SEPARATION AND DETECTION OF PROTEINS

SDS-PAGE

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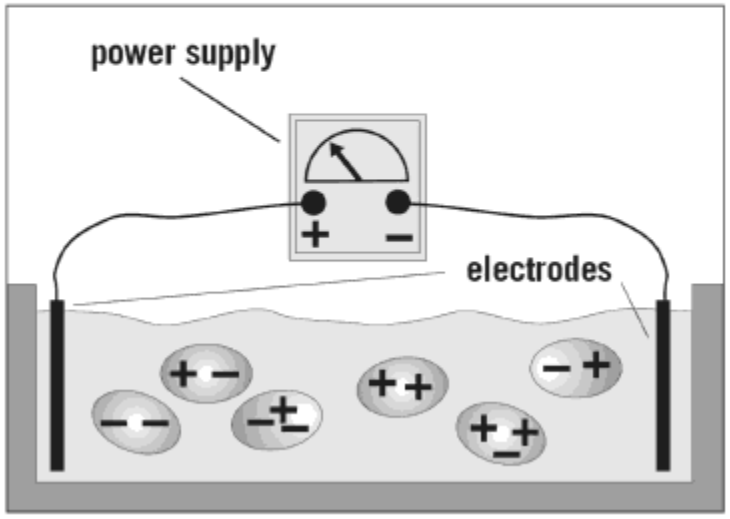
sodium dodecylsulphate-polyacrylamide gel electrophoresis

-method for separation of proteins according to their molecular weight

Principle



Proteins move in the electric field. Their relative speed depends on the charge, size, and shape of the protein

Why Use Acrylamide Gels to Separate Proteins?

- Acrylamide gel: tight matrix
- Smaller pore size than agarose
  - average **amino acid** = **110 Da**
  - Ideal for protein separation

Various steps of SDS- PAGE experiment

Prepare polyacrylamide gels solution from the gel stock (30%) for resolving gel to make final concentration of 12.5%.

After polymerization of resolving cast the stacking gel and place comb over it to prepare wells.

Add samples to the sample buffer.

Heat to 95°C for 4 minutes.

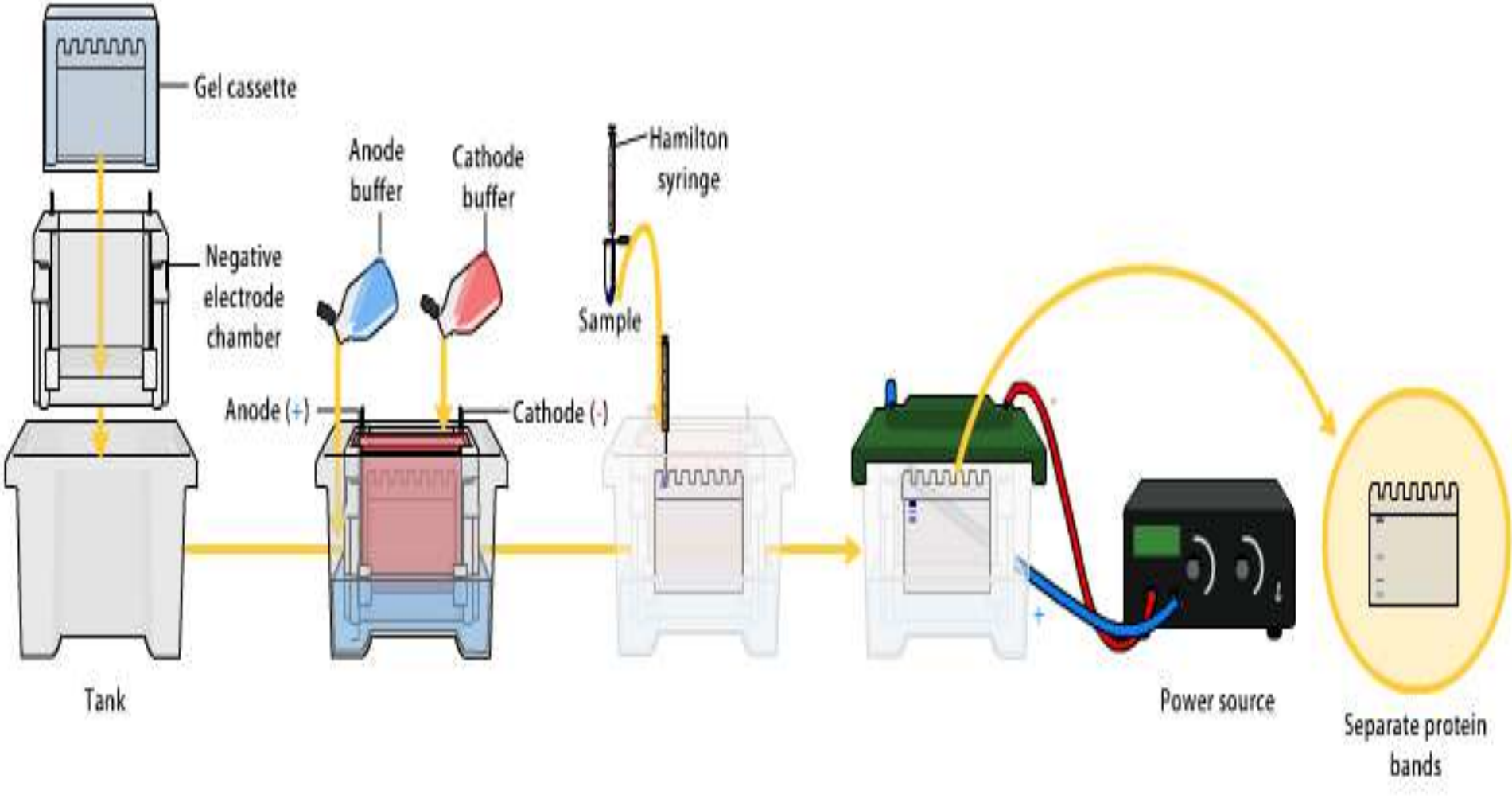
Load the samples onto polyacrylamide gel.

Run 200 volts for 30-40 minutes.

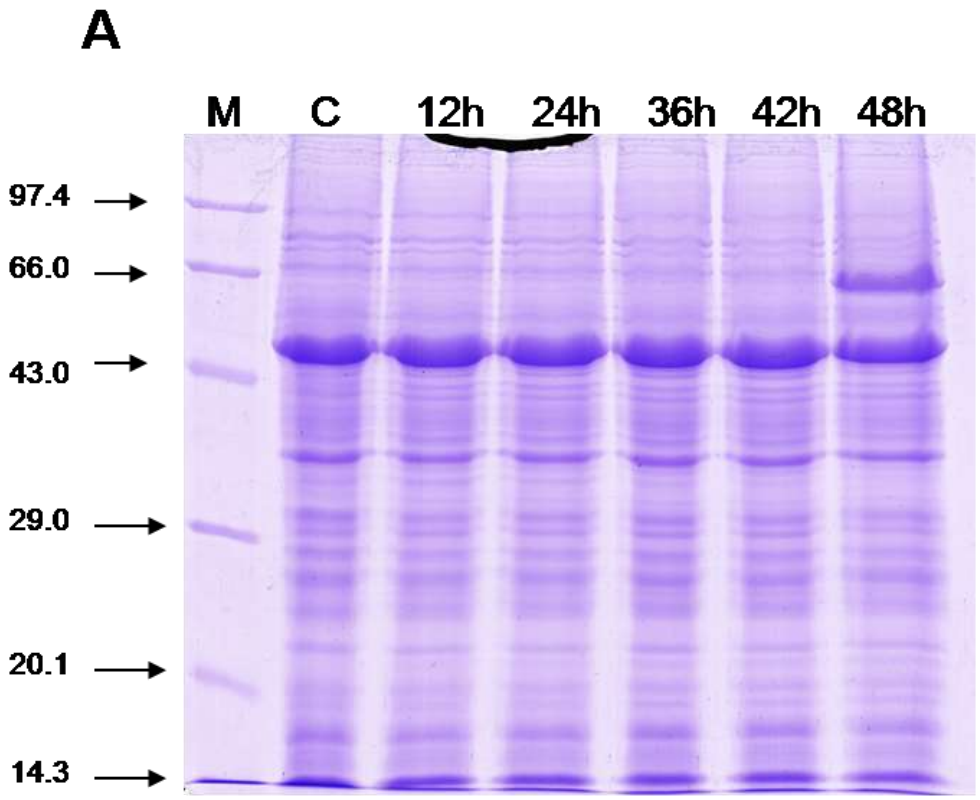
Stain in Coomassie Blue stain (CBB)

Destained

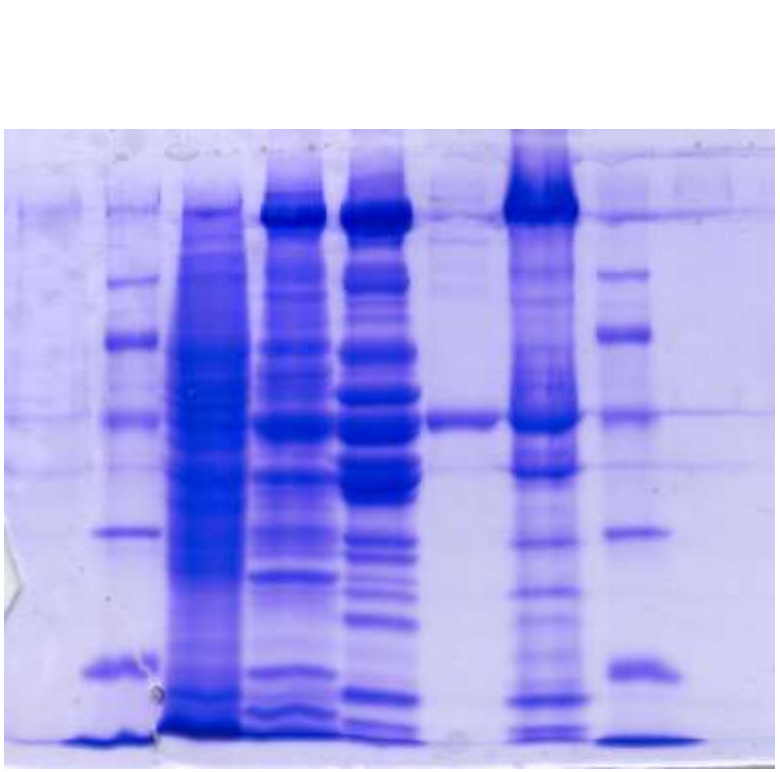
Identify molecular markers, and the separated proteins.



Schematic representation of the various steps involved in SDS-PAGE



Plant protein (rice) isolated after salt treatment



Protein Isolated form E.coli

SDS-PAGE gel stained with CBB. The protein isolated form different source show different profile (example A &B). Protein from same species under different condition show difference in profile (example A, 48 hour)

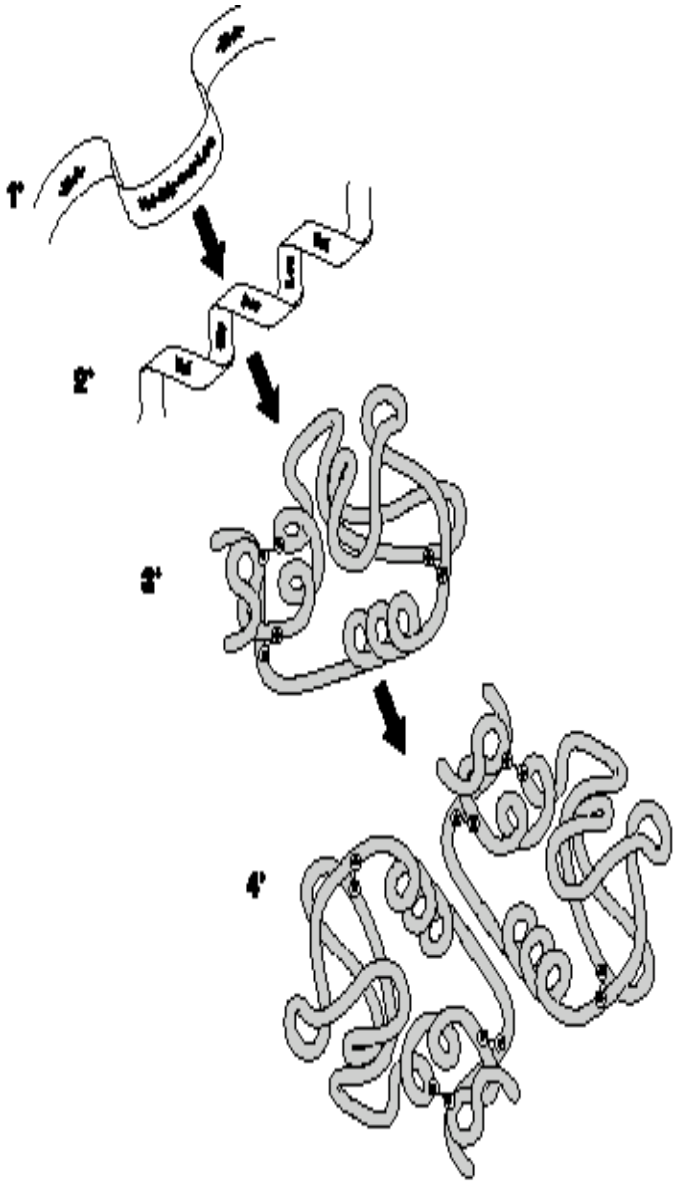
Levels of Protein Organization

Primary structure = linear chain of amino acids

• **Secondary structure** = domains of repeating structures, such as β-pleated sheets and α-helices

• **Tertiary structure** = 3-dimensional shape of a folded polypeptide, maintained by disulfide bonds, electrostatic interactions, hydrophobic effects

• **Quaternary structure** = several polypeptide chains associated together to form a functional protein



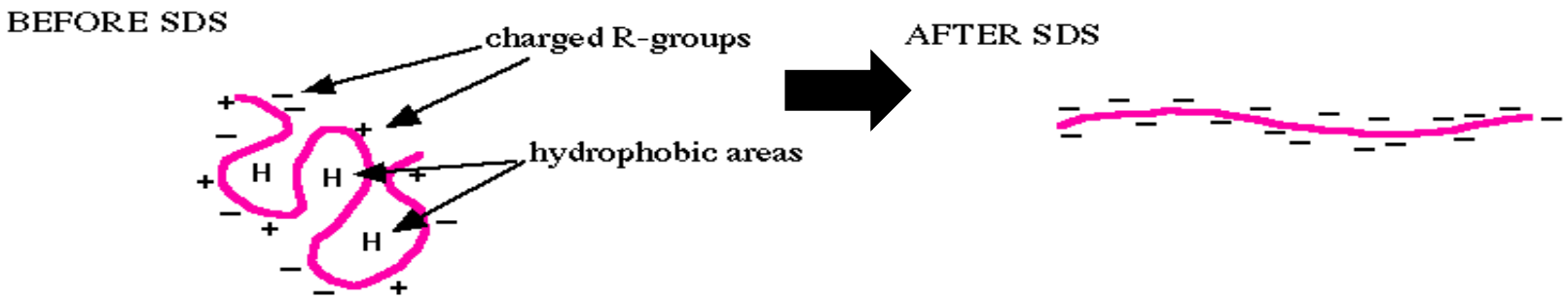
Purpose of SDS in PAGE

1. SDS disrupts some of the non-covalent interactions that stabilize protein quaternary and tertiary structures, facilitates de-naturation.

2. SDS also has a negative electrical charge and binds to proteins in a constant mass ratio of **1.4 : 1,** so that the total amount of detergent bound is directly proportional to the molecular weight of the protein.

3. The 'coating' of negatively charged SDS overwhelms the inherent charges of protein molecules and gives them a uniform charge to mass ratio.

4. This allows proteins to be separated on the basis of their relative sizes.

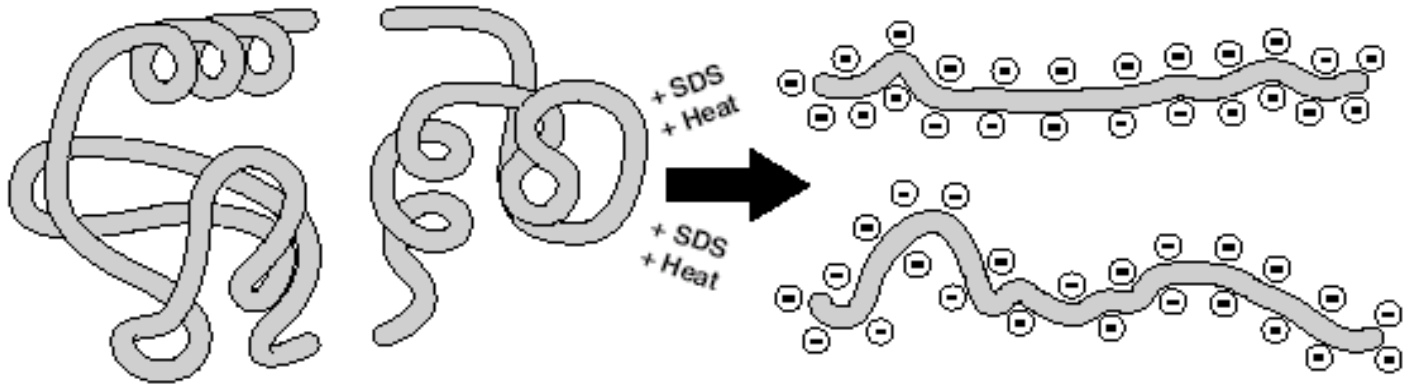


Why it is necessary to boil the protein sample with sample buffer

-Proteins denatured by heating them in a sample buffer containing sodium dodecyl sulphate (SDS)

-The proteins no longer have any secondary, tertiary or quaternary structure.

-Resultant proteins take on a rod-like shape and a uniform negative charge-to-mass ratio proportional to their molecular weights



Migration of proteins (boiled with sample buffer) in electric field:

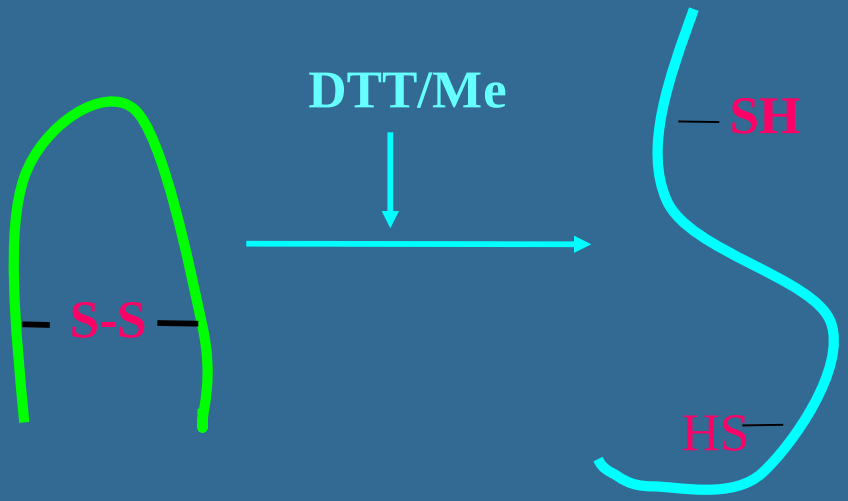
-Negatively charged proteins move towards the **positive pole**.

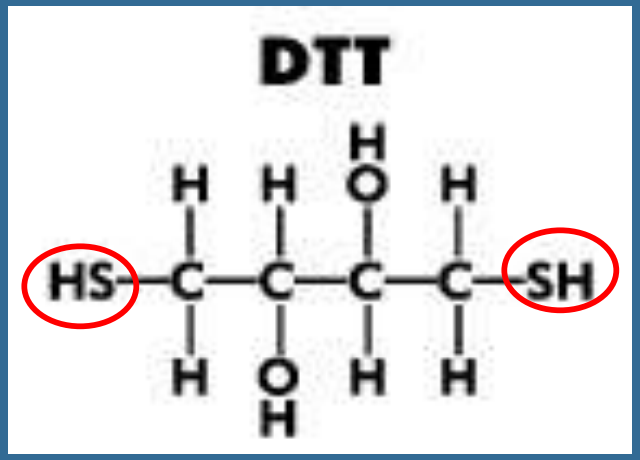
-Migration of proteins:

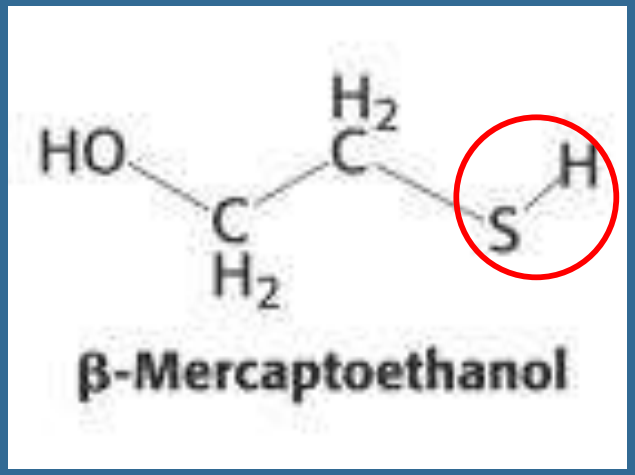
Directly proportional to the overall charge of proteins

Inversely proportional to protein size (molecular weight)

What is the use of DTT/β-Me In SDS-PAGE

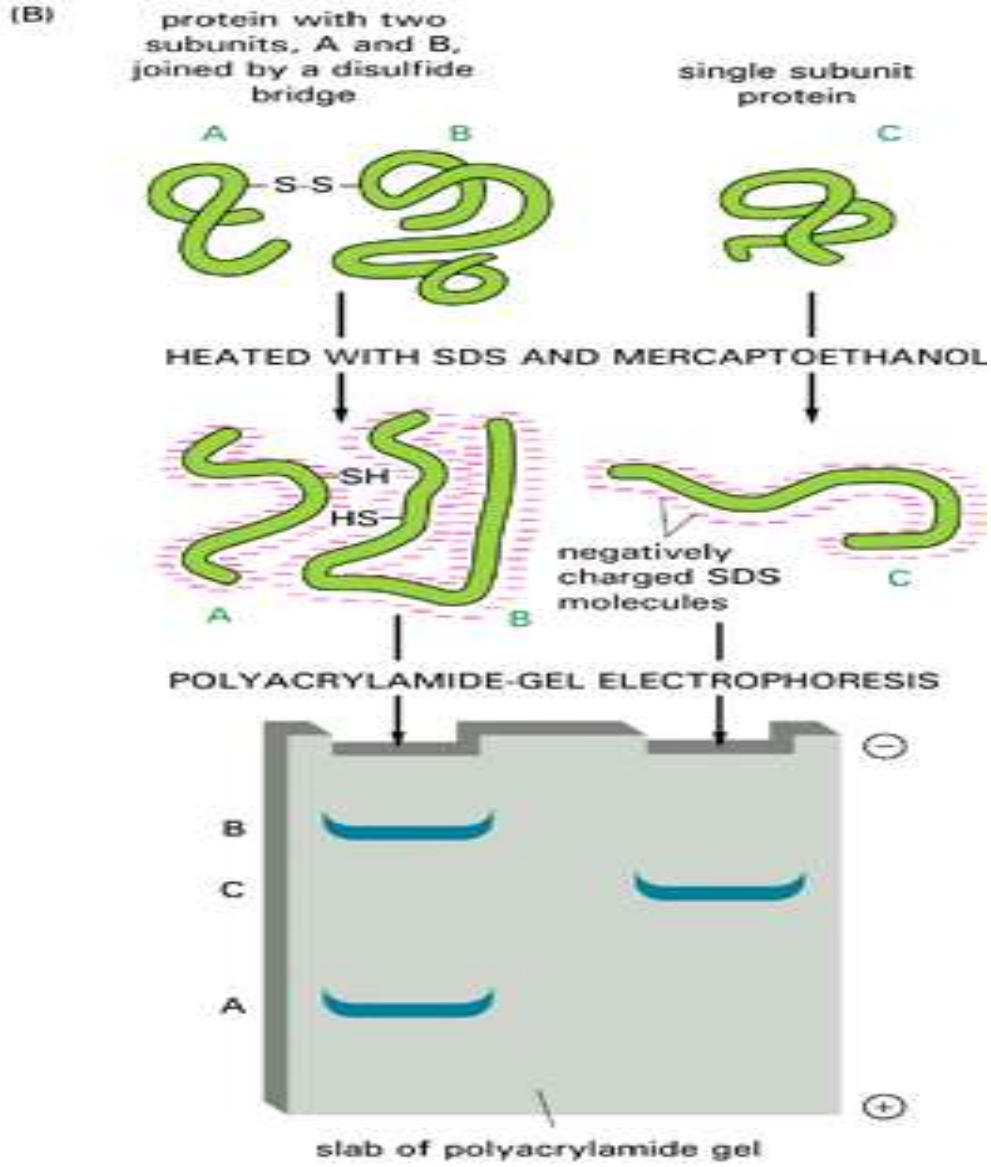


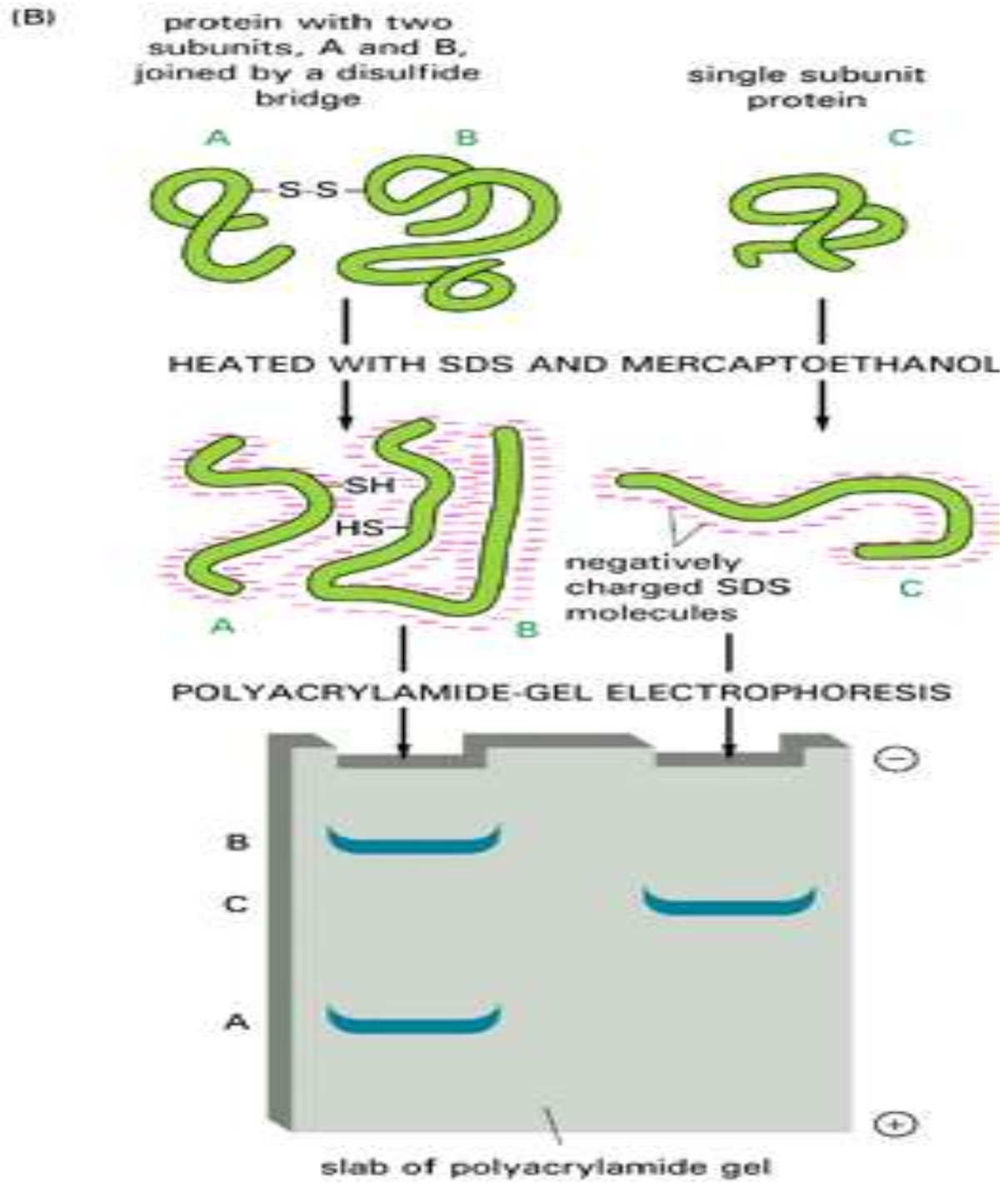




How does an SDS-PAGE gel work?

- **Negatively charged proteins move to positive electrode**
- **Smaller proteins move faster**
- **Proteins separate by size**



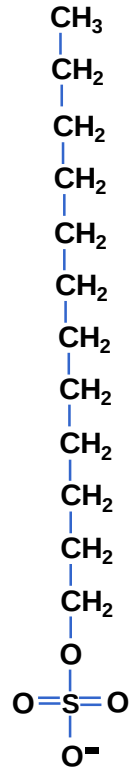

(B)
protein with two subunits, A and B, joined by a disulfide bridge
single subunit protein
A
B
C
S-S
HEATED WITH SDS AND MERCAPTOETHANOL
SH
HS
A
B
negatively charged SDS molecules
C
POLYACRYLAMIDE-GEL ELECTROPHORESIS
B
C
A
slab of polyacrylamide gel

What is in the Sample Buffer?

Tris buffer to provide appropriate pH
SDS (sodium dodecyl sulphate) detergent to dissolve proteins and give them a negative charge
Glycerol to make samples sink into wells
Bromophenol Blue dye to visualize samples

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

- **SDS** (Sodium Dodecyl Sulfate) detergent
  - –solubilizes and denatures proteins
  - –negative charge to proteins
- **Heat** denatures proteins



SDS

Protein Size

- Size measured in daltons (Da) or kilodaltons (kDa)
- Dalton = atomic mass unit

  = corresponds to mass of hydrogen molecule (1.66×10^{-24} gram)

  = defined also as 1/16 of the mass of an atom of oxygen
- Average amino acid = 110 Da

  Average nucleotide pair = 649 Da