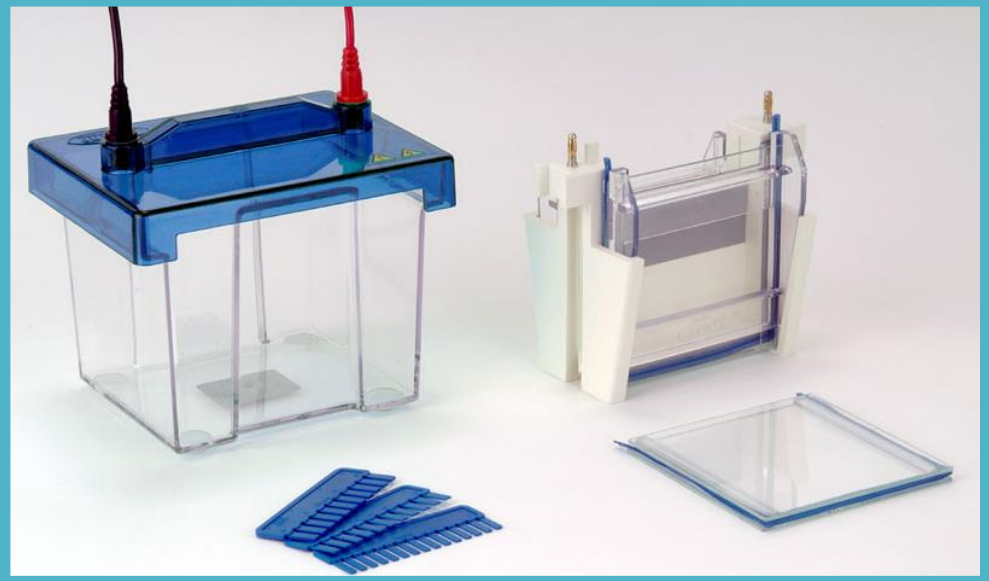
SDS-polyacrylamide gel electrophoresis



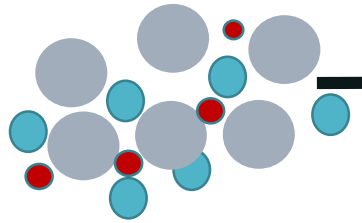
Protein Isolation and Purification

Protein purification is a series of processes intended to isolate one or a few proteins from a complex mixture, usually cells, tissues or whole organisms.

Whole Tissue

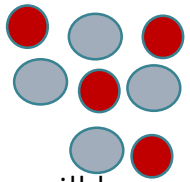
Tissue homogenization

Cell debris and other proteins are removed by centrifugation and other protein could be removed by salting out

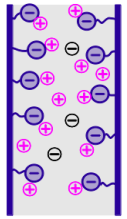


You will have a mixture of proteins that have different charges, Sizes , and other properties

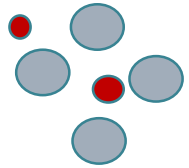
Gel filtration chromatography



You will have a mixture of proteins that have ionizable molecule similar Sizes ,



Ion exchange chromatography



How to make sure that your protein is pure? And also how to characterize it?

During the course of protein purification, and to determine the molecular weight, researchers are more likely to use gel electrophoresis

Sodium Dodecyl Sulfate-PolyacrylAmide gel Electrophoresis (SDS-PAGE) is a low-cost, reproducible and rapid method for: qualifying, comparing , characterizing proteins[e.g. determining MW of proteins] and checking purity of protein samples.

This method separates proteins based primarily on their molecular weights.

Gel electrophoresis

In general, fractionation by gel electrophoresis is based on differences in size, shape and net charge of macromolecules.[in molecules native condition using native gel electrophoresis]

Systems where you separate proteins under native conditions cannot distinguish between these effects and therefore proteins of different sizes may have the same mobility in native gels.

In SDS PAGE this problem is overcome

To separate molecules according **only** to their molecular weight first we must:

1. Eliminate the effect of shape: (by adding SDS and beta-mercaptoethanol)

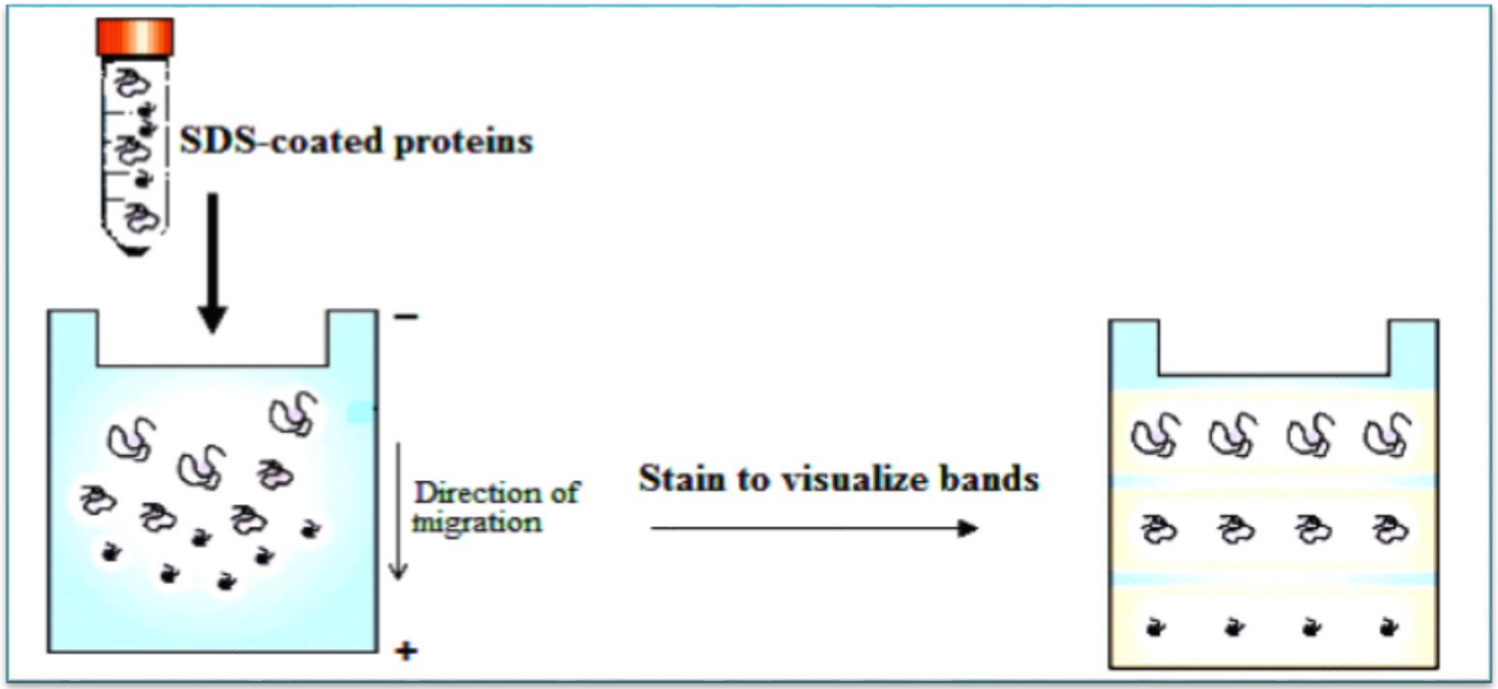
to get them to be linear so that the proteins no longer have any secondary, tertiary or quaternary structure (i.e. we want them to have the same linear shape)

2. Eliminate the effect of charge (by adding SDS)

all must have same type of charge and charge to mass ratio

The electrophoretic Mobility of the molecules is now considered to be a function of their sizes i.e. the migration of the SDS-treated proteins towards the anode[+] is aversely proportional to the logarithms of their molecular weights, or more simply expressed:
Small proteins migrate faster through the gel.

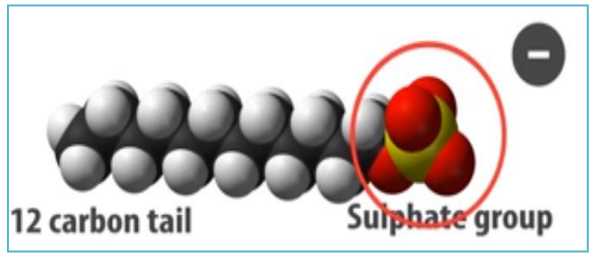
[Compare this with the situation in ion exchange.]



SDS PAGE-Electrophoresis

SDS PAGE-Electrophoresis

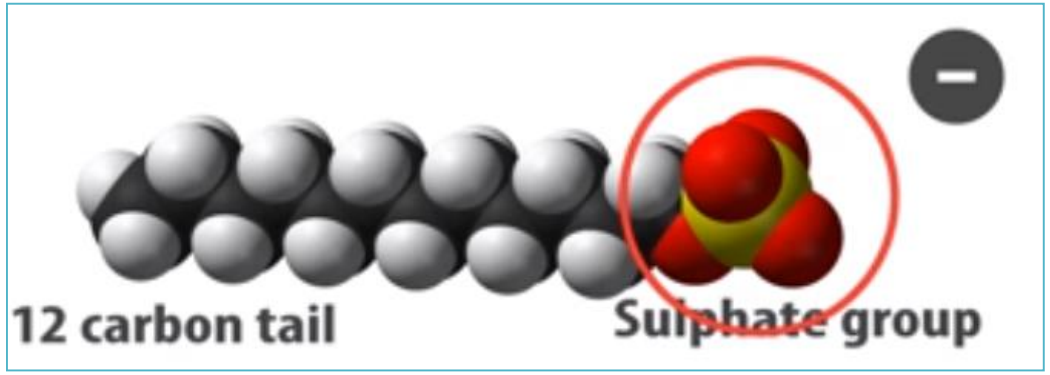
- Sodium dodecyl sulphate (SDS)
  - A molecule that has a carbon tail (neutral charge)
  - Sulphate group is negatively charged



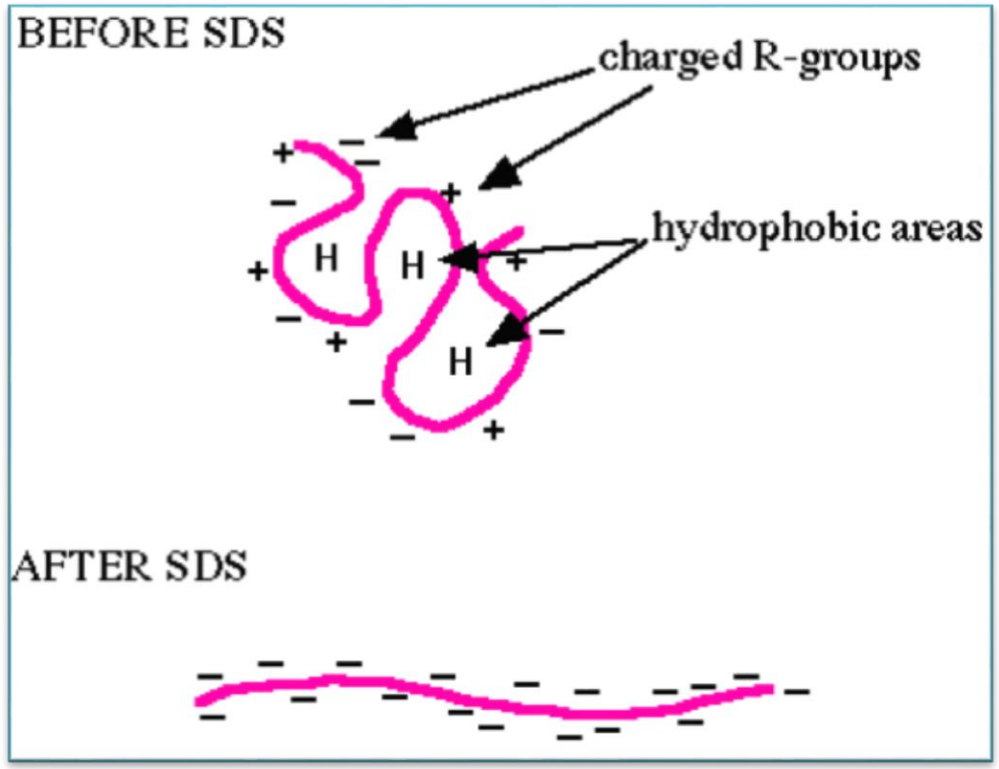
- Gel
  - Polyacrylamide
    - -Stacking,
    - -Separation
- Practical

Sodium dodecyl sulphate (SDS)

- A molecule that has a carbon tail (neutral charge)
- Sulphate group is negatively charged



Its effect on proteins



2-Polyacrylamide gel [Acrylamide stock]:

- The polyacrylamide gel is formed by co-polymerization of acrylamide and a cross-linking
By N,N'-methylene-bis-acrylamide.

-***To polymerize the gel*** a system consisting of ammonium persilfate (initiator) and tetramethylene ethylene diamin (TEMED) is added[catalyst].

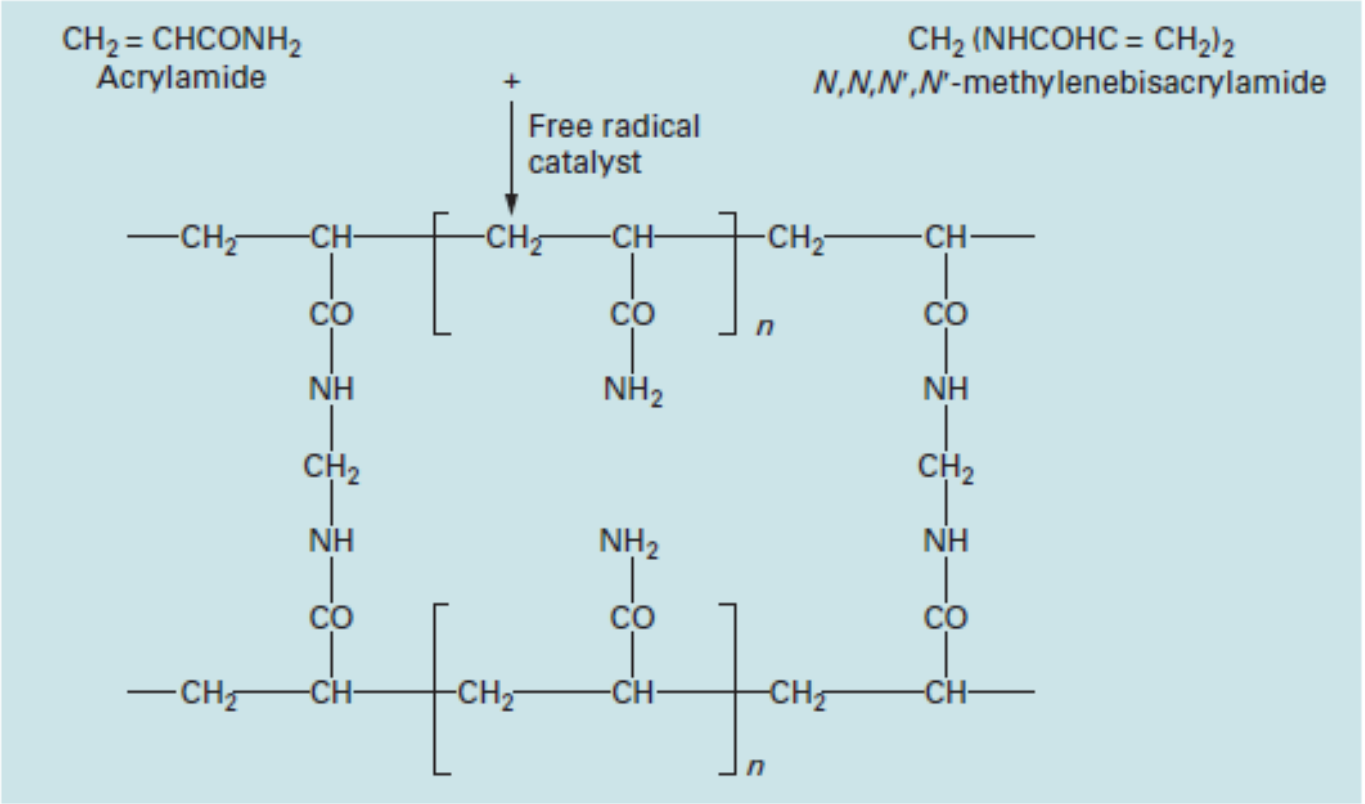


Fig. 10.5 The formation of a polyacrylamide gel from acrylamide and bis-acrylamide.

Principle:

-In denaturing protein electrophoresis, the addition of SDS to the electrophoresis buffer uniformly coats the proteins with negative charges, equalizing the charge to mass ratio for all proteins.

-A Concurrent treatment with a disulfide reducing agent such as β-mercaptoethanol or DTT (dithiothreitol) further breaks down the macromolecules into their subunits.

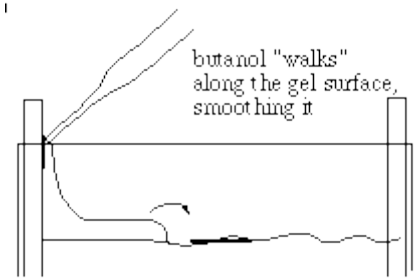
-So, the proteins samples are having uniformed structure and charge → the separation will depend on their molecular weight only.

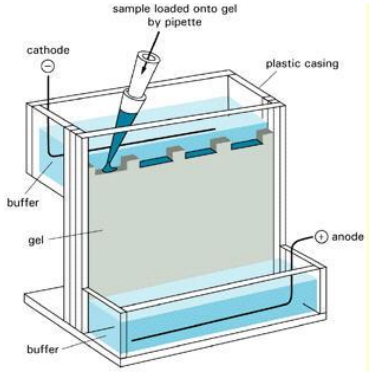
-Small proteins migrate faster through the gel under the influence of the applied electric field.

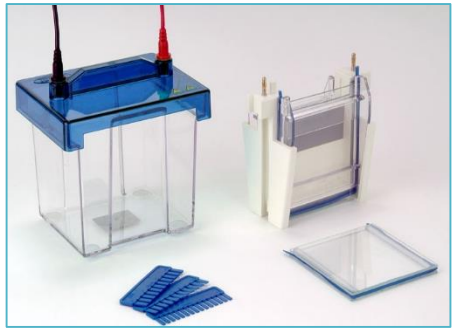
Note: The number of SDS molecules that bind is proportional to the size of the protein, thereby in the electrical field, protein molecules move towards the anode (+) and separated only according to their molecular weight.

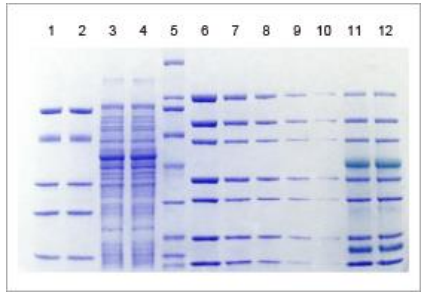
SDS-PAGE steps

Gel preparation → Load the running buffer → Loading the sample → Running the gel → Visualize the sample









1-Electrophoresis components

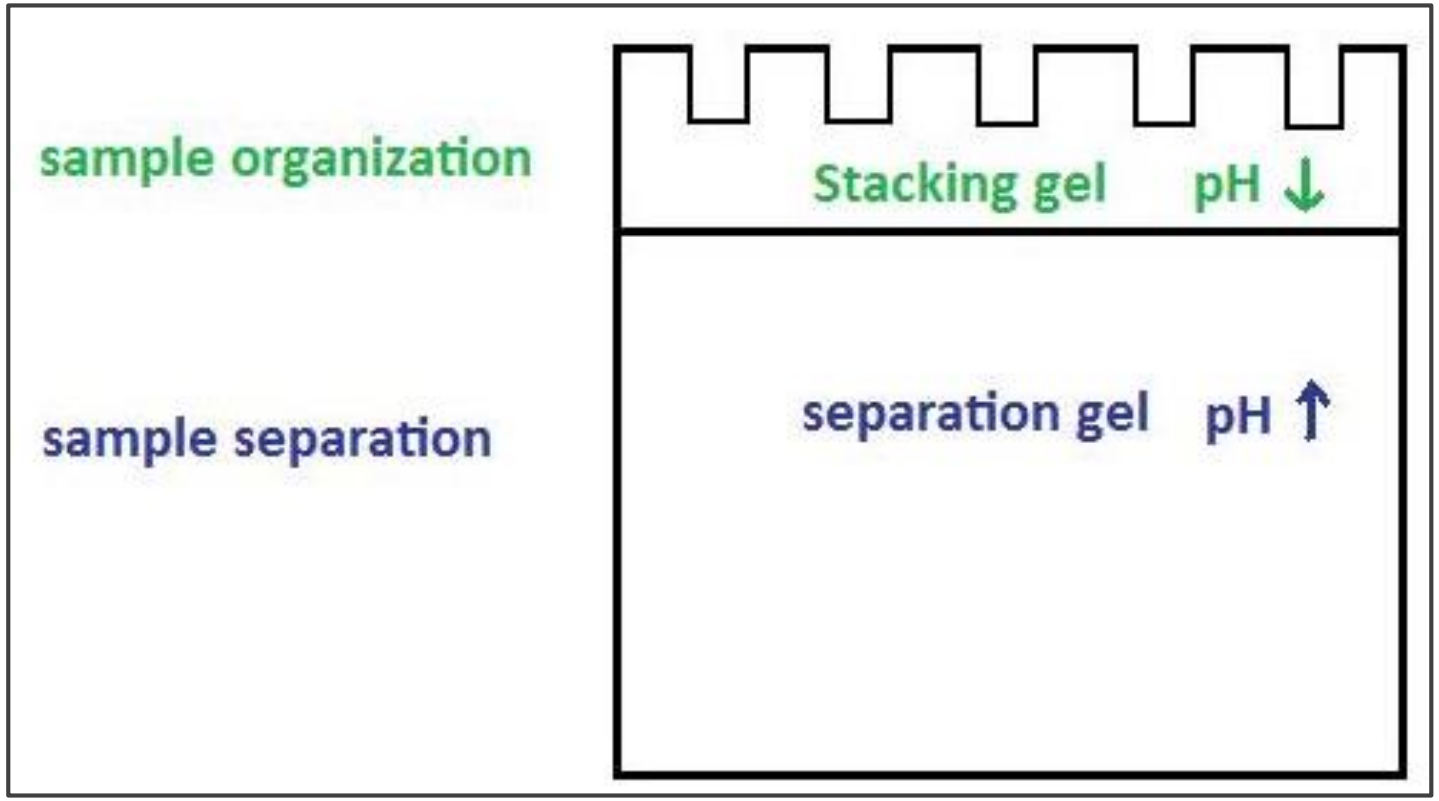
1. **Lid and tank**
2. **Combs**
3. **Spacer plates**
4. **Short plates**
5. **Casting frame**
6. **Sample loading guides**
7. **Casting stand**

2-Gel preparation

- Stacking and separation are the same components but differs in concentration and pH

- The polyacrylamide gel is formed by co-polymerization of acrylamide and a cross-linking by N,N'-methylene-bis-acrylamide.

- To polymerize the gel a system consisting of ammonium persulfate and tetramethylene ethylene diamin (TEMED) is added.



2-Gel preparation

Separation gel

Stock solutions	Volume of stock solution required to make 12% polyacrylamide gel
1.5 M Tris/HCl, pH 8.8	2.0 ml
Acrylamide stock	3.2 ml
Water	2.8 ml
10% SDS	80 µl
10% Ammonium persulphate (fresh)	100 µl
TEMED	20 µl

Stacking gel

Stock solutions	Volume of stock solution required to make 4% polyacrylamide gel
0.5M Tris/HCl, pH6.8	1.0 ml
Acrylamide stock	1.0 ml
Water	3.0 ml
10% SDS	80 µl
10% Ammonium persulphate (fresh)	100 µl
TEMED	20 µl

- What is the difference between separation and stacking gel?
- How you will pour two kinds at the same time?
- Which one you should prepare first?

3-Loading the sample and Running the gel

1.Sample preparation, using the disruption buffer which contain:

-10% (w/v) SDS [?]

-1M Tris/HCl, pH 6.8

-Glycerol [?]

-β-Mercaptoethanol [?]

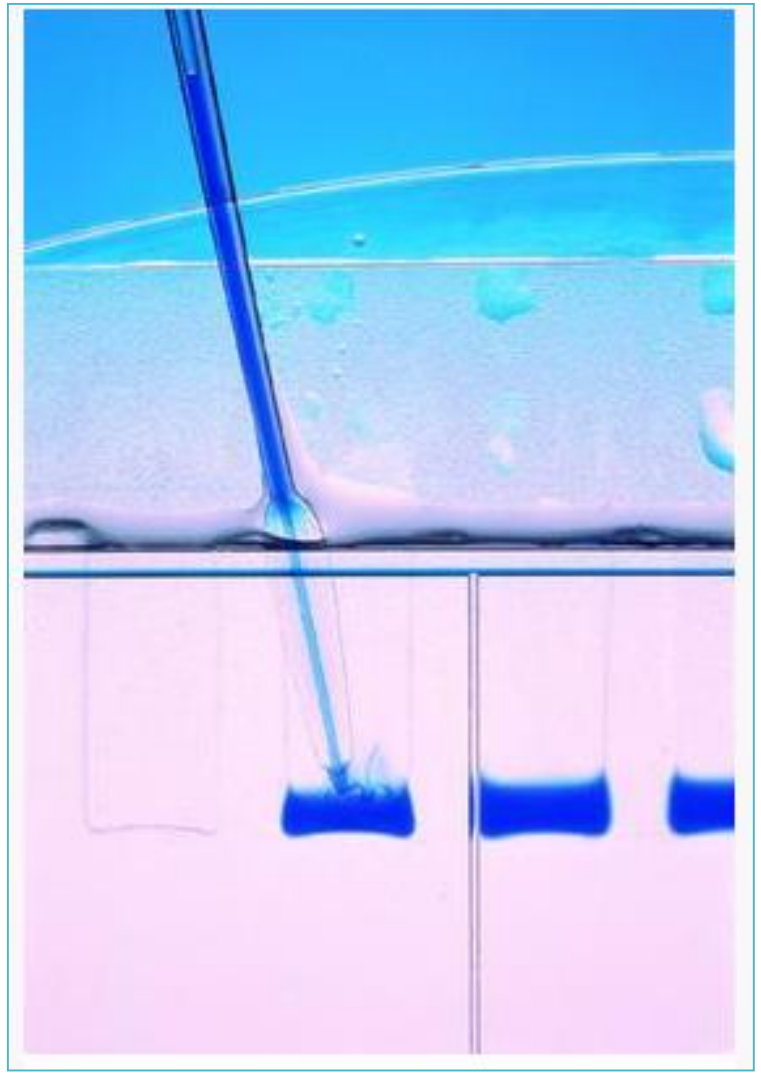
-Bromophenol blue [?]

-with distilled water

[40µl of protein sample + 10 µl of disruption buffer→ boil the mixture 3minets at 99℃ .]

Do not forget to include the ladder

Add…….of the sample



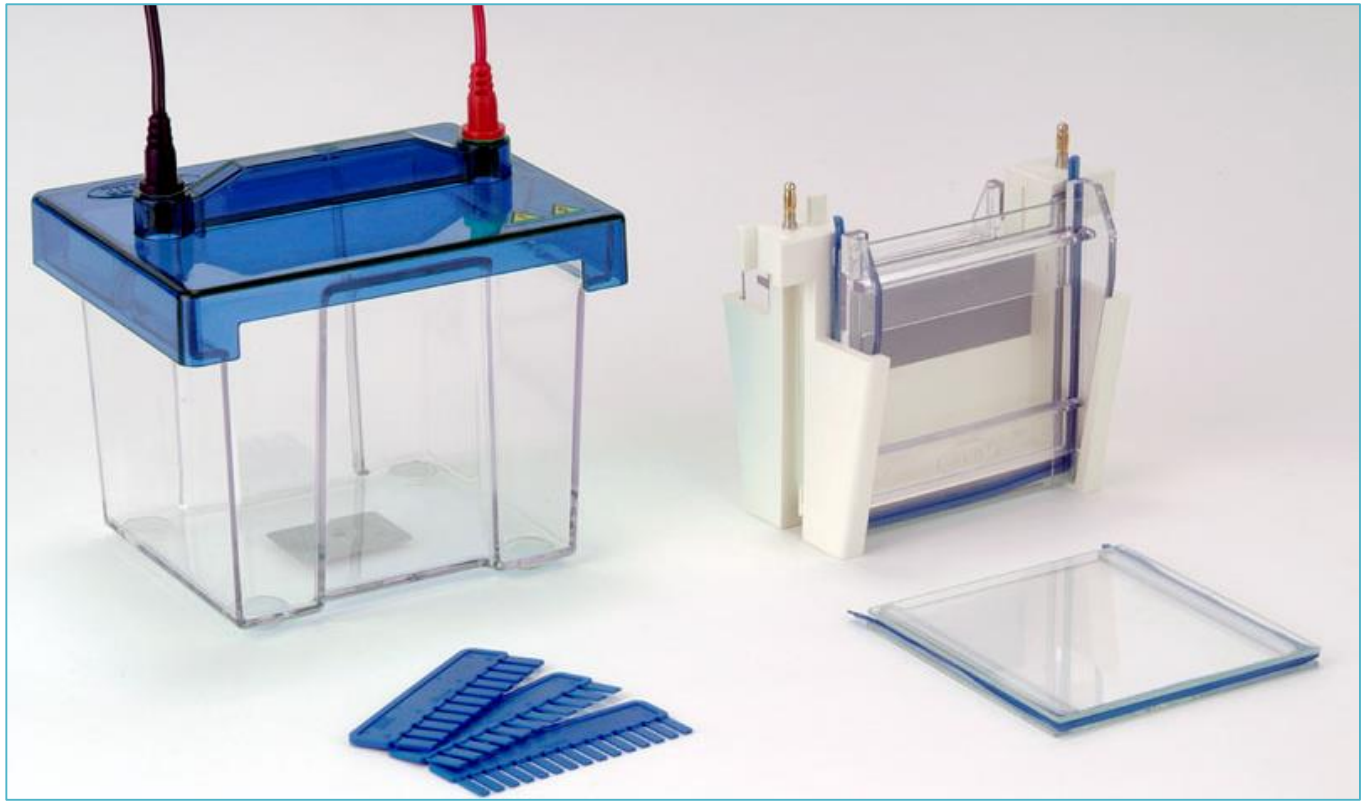
3-Loading the sample and Running the gel

4- SDS-PAGE, Running buffer :

Tris

Glycine

SDS



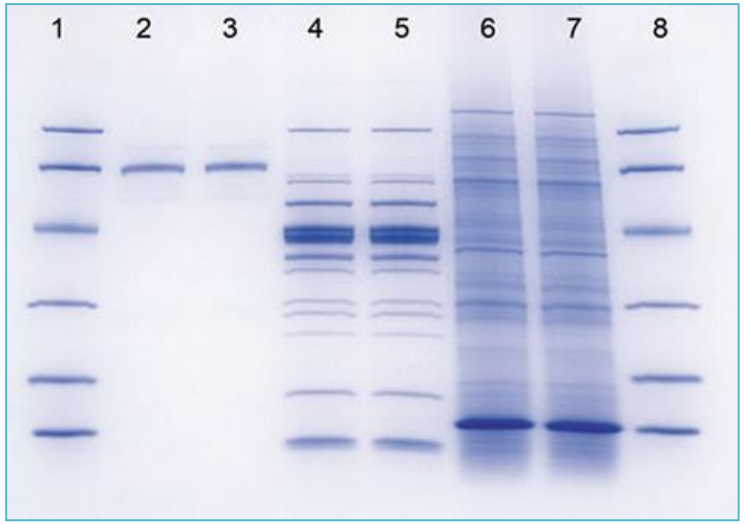
4-Visualizing the bands

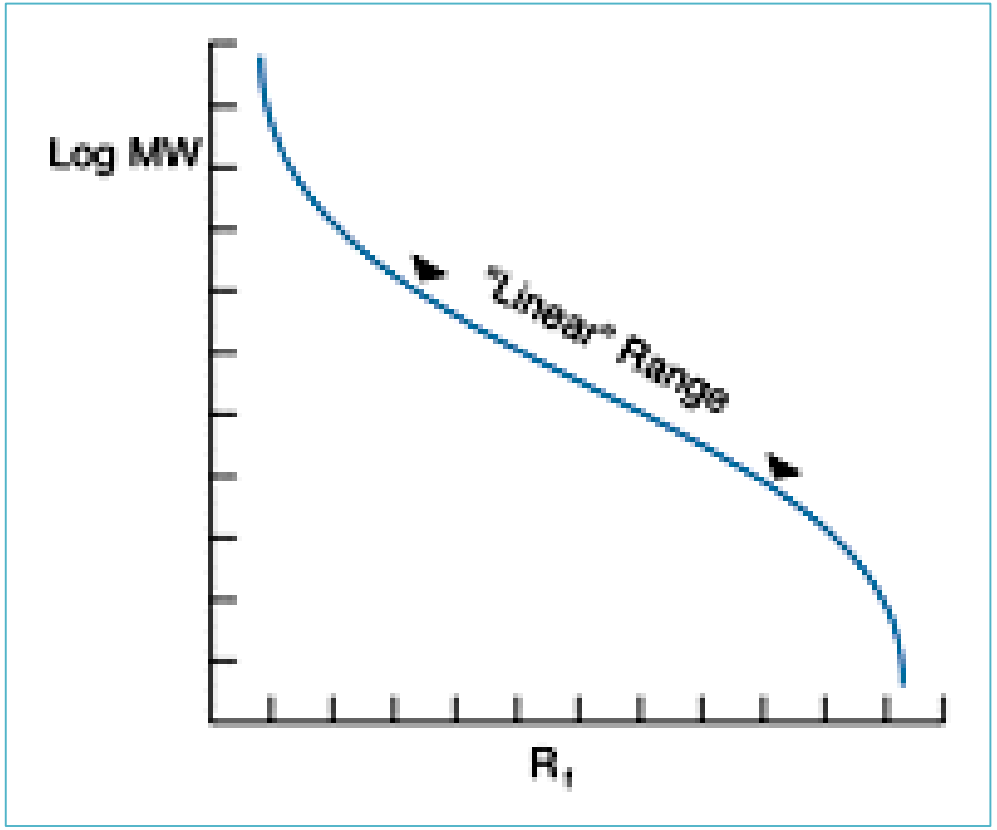
5-SDS-PAGE, Stain: [?]

Glacial acetic acid
Methanol
Coomassie brilliant blue R 250 [?]
Made up to 1L with distilled water.

6-SDS-PAGE, de-stain:

Glacial acetic acid
Methanol
Made up to 1L with distilled water.





graph of log MW vs. Rf is sigmoidal, it is nearly linear for a range of molecular weights excluding very small and very large M wt

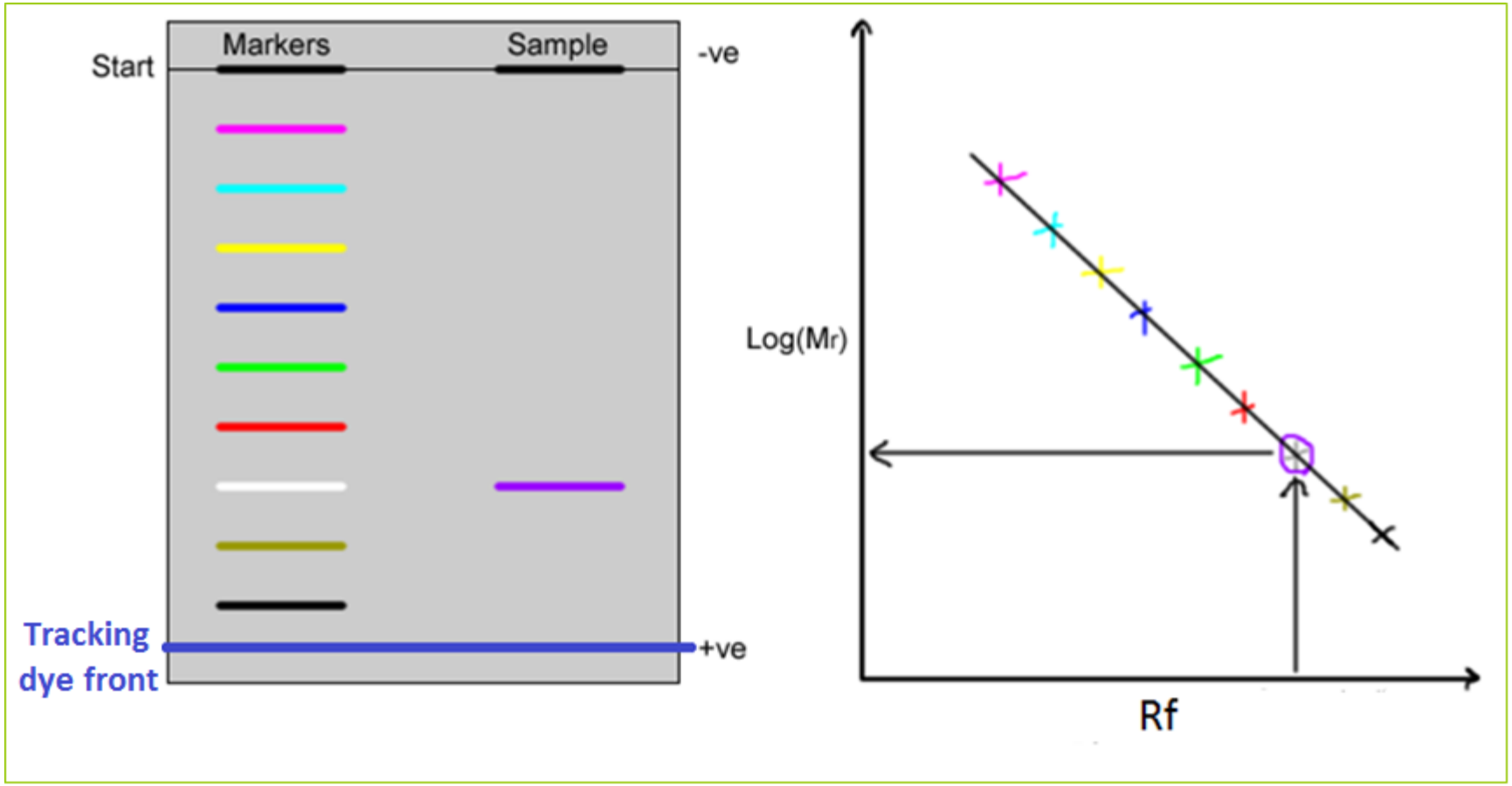
(Figure has been taken from http://www.nationaldiagnostics.com/article_info.php/articles_id/55)

-In practice the proportionality of log(MW) vs. Rm holds true for most proteins, provided they are fully denatured, and provided the gel percentage has been chosen to match the molecular weight range of the sample.

-In fact, the actual plot of log(MW) vs. Rf is sigmoidal (previous Figure), because at high MW, the sieving effect of the matrix is so large that molecules are unable to penetrate the gel, while at low MW, the sieving effect is negligible, and proteins migrate almost at their free mobility, which in SDS is independent of MW.

7-Analysis:

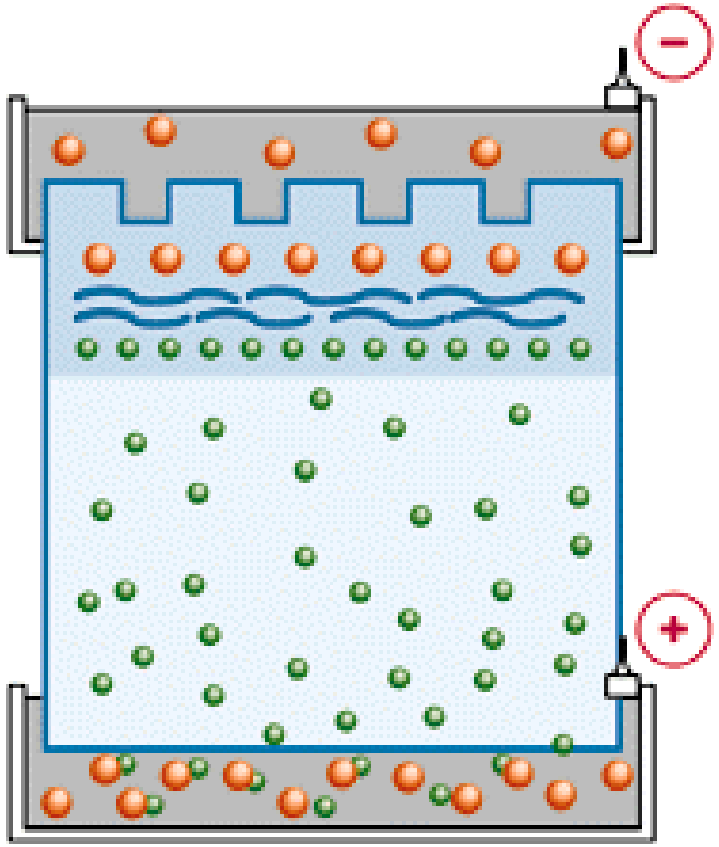
Molecular weight determination.

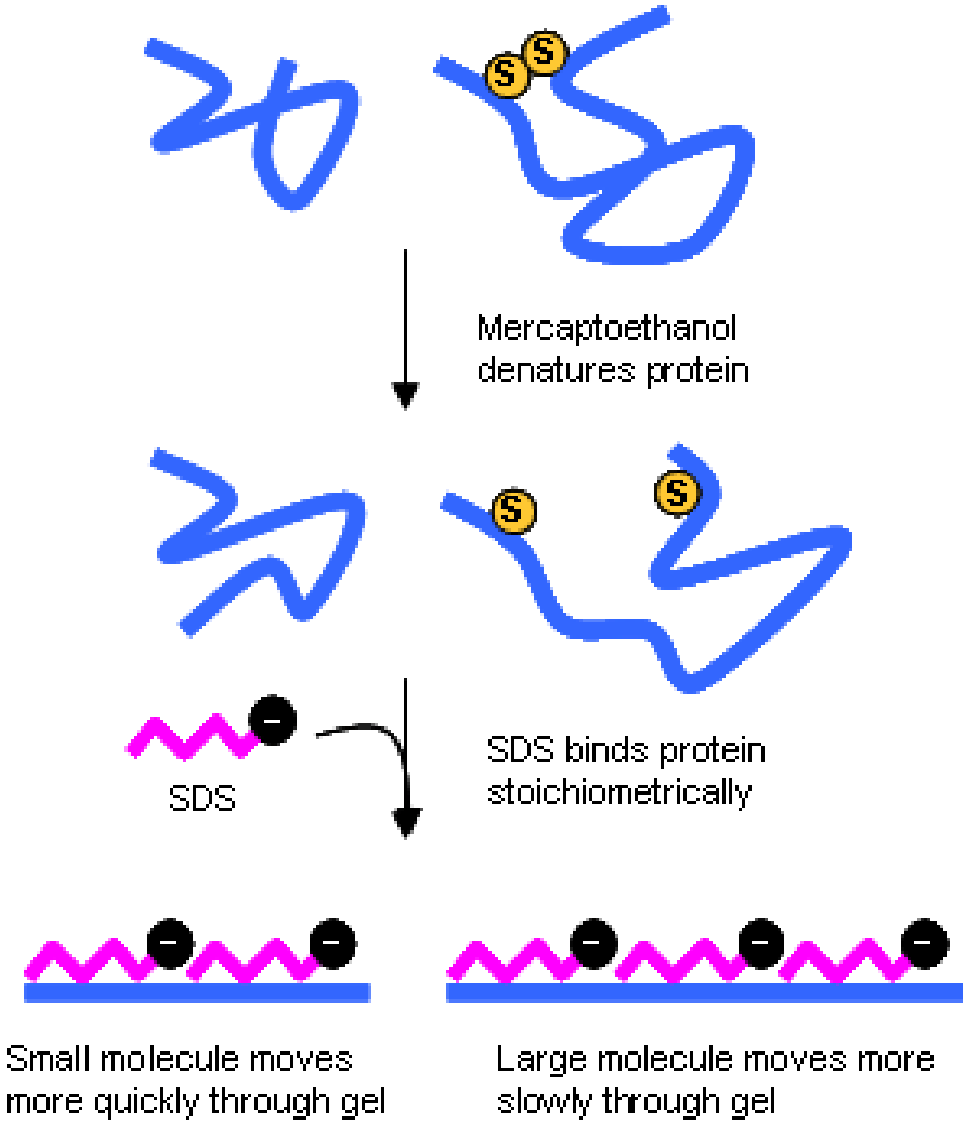


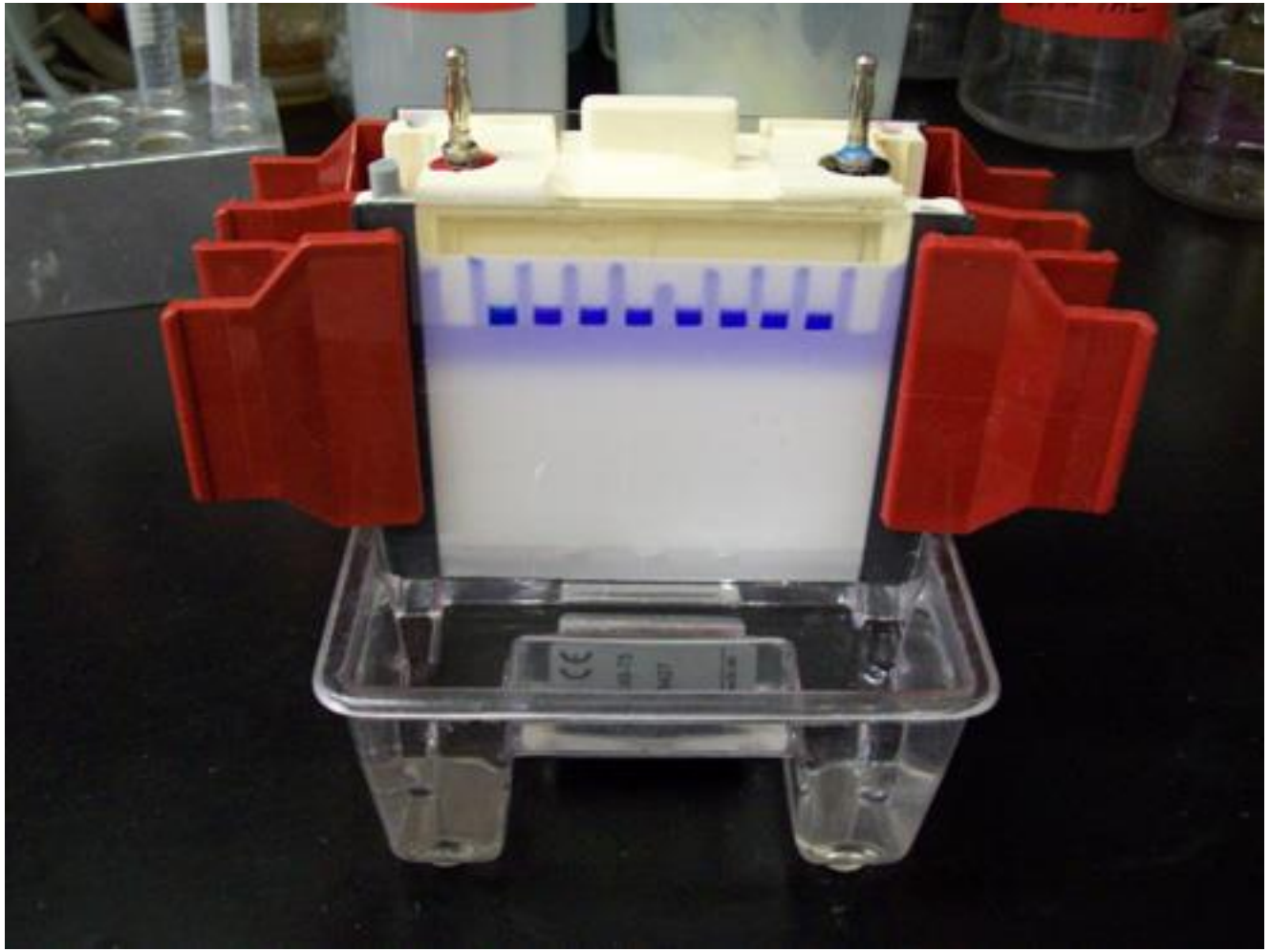
- Rf = $\frac{\text{Distance of migration of sample}}{\text{Distance moved by tracking dye}}$

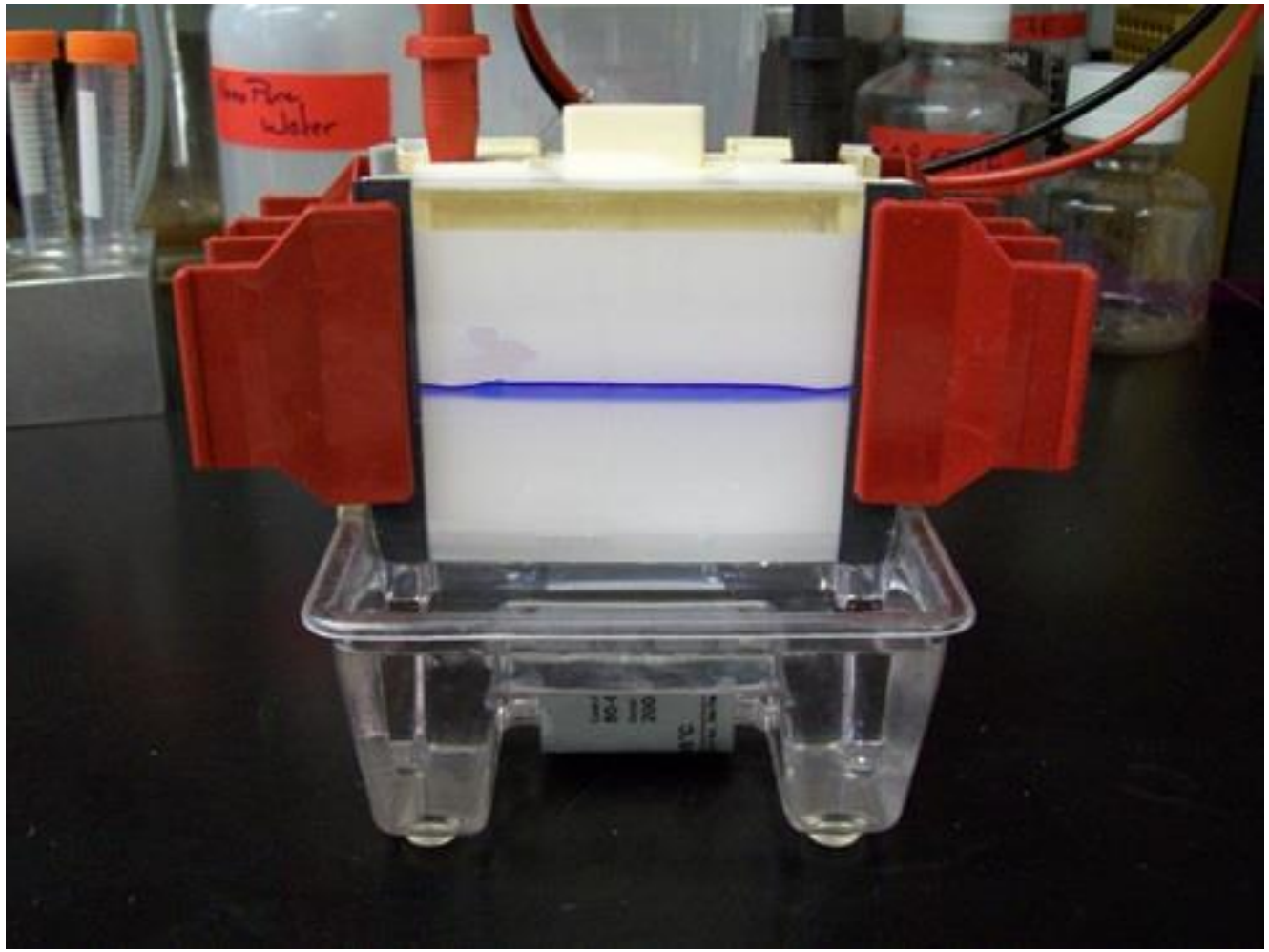
Home work

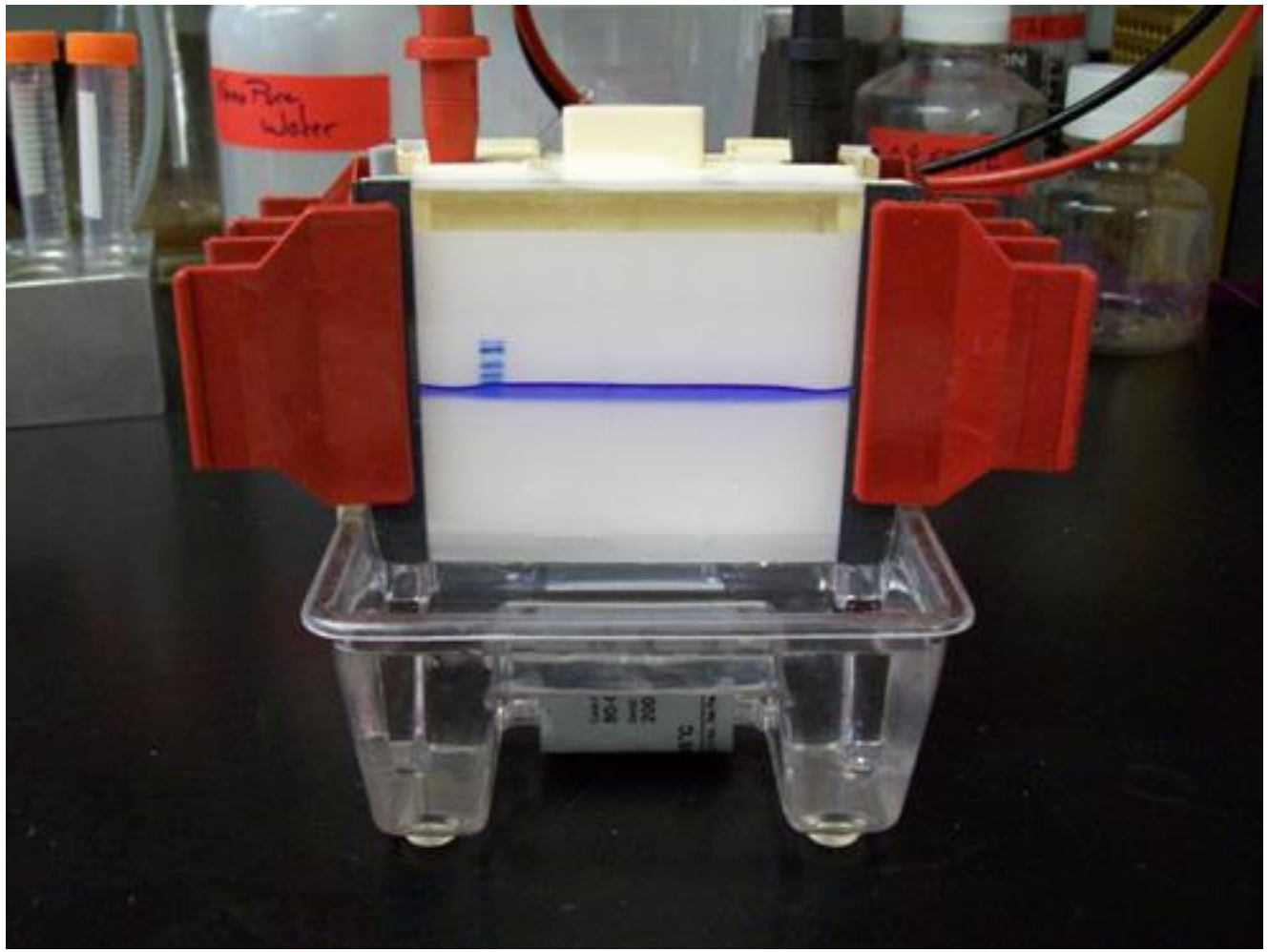
Why glycine should be added in the buffer

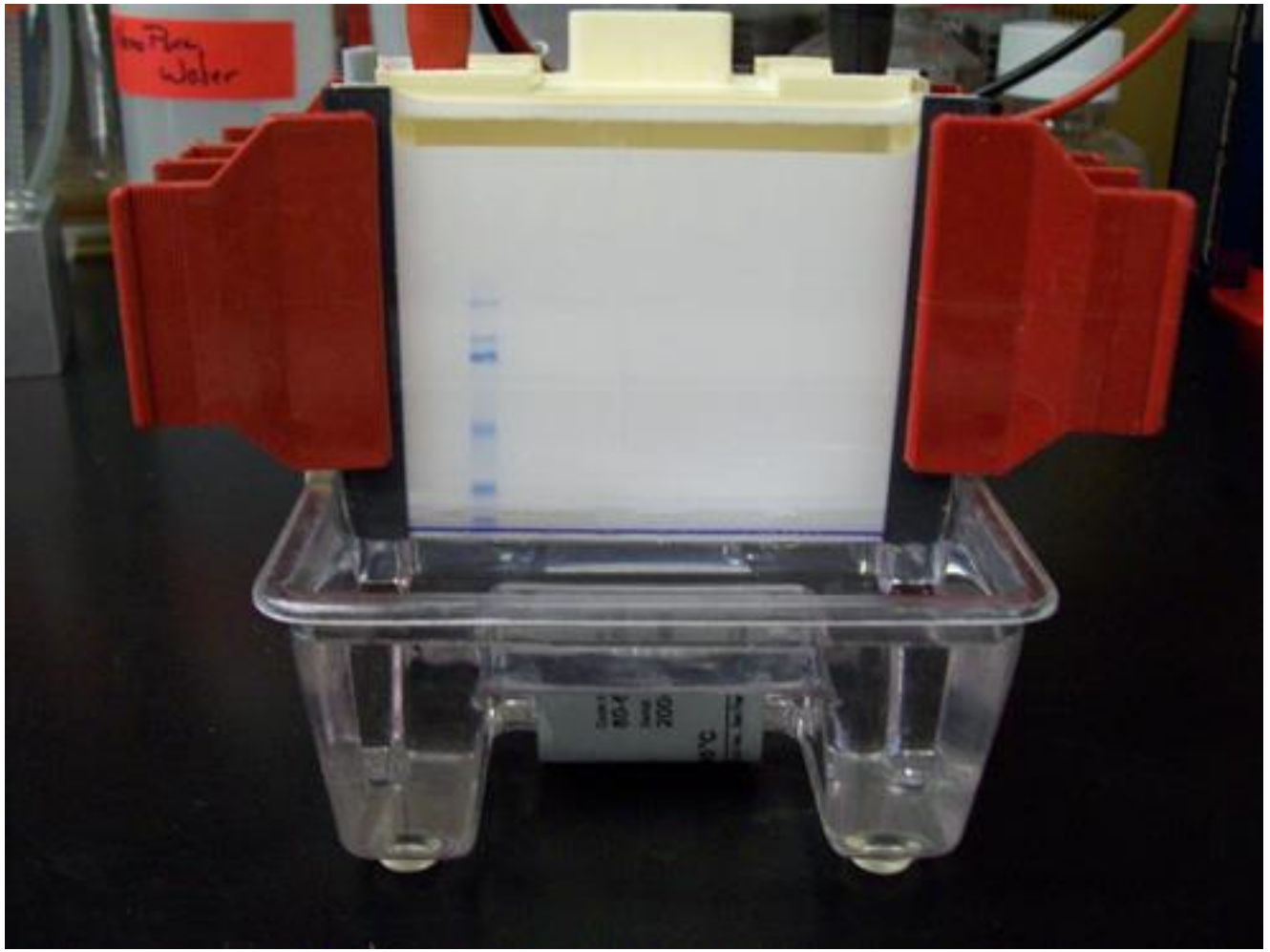


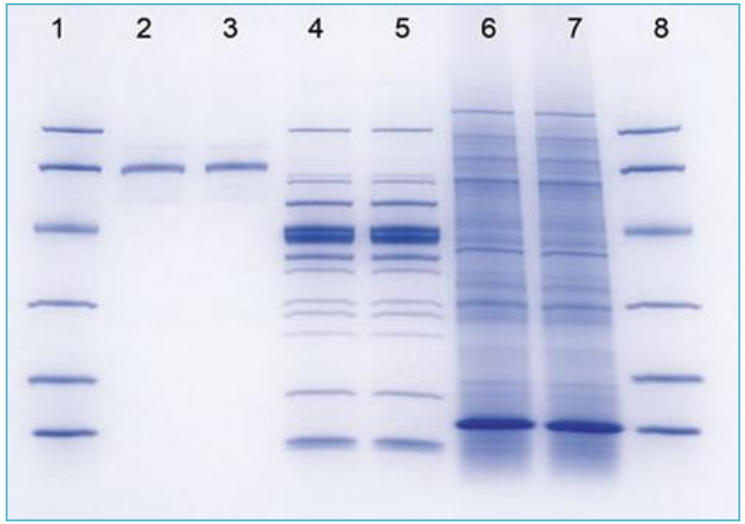

S
S
Mercaptoethanol
denatures protein
S
S
SDS
SDS binds protein
stoichiometrically
Small molecule moves
more quickly through gel
Large molecule moves more
slowly through gel








Water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