Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

What is SDS-PAGE?

SDS-PAGE is an electrophoresis method that allows protein separation by mass. The medium (also referred to as ′matrix′) is a polyacrylamide-based discontinuous **gel**. In addition, **SDS** (**sodium dodecyl sulfate**) is used. About 1.4 grams of **SDS** bind to a gram of protein, corresponding to one **SDS** molecule per two amino acids.

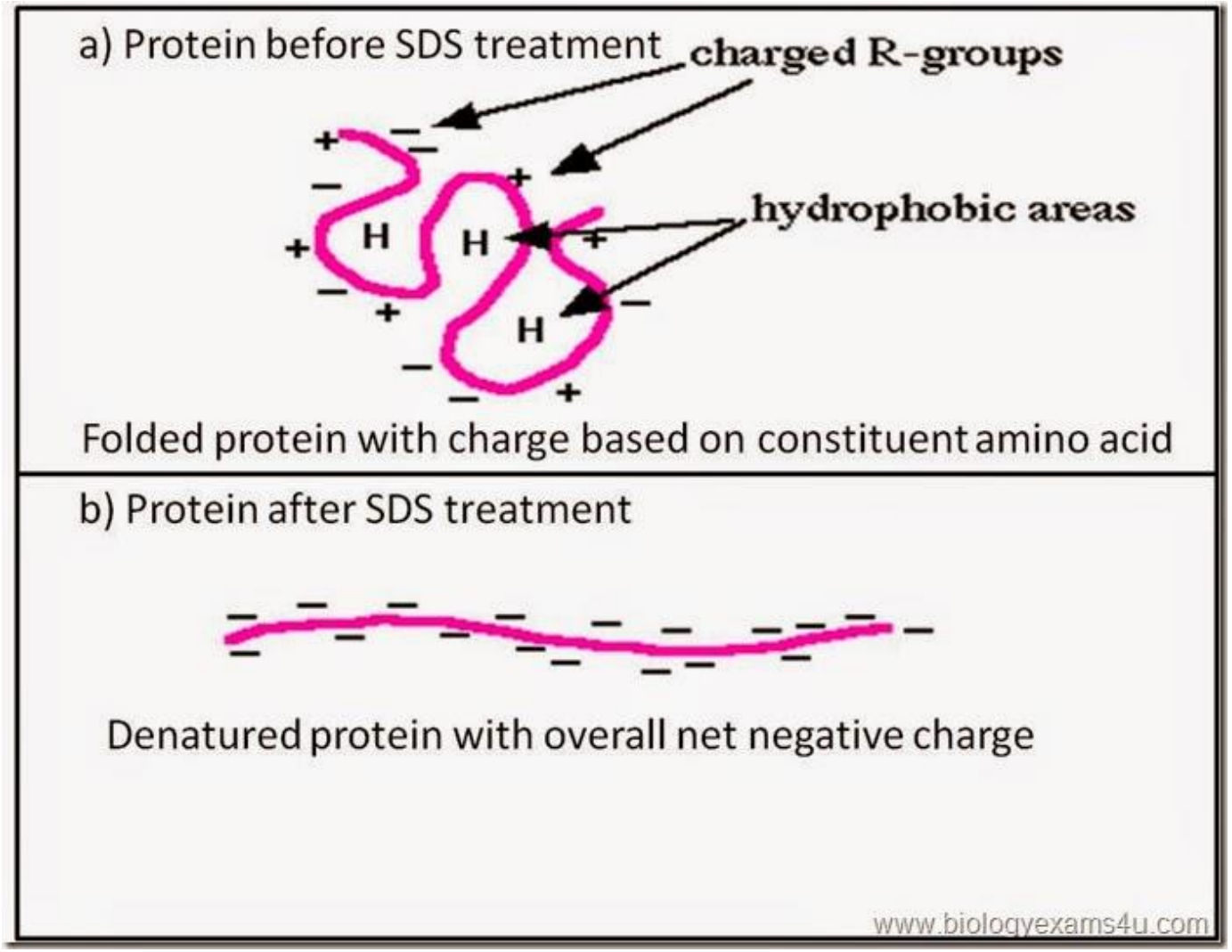
Principle

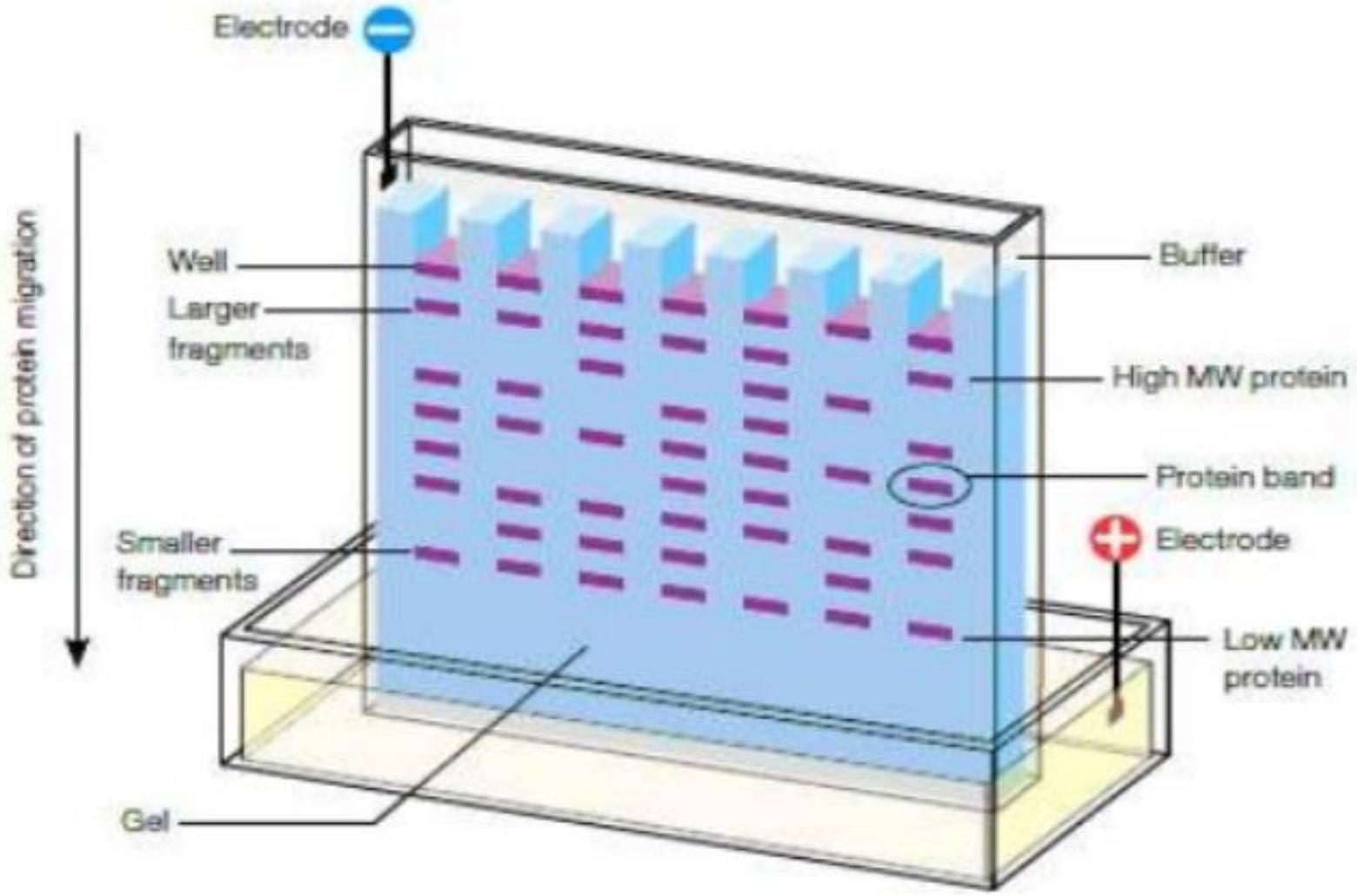
A reducing agent such as mercaptoethanol or dithiothreitol (DTT) (in the presence of a detergent i.e. SDS) breaks down the disulfide bridges that are responsible for protein folding; and a detergent such as SDS imparts negative charge to the proteins thereby linearizing them into polypeptides. Polyacrylamide provides a matrix for the polypeptides to run. Polypeptides run towards the positive electrode (anode) through the gel when electric field is applied. Electrophoretic mobility of the proteins depends upon 3 factors:

- **Shape** – All the proteins are in the primary structure after the treatment with a reducing agent. So, shape doesn't affect the protein separation.
- **Charge** – All the proteins are negatively charge proportional to their molecular weight after treatment with SDS. So charge doesn't affect the separation.
- **Size**– proteins get separated solely on the basis of their molecular weight.

Smaller polypeptides move faster because they have to face less hindrance, larger ones move slower because of greater hindrance. Hence proteins get separated ONLY on the basis of their mass

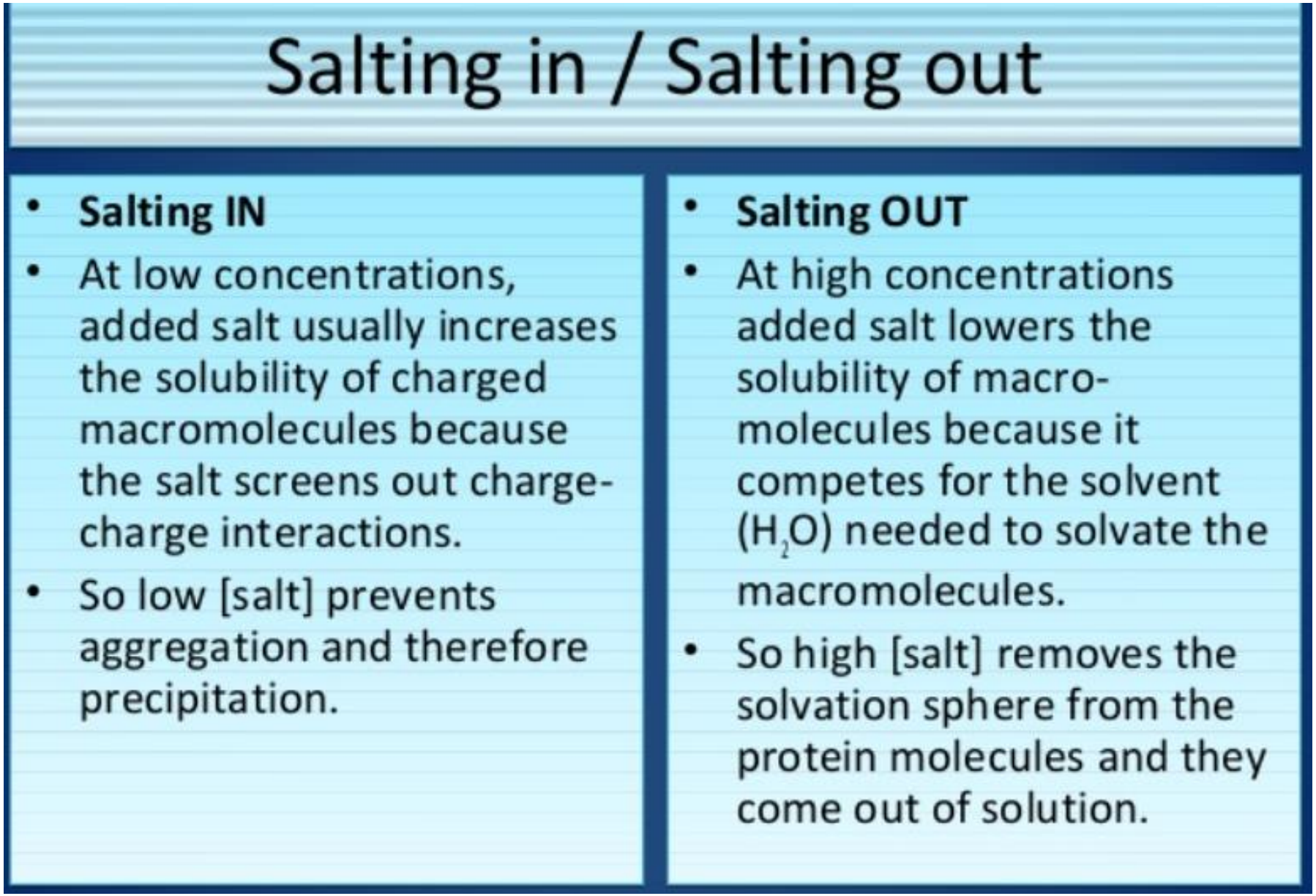
Function of SDS





SDS-PAGE Apparatus

Ammonium sulfate precipitation



General protocol for protein purification

- Taking the intact Tissue.
- Homogenisation
- Getting rid of debris and insoluble stuff
- Precipitation of protein with the salt(salting – out)
- Getting rid of salt excess by dialysis
- Further purification by column and ion exchange chromatography ,
- Finding out the exact molecular weight by Column chromatography and by SDS-Gel-electrophoresis

Fractional Precipitation ("salting out")

- Proteins require H_2O molecules interacting with surface groups, in order to stay in aqueous solution (hydration).
- Salting out usually uses increasing concentrations of ammonium sulfate [$(NH_4)_2SO_4$] to compete with the protein groups for the available H_2O.
- Like all purification methods, salt fractionation has to be worked out empirically for each protein of interest
- **Every protein in the solution has its own solubility limits in ammonium sulfate, *independent of the other proteins in the mixture.***

Why choosing $(NH_4)_2SO_4$ for precipitation?

- Has a wide range of application
- Very effective to ppt out water soluble proteins.
- These ions have stabilizing effect on protein
- You can do sequential ppt of your desired protein depending upon its molecular weight.
- Proteins are readily stored as ammonium sulfate ppt.