# UNIT-3



## INTRODUCTION TO PROTEIN

### Structure

proteins are unbranched polymers constructed from 22 standard α-amino acids they have four levels of structural organization, primary structure the amino acid sequence is specified by genetic information. As the polypeptide chain folds, it forms certain localized of adjacent amino acids that constitute Secondary Structure. The overall three dimensional shape that a polypeptide assumes is called the tertiary structure. protein that consist of two or more polypeptide chains (OR subunit) are said to have quaternary structure.

### # Primary Structure

The primary structure (1° structure) of a polypeptide is its amino acid sequence. The amino acid are connected by peptide bonds. primary structure of polypeptide determines the higher levels of structural organization.

### # Secondary Structure

The most common types of Secondary structures (2°) are the α-helix and β-pleated sheet. Both α-helix and β-pleated sheet patterns are stablized by hydrogen bonds b/n the Carbonyl and N-H groups in the polypeptide backbone.

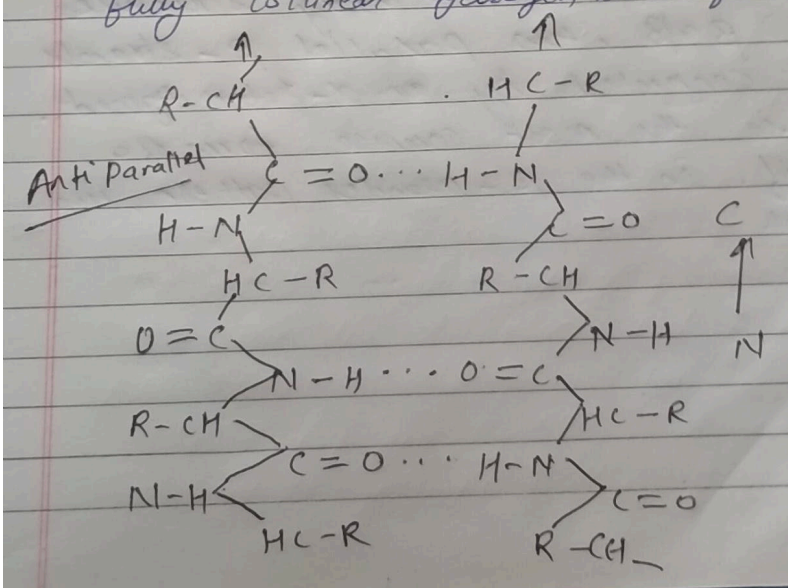
# α- Helix

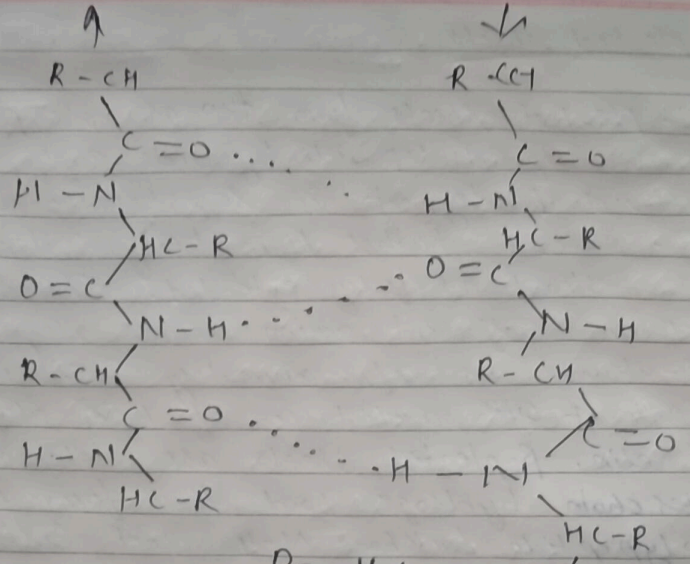
The α- Helix is a rigid, rod like structures that form when a polypeptide chain twists into a helical conformation. The screw sense of α-helix can be right handed (clock-wise) or left handed (counter clock wise) However right handed helices are energetically more favorable. In almost all proteins, the helical twist of the α-helix is a right handed.

# There are 3.6 amino acid residues per turn of the helix and the pitch (the distance b/n corresponding points per turn) is 0.54nm. Each residue is related to the next one by a rise of 1.5Å (0.15nm) along the helix axis A single turn of α-helix involves 13 atoms from O to the H of the H bond. for this reason, the α- helix is refered to as the 3.6 helix. Length of α-helix is usually 10-15 amino acid residues. Intrachain hydrogen bonds form between the N-H group of each amino acid and the Carbonyl group of the amino acid four residues away.

## β-pleated sheets

β-pleated sheets form two or more polypeptide chains segments up side by side. Each individual segment is referred to as β-strand. Rather than being coiled each strand is fully extended. The distance between adjacent amino acids along a β-strand is approximately 3.5Å in contrast with a distance of 1.5Å along an α-helix. β-pleated sheets are stablized by interchain hydrogen bond that form bet the polypeptide backbone N-H and Carbonyl groups of adjacent strands. Adjacent strand can be either parallel or anti parallel. In parallel β-pleated sheet structures, the polypeptide chains are arranged in same direction, However in antiparallel runs in opposite direction. Antiparallel β-pleated sheet are more stable than parallel. because fully collinear hydrogen bonds form.





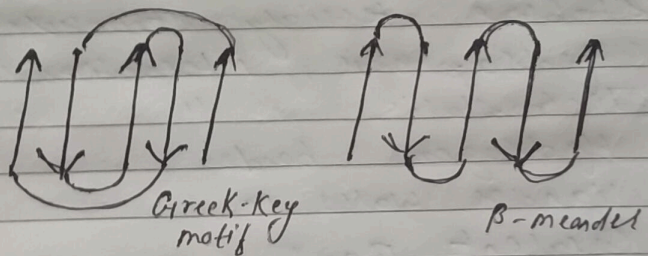
Parallel

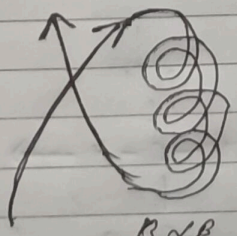
# Supersecondary structure :—

Many globular proteins contain combinations of α-helix and β-pleated sheet-secondary structures. These patterns are called supesssecondary structures. In the βαβ, two parallel β-strands are connected by an α-helix segment. This is the most common form of a motif. In the β-meander pattern, two parallel.

β- strands are connected by polar amino acids and glycines to effect an abrupt change in the direction of polypeptide chain. Called reverse or β- turns.

# In αα- motif two successive α-helix separated by a loop or non-helical segment become enmeshed of the compatible chains. When an antiparallel β- strand folds back on itself in a pattern that resembles a common Greek pottery design the motiff is called the Greek key.

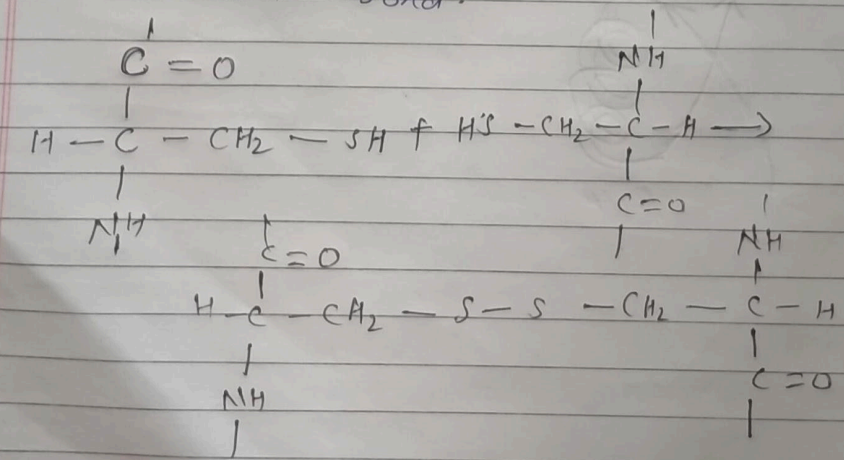


# Tertiary structure

The term tertiary structure (3° structure) refers to the unique three dimensional conformations that globular proteins assume as a consequence of interactions b/n the side chain in their primary structure. All information needed to fold the protein into its native 3° structure is contained within the primary structure of the peptide chain itself. The following types of covalent and non-covalent interactions stabilize tertiary structure.

1. Hydrophobic interactions.
2. Electrostatic "
3. Hydrogen bonds
4. Vander walls "
5. Covalent Bond.



Structure of Cystine

# Quastnary Structure :—

Many proteins like haemoglobin are composed of two or more polypeptide chains (i.e multimeric or oligomeric) and each polypeptide chain is called a subunit. Subunits in a multimeric protein may be identical or quite different. Polypeptide subunits assemble to form Quastnary structure. and are held together by non-covalent interactions such as hydrophobic interactions, electrostatic and hydrogen bonds as well as covalent cross links.

# Determination of molecular weight protein :—

The avgesage molecular wt ($\frac{1}{12}$ / $^{12}$C-atom) of a standarded amino acid is nearest to 128. When an amino acid participates in the formation of a polypeptide one molecule of water (m.wt 18) is removed during peptide bond formation. Thus the avesage molecular wt of an amino acid residue in a polypeptide is considered about 110 (128-18) Alternatively, this quantity may be expressed in terms of molecular mass. ~~denoted~~ by expressed unit daltons

(09)

# Denaturation of protein.

The primary structure of a protein is determined by the sequence of amino acids, but the secondary and tertiary structures of proteins define their natural or native conformation which are often folded. Denaturation is a process in which proteins lose their native conformations.

# Denaturation includes the breaking of non-covalent H-bond, van der walls force of interaction and covalent bond denaturation usually does not include the breaking of peptide bonds. Denaturation results is a loss of activity.

# #CHEMICAL PROPERTIES OF PROTEIN

Chemical Reactions of protein

1. Arginine Residue
2. Glutamic and Aspastic Acid Residues
3. Cystine Residue
4. Cysteine "
5. methionine "
6. Histidine "
7. Tryptophan "
8. Tyrosine "

# The chemical modification of protein is of importance for a number of seasons.

# A - It provides derivaties suitable for sequence analysis,

# B - Identifies the reactive groups in active sites of an enzyme

# C - Enables the binding of protein to a carriers. and

# D - Provides changes in protein properties which are important in food processing.

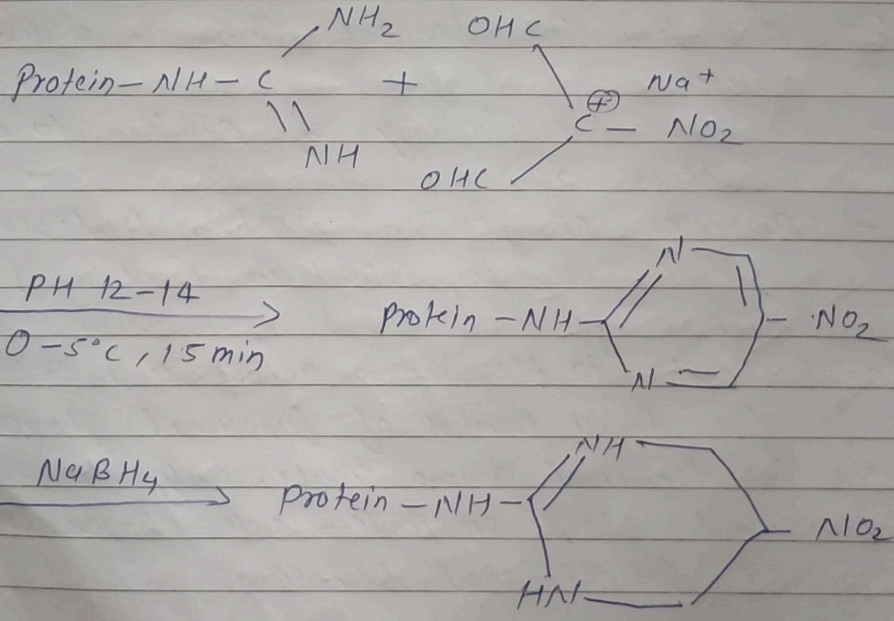
# In contrast to free amino acids and except for the small number of functional groups on the terminal amino acids, only the functional groups on protein side chains are available for chemical reactions.

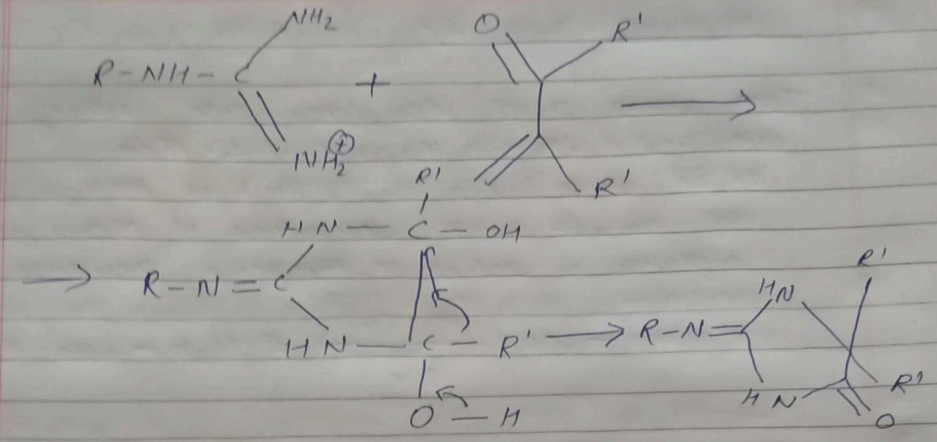
(1) Arginine Residue

→ The arginine residue of proteins reacts with α- or β- dicarbonyl compounds to form cyclic derivatives.

→ The nitropyrimidine derivative absorbs at 335 nm. The arginyl bond of this derivative is not cleaved by trypsin but it is cleaved in its tetrahydro form, obtained by reduction with $NaBH_4$.

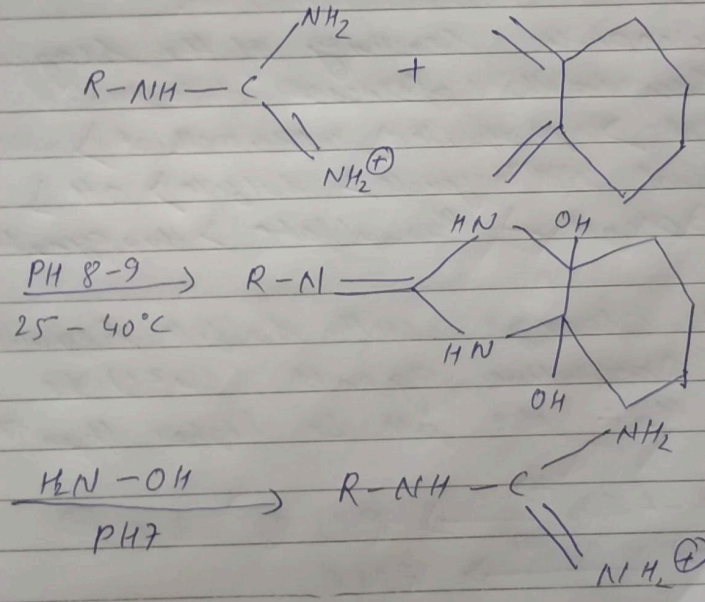
→ In the reaction with benzil, an iminoimidazolidone derivative is obtained after a benzilic acid rearrangement.





# Reaction of the arginine residue with 1,2 cyclohexanedione is highly selective and proceeds under mild conditions.

# Regeneration of the arginine residue is again possible with hydroxylamine.



(2) Glutamic and Aspartic Acid Residue

# These amino acid residues are usually esterified with methanolic HCL. There can be side reactions, such as methanolysis of ~~cyanide~~ amide derivatives of N,O-acyl migration in serine or threonine residues.

$$Protein-COOH \xrightarrow[0^\circ C]{CH_3OH/HCl} protein-COOCH_3$$

(3) Cystine Residue

Cleavage of cystine is possibly by a nucleophilic attack.

$$Protein-S-S-Protein + Y^{\ominus} \longrightarrow protein-S-Y + protein-S^{\ominus}$$

# The nucleophilic reactivity of the reagents decreases in the series.

Hydroxide > phosphite > alkanethiol > thiophenol and cyanide > sulfite > OH > p-nitrophenol > thiosulfate > thiocyanate.

Complete cleavage with sulfite requires that oxidative agents ($Cu^{2+}$) be present & that the pH be higher than 7.

$$RSSR + SO_3^{2\ominus} \longrightarrow RSSO_3^{\ominus} + RS^{\ominus}$$

$$2RS^{\ominus} \xrightarrow{Cu^{2\oplus}} RSSR$$

(4) Cysteine Residue

- A number of alkilating agents yield derivatives which are stable under the conditions for acidic hydrolysis of proteins.

# The reactions with ethylene imine giving an S-aminoethyl derivative and hence an additional linkage position in the protein for Hydrolysis by trypsin.

# Lodoacetic acid, depending on the PH, can react with cysteine, methionine lysin and histidine residue.

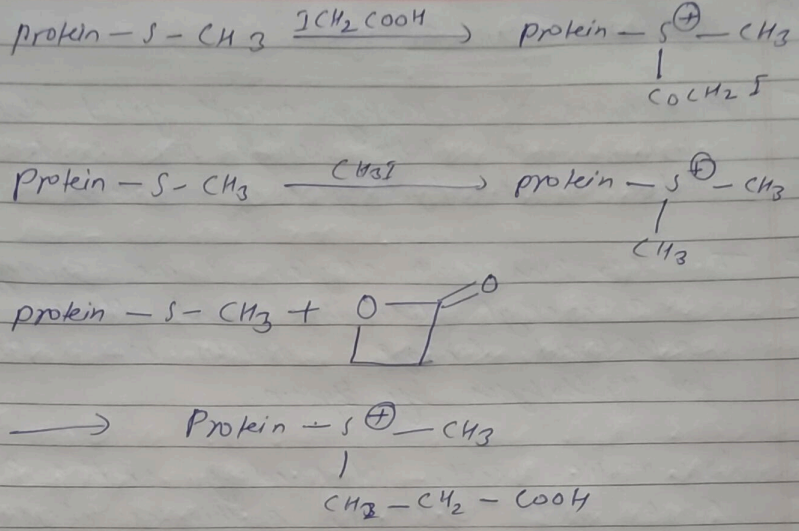
$$\text{Protein} - SH \xrightarrow{ICH_2COOH} \text{protein} - S - CH_2 - COOH$$

(5) Methionine Residue

# Methionine Residues are oxidised to Sulfoxides with hydrogen peroxide

# The Sulfoxide can be reduced, regenerating methionine using an excess of thiol reagent.

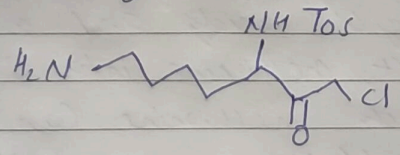
# $\alpha$- Halogen Carboxylic acids and alkyl Halogenides convert methionine into Sulfonium derivatives from which methionine can be regenerated in an alkaline medium with an excess of thiol reagent.

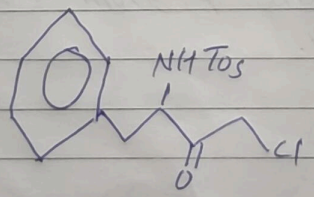



(6) **Histidine residue**

# Selective modification of Histidine residues present on Active sites of Serine proteinases is possible.

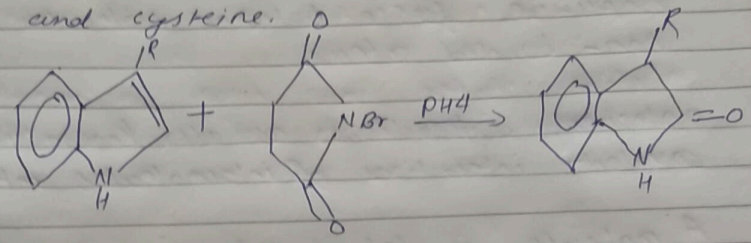
# Substrate analogues such as Halogenated methyl Ketones inactive such enzymes.



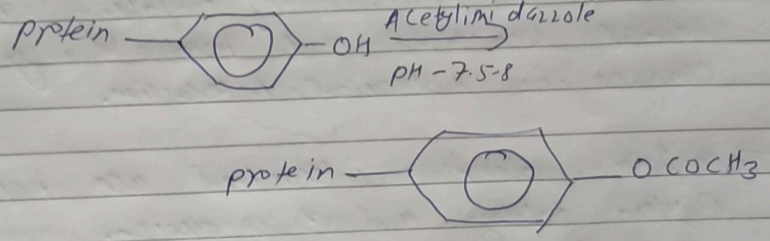
(7) Tryptophan Residue

— N-Bromosuccinimide Oxidizes the Aryptophan side chain and also tyrosine, Histidine, and cysteine.




(8) Tyrosine Residue

# Selective acylation of tyrosine can occur with acetylimidazole as a reagent.




The End

# PHYSICAL

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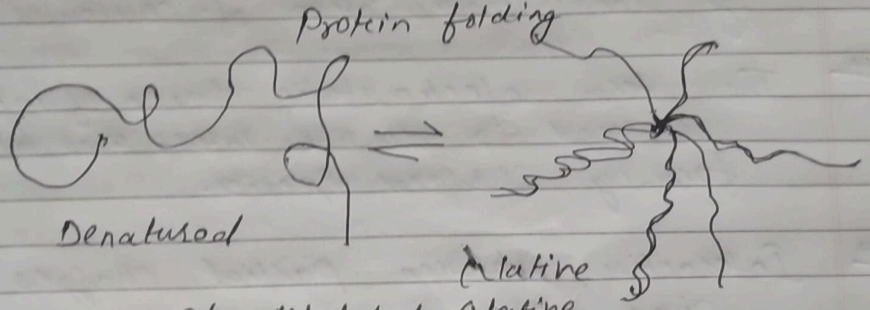
Interaction that determine the property of proteins

- Molecular interaction are attractive or repulsive force between molecules and non-bonded atoms.
- Molecular interactions are also known as non-covalent interactions or intermolecular interactions.
- A molecule is set of atoms that associates tightly with covalent bond. and it does not dissociate or lose its structure, when it interact with its environment.
- Covalent bonds remain intact, when proteins unfold. The processes of unfolding are not chemical reactions and involve only changes in molecular interaction.
- Therefore, molecular interactions are important in protein folding and drug design.

## Native States

- When proteins fold into 3D globular structures, they are called in Native states.
- The proteins Native states and their assemblies are stabilized by several molecular interaction viz, electrostatic force, Vander Waal interaction, hydrogen bond and hydrophobic interactions.
- In biological systems, proteins assembles with DNA, RNA membranes and with other proteins to perform specific function.




## Balance B/n ~~Natured~~ Native And Denatured State :—

- Molecular interactions stabilize both the folded state and denatured state.

Large no. of intra-molecular interactions within a protein native state are opposed by intermolecular interaction in the denatured state with surrounding water molecules.

# On balance, native biological macromolecules and assemblies are marginally stable.

# A small perturbations can tip the balance from folded state to unfolded state.

# Native states have low conformational entropy compare to denatured state.

## # SHORT-RANGE-INTERACTION

# All molecular interactions are fundamentally electrostatic in nature and can be described by some modification of COULOMB'S Law.

# The term electrostatic force of interaction is used to describe interactions b/n formally charged species.

# Interactions between partial charges are given other names like hydrogen bond, van der waal interaction.

# When two atoms are close together the occupied orbitals on the atom surfaces overlap, causing electrostatic repulsion b/n surface electrons.

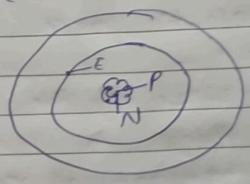
# This repulsive force between atoms acts over a very short range, but is very large when distances are too short.

# Electrostatic force

All matter is made up of atoms

Atoms contain

(i) proton (+)
(ii) Neutrons (0)
(iii) Electrons (-)




# ~~Electric charge~~

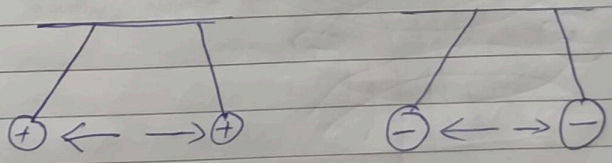
~~All~~

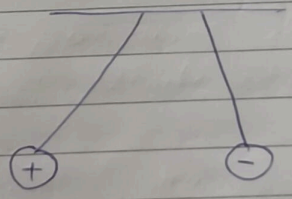
# Law of electric charge

(i) The law of electric charges states that like charges repel and opposite charges attract.

(ii) Protons are positively charged and electrons are Negatively charged

(iii) Without this attraction, electrons would not be held in atoms.



# Electric force

The force between the charged object is an electric force.

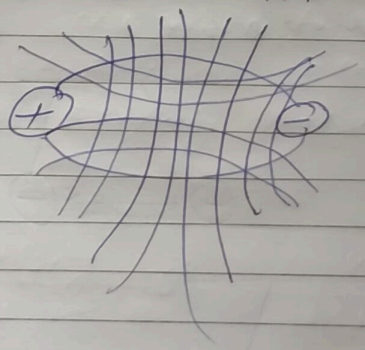
The size of the electric force depends on 2 things.

(I) The amount of charge ( greater the charges greater the force)

(II) The distance b/n charge ( the fusthes the distance, the less the force).

# Electric field

# An electric field in the region around a charged object where electric forces can be exerted on another charged object. (Repelled or attracted)



# Coulom's Law – Gives the electric force b/n two point charges.

$$F = k\frac{q_1 q_2}{r^2}$$

$k = 9.0 \times 10^7$

$q_1$ = charge, (charge on mass)

$q_2$ = $m_2$ ( " )

$r$ = distance

The electric force is much stronger than the gravitional force.

$$F_g = G\frac{m_1 m_2}{r^2}$$

$$G = 6.67 \times 10^{-11}\ \frac{Nm^2}{Kg^2}$$

# Charged object

Atoms do not have a charge because the numbers of electrons and protons cancel each other.

$3P^+ + 3e^- = 0$

# Atoms either gains or loss electrons

There are 3 ways objects can be charged

(i) Friction

(ii) Conduction

(iii) Induction

# Friction

# Charging by friction occurs with electrons are wiped from one object onto another.

→ e.g — If you use a cloth to rub a plastic rules electrons move from the cloth to the rules. The rules gains electrons and the cloth loses electrons.

# Conduction

# Charging by conduction happen when electrons move from one object to another through direct contact.

e.g — Suppose you touch an uncharged piece of metal with a positively charged glass rod. electrons from the metal will move to the glass rod. The metal loses electrons and becomes positively charged.

# Induction

Charging by induction happens when charges in an uncharged object are rearranged without direct ~~contact~~ contact with a charged object.

→ ex — If you charge up a ballon through friction and place the ballon near pieces of papers, the charges of the papers will be rearranged and the papers will be attracted to the ballon.

## Van der Walls Interactions:—

It is the sum of the attractive or repulsive forces between molecules.

These were named after Johannes Diderik Van der Walls

Characteristics

# They are weaker than normal covalent bonds.

# Vdw forces are additive and cannot be saturated.

# They have no directional characteristics.

# They are all short range forces and hence only interaction b/n nearest need to be considered instead of all the particles. The greater is the attraction if the molecules are closer due to Vdw forces.

# Vdw forces are independent of temperature except dipole - dipole interactions.

# force b/n two permanent dipoles.

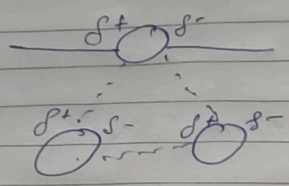
# force b/n a permanent dipole and a corresponding induced dipole.

# force b/n two instantaneously induced dipoles.

# Dipole - Dipole

Two polar molecules align so that $\delta+$ and $\delta-$ are matched

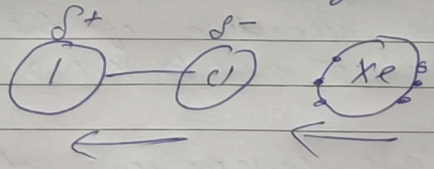
ex- ethane ($C_2H_6$) Vs Fluoromethane ($CH_3F$)




# Dipole - Induced Dipole

A dipole can induce a temporary dipole to form in a non-polar molecule.

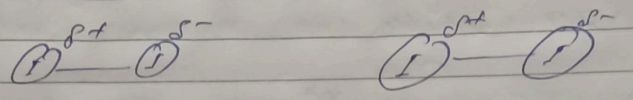
Then molecules then line up to match $\delta+$ and $\delta-$ charges




# Dispession forces

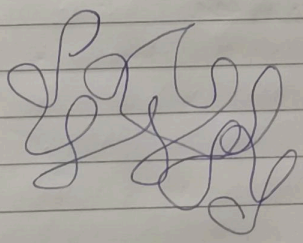
A temporary dipole forms in a non-polar molecule which leads to a temporary dipole to form in another non-polar molecule.

Dispession is the only intermolecular attraction that occur b/n non-polar molecules.




## Significance

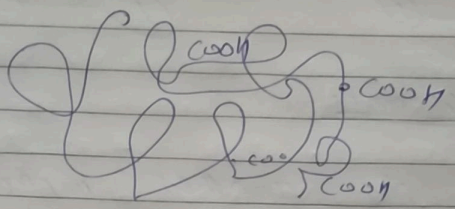
V dI is another important bonding type that helps stabilizing the protein structure. In the coiled-coil protein These is interaction b/n side chain in alpha helix. If these repeating Residues are hydrophobic, such as Leucine. Van der Walls interaction will be formed to stabilize this protein structure.



Graphenes Bonding forces in Graphite. Weak forces between graphenes suggest that they are the van der walls forces.

In polymer formation

- many polymeric chain are held linked by vander walls forces and get stable.




used by some Animal.

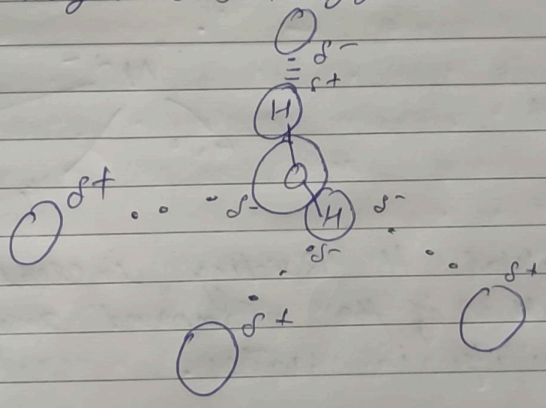
# Gecko Lizard use these forces to climb on the walls.

# Some other animal use animal to walk on water.

# Hydrogen Bond

A hydrogen bond (or H-bond) is a primarily electrostatic force of attraction between a hydrogen atom which is covalently bound to more electronegative atom or group, and another electronegative atom bearing a lone pair of electrons — the hydrogen bond acceptor. Such an interacting system is generally denoted D — H ··· A, where the solid line denotes a polar covalent bond and the dotted or dashed line indicates the hydrogen bond.

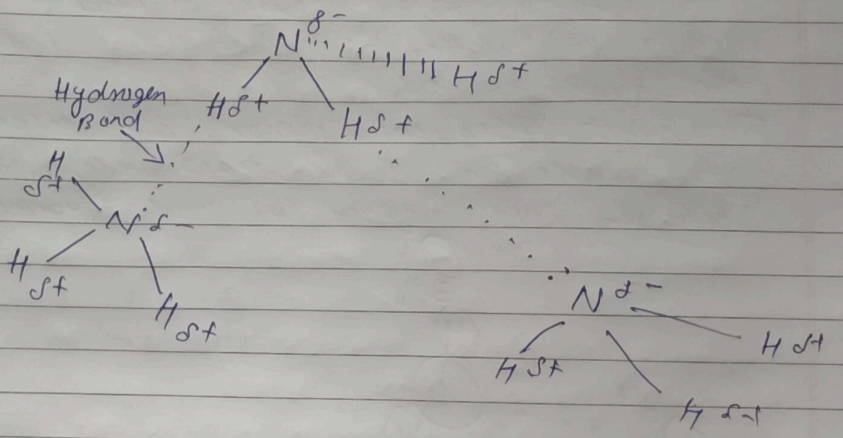
The most frequent donor and acceptor atoms are the second row elements Nitrogen (N), Oxygen (O) and Fluorine (F)



# TYPES

(I) INTERMOLECULAR H-BOND
(II) INTERA MOLECULAR H-BOND

(I) Intermolecular H-Bonding — This type of bonding is between two or more same or different molecules when combine together to form a dimer or polymer respectively and leads to a phenomenon called association.

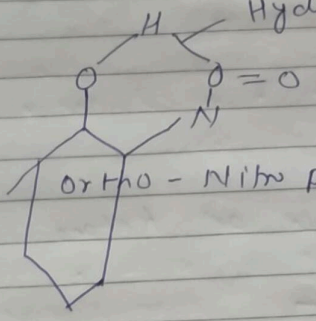



(II) # Intermolecular H Bonding increase the boiling points of the compound and also its solubility in water.

# Intramolecular H-Bonding :-

# This type of Bonding occurs within two atoms of the same molecule and leads to a phenomenon called chelation.

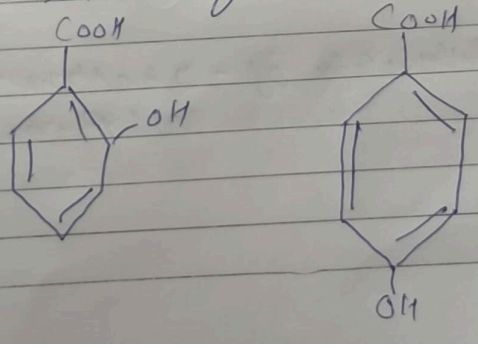
# This type of hydrogen binding frequently occurs in organic compound and result in the cyclisation of the molecule




Ortho - Nitro phenol

# This type of H-Bonding decreases the boiling point of the compound and also its solubility in water.

# The large acidity is due to the intramolecular H bonding which is capable of stabilizing the salicylate ion.



# Intramolecular H-Bonding is weaker than intermolecular H-Bond.

# Application of Hydrogen BOND :—

(1) melting point and Boiling point :—
Intermolecular hydrogen bond resulting in the association molecule raises mp & BP & decreased in intermolecular H-Bond.

$H_2O > H_2S$
$NH_3 > PH_3$
$R-OH > R-SH$
$R-OH > R-OR$

Alcohol > Aldehyde or Ketone

(2) Solubility of water :—

Compounds whose molecule can form hydrogen bond with water molecule are soluble in water.

# Intermolecular hydrogen bond decreases solubility of the compound in water.

ex — solubility of — o-nitrophenol
m & p-nitrophenol

# Propesty

H-bond shifts the positions of bands in Ultraviolet, Infrared and H.I NMR spectra.

# Hydrogen Bond in paints dyes & textile material.

# H-Bond in clothing material

# Cleaning action of soaps and detergent.

# H-Bond in Carbohydrate

# H-Bond in DNA.

# Hydrophobic Interaction

## Introduction

Hydrophobic interaction describe the relation between wates and hydrophes.

Hydrophes are hon-polers molecule and usually have a long chain of Carbons that do not interact with water molecule.

The miking of fat and water is a good example of this interaction.

## Define

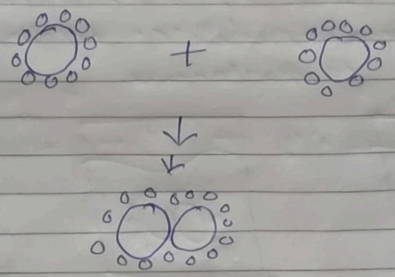
The hydrophobic effect is the observed tendency of hon-polar substances to aggregate in an aqueous soln and exclude wates molecule.

# The tendency of hon polar molecule in a polar solvent (water usually) to interact with one another is called hydrophobic effect.

# The interaction b/n the hon polar molecule are called hydrophobic interaction.

# Causes of Hydrophobic Interaction:—

# The non-polar substance like fat molecules tend to clump up together rather than distributing itself in a water medium, because this allow the fat molecules to have minimal contact with water.



A/c to Gibb's formula:

$$\Delta G = \Delta H - T\Delta S$$

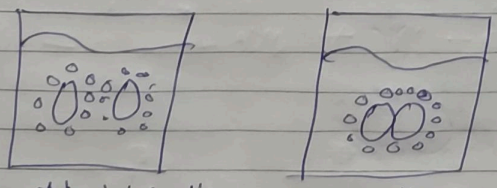
# Formation of HYDROPHOBIC INTERATION:—

# The mixing hydrobes and water is not spontaneous, however, hydrophobic interaction b/n hydrophobes and spontaneous.

# When hydrobes come together and interact with each other, enthalpy increase ($\Delta H$ is positive) because some of hydrogen bonds that form that clathrate cage will be broken.

# Tearing down a portion of clathrate cage will cause the entropy to increase (ΔS is positive) since forming it decreases the entropy.

# A/C to equation (1)
If ΔH is a small positive value and ΔS is large positive value then ΔG will be negative hence hydrophobic interaction is spontaneous.



No interaction



Hydrophobic interaction

| | |
|---|---|
| ΔH = Negative | ΔH = positive |
| ΔS = Negative | ΔS = " |
| ΔG = positive | ΔG = Negative. |

# Strength of Hydrophobic interaction :-

1) Temp :- Strength of hydrophobic interaction increases with temp.

2) No. of Carbons In Hydrophobes :-
molecules with greater no. of Carbon will have the strongest hydrophobic interaction.

# SDS PAGE

(3) shape :- aliphatic organic molecules have stronger interaction than aromatic compound.

# SDS PAGE GEL - electrophoresis :-
SODIUM DODECYL SULPHATE
poly ACRYmide GEL.

electrophoresis :- is a laboratory technique for separating molecules based on their charge.

## SDS - PAGE

The purpose of this method is to separate proteins according to their size, and no other physical feature.
and it is a step in western blot.

## The Gel (MATRIX)

The gel (matrix) itself is composed of either agarose or polyacrylamide.

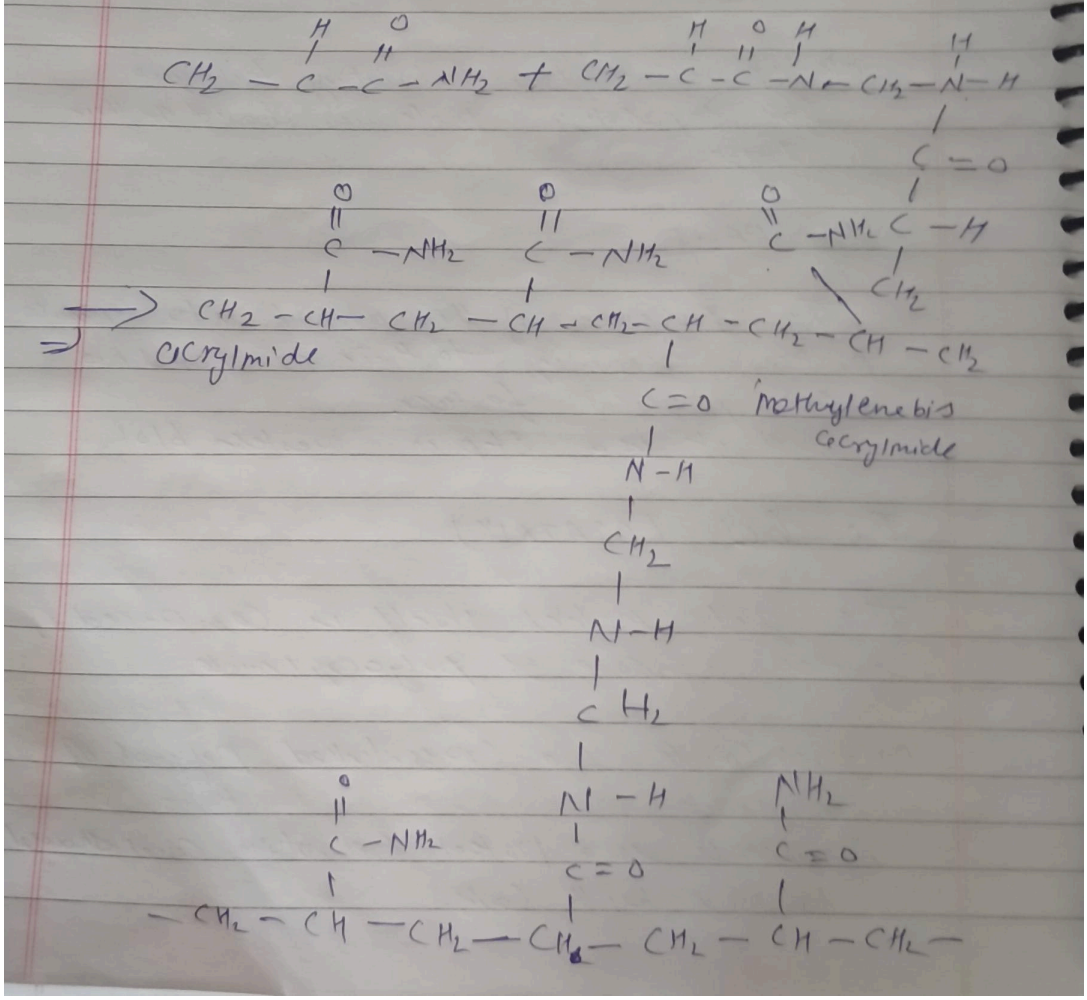
polyacrylamide is a cross-linked polymer of acrylamide.
Acrylamide is a potent neurotoxin and should be handled with care.

# POLYACRLAMIDE GELS

# Have smaller pores than agarose therefore high degree of resolving power.

# Can separate DNA fragments which range in size from 10–500 bp.

# poly acrylamide gel electrophoresis is also used to separate protein molecules.



# Polymerization of ACRYLMIDE

# Cross-linked polyacrylmide gel are formed from the polymerization of acrylmide monomers in the presence of smaller amounts of N,N'

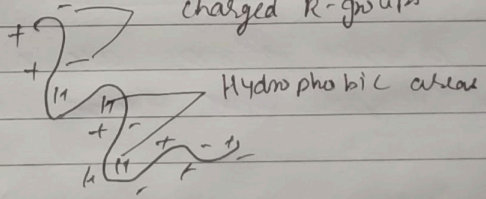
# Bisacrylmide is the most frequently used cross linking agent for polyacrylmide gels.

# SODIUM DODECYLSULPHATE:—

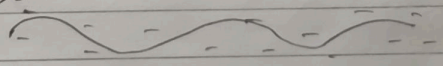
# SD is a detergent that can dissolve hydrophobic molecules but also has a negative charge attached to it.

# If SDS is added to proteins, they will be ~~solubilized~~ soublized by the detergent, plus all the proteins will be covered with many negative charges.

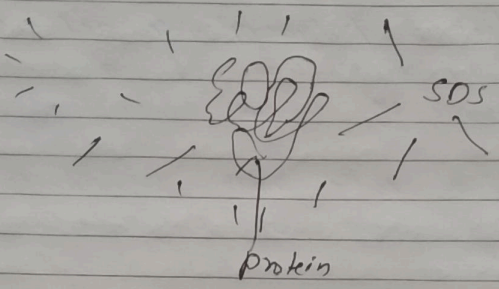
Before SDS




After SDS



# SDS And proteins




SDS And Proteins.

- SDS hun polar chains arrange themselves on protein and destroy Secondary tertiary and quattanary structure.

- So much SDS binds to proteins that the negative charge on the SDS drowns out any net charge on protein side chain.

- In the presence of SDS all proteins have Uniform shape and charge per ~~on~~ unit length.

# Components of the system:—

# DC power source, Reservoir tank, Glass plates spacers and combs.

# Support medium
Gel (polyacrylmide)

# Buffer system
Higher Buffer Capacity

# molecules to be separated
proteins
(Nucleic Acids

# PROCEDURE

# Prepare polyacrylmide gels.
# Add diluted samples to sample buffer.
# Heat to 95°c for 4 min.
# Load the samples onto polyacrylmide gel.
# Run at 200 volts for 30-40 min strain.

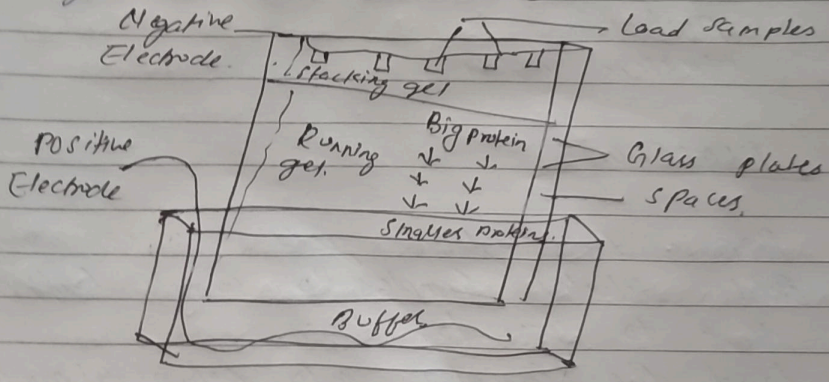
# GEL PREPARATION :—

Mix ingredients GENTLY in the order shown above, ensuring no air bubbles form.

pour into glass plate assembly carefully.

Overlay gel with isopropanol to ensure a flat surface and to exclude air.

Wash off isopropanol with water after gel has set (±15 min)




An illustration of an apparatus used for SDS PAGE

Sample Buffes :-

# Tris Buffer to provide appropriate pH.
# SDS detergent to dissolve proteins and give them a negative charge.
# Glycerol to make samples sinks into wells.
# Bromophenol Blue dye to visualize samples.

Run at 200 volts for ~30-40 min

Running Buffes. pH-8.3

Tris - Base - 12.0 g
Glycine - 57.6 g
SDS - 4.0 g

distilled water to 4 litres.