Polyacrylamide Gel Electrophoresis (PAGE)

The migration of charged proteins in an electric field can be called as process of protein **electrophoresis.**

These procedures are of many types and it is a general misconception that they are used for large scale purification. But in reality, we do not generally use electrophoresis methods to purify proteins in large amounts.

This is for the reason that simpler and more efficient cum cost effective alternatives are usually available. Also the electrophoretic methods generally cause adverse effects on the structure and thus the function of proteins.

Electrophoresis is highly useful as an analytical method. This is possible because electrophoresis as a method in current form provides certain advantages such as ability to visualize proteins and their separation. This allows a researcher/worker to estimate quickly the number of different proteins in a mixture or the degree of purity of a particular protein preparation.

Electrophoresis is also used regularly for determination of crucial properties of a protein such as its isoelectric point and approximate molecular weight.

For determination of approximate molecular mass, we use PAGE. This can be used to find out the approx. molecular mass for native proteins via native-PAGE and determination of approx. molecular mass and subunit composition (if any) for a given protein via SDS-PAGE.

Electrophoresis of proteins is generally carried out in gels made up of the cross-linked polymer polyacrylamide. The polyacrylamide gel acts as a molecular sieve, slowing the migration of proteins approximately in proportion to their charge-to-mass ratio.

Migration may also be affected by protein shape. In electrophoresis, the force moving the macromolecule is the electrical potential, *E.* The electrophoretic mobility of the molecule, μ, is the ratio of the velocity of the particle molecule, *V,* to the electrical potential.

Electrophoretic mobility is also equal to the net charge of the molecule, *Z,* divided by the frictional coefficient, *f,* which reflects in part a protein's shape.

Thus: $\mu = \frac{v}{E} = \frac{z}{f}$

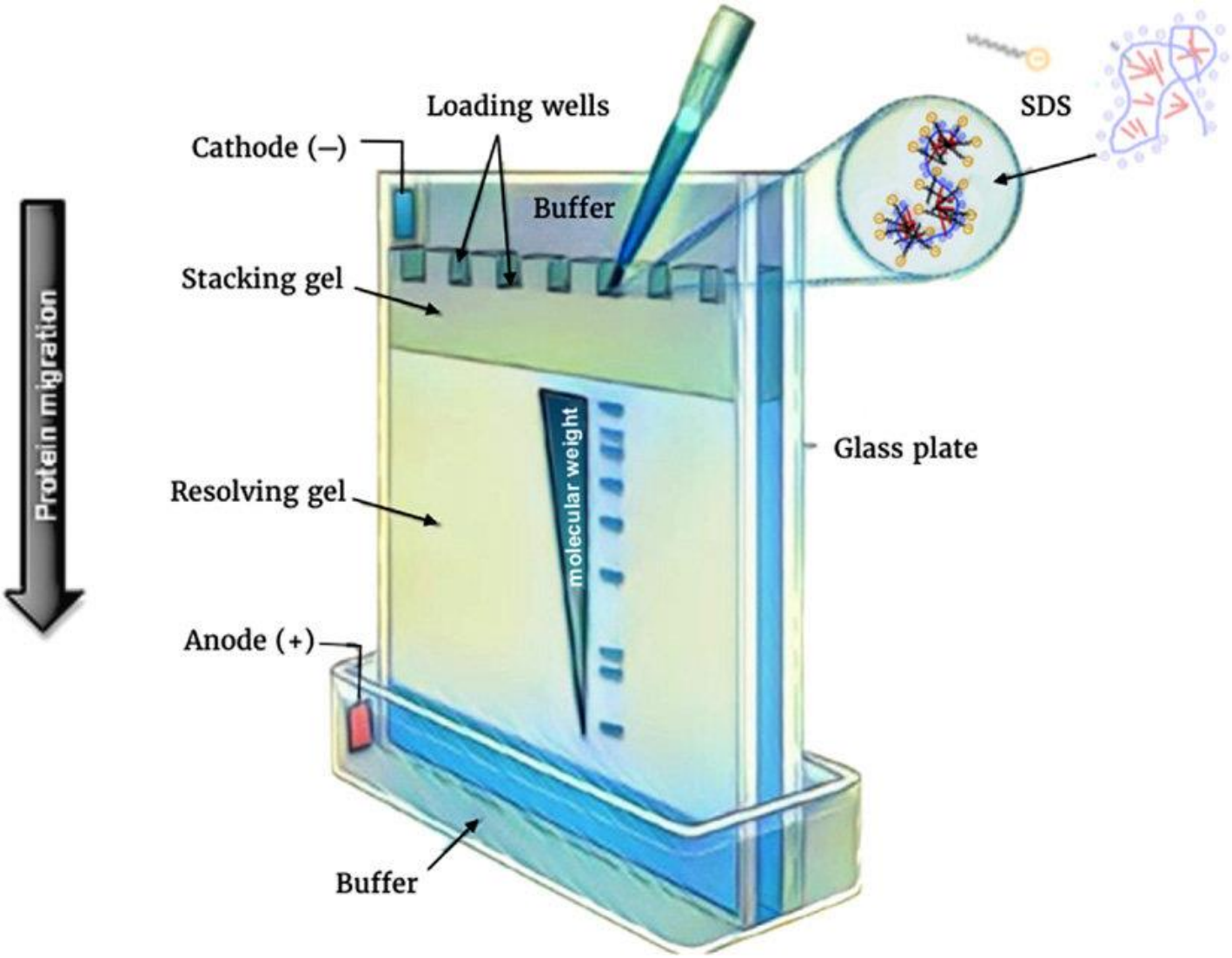
The migration of a protein in a gel during electrophoresis is therefore a function of its size and its shape.

Components of the acrylamide gel: To make the polyacrylamide gel we need following components:

1. Acrylamide as monomer
2. Bis-acrylamide as cross-linker
3. Ammonium persulfate or APS as initiator which provides free radicals to initiate the polymerization reaction between components 1 & 2.
4. TEMED as promoter which promotes the free radical formation by APS so that reaction is initiated.
5. Buffer: Tris - HCl with pH = 8.8 for resolving gel and Tris - HCl with pH = 6.8 for stacking gel.
6. SDS detergent is added while making the gel if we are going to perform SDS-PAGE on our sample. But, if we are going to perform the Native-PAGE on our samples then we **DO NOT USE SDS in the gel mix and any of the buffers.**

Stacking gel: This is low percentage part of the gel, usually, 4% - 5% and therefore has larger pore size. It is made above the resolving gel & is used to ensure that sample has completely settled down i.e. stacked completely before it enters the resolving gel.

Resolving gel: This gel has higher percentage than stacking gel but exact percentage is not fixed. It is decided by the investigator based on the sample. If the protein size is small then we need gel with small pore sizes, thus, we have higher percentage gels made. On the other hand, if we have larger proteins then we need bigger sized pores and we make a low percentage gel. But this low percentage will be greater than the one used for stacking gel.



Why we use SDS?? There is a detergent **sodium dodecyl sulfate (SDS)** which is used in the preparation of gel, buffers and sample while one is going to perform the SDS-PAGE.

SDS binds to most proteins in amounts roughly proportional to the molecular weight of the protein, about one molecule of SDS for every two amino acid residues.

The bound SDS contributes a large net negative charge & makes the intrinsic charge of the protein insignificant and provides each protein with a similar charge-to-mass ratio.

Also, the native conformation of a protein is altered when SDS is bound, and most proteins assume a similar shape.

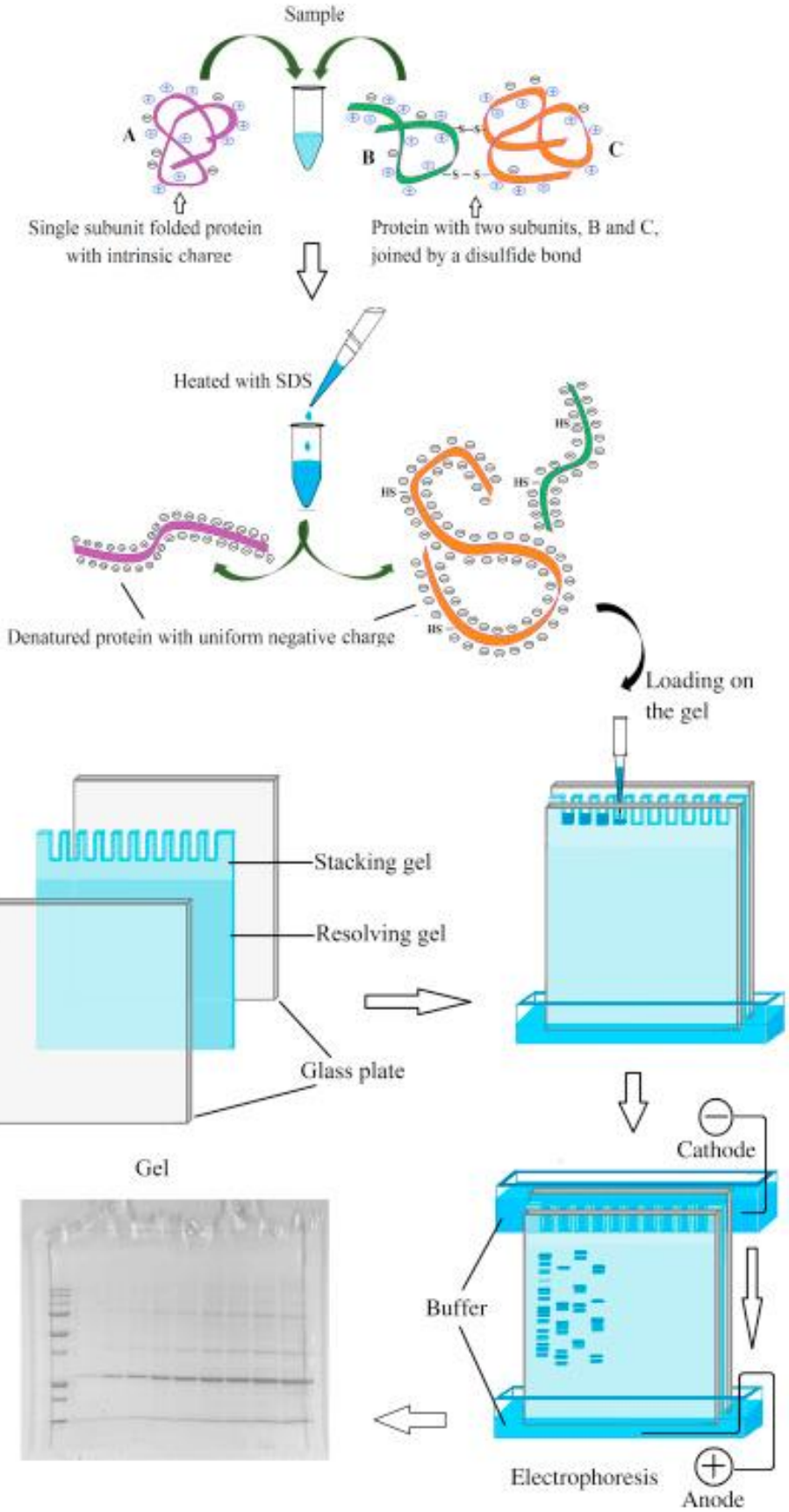
Electrophoresis in the presence of SDS therefore separates proteins on the basis of mass (molecular weight), with smaller polypeptides migrating more rapidly and are found near the gel front.

Sample buffer: This is used for sample preparation and is prepared in Tris - HCl of pH 6.8 and it contains:

- Glycerol to add density so that sample can settle down easily in the bottom of the well.
- Bromophenol blue as tracking dye so that gel front can be tracked & one can monitor the progress of gel run.

- SDS is added when we are doing SDS – PAGE. Its role is discussed in the previous section.
- β - mercaptoethanol is added to the sample buffer if we need to perform reducing SDS – PAGE. This is the most common form of PAGE performed in the laboratories.

Figure showing workflow for SDS – PAGE is given below. Please refer to it for relating all the steps discussed above. Only staining step is discussed after the figure.



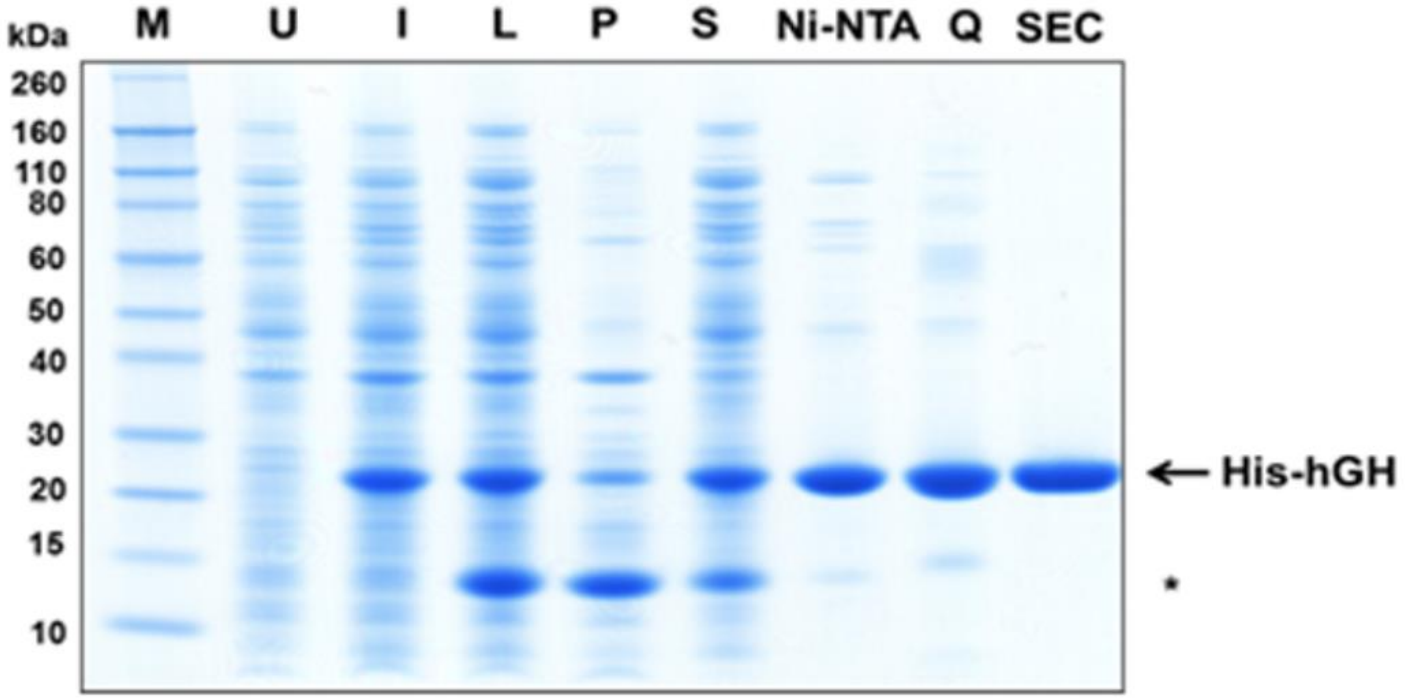
Staining the gel: Once the gel run is complete, we take it out from the apparatus and proceed with staining the gel.

Staining is essential so that we can identify the positions where protein is present in the gel and compare them with markers for analysis.

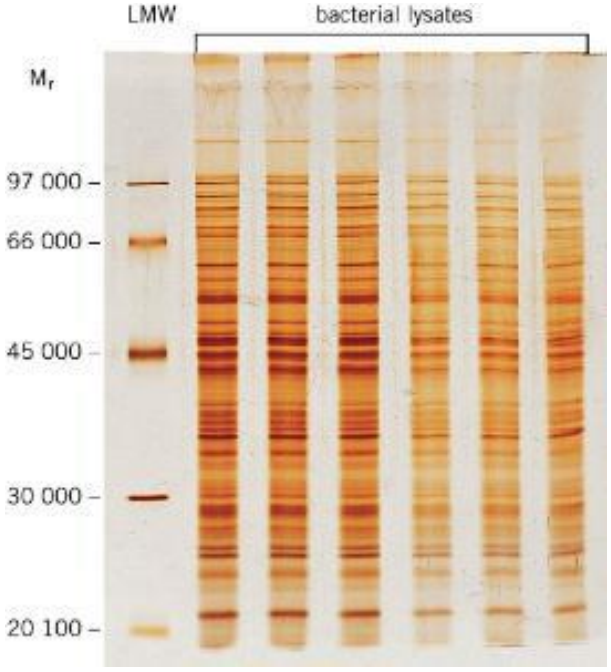
For staining, we use dyes that bind proteins but do not bind the gel. This, helps generates the contrast.

Most commonly used stain is CBB or Coomassie Brilliant Blue. It stains the proteins blue and can detect bands having up to 100 ng of proteins.

For staining the gel is soaked in the staining solution for a given amount of time and then is placed in the de-staining solution to remove the extra stain. The final gel will look as is shown in figure below after de-staining.



Another method of staining is called as silver staining. This method is highly sensitive and can detect proteins up to 1 ng in each band. The gel after silver staining has been shown in the figure below.

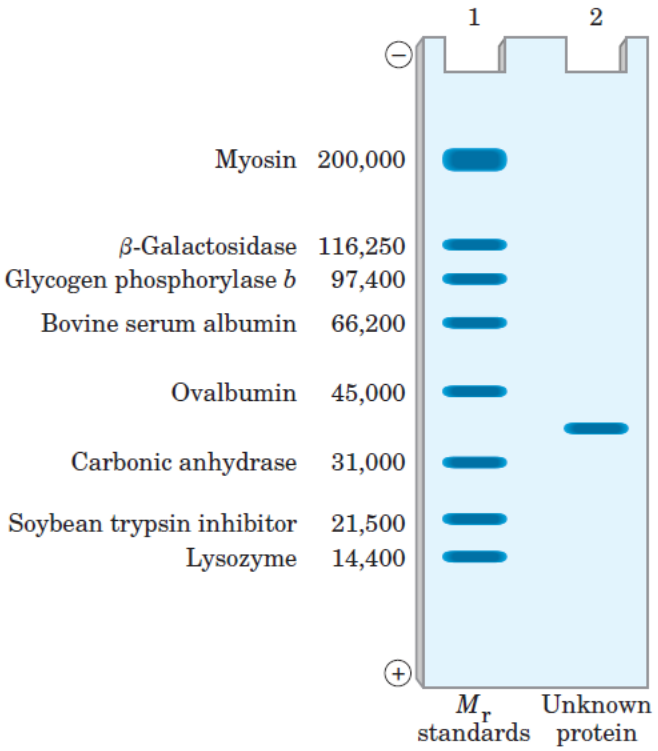


Role of native page in determination of the approx. molecular mass of proteins

The electrophoretic mobility of a protein on an SDS polyacrylamide gel is related to its molecular weight, *M*r.

If we take a look at the figure below, we can see following:

- Standard proteins of known molecular weight are subjected to electrophoresis (lane 1).
- These marker proteins can be used to estimate the molecular weight of an unknown protein (lane 2).



A plot of log *M*r of the marker proteins versus relative migration during electrophoresis is linear, which allows the molecular weight of the unknown protein to be read from the graph as is shown below.