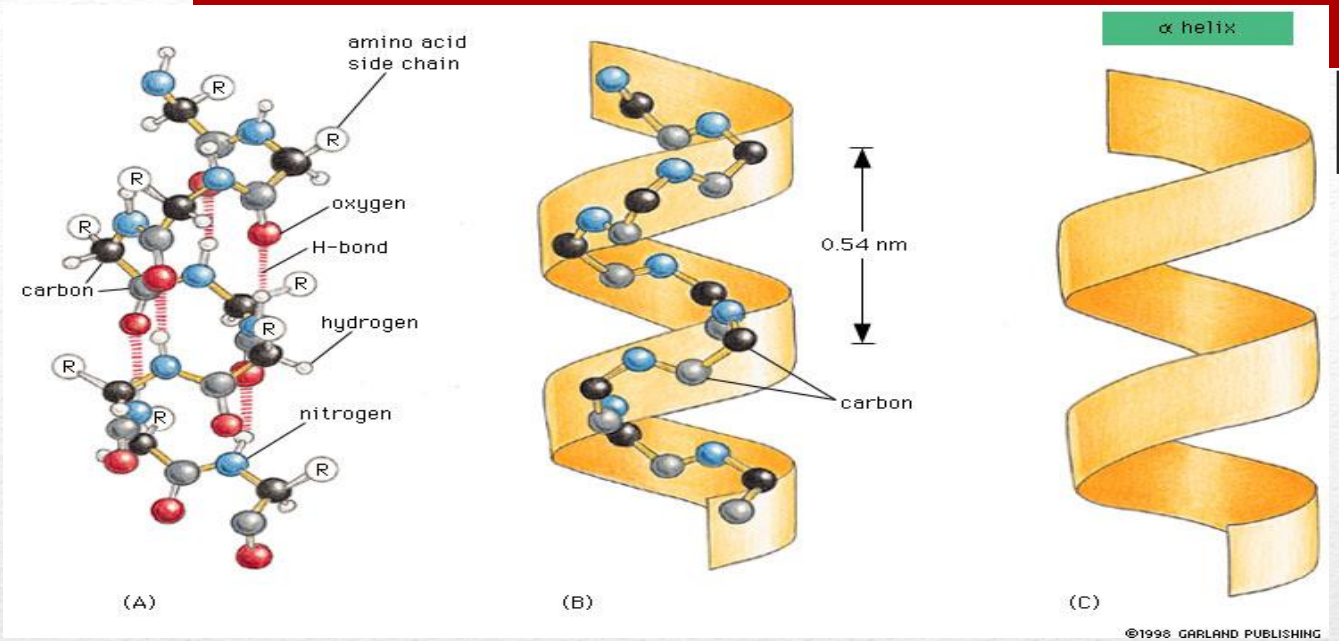
An alpha helix has

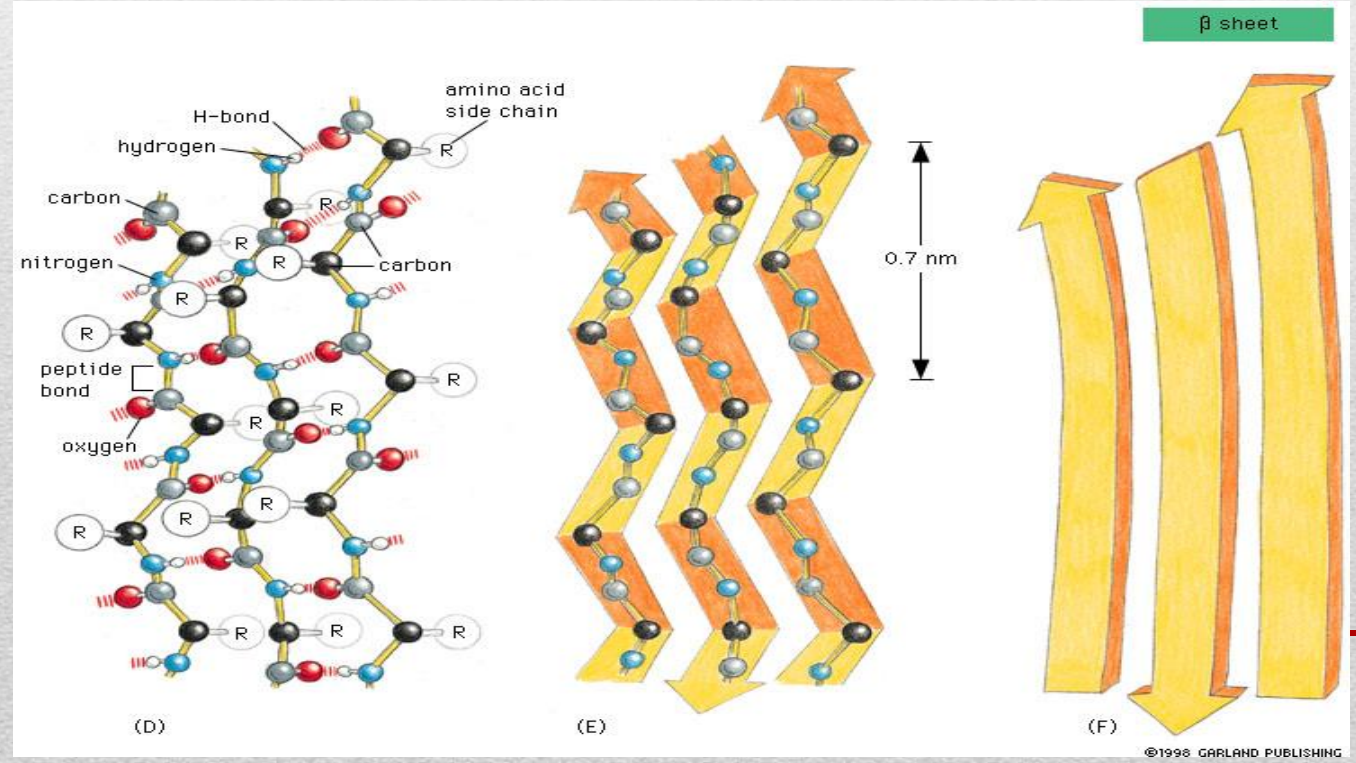
- A coiled shape held in place by hydrogen bonds between the amide groups and the carbonyl groups of the amino acids along the chain.
- Hydrogen bonds between the H of a –N-H group and the O of C=O of the fourth amino acid down the chain.

Secondary Structure – Alpha Helix



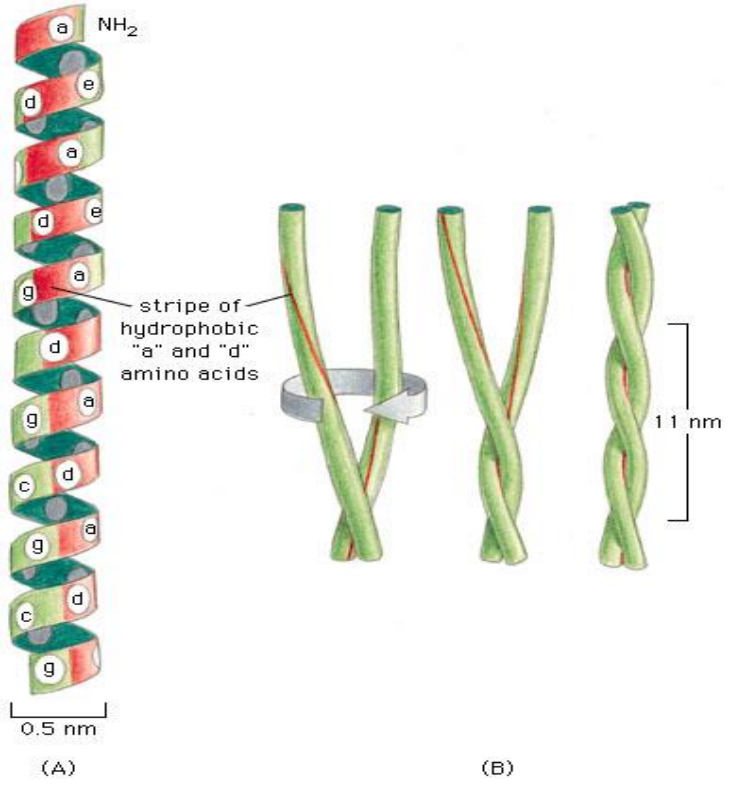
Protein Folding





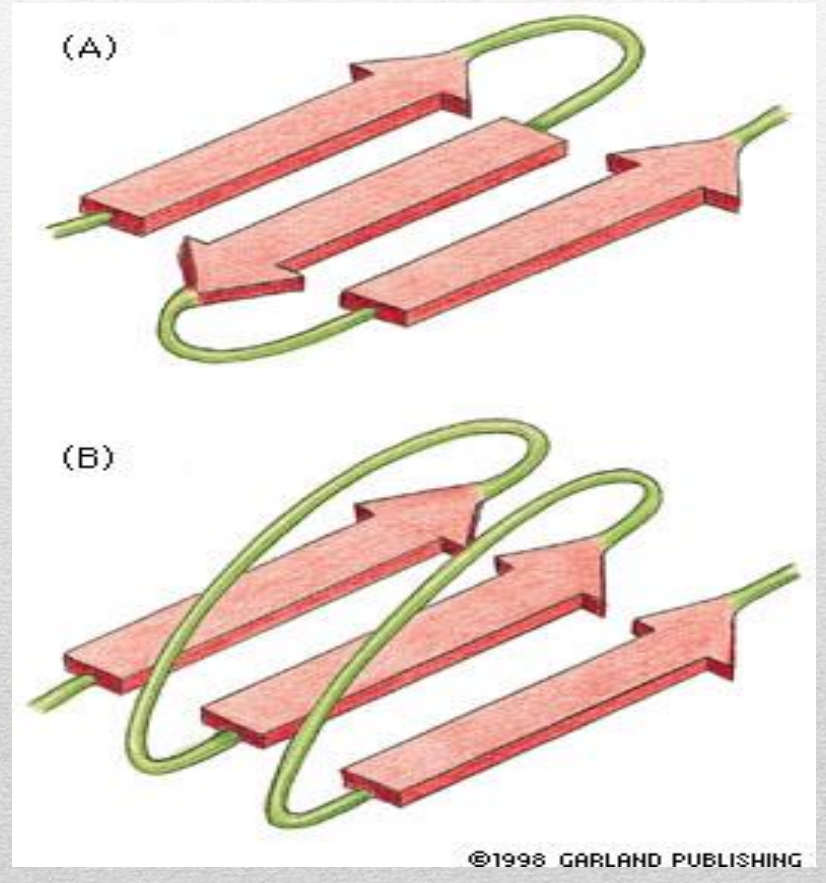
- 2 regular folding patterns have been identified – formed between the bonds of the peptide backbone
- α-helix – protein turns like a spiral – fibrous proteins (hair, nails, horns)
- β-sheet – protein folds back on itself as in a ribbon –globular protein

α Helix



- Formed by a H-bond between every 4th peptide bond – C=O to N-H
- Usually in proteins that span a membrane
- The α helix can either coil to the right or the left
- Can also coil around each other – coiled-coil shape – a framework for structural proteins such as nails and skin

β Sheets



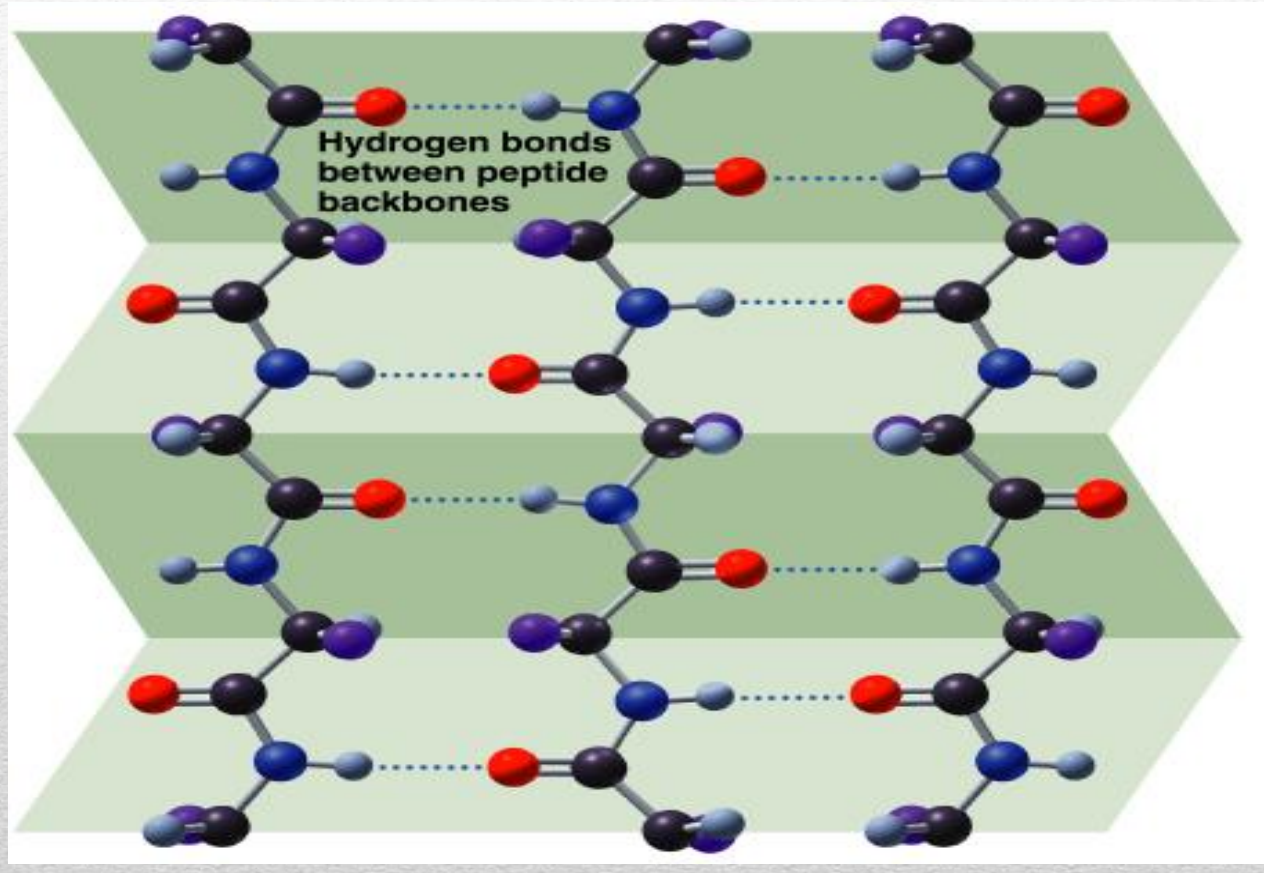
- Core of many proteins is the β sheet
- Form rigid structures with the H-bond
- Can be of 2 types
  - Anti-parallel – run in an opposite direction of its neighbor (A)
  - Parallel – run in the same direction with longer looping sections between them (B)

Secondary Structure – Beta Pleated Sheet

A beta-pleated sheet is a secondary structure that

- Consists of polypeptide chains arranged side by side.
- Has hydrogen bonds between chains.
- Has R groups above and below the sheet.
- Is typical of fibrous proteins such as silk.

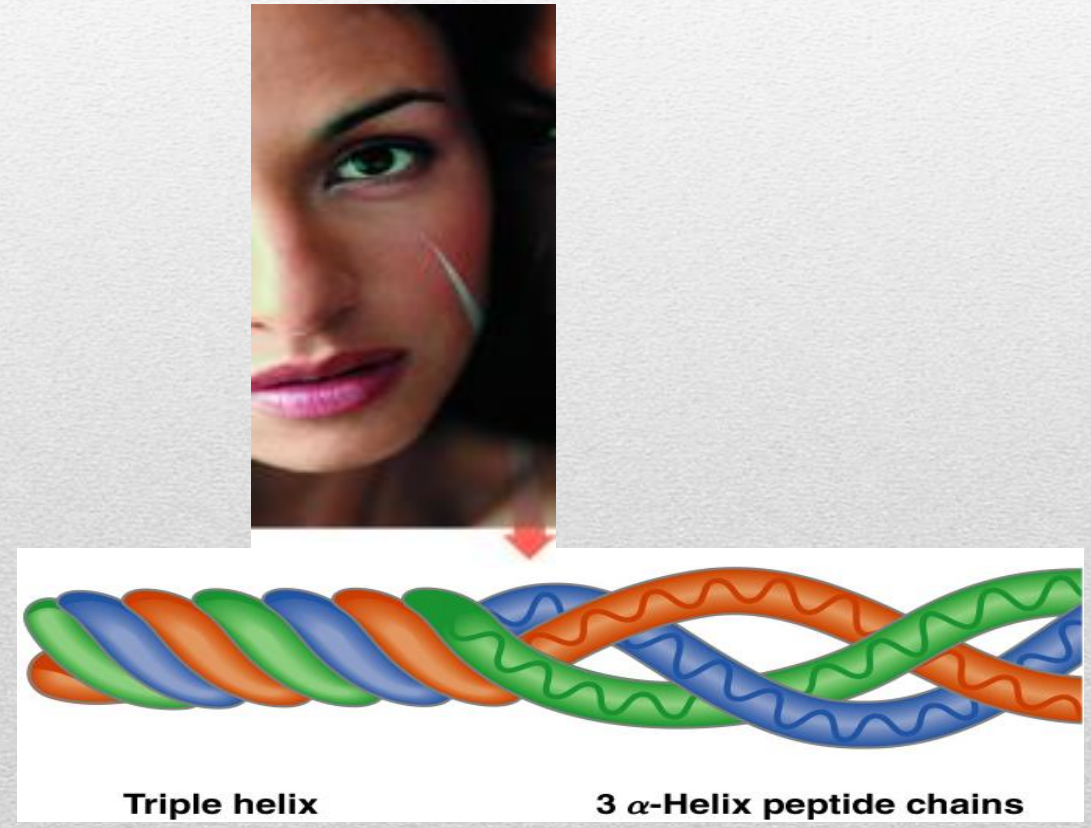
Secondary Structure: β-Pleated Sheet



Secondary Structure: Triple Helix

A triple helix

- Consists of three alpha helix chains woven together.
- Contains large amounts glycine, proline, hydroxy proline, and hydroxylysine that contain –OH groups for hydrogen bonding.
- Is found in collagen, connective tissue, skin, tendons, and cartilage.



Tertiary Structure

The tertiary structure of a protein

- Gives a specific three dimensional shape to the polypeptide chain.
- Involves interactions and cross links between different parts of the peptide chain.
- Is stabilized by

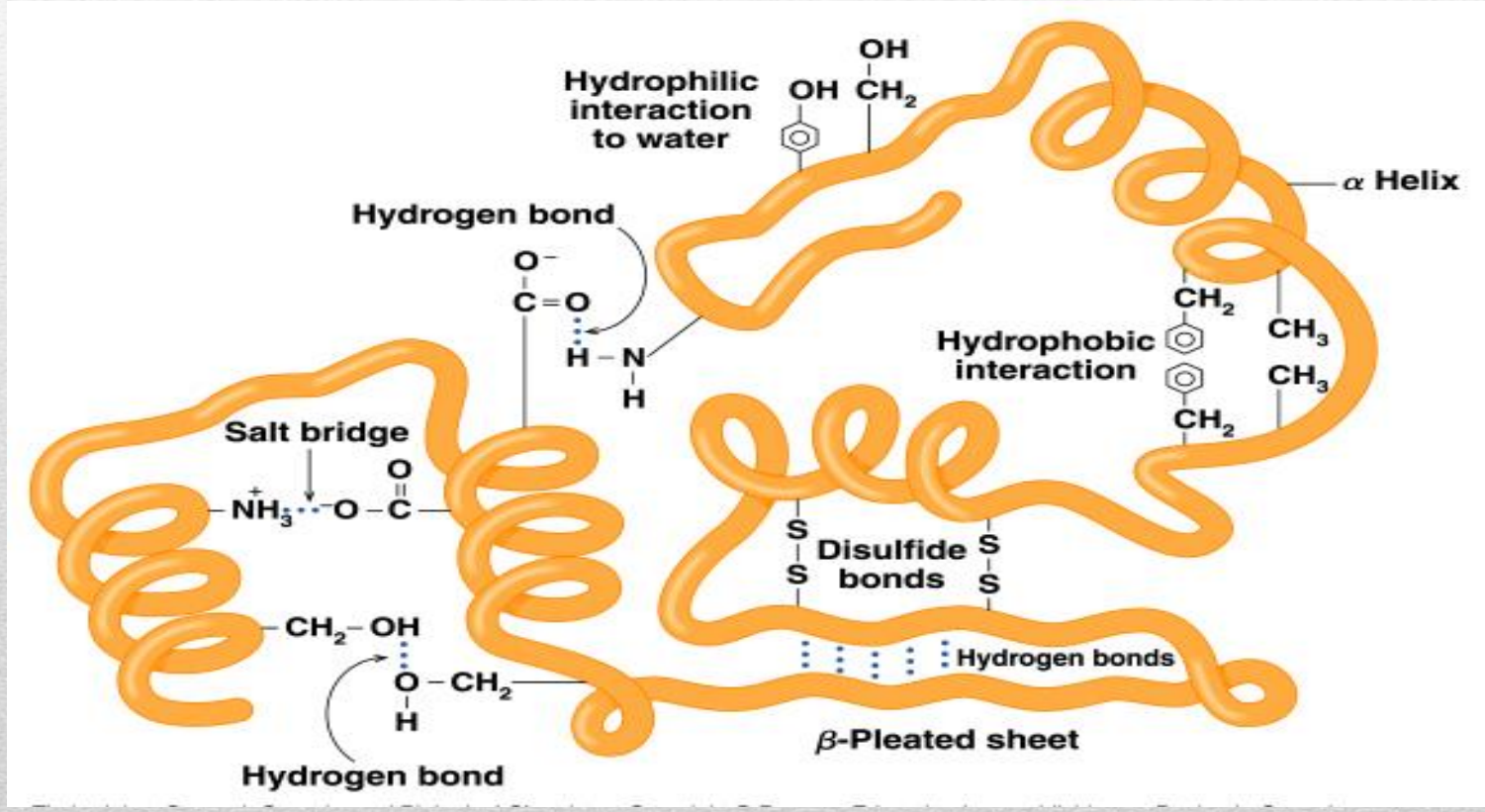
  Hydrophobic and hydrophilic interactions. Salt bridges.

  Hydrogen bonds.

  Disulfide bonds.

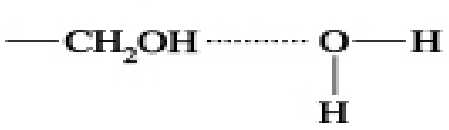
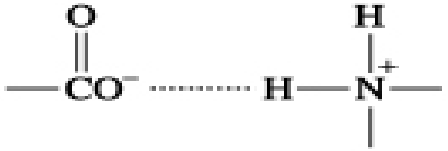
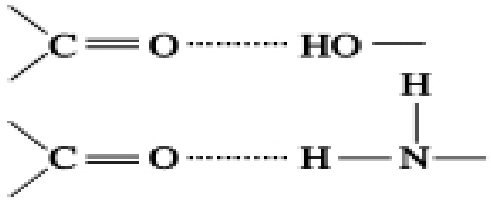
Tertiary Structure

- The interactions of the R groups give a protein its specific three-dimensional tertiary structure.

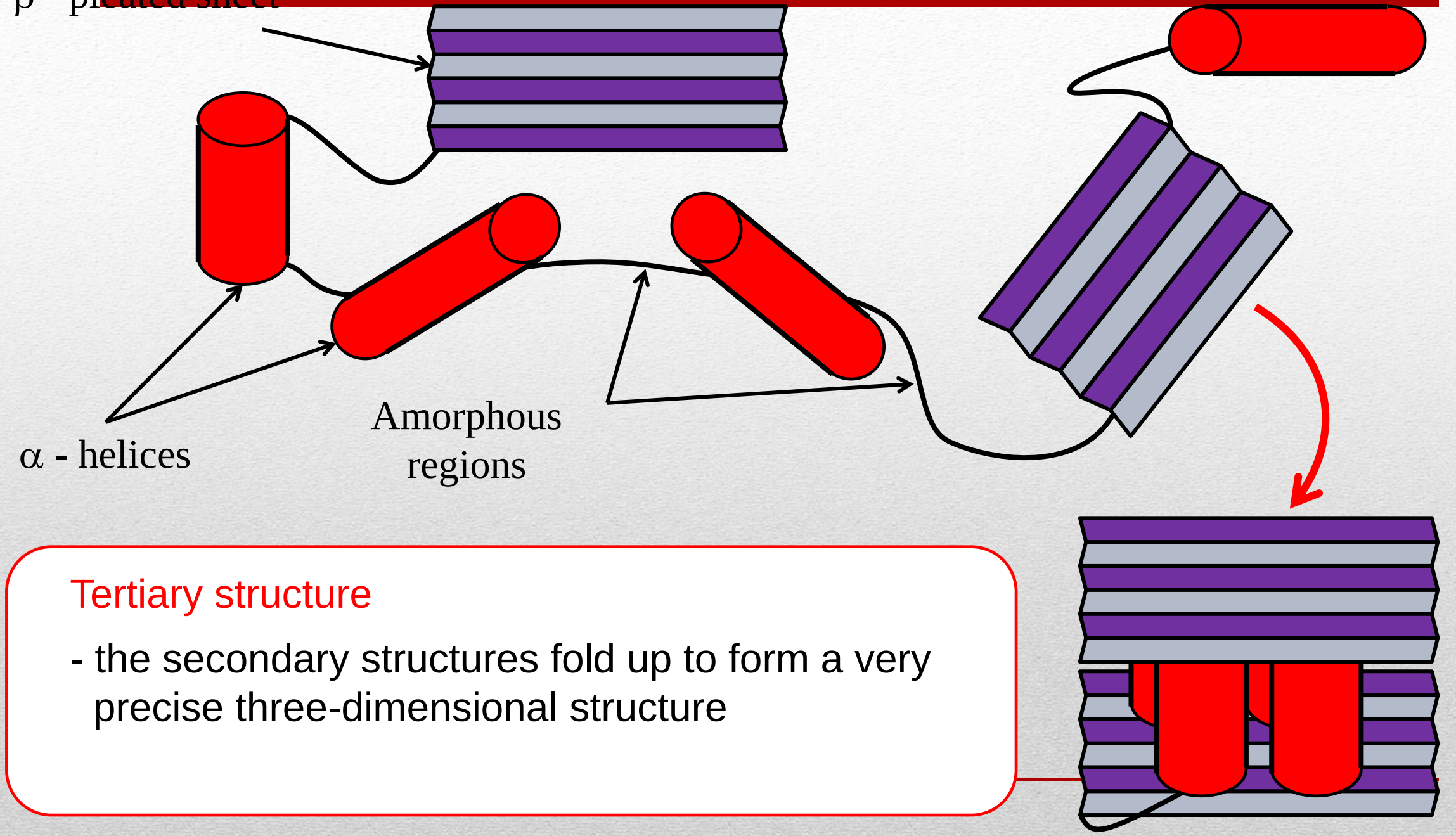


Tertiary Structure

TABLE 19.5 Some Cross-Links in Tertiary Structures

	Nature of Bonding	Example
Hydrophobic interactions	Attractions between nonpolar alkyl and aromatic groups form a nonpolar center that is repelled by water	
Hydrophilic Interactions	Attractions between polar or ionized R groups and water on the surface of the tertiary structure	
Salt bridges	Ionic interactions between ionized R groups of acidic and basic amino acids	
Hydrogen bonds	Occur between polar side groups of amino acids	
Disulfide bonds	Strong covalent links between sulfur atoms of two cysteine amino acids	

β - pleated sheet



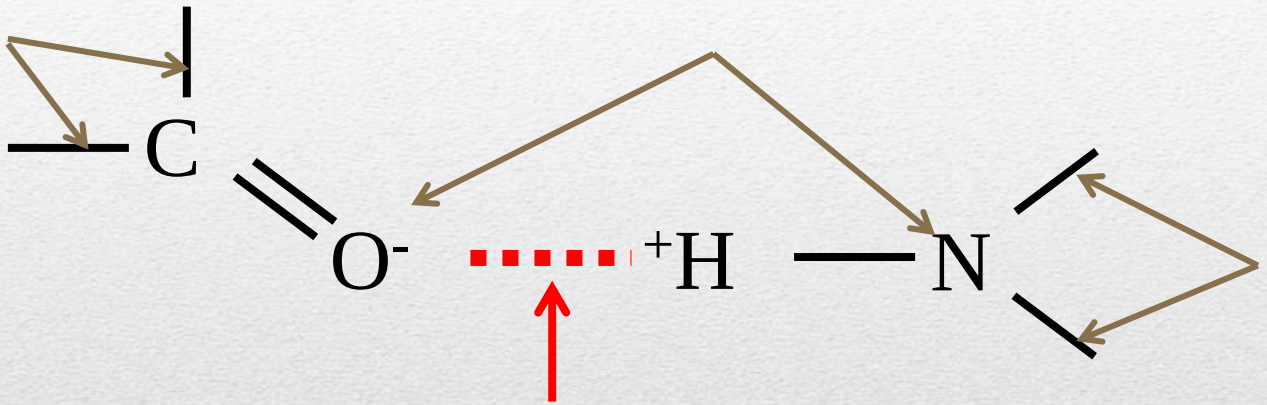
Tertiary structure

- the secondary structures fold up to form a very precise three-dimensional structure

Hydrogen bonds

Shared electrons spend longer at these atoms, forming a slight negative charge

bonds to molecule



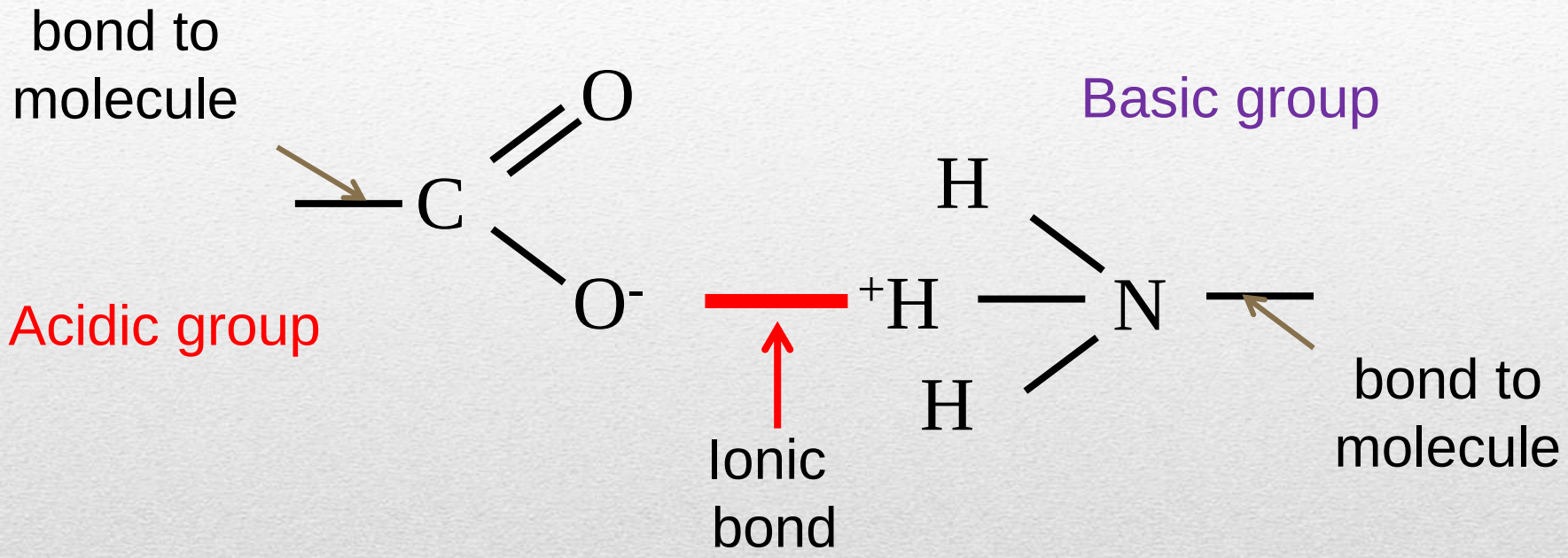
bonds to molecule

hydrogen bond

Hydrogen bonds form between these polar groups

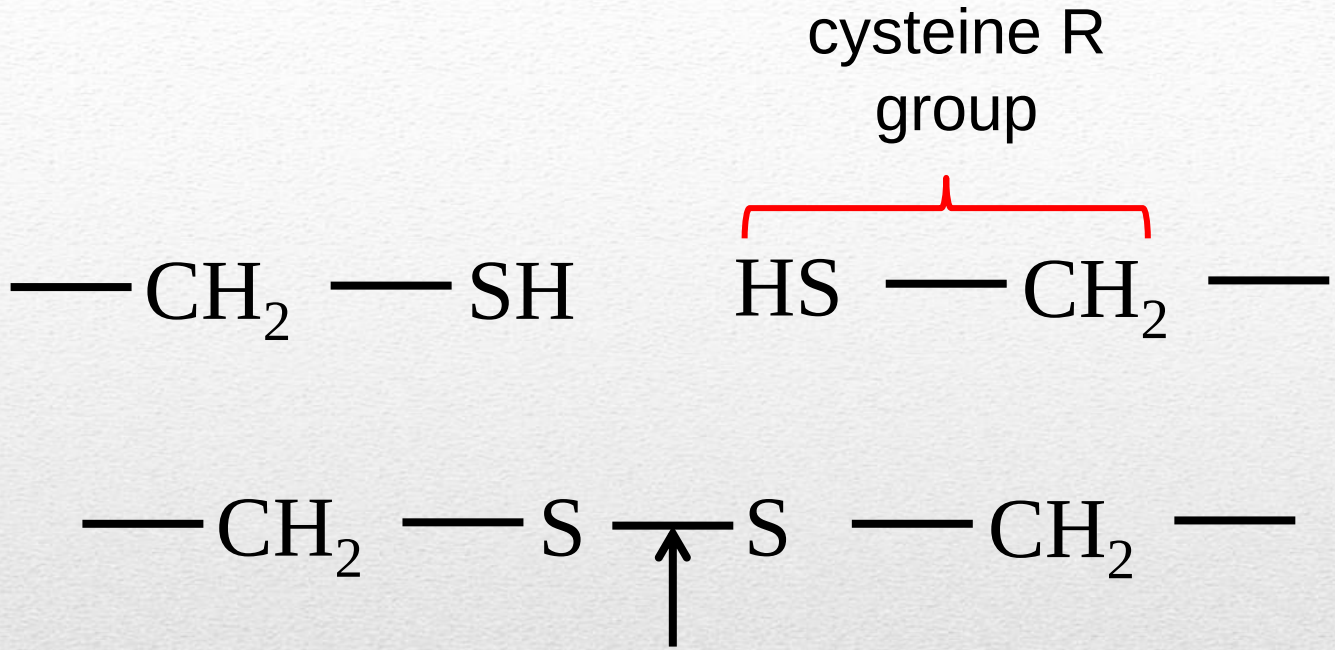
High temperatures and altered pH can split these bonds

Ionic bonds



Ionic bonds can be split by changing the pH

Disulphide bonds



(covalent)

Disulphide bonds can be split by reducing agents

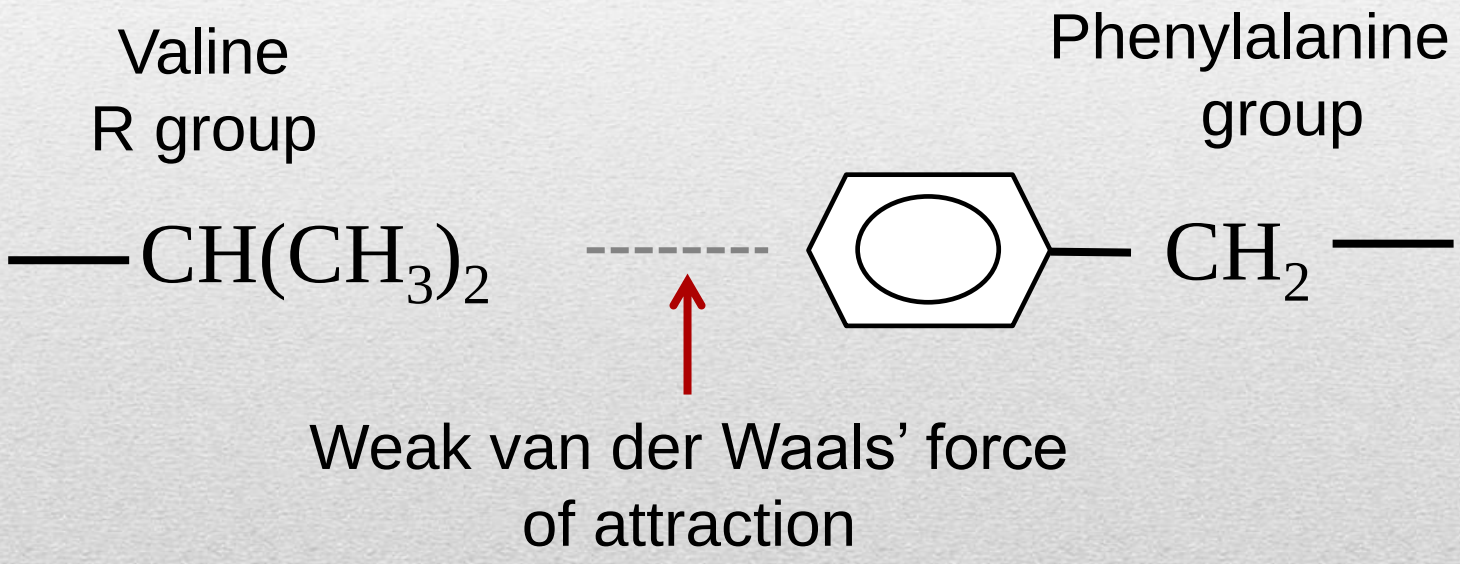
Polar and Non Polar

- The arrangement of the atoms in some molecules is such that one end of the molecule has a positive electrical charge and the other side has a negative charge. If this is the case, the molecule is called a polar molecule, meaning that it has electrical poles. Otherwise, it is called a non-polar molecule.
- Whether molecules are polar or non-polar determines if they will mix to form a solution or that they don't mix well together. Also, polar molecules are water soluble, while non-polar molecules are fat soluble.

van der Waal's forces

These are weak forces of attraction between **non-polar** groups

Water excluded from these *hydrophobic* side chains helps keep the side chains together



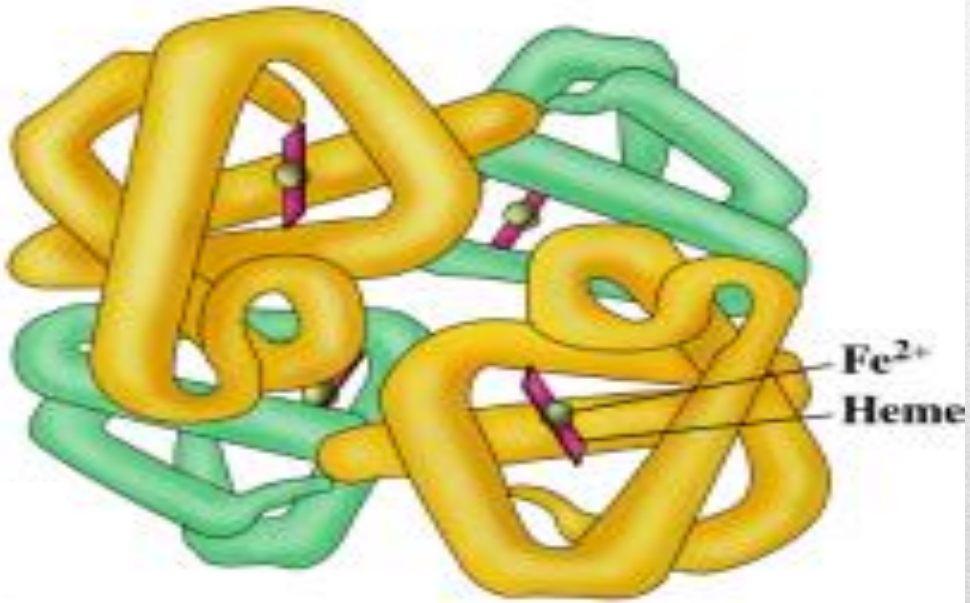
These forces can be split by a rise in temperature

Quaternary Structure

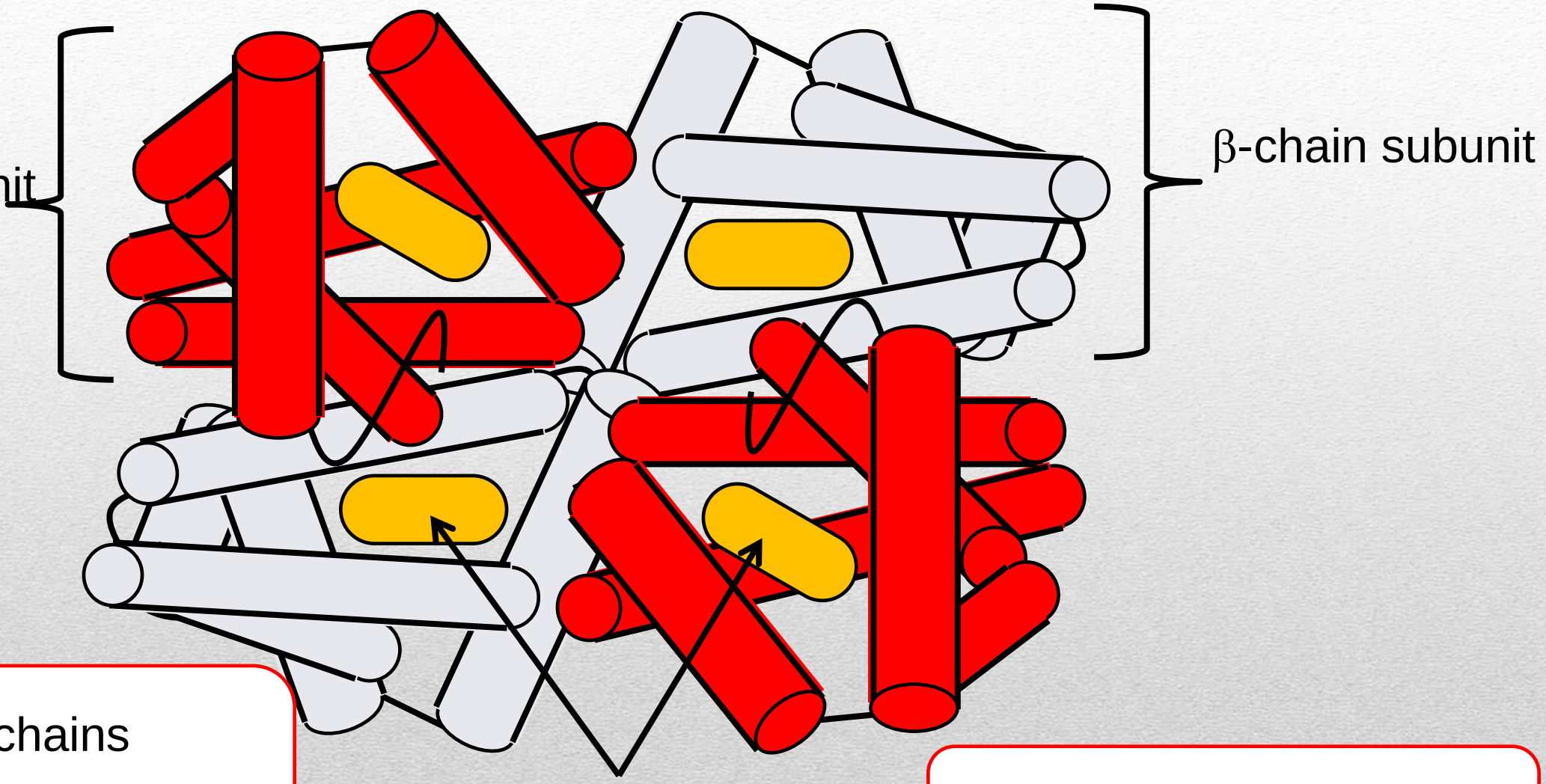
The quaternary structure

- Is the combination of two or more tertiary units.
- Is stabilized by the same interactions found in tertiary structures.
- Of hemoglobin consists of two alpha chains and two beta chains. The heme group in each subuni picks up oxygen for transport in the blood to the tissues.

hemoglobin



Haemoglobin is an example of a *globular* protein with quaternary structure



4 polypeptide chains
- 2 α -subunits
- 2 β -subunits

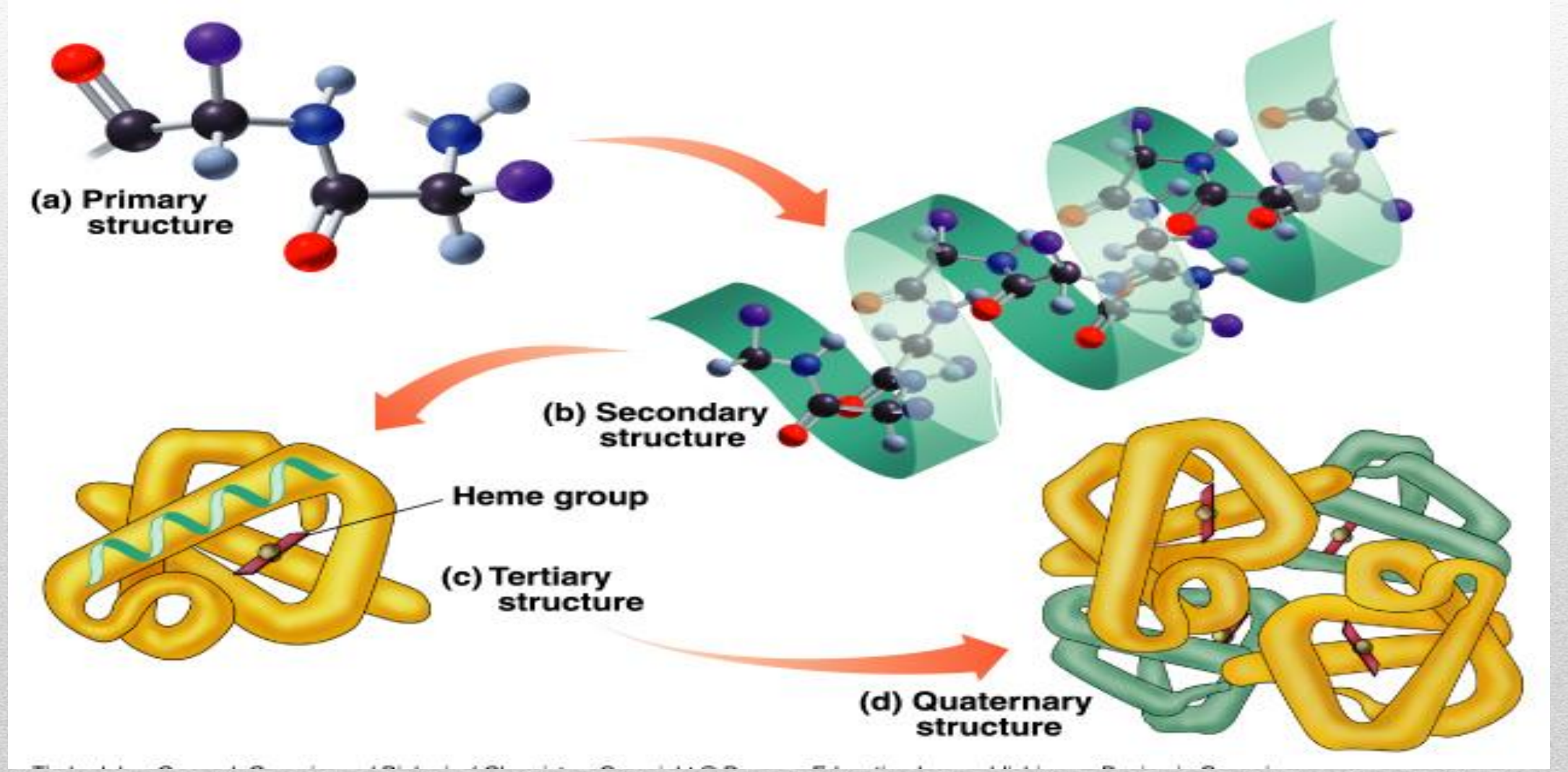
4 haem prosthetic groups

Summary of Protein Structure

TABLE 19.6 **Summary of Structural Levels in Proteins**

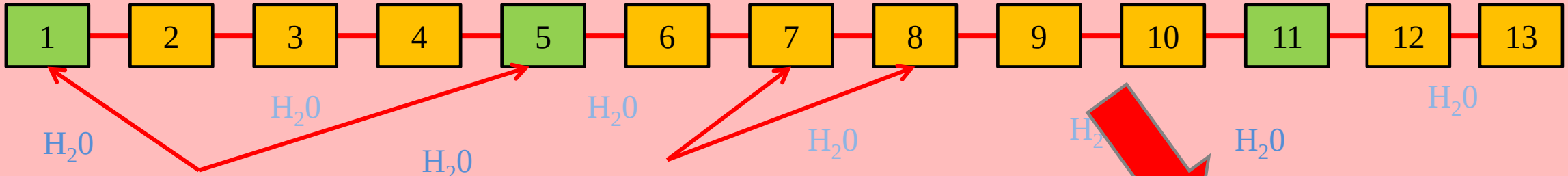
Structural Level	Characteristics
Primary	The sequence of amino acids
Secondary	The coiled α-helix, β-pleated sheet, or a triple helix formed by hydrogen bonding between peptide bonds along the chain
Tertiary	A folding of the protein into a compact, three-dimensional shape stabilized by interactions between side R groups of amino acids
Quaternary	A combination of two or more protein subunits to form a larger, biologically active protein

Summary of Protein Structures



Globular proteins

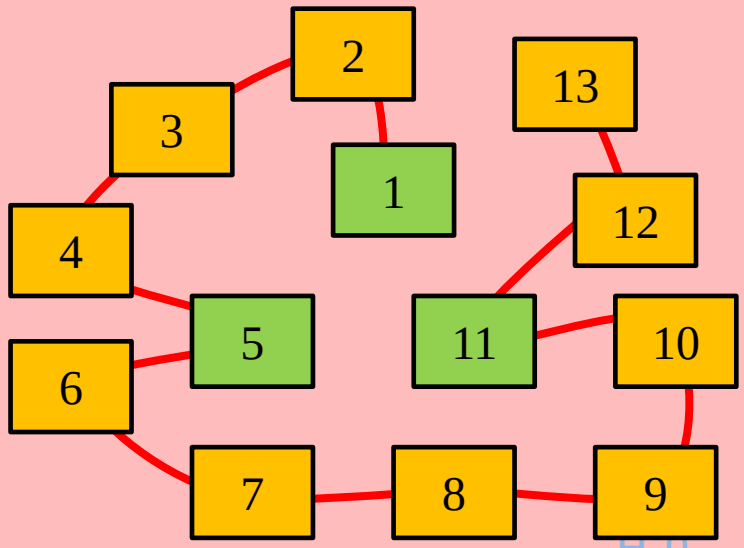
NOTE: the cell is an *aqueous* environment



H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O H_2O

Hydrophobic R groups

Hydrophilic R groups



Globular proteins form a spherical mass with a specific 3-D shape (tertiary and quaternary structure)

They fold up so that *hydrophilic* groups are on the outside and *hydrophobic* groups are inside the molecule

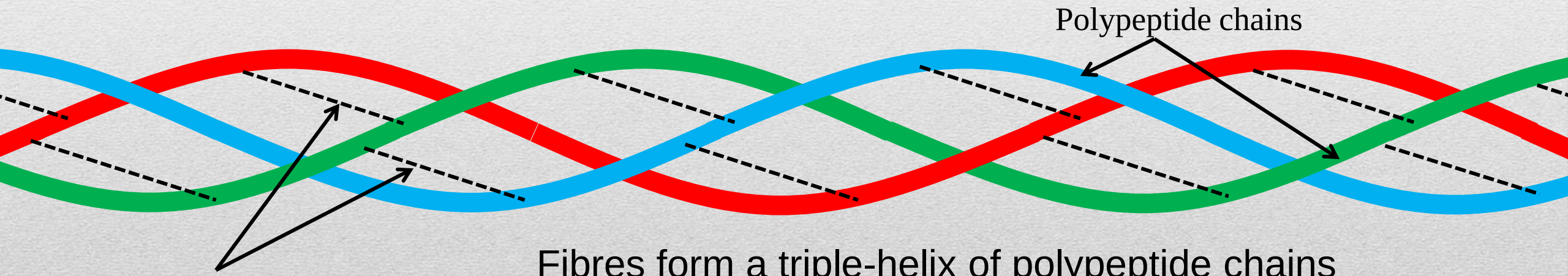
Fibrous Proteins

Fibrous proteins

- Fibrous protein molecules form long chains or fibres
  (they have primary, secondary, tertiary and quaternary structure)

- Their fibrous nature makes them insoluble in water...
- ... this makes them useful for structure and support

Collagen found in skin, teeth, bones, tendons, blood vessel walls



Fibres form a triple-helix of polypeptide chains

These chains are held together by hydrogen bonds

STRUCTURE FUNCTION RELATIONSHIP OF PROTEINS

- The R-groups of AA dictate structure-function relationships of peptides and proteins.
- The hydrophobic amino acids will be found in the interior of proteins shielded from direct contact with water.
- The hydrophilic amino acids are generally found on the exterior of proteins as well as in the active centers of enzymatically active proteins.
- It is the nature of amino acid R-groups that allow enzyme reactions to occur.
- The imidazole ring of histidine allows it to act as either a proton donor or acceptor at physiological pH. Hence, it is frequently found in the reactive center of enzymes.
- Equally important is the ability of histidines in hemoglobin to buffer the H^+ ions from carbonic acid ionization in red blood cells. It is this property of hemoglobin that allows it to exchange O_2 and CO_2 at the tissues or lungs, respectively.

STRUCTURE FUNCTION RELATIONSHIP OF PROTEINS

- The primary alcohol of serine and threonine as well as the thiol (-SH) of cysteine allow these amino acids to act as nucleophiles during enzymatic catalysis. Additionally, the thiol of cysteine is able to form a disulfide bond with other cysteines:
- This simple disulfide is identified as cystine. The formation of disulfide bonds between cysteines present within proteins is important to the formation of active structural domains in a large number of proteins. Disulfide bonding between cysteines in different polypeptide chains of oligomeric proteins plays a crucial role in ordering the structure of complex proteins, e.g. the insulin receptor.

PROPERTIES OF PROTEINS: Physical

- Proteins are generally tasteless, odourless compounds.
- They are generally soluble in water, dilute acids and alkalies and weak salt solution.
- They are optically active.
- They are ampholyte in nature.
- They possess colloidal properties because of their colloidal nature.
- Viscosity of a protein solution varies with kind and concentration of proteins. A solution of protein is least viscous at its pI.
- Vegetable proteins are easy to crystallize.

Denaturation

Denaturation means

- Disorganization of native protein molecule by which the configuration or regular arrangement of the protein molecule is altered to an irregular diffuse arrangement.
- Thus, in simpler words denaturation means unfolding of protein where all secondary, tertiary and quaternary (if present) structures are lost and protein is in its primary structure only. It is simple unfolded state.

Denaturation

Denaturation by

- Physical Agents
- Chemical Agents

- 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.

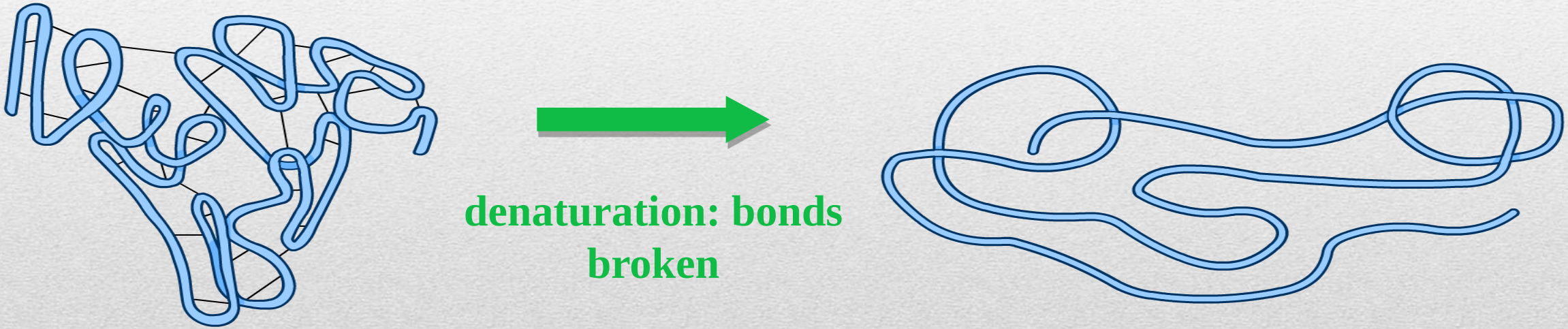
Denaturation

Denaturation leads to changes

- Physical changes
- Chemical changes
- Biological changes
- **Physical changes** include: decreased solubility, altered surface tension, increased viscosity and inability to be crystallized.
- **Chemical changes** include: splitting of all holding forces of peptide like H bond, disulphide bond, Vander Waals forces, salt linkage etc. that results in unfolded and uncoiled protein chains. Some active chemical group may lose their activity like sulphydril group of cysteine, disulphide group of cystine and cysteine and phenolic group of tyrosine.
- **Biological changes** include increased digestivity, lost activity of enzymes and hormones, altered antibodies formation etc.

Denaturing proteins

If the bonds that maintain a protein's shape are broken, the protein will stop working properly and is **denatured**.



denaturation: bonds broken

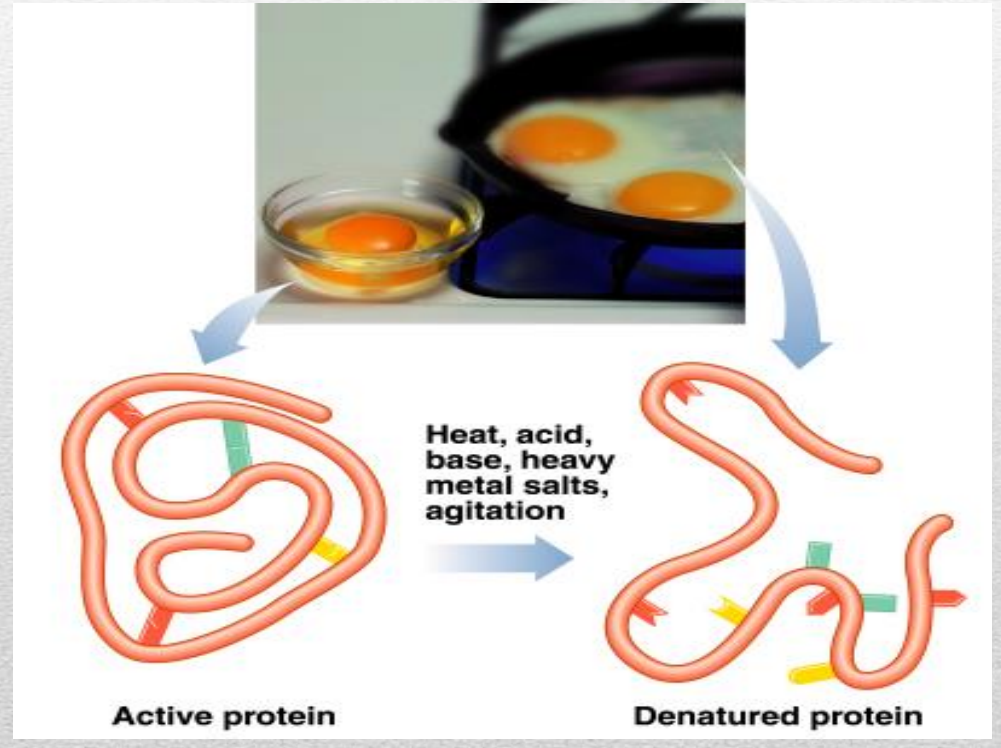
Changes in temperature, pH or salt concentration can all denature a protein, although the specific conditions will vary from protein to protein.

Fibrous proteins lose their structural strength when denatured, whereas globular proteins become insoluble and inactive.

Applications of Denaturation

Denaturation of protein occurs when

- An egg is cooked.
- The skin is wiped with alcohol.
- Heat is used to cauterize blood vessels.



Precipitation

- Protein precipitation is a method used to extract and purify proteins held in a solution.
- Occurs when the charges of a metaprotein (denatured protein) is brought up to its isoelectric point.
- At the isoelectric point, the solubility of proteins is lowest, that help them precipitate.
- Also called as flocculation.
- Precipitation is a reversible process,

Methods of precipitation

- Salting out
- Fractionation by solvant
- Positive ions
- Negative ions
- Antibodies

Salting Out

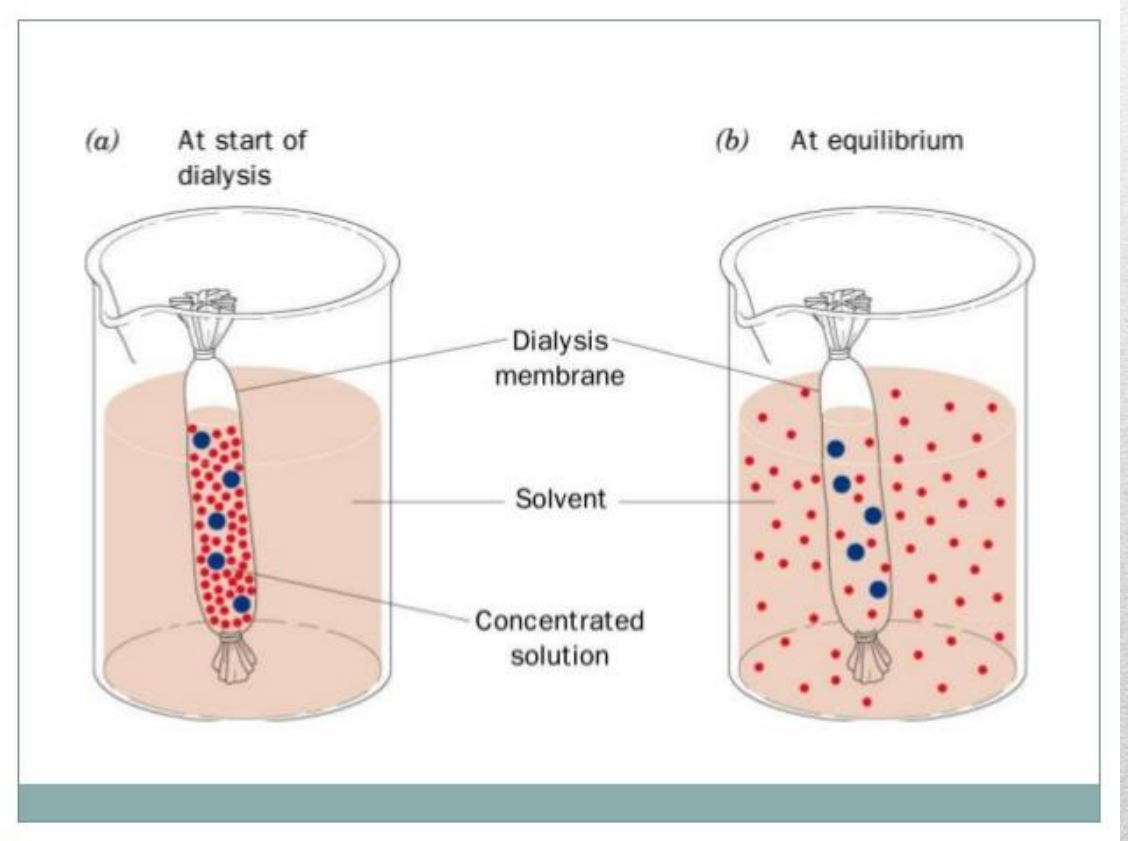
- Process of precipitation of proteins **by addition of a neutral salt solution**.
- It is a most common method for precipitation of proteins.
- The salt most frequently used is ammonium sulfate.
- The interaction of the salt ions with water molecules removes the water barrier between protein molecules, allowing the hydrophobic parts of the protein to come into contact. This results in the protein molecules aggregating together and precipitating out of solution.
- As a general rule, the higher the molecular weight of the protein, the lower the concentration of the salt that is required to cause precipitation, so it is possible to separate a mixture of different proteins in solution by gradually increasing the salt concentration, so that different proteins precipitate at different stages, a process known as fractional precipitation.

Salting Out

- Depending on the concentration of salt solution used, the salting out may either
  - **Half Saturation,** where smaller concentration of salt is used; or it may be a
  - **Full Saturation** where higher concentration of salt is used.
- By half saturation, thus larger proteins can be precipitated, whereas, for smaller proteins, full saturation is required.

Dialysis

- Process for removal of salts
- Always followed by salting out



Fractionation by solvants

- Problems of salt in protein during Salting out
- Solvents like ethanol is used at lower temperature and vacuum.
- Organic solvents reduce the dielectric constant, which in this context can be regarded as a measure of the polarity of a solvent.
- A reduction in polarity means there is less of a tendency for solvent molecules to cluster around those of the protein, so that there is less of a water barrier between protein molecules and a greater tendency toward protein precipitation.
- Many organic solvents interact with the hydrophobic parts of protein molecules, causing denaturization; however, some, such as ethanol and dimethyl sulfoxide (DMSO), do not.

Addition of positive ions

- Proteins can also be precipitated by positive ions like Zn^{++}, Hg^{++}, Cu^{++}, Pb^{++} etc.
- Due to its amphoteric nature, when the pH of solution is more than its pI, the proteins assume a negative charge. So, if positive ions are added at that pH, proteins form metal proteinate and are precipitated.

Addition of negatively charged ions

- Complex alkaloidal reagents, such as picric acid, phosphotungstic acid, tannic acid, sulphosalicylic acid etc. are negatively charged.
- These negatively charged ions precipitate proteins in acidic medium (acidic to pI) and form protein salts.

Precipitation by Antibodies

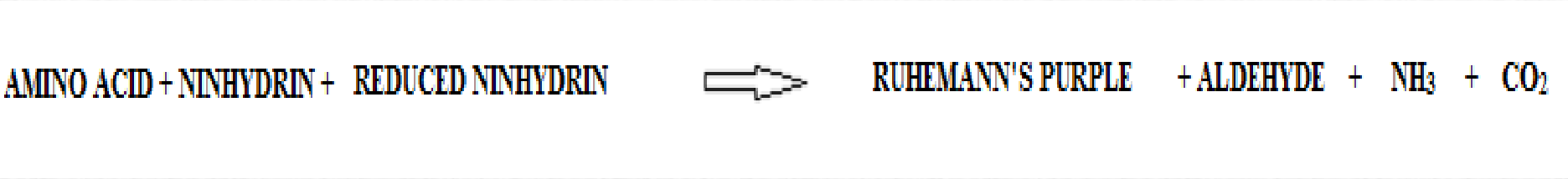
- Antibodies produced against a particular protein can be used to remove that protein from solution by binding to it.
- By using antibodies, proteins can be separated from a solution rather than precipitation. This technique requires certain other techniques like chromatography, immune assay etc.

Coagulation

- Precipitation by heat
- Irreversible

COLOUR/ SPECIAL REACTION OF PROTEINS

Ninhydrin Reaction



- This reaction gives blue colour. When proteins are boiled with ninhydrin (Triketo hydrindene hydrate), amino acids are oxidatively deaminated and produce keto acid and NH_3. An imino acid is produced as intermediate.
- Keto acid formed in oxidative deamination is decomposed by heat into aldehyde and CO_2 and ninhydrin is reduced to hydrindantin. Reduced ninhydrin (hydrindantin) reacts with ammonia and some of ninhydrin to form **Ruhemann's purple** that gives blue colour.

Biuret Reaction

- Biuret reaction is given by substances containing two $–CONH_2$ groups joined together with carbon or nitrogen atom.
- Biuret ($NH_2CONH.NHCONH_2$) is a compound that is formed by condensation of two molecules of urea after heating. It was seen that when biuret is reacted with alkaline copper sulphate solution, it gives colour. Thus the reaction is called as “Biuret reaction”. Biuret reaction is also given by substances that contain $–CH_2NH_2$, $–C(NH)NH_2$, $–CSNH_2$ and –CONH groups. As proteins contain –CONH group (peptide bond), they give biuret reaction.
- Biuret test is actually due to coordination of cupric ions with the unshared electron pairs of peptide nitrogen and oxygen of water to form a coloured coordination complex.

Xanthoproteic Reaction

- This reaction is also known as yellow protein reaction and is specific for the presence of aromatic amino acids in protein.
- Protein containing aromatic nucleus react with concentrated nitric acid to give yellow colour which is due to nitration of phenyl ring to give nitro substitution compounds. These substances turn orange in alkaline medium due to salt formation.

Millon's test for tyrosine

- Millon's reagent is solution of mercurous and mercuric nitrate containing nitric acid.
- Proteins react with Millon's reagent and if tyrosine is present, they form white precipitate. This white precipitate turns red when heated. The test is used for identification of tyrosine in proteins, though the test is not specific as phenols also give this test.

Hopkins Cole reaction

- This test is also known as Glyoxylic acid reaction and is used for the identification of tryptophan in proteins.
- The test is specific of indole ring found in tryptophan. When proteins having tryptophan is mixed with glyoxylic acid ($CHO.CH_2OH$) and layered with concentrated sulphuric acid, there is formation of a violet ring at the junction.

Nitroprusside reaction

- This reaction identifies the presence of free sulfhydril (--SH) group in the protein. Thus the test identifies cysteine.
- Proteins having free –SH group react with sodium nitroprusside in ammonical solution and produce reddish colour. There may be chances that some proteins that give this test negative may give positive result after denaturation; which means liberation of free –SH group.

Sakaguchi Reaction

- Sakaguchi test is done to identify arginine.
- The test is specific for the “guanidine” group of arginine. In alkaline medium, guanidine group of arginine combines with α Naphthol to form a complex that is oxidized by sodium hypochlorite to form an intense red colour.

Unoxidized Sulphur test

- This test is done to detect the presence of sulphur containing amino acids.
- This test is also called as lead sulphide test. When proteins are boiled with strong alkali, they form sodium sulphide. When lead acetate is added in this mixture, black to brown precipitate is formed of lead sulphide.

FEW PROTEINS

SIMPLE PROTEINS

- **Albumins and Globulins:**
- They are the proteins that contain most of the amino acids. Both are coagulated by heating. They differ in their solubility; albumin is soluble inwater, whereas, globulin is insoluble in water. Both are soluble in neutral salt solution and alkalies. Albumin is smaller in size and require full saturation for precipitation, whereas, globulins are larger and can be precipitated by half saturation. These proteins are present in egg albumin, muscles, serum, milk, cereals, peas, beans etc.

- **Glutelins and Gliadins**
- They are present in cereals especially wheat. Glutelins have large amount of glutamic acid. Gliadins have higher concentration of pralines, so they are also known as prolamines.
- Gliadin is insoluble in water and absolute alcohol, but soluble in dilute alcohol, dilute acids and alkalies.

- **Sceleroproteins or Albuminoids**
- These proteins are characterized by their great stability and insolubility in water and salt solution. They are called as albuminoids as they are similar to albumins.
- These proteins form most of supportive structure of animals e.g. collagen in cartilage, white fibrous connective tissue, osein in bones, dentine in teeth, keratin in hair, nails, hooves and horns.

- **Histones:**
- They are complex than prolamines. Histones are basic proteins and contain large amount of histidine. They are soluble in water but insoluble in ammonium hydroxide. They are not coagulated readily by heat. These proteins are present in hemoglobin, nucleoproteins and in sperm of fish.

- **Protamines:**
- Protamines are simplest of proteins as they contain only 8 amino acids. They are soluble in water but not coagulated by heat. These proteins are basis in nature due to presence of arginine. They are found in association with nucleic acid in sperm cells of certain fishes.

CONJUGATED PROTEINS

- **Nucleoproteins:**
- Nucleoproteins contain nucleic acid and proteins. In proteins, histones and peotamines are found. They are present in protoplasm and nuclei and are constituent of chromatin.

- **Phosphoproteins:**
- They contain phosphoric acid as non protein part. Examples are casein found in milk and vitelline found in eggs. They have about 1% of phosphorus. They are sparingly soluble in water and dilute acids but readily soluble in dilute alkalies.

- **Glycoproteins and Mucoproteins:**
- In these proteins, non protein part is carbohydrate. The carbohydrate generally is a mucopolysaccharide which contain hyaluronic acid and chondroitin sulphate. Mucoproteins contain more than 4% hexosamine but glycoproteins contain less than 4% hexosamine.
- They are important constituents of ground substance of connective tissue and present in tendons, bones, synovial fluid and mucin, mucoids and certain protein in protoplasm. They are also found in gonadotropic hormones.

- **Chromoproteins:**
- They have certain heterocyclic compounds like porphyrins as prosthetic group. Porphyrins combine with metal giving rise to coloured proteins. Example sinclude: hemoglobin, melanoprotein, flavoprotein, cytochrome, chlorophyll etc.

- **Metalloproteins:**
- They contain metals as prosthetic group e.g. Ferritin (have Fe), Ceruloplasmin (have Cu), Carbonic Anhydrase (have Zn).

- **Lipoproteins:**
- Lipoproteins contain lipids as prosthetic group.

DERIVED PROTEINS

- They are derived from above two classes of proteins either by action of acid, alkalies or enzymes. They are the result of partial to complete hydrolysis of proteins. Different stages of derived proteins are characterized based on their solubility and precipitation.

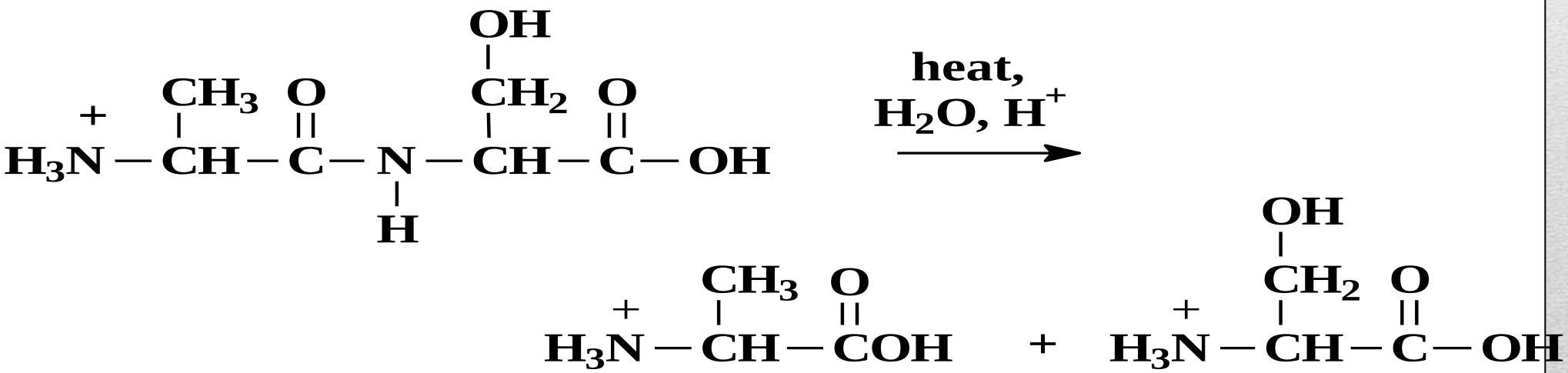
Protein Hydrolysis

Protein hydrolysis

- Splits the peptide bonds to give smaller peptides and amino acids.
- Occurs in the digestion of proteins.
- Occurs in cells when amino acids are needed to synthesize new proteins and repair tissues.

Hydrolysis of a Dipeptide

- In the lab, the hydrolysis of a peptide requires acid or base, water and heat.
- In the body, enzymes catalyze the hydrolysis of proteins.



Derived proteins

- **Primary Derivatives:**
- They are derived at an early stage of hydrolysis. They are also called as metaprotein and are actually denatured protein. They are heat coagulable.

Secondary Derivatives

They are obtained at a later stage during hydrolysis.

- **1. Proteoses:**
- They are not coagulated by heat but precipitated by saturated salt solution. They are either:
  - **Primary proteoses:** which are larger and require half saturation for precipitation; or
  - **Secondary proteoses:** which are smaller insize and thus require full saturation for precipitation.

- **2. Peptones:**
- They are soluble in saturated salt solution; so can’t be precipitated even by full saturation. They are not coagulated by heat.

Secondary Derivatives

They are obtained at a later stage during hydrolysis.

- **3. Peptides:**
- They have two or more amino acids. They are not coagulable and neither can be precipitated.

- **4. Diketopiperazines:**
- They are cyclic anhydride of two amino acids.