



# CSIR-NET

Council of Scientific & Industrial Research

**LIFE SCIENCE**

**VOLUME – 1**

**MOLECULES & THEIR INTERACTION  
RELEVANT TO BIOLOGY**



## CONTENT

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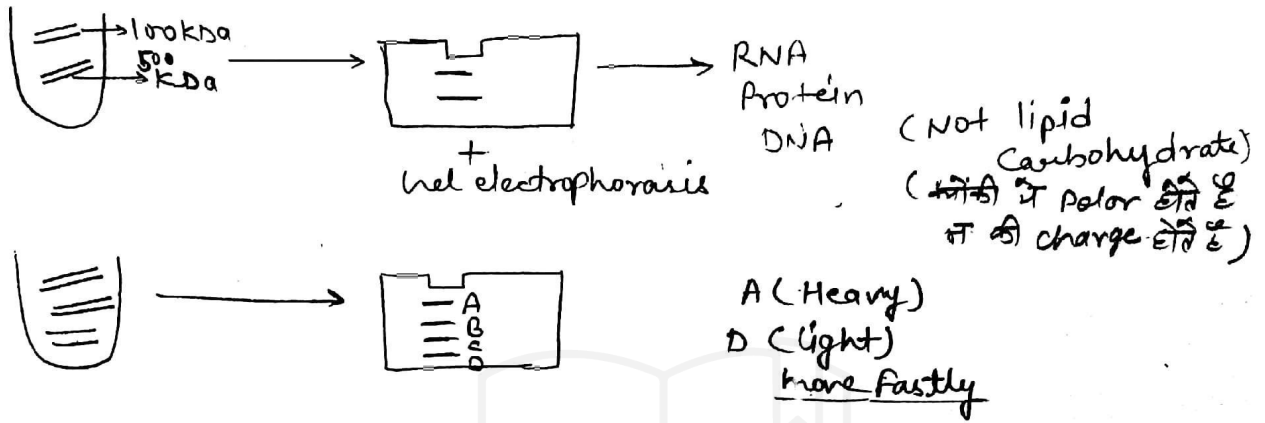
### MOLECULES AND THEIR INTERACTION RELEVANT TO BIOLOGY

#### (Tool and Techniques)

1. Fundamental (Gel ELECTROPHORESIS)	1
2. Bonding	9
3. Fluorescent	25
4. Autoradiography	34
5. DNA Foot PRINTING	35
6. Affinity	40
7. DNA, DNA POLYMERASE	45
8. Domain, Motif, GENE	59
9. Chromatography (Technique)	63
10. Single NUCLEOTIDE POLYMORPHISM	93
11. Gel ELECTROPHORESIS	94
12. Restriction ENDONUCLEASE DIGESTION	111
13. Blotting	116
14. Microscopy	135
15. Cell SORTER	160
16. PCR	164
17. Recombinant DNA Technology (RDT)	172
18. Protein Tagging	201
19. C-DNA LIBRARY	205
20. Protein SEQUENCING	207
21. DNA SEQUENCING	211
22. Microarray	222
23. Chip ON CHIP	225

## FUNDAMENTAL

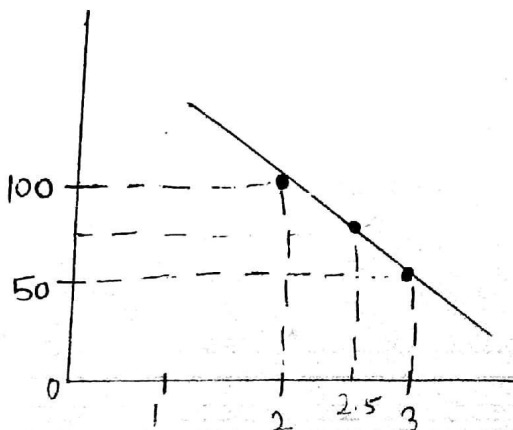
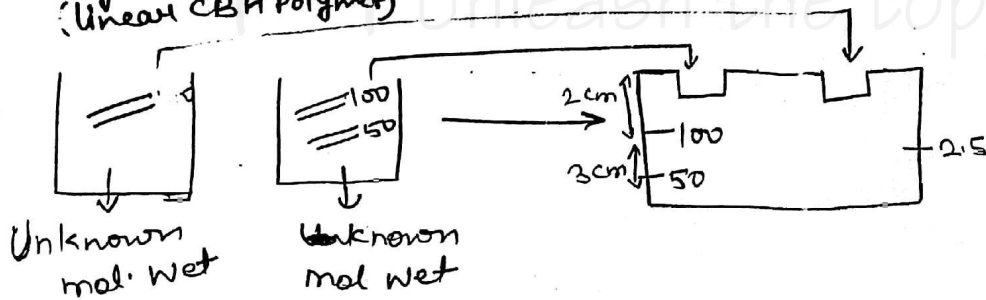
:- gel electrophoresis greek word which means  
"gel" - semisolid → porous  
borne by electricity.  
Electro + phoresis → movement



- gel electrophoresis is a movement of charge particles under electric field. Separate acc<sup>n</sup> charge & size
- gel electrophoresis separate the molecule.
- gel electrophoresis is applicable only for DNA, RNA & Protein not for carbohydrate & lipid. (cellulose acetate)  
DNA migrate to Anode.

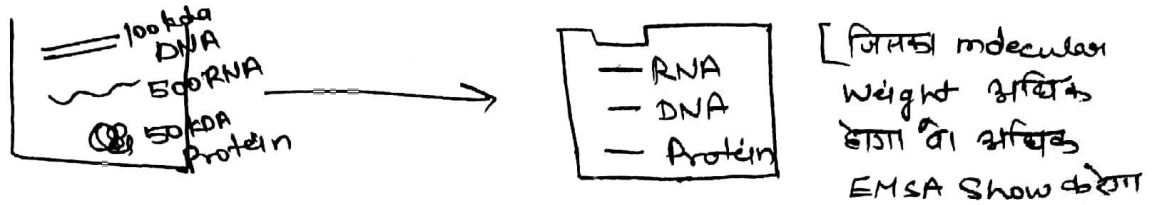
→ 2 types -

- ① Agarose (Linear CBH Polymer)
- ② PAGE (Not solid)



(molecular weight plot use)

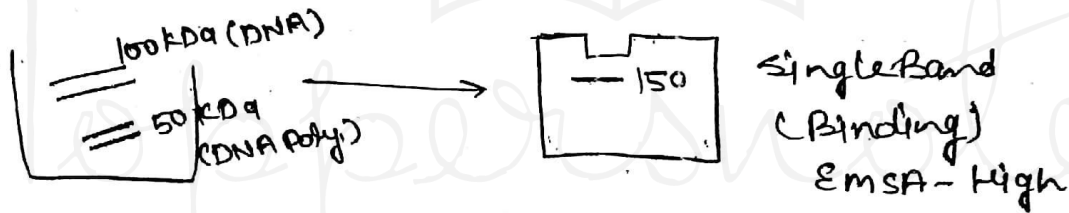
- > Band represent the matter!
- > gel electrophoresis separate the RNA from DNA, RNA from protein.



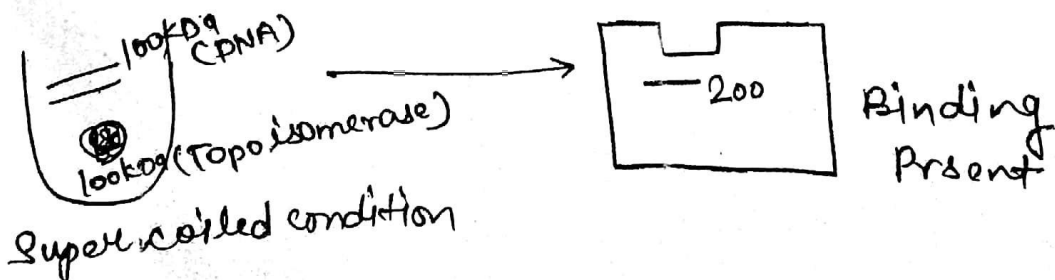
### EMSA - Electrophoretic mobility shift Assay

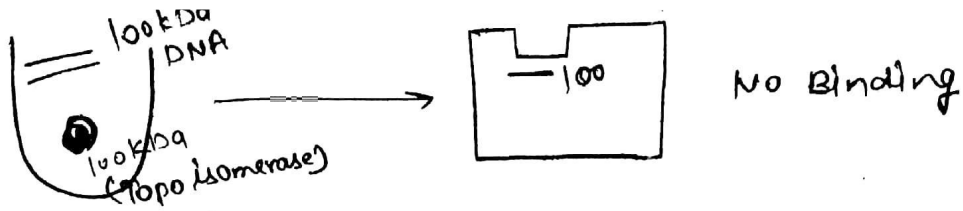
DNA की Binding following enzyme से हो सकती है।

- + DNA Poly.
- + RNA Poly.
- + Helicase
- + Topoisomerase



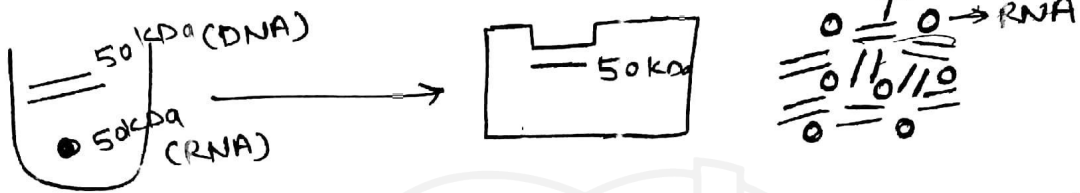
→ यदि DNA व Enzyme को electrophoresis करवाने पर single Band प्राप्त होता है तो उसमें Binding Present होती है। और EMSA High होता है।



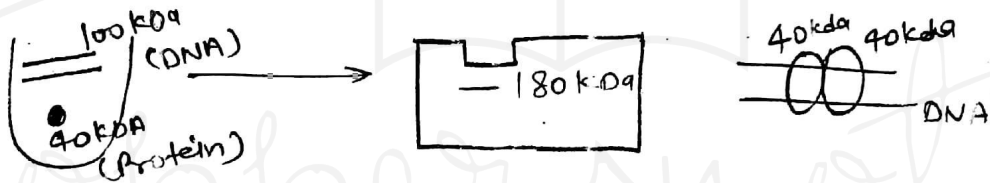


Relax condition

→ Topoisomerase Supercoiling में ही Bind होता है।  
Relax condition में DNA के साथ Bind नहीं होता।



→ If molecular weight is same of DNA & RNA then it is not differentiated But Binding is absent



→ In this situation many proteins are binded with DNA.

→ DNA is Helical

PAGE - Polyacrylamide gel electrophoresis

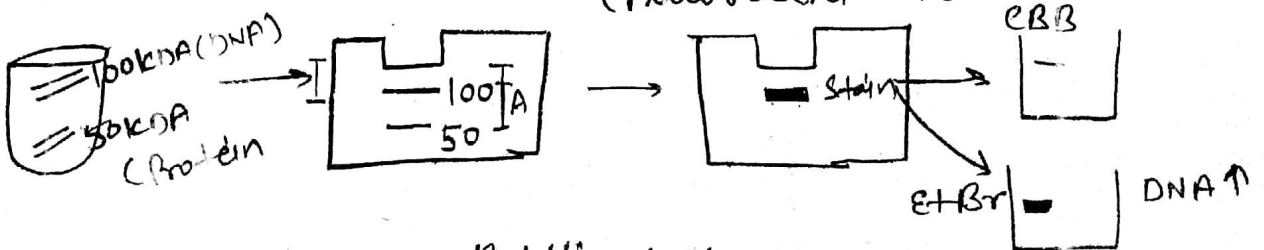
→ Enzymes binds with their substrate.

→ DNA Poly. binds with DNA.

→ RNA Poly. also binds with DNA.

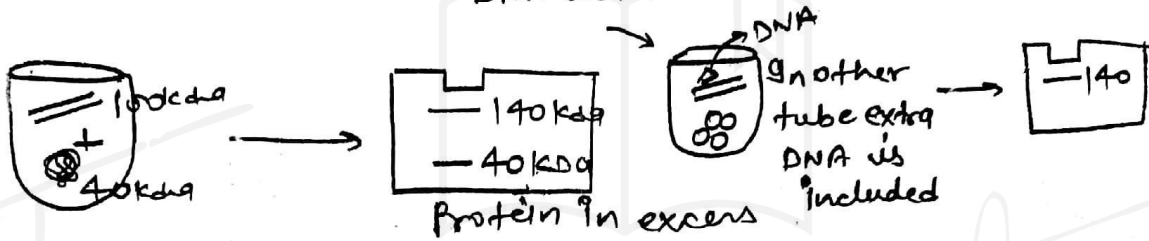
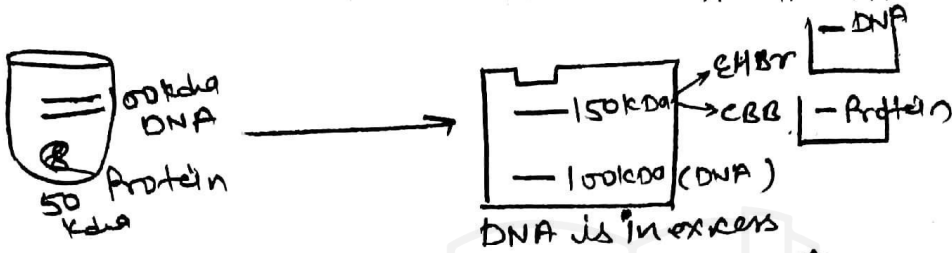
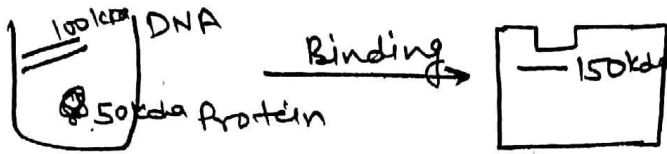
CBB - Blue - Protein

EtBr - Red - DNA orange  
(Fluorescent dye)

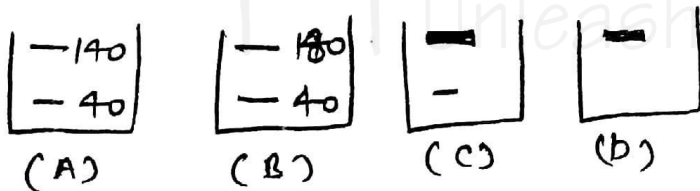


CBB - Coomassie Brilliant Blue

↑ DNA  
↑ RNA  
molecular height is



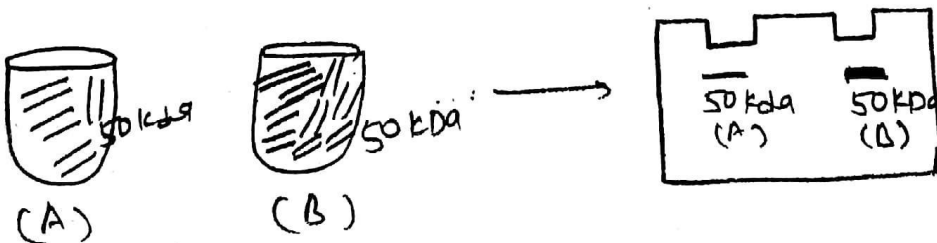
Experiment :-



DNA in excess amount

Protein in excess

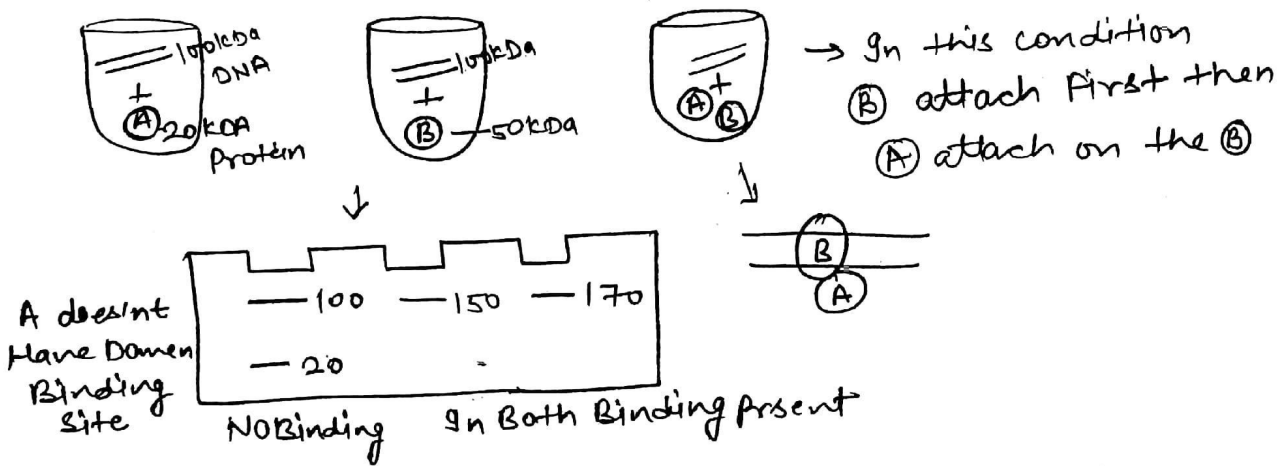
DNA included



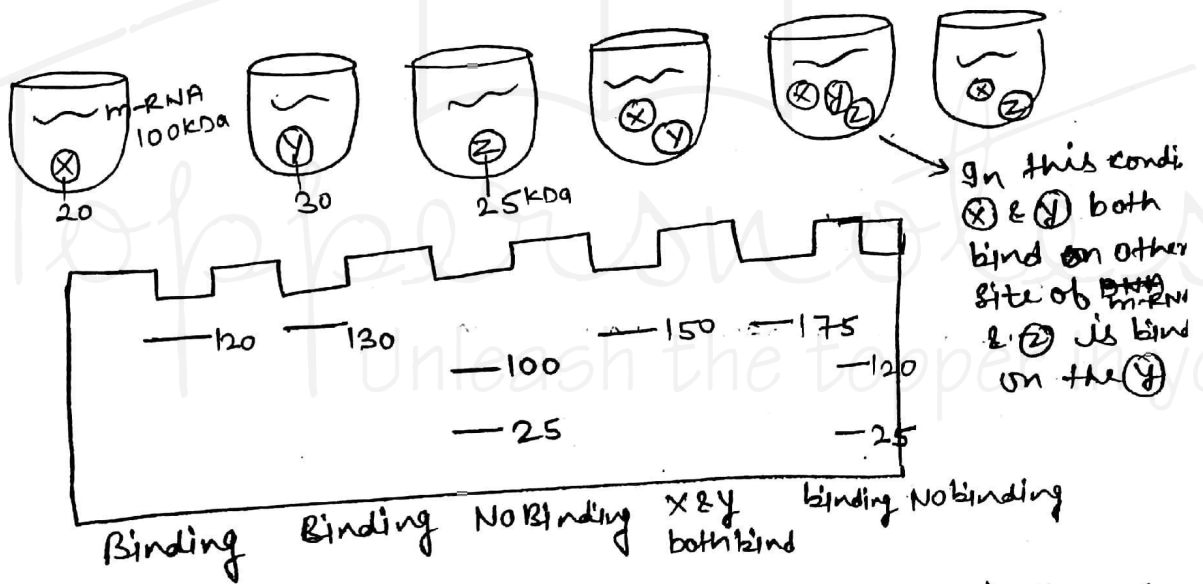
(B) DNA amount is high but molecular weight is same

→ In this stage thickness is high

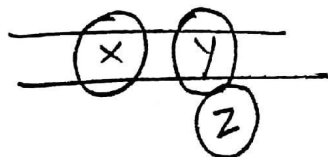
→ Thickness indicate concentration, shifting of Band indicate binding.



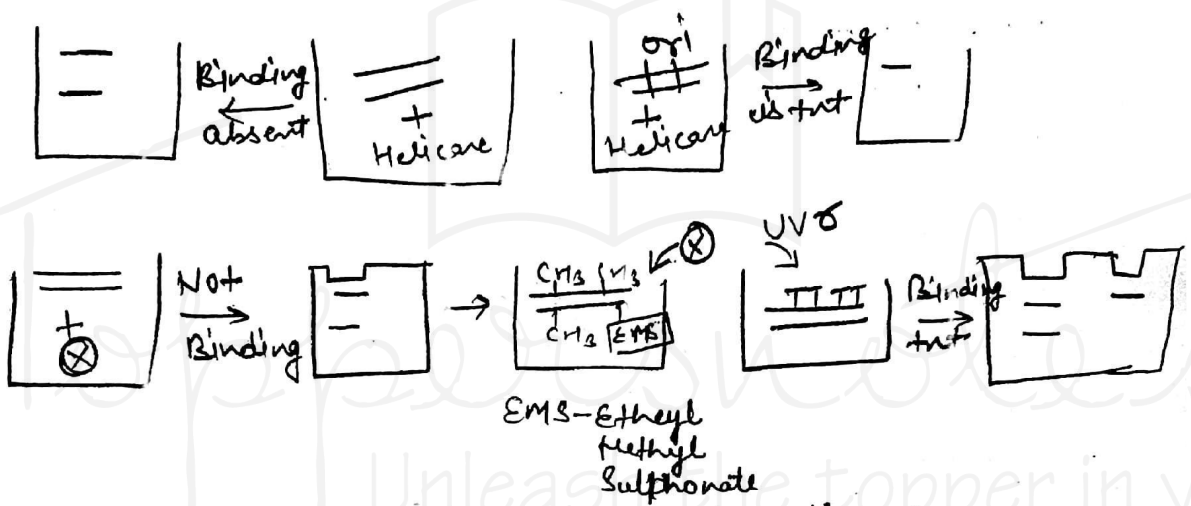
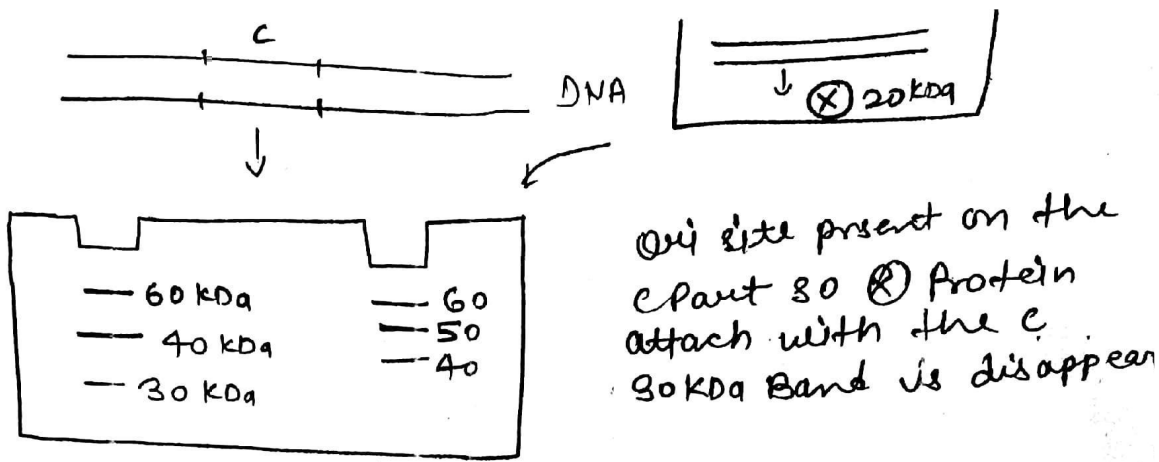
- DNA, DNA Not Bind together
- Hexokinase also Not Bind with other Hexokinase
- all Hexokinase Not interact together.



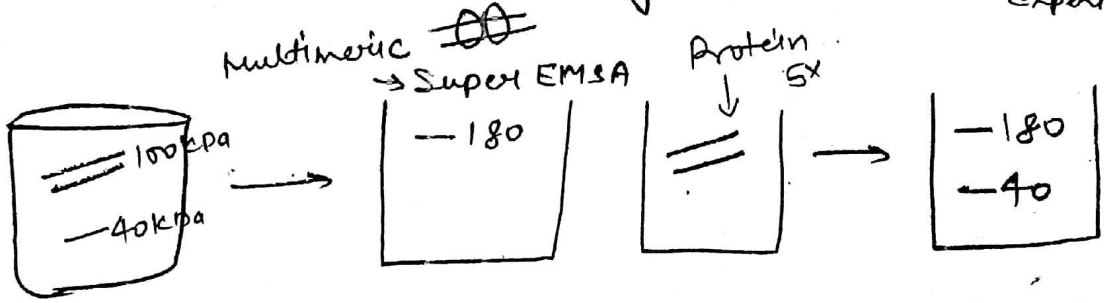
→ Molecular weight is diff and domain site is present then it's bind different site of m-RNA



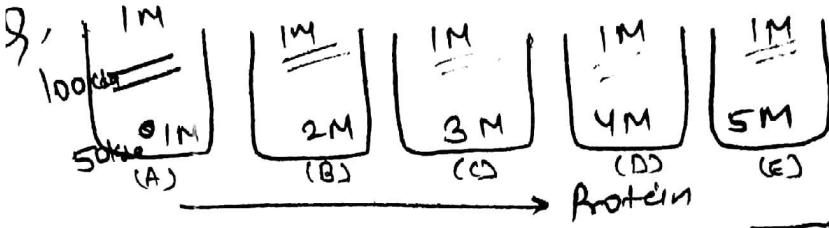
→ DNA, RNA have always Negative charge (-ve) & Proteins are ampholytes (so +ve, -ve both)



- These  $\otimes$  protein Bind with Thymine dimer
- The molecular weight of molecule must be known to us before performing EMSA or before EMSA Experiment



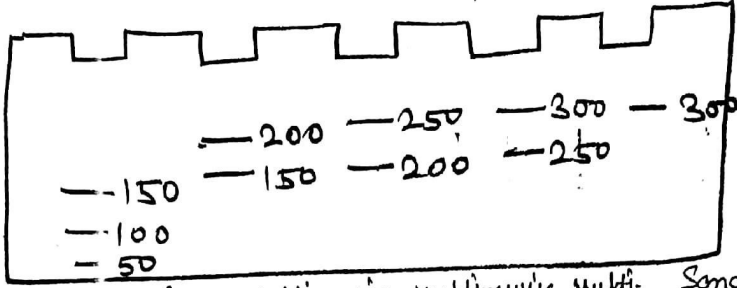




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Sandwich



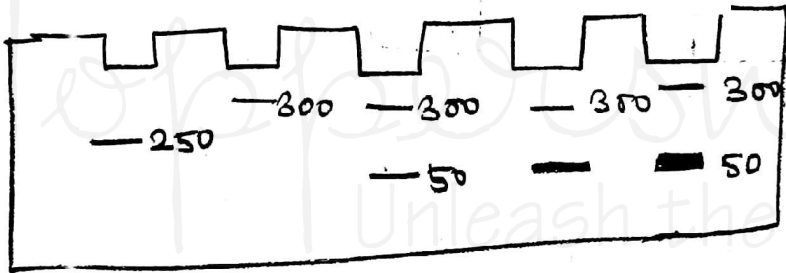
→ Monomeric  
→ DNA: RNA ↑

Multimeric Multimeric Multi-meric

Sandwich

Sandwich

Multimeric binding



Sandwich

0

00











(low concn of protein)

→ जब Protein concentration कम है वह excess amount में DNA के साथ Bind होती है - sandwich binding कहलाती है

→ All gel based questions can be solved by remembering two questions in mind.

① Concentration --■

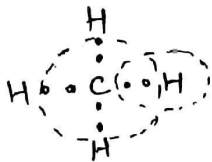
② Position of band --

Super coiled	+	+	+	+	+	NAD <sup>+</sup> - Inhibitor Protein c - Activator
Relax	+	+	+	+	+	
Protein X	-	+	+	+	+	
ATP	-	-	+	+	+	
Protein c	-	-	+	+	+	
NAD <sup>+</sup>	-	-	-	+	+	
Relax						
Super coiled						
			ATP Bind			
			With Relax			
			DNA			

→ Topoisomerase change the topology of DNA

→ Supercoiled form of DNA is converted into relax DNA.

# BONDING



- Non-covalent  $\Rightarrow$  weak interaction  
1-5 kcal/mol energy req.
- ① Electrostatic = Ionic
  - ② Hydrogen bonding
  - ③ Hydrophobic
  - ④ Van der Waals

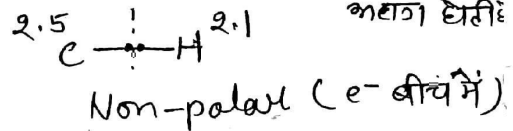
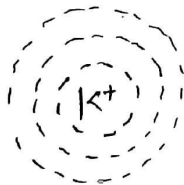
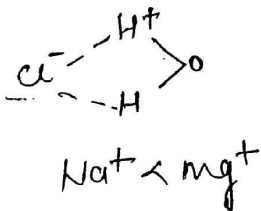
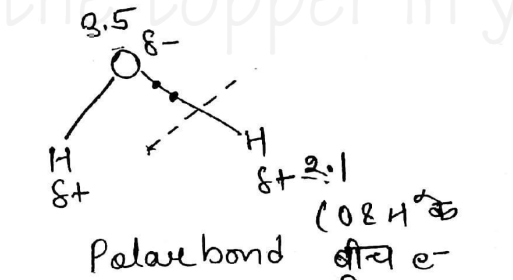
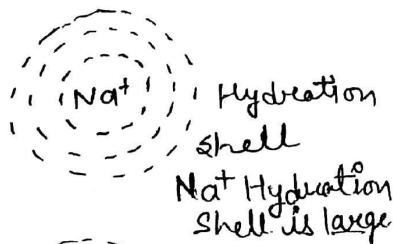
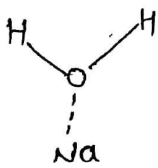
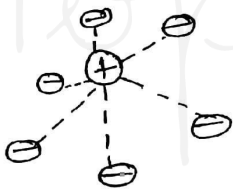
- $\rightarrow$  Present between two atoms
- $\rightarrow$  Strong bonding (stable in nature)
- $\rightarrow$  Form in High temp, Enzyme use
- $\rightarrow$  Covalent bonding is sharing of electron.
- $\rightarrow$  Atoms form the molecule (2 type Polar & Non-polar)

## Non-covalent bonding

① Electrostatic :- (Temporary)

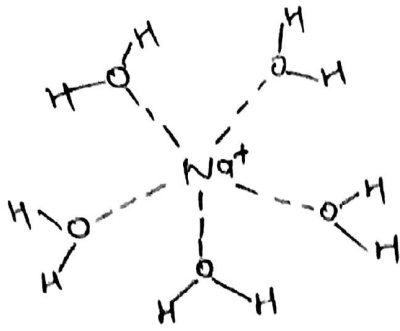
$$F = \frac{Kq_1q_2}{r^2}$$

$K^+$  की size  $Na^+$  से ज्यादा होती है But hydration shell  $Na^+$  के ज्यादा बनता है



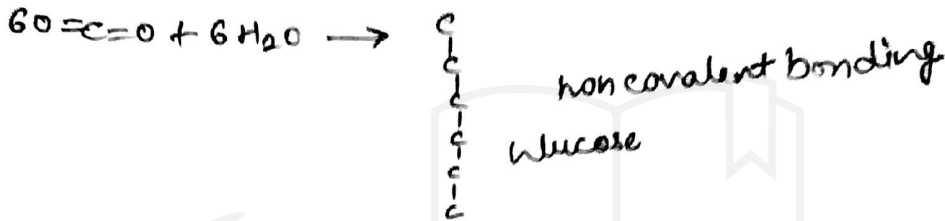
Hydration shell :- It is a shell of water molecules that surrounds the ions.

$\rightarrow$  It is a cage of water molecule.

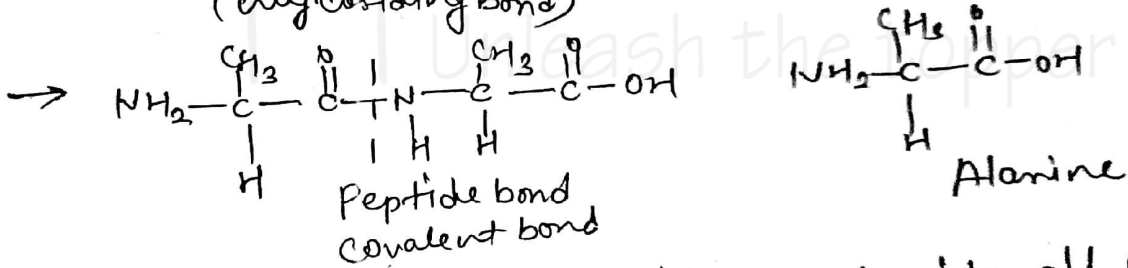
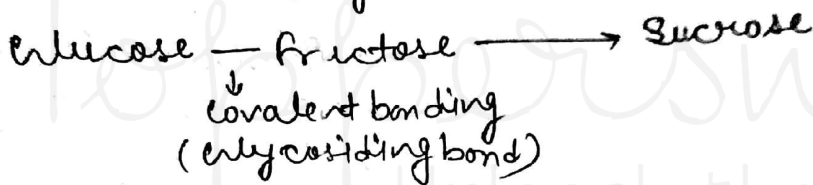


Hydration shell

→ DNA, RNA, ATP, etc are no stage. All are molecules  
GTP, TTP, CTP



Glucose - Fructose (Non-covalent bonding)  
No stage



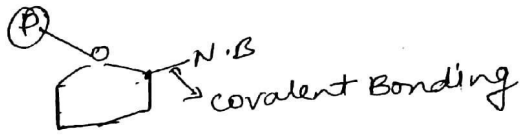
→ Purin, Pyrimidine, Nucleotide & Nucleoside all are no 'stage'

→ All are molecules and molecules form matter

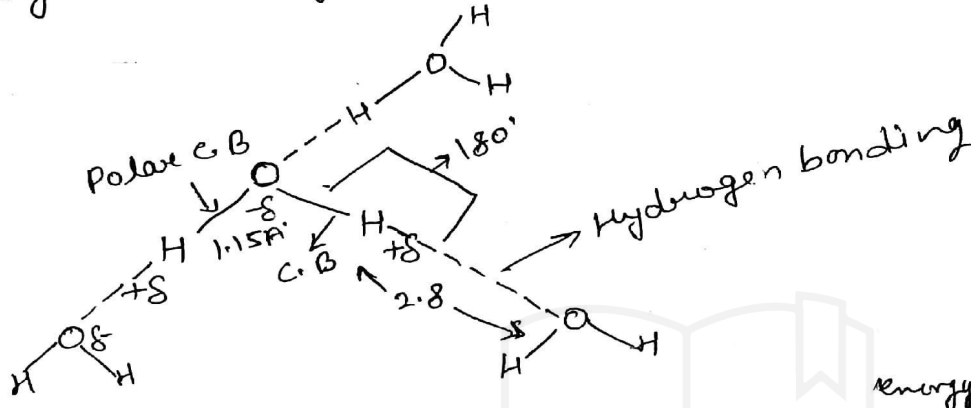
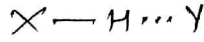
→ Virus are no any stage. Many virus form a Crystal.

→ According to inter molecular Distance various forms are found Solid, liquid, gas.

99<sub>1</sub> - 99<sub>2</sub> - 99<sub>3</sub> - 99<sub>4</sub> - Peptide



② Hydrogen bonding :-



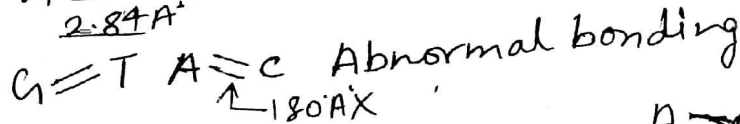
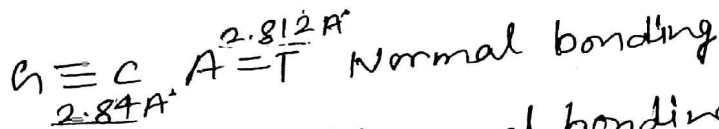
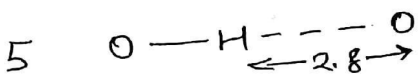
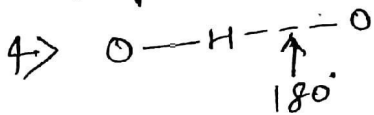
energy - 5 kcal mol<sup>-1</sup>

→ Conditions -

1) H is must

2) FON  
 4 3.5 3.0  
 H-F    H-O    H-N

3) Hydrogen must be present b/w two highly electro-negative atoms



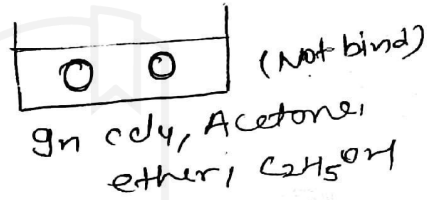
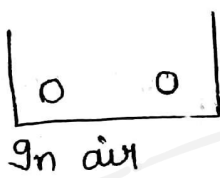
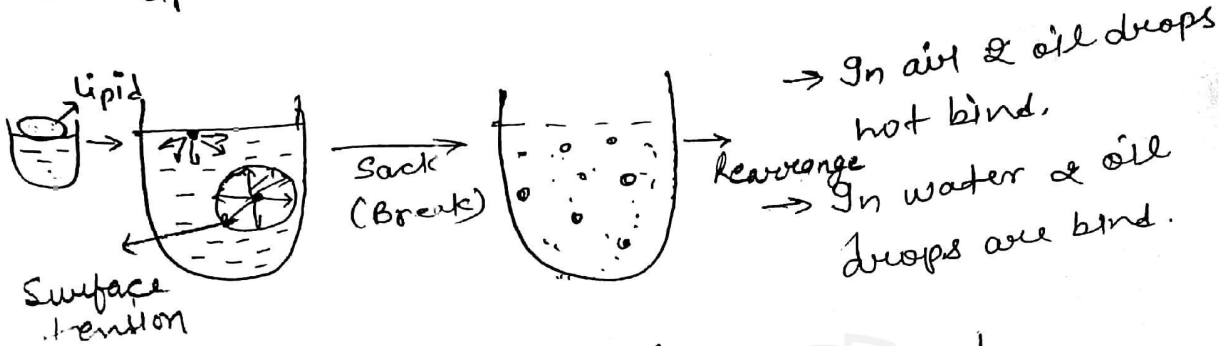
1 Twin = 10bp = 360°  
 1bp = 36°

1 Twin = 12bp = 360°  
 1bp = 30°

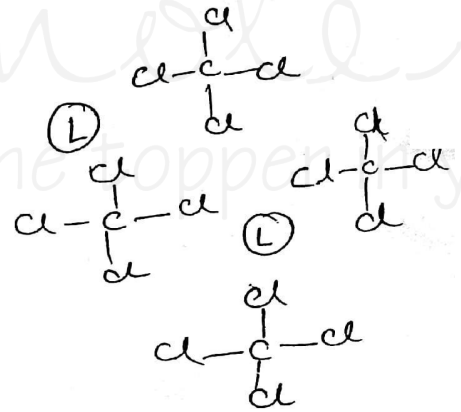
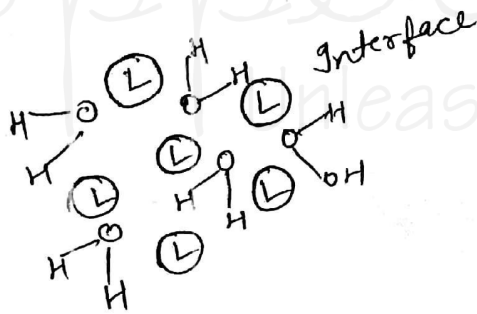
$A = C$  The angle and distance is major by X-ray crystallography

### 3) Hydrophobic Interaction :-

- Carbohydrate is a polar molecule because Polyhydroxy aldehyde & Ketone is present
- Lipid is Non-polar.



→ Water is must for hydrophobic force.

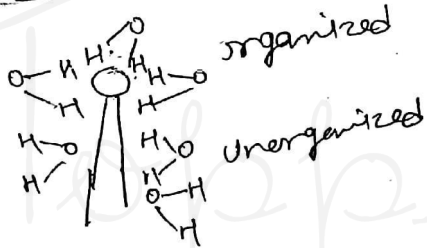
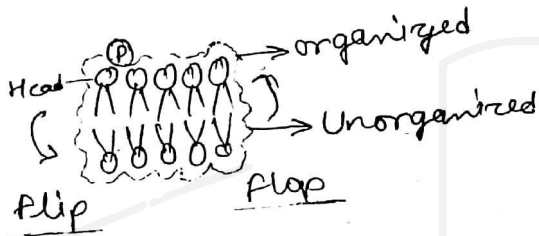


- The water molecules are randomly oriented around the lipid droplet,
- Hydrophobic force is tendency of water molecule to form bigger cage.
- The water molecule which are present at the interface (2 lipid) They come out on surface in order to form perfect H-bonding with other water molecules because

of this outward movement of water a vacuum is generated and becoz of the vacume molecules/ matter come close to each other.

→ Water molecule that not exist b/w lipid layer & lipid molecule.

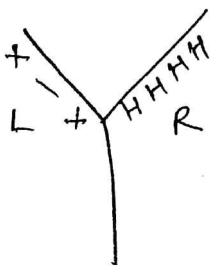
→ Nitrogenous base, Glucose-6-P<sub>1</sub> & Phospholipid  
 → H<sub>2</sub>O present the both side of plasma membrane.



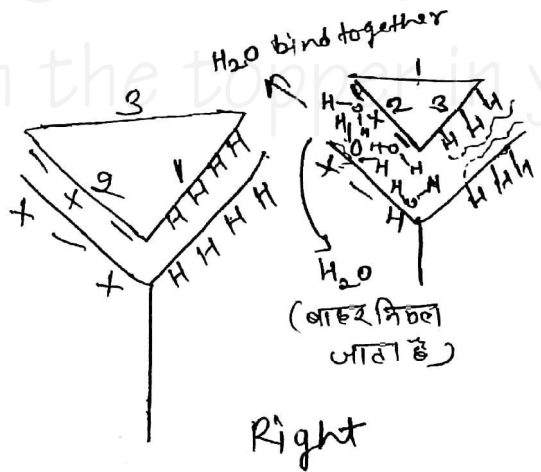
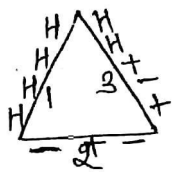
→ H<sub>2</sub>O lipid bilayer cross the lipid bilayer  
 → O<sub>2</sub> & CO<sub>2</sub> is soluble in lipid

→ Alcohol also cross the lipid bilayer  
 → All str are sd

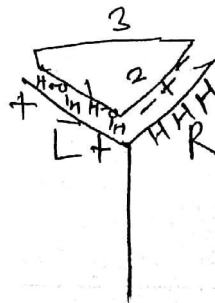
ex-



Receptor



Right

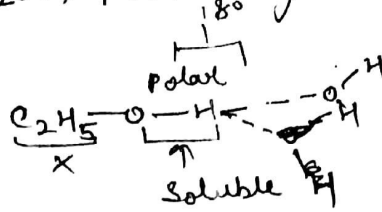
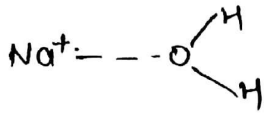


Wrong

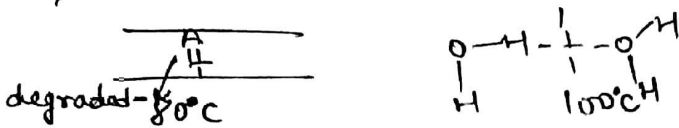
(Kcl is not diff into ions in H<sub>2</sub>O becoz Kcl lattice energy is very high)

★ Solubility :-

→ Alcohol is soluble in  $H_2O$ , formaldehyde also.  $H-C(=O)-H$

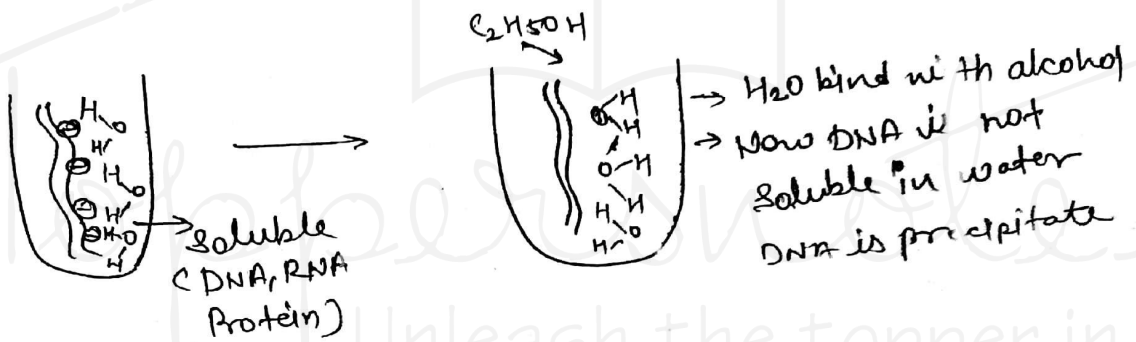


→ Alcohol is good solvent.



30 ml + 70 ml → 97 ml (3 ml reduce)  
 Alcohol  $H_2O$

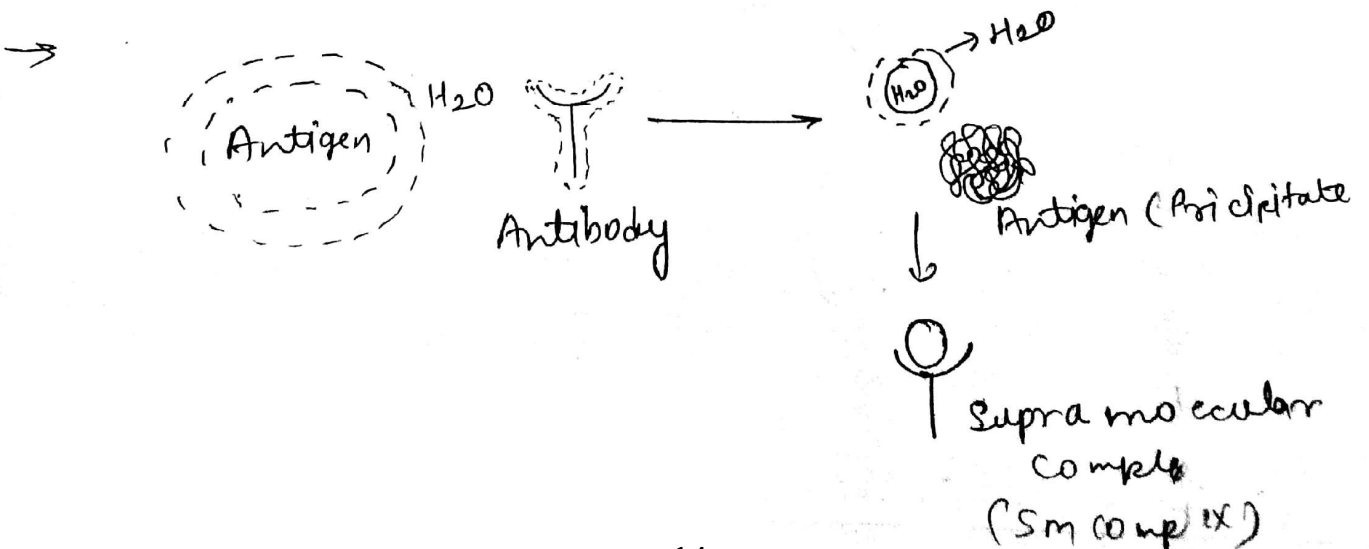
300 ml + 700 ml → 970 ml



→ DNA more soluble & RNA is less soluble.

→ Solubility = Interaction with water

→ Alcohol form perfect H-bonding with water.  
 (All conditions are met)

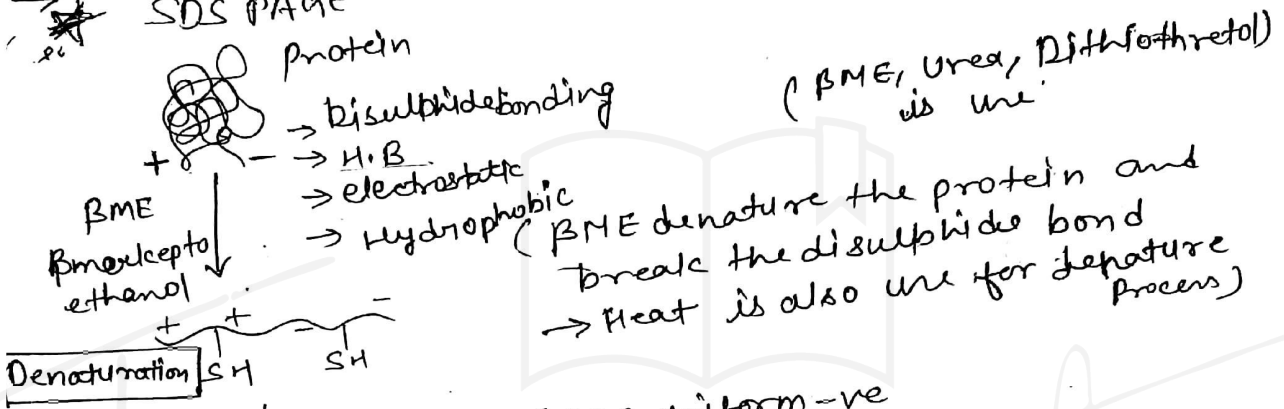




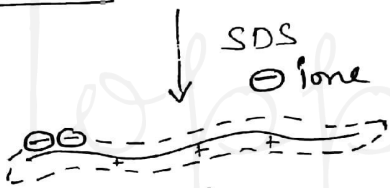
→ Antigen के चारों ओर water होता है व Antibody के चारों ओर भी water होता है। जिसमें H<sub>2</sub>O का एक network Antigen & Antibody. Supramolecular complex कहते हैं।

→ DNA, RNA, Protein, CBH, Lipid is macromolecule  
one molecule bind with another macromolecule & Form SM complex.

→ SDS PAGE

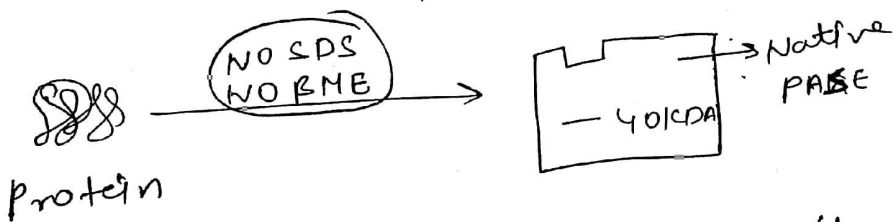
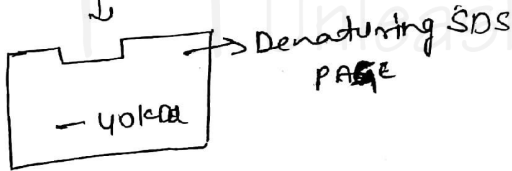


(BME denature the protein and break the disulfide bond → Heat is also use for denature process)



(SDS uniform -ve mask the +ve)

(SDS - Sodium dodecyl Sulphate)



→ Direct use the SDS before denature the protein then Heat is use

