SODIUM DODECYL SULPHATE -POLYACRYLAMIDE GEL ELECTROPHORESIS [SDS-PAGE]

Lab#8

BCH 333

SDS-PAGE:

- SDS-PAGE: **S**odium **D**odecyl **S**ulfate-**P**olyacryl**A**mide **G**el **E**lectrophoresis.
- The separation of macromolecules in an electric field is called electrophoresis.
- Separates proteins by electrophoresis using a discontinuous polyacrylamide gel.
- SDS-PAGE aims to separate and identify proteins according to their molecular weight.

- Applications:
  - Quantifying.
  - Comparing (relative abundance).
  - Characterizing proteins (e.g., determining MW of proteins).
  - Checking the purity of protein samples.

Principle of SDS-PAGE:

- In general, fractionation by gel electrophoresis is based on **differences in size, shape and net charge of macromolecules** (in molecules native conditions using native gel electrophoresis).
- **Native gel electrophoresis,** 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 by:

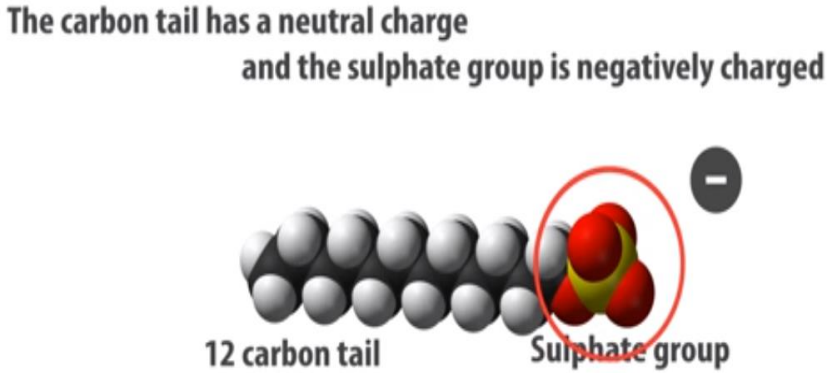
1. Using an anionic detergent, Sodium Dodecyl Sulfate (**SDS**), which binds strongly to most proteins.
2. Using disulfide reducing agent such as **β-mercaptoethanol** or **DTT** (dithiothreitol).

⟶ Proteins are unified in charge and shape, thus they separate based on **size**

Principle of SDS-PAGE:

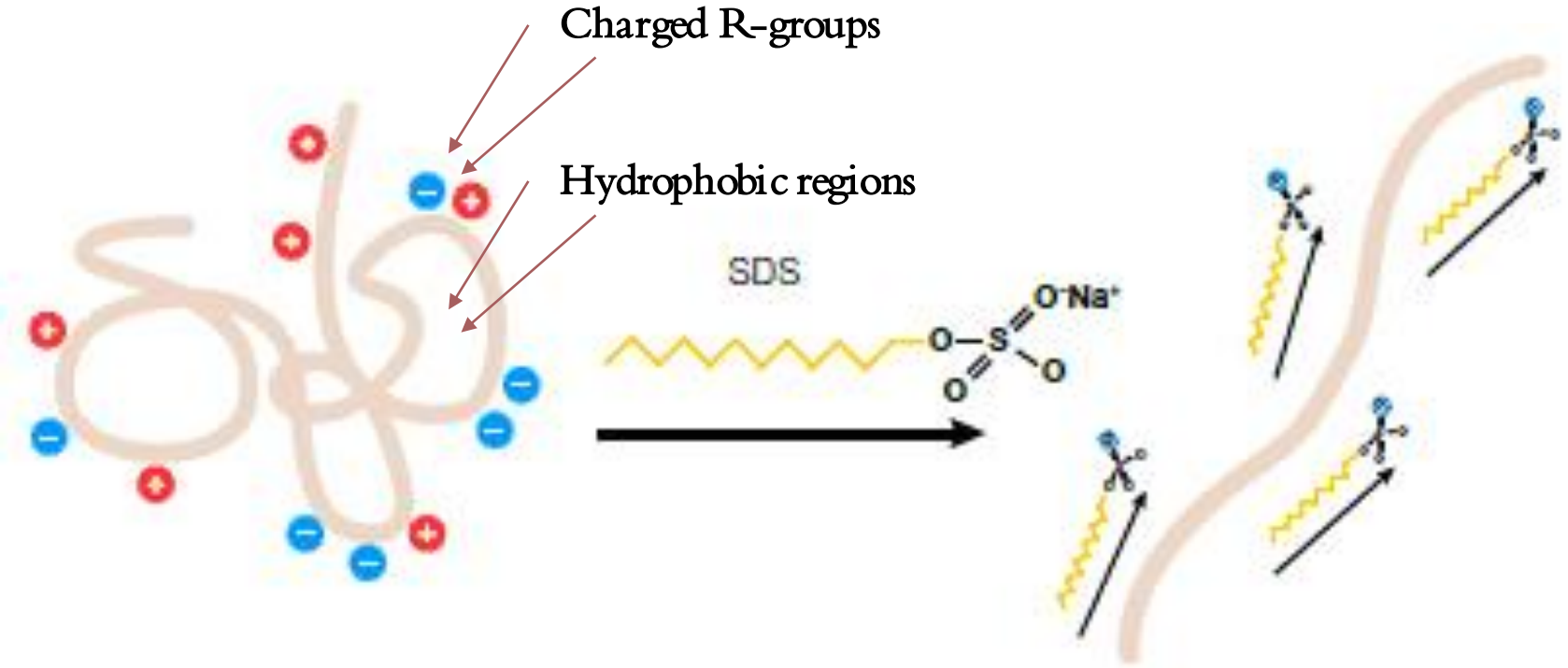
- Sodium Dodecyl Sulfate (SDS) is a **detergent** which **denature proteins** by binding to the hydrophobic regions, all non-covalent bonds will disrupt, and the proteins acquire a **negative net charge.**
- A Concurrent treatment with a **disulfide reducing** agent such as β-mercaptoethanol or DTT (dithiothreitol) further breaks down the strong bound disulfide.

➔So, the proteins samples are having uniformed structure and charge (-ve).

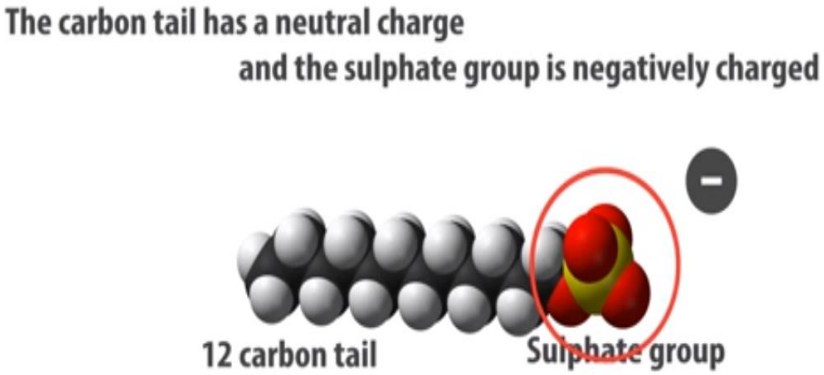


Molecular structure of SDS

Effect of SDS



Effect of SDS on the conformation and charge of a protein. Source: bio-rad.com



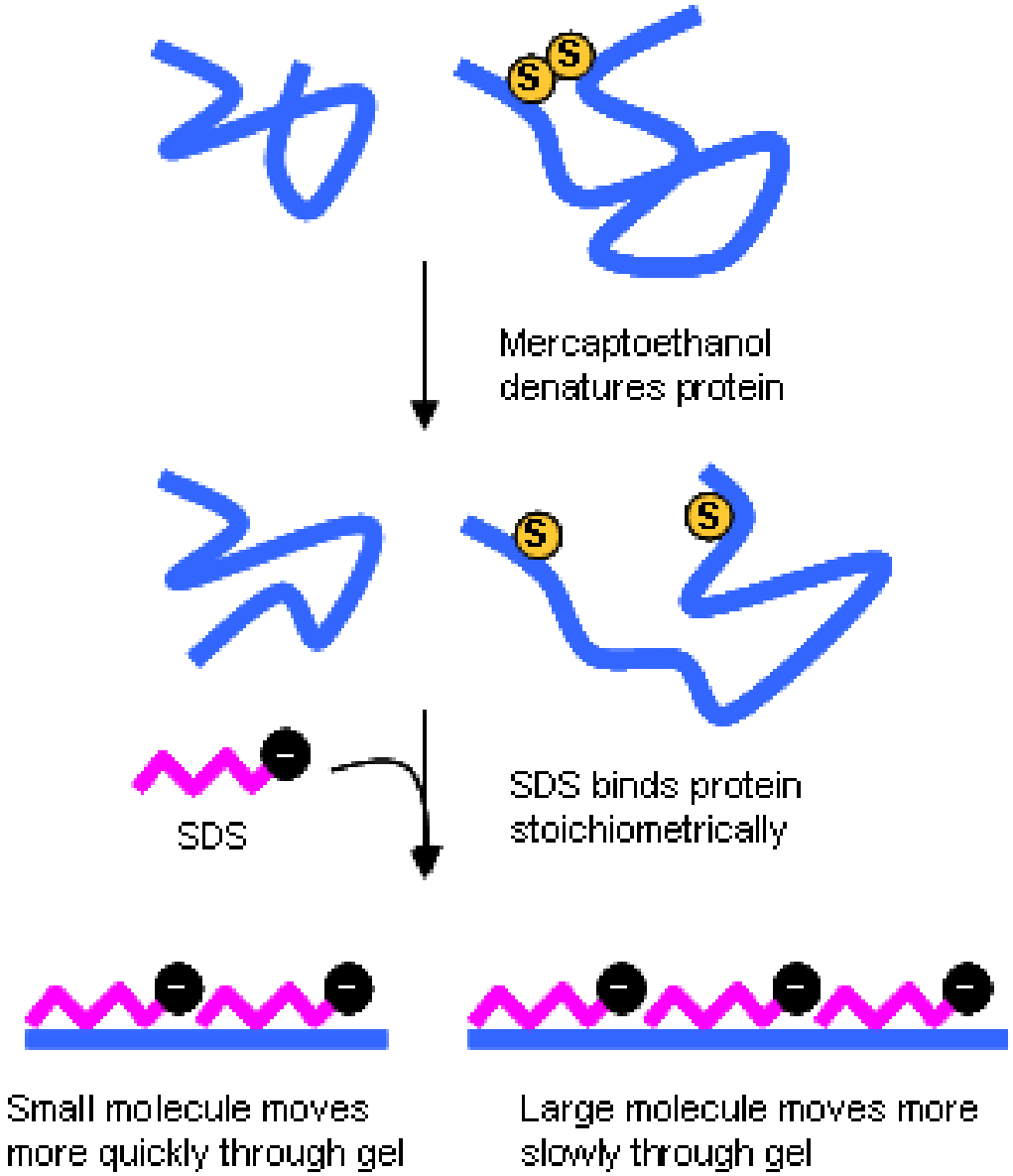
Molecular structure of SDS

(A) SDS PAGE

(B) Conventional native PAGE

SDS vs native PAGE

Effect of β-mercaptoethanol



Effect of β-mercaptoethanol

Principle of SDS-PAGE:

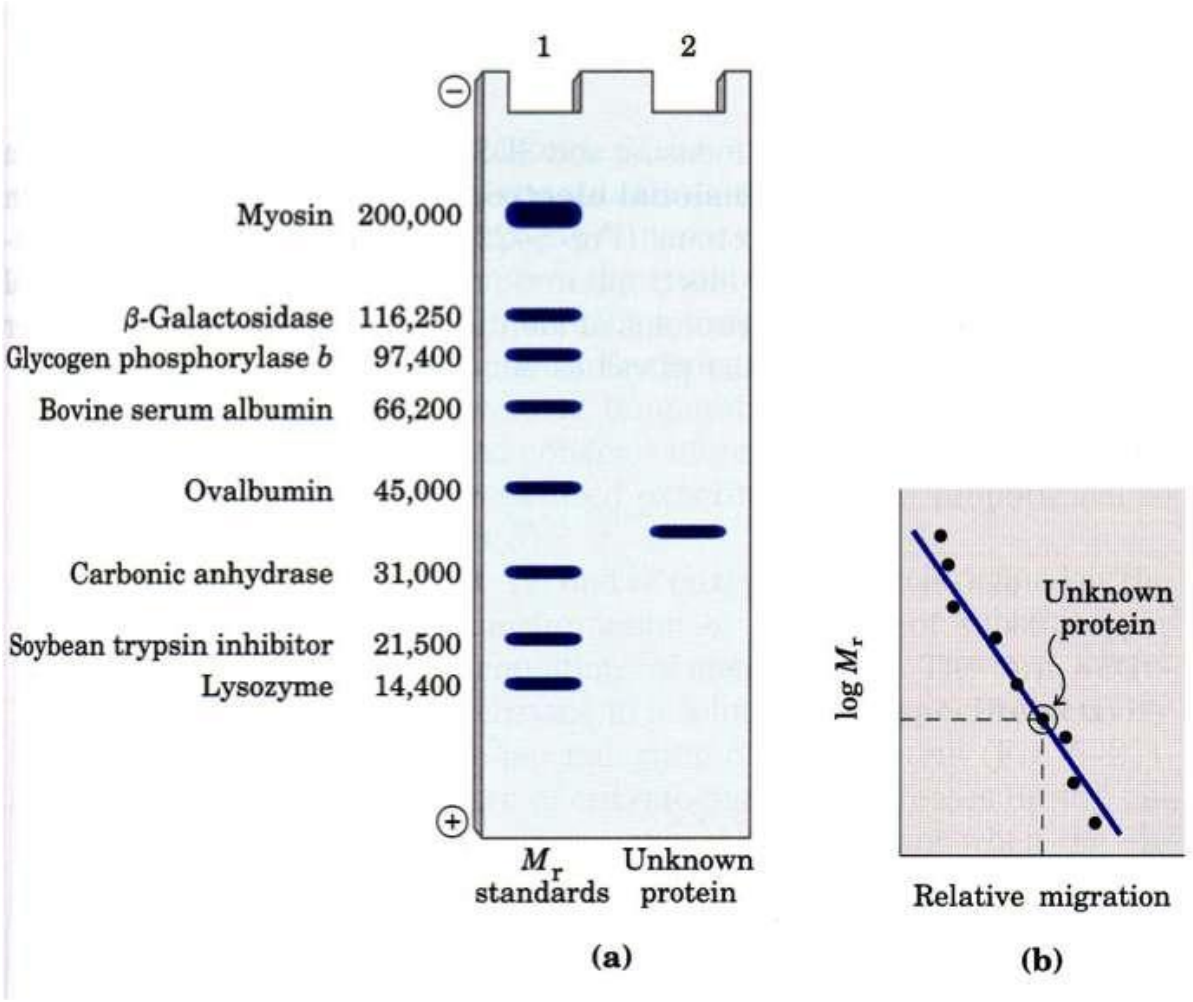
- Because the **charge-to-mass ratio is nearly the same** among SDS-denatured polypeptides, the final separation of proteins is dependent almost entirely on the differences in relative molecular mass of polypeptides (separation will depend on their molecular weight only).

➔i.e The electrophoretic mobility of the molecules is now considered to be a function of their size (the protein samples are having uniformed **structure** and **charge** → the separation will depend on their molecular weight only).

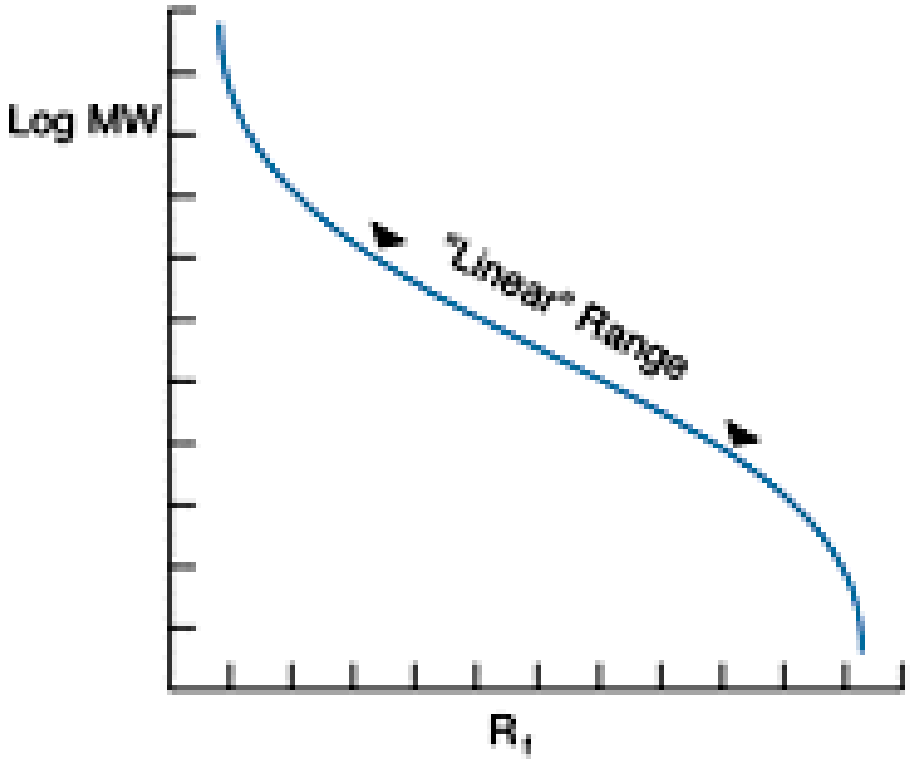
- Polyacrylamide gels **restrain larger** molecules from migrating **as fast as smaller molecules.**
- Thus, small proteins migrate faster through the gel under the influence of the applied electric field, whereas large proteins are successively retarded, due to the sieving effect of the gels.

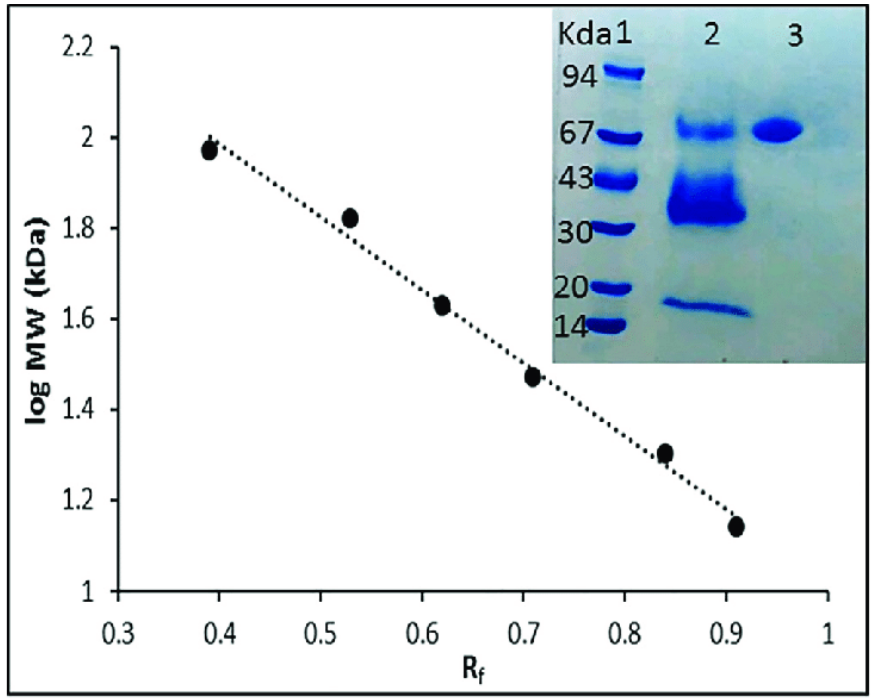
➔i.e the migration of the (SDS-treated proteins), towards the anode (+) is inversely proportional to the logarithms of their molecular weights, or more simply expressed:

Small proteins migrate faster through the gel



SDS-treated proteins have very similar charge-to-mass ratios and similar shapes. During SDS-PAGE, the rate of migration of SDS-treated proteins is effectively determined by **molecular weight**.





Graph of log MW vs. R_f is sigmoidal; it is nearly linear for a range of molecular weights excluding very small and very large MW.

Practical part

OBJECTIVES:

- To separate and calculate the molecular size of proteins by comparing the separated bands with a known standard molecular weight marker.

SDS-Polyacrylamide Gel Electrophoresis preparation

1. Sample Preparation:

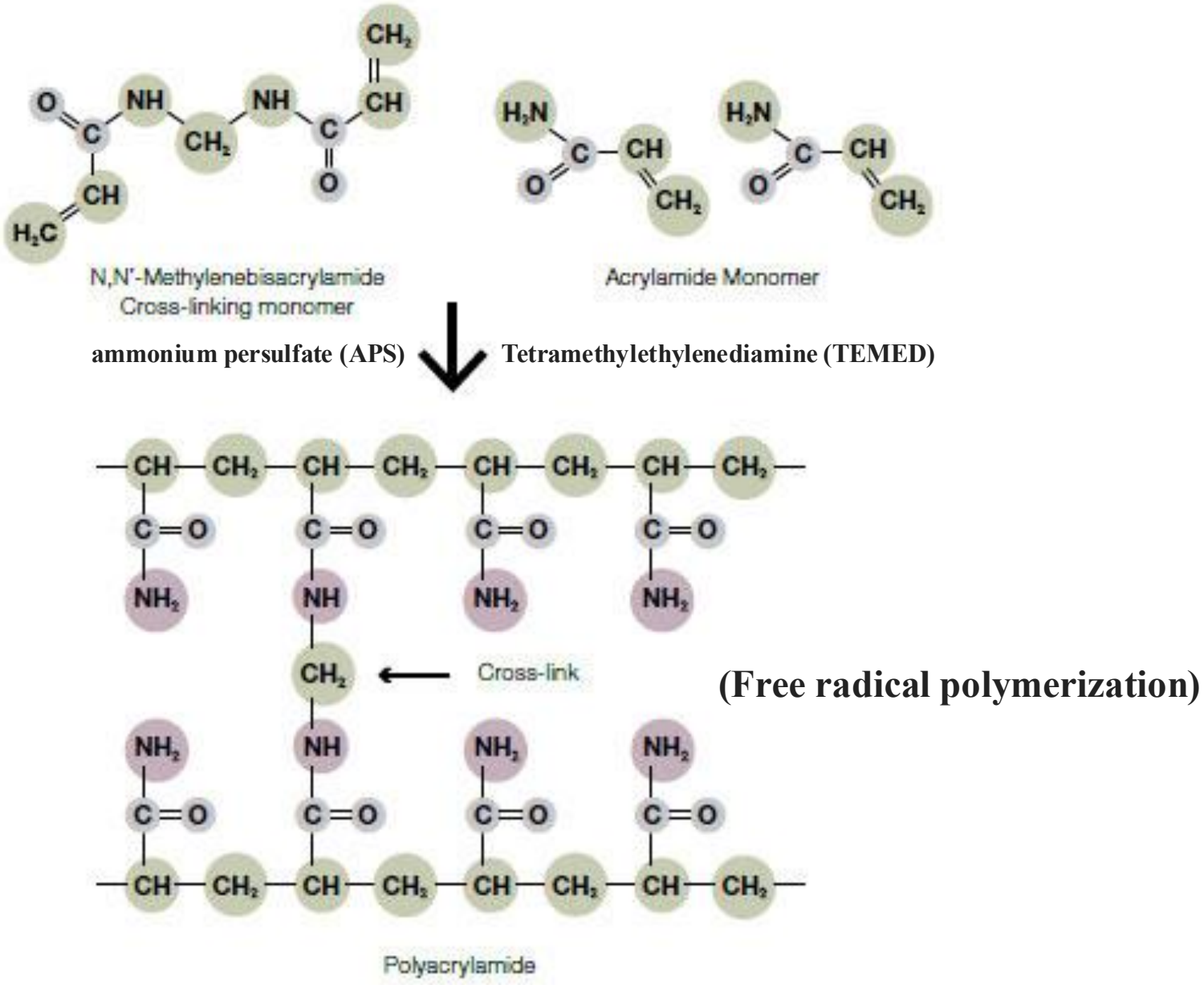
40 µl of protein sample + 10 µl of disruption buffer ➔ boil the mixture **3 min at 99 ℃**, why?

- **Disruption buffer [loading buffer] contains:**
  - 10% (w/v) SDS [?]
  - 1M Tris/HCl, pH 6.8
  - Glycerol [?]
  - β-Mercaptoethanol [?]
  - Bromophenol blue [?]

2. Polyacrylamide Gel (Acrylamide stock):

- The polyacrylamide gel is formed by co-polymerization of acrylamide and **cross-linking** by N, N'-methylene-bis-acrylamide "bis-acrylamide ".
- Polyacrylamide = acrylamide + bis-acrylamide.

- **To polymerize the gel a system:**
- 1- Ammonium PerSulfate (APS) [initiator].
- 2-TetraMethylene Ethylene Diamine (TEMED) [catalyst].



(Free radical polymerization)

Polymerization of acrylamide monomers and bis-acrylamide. Source: bio-rad.com

2.Polyacrylamide Gel:

- It is composed of **two** gels layers:

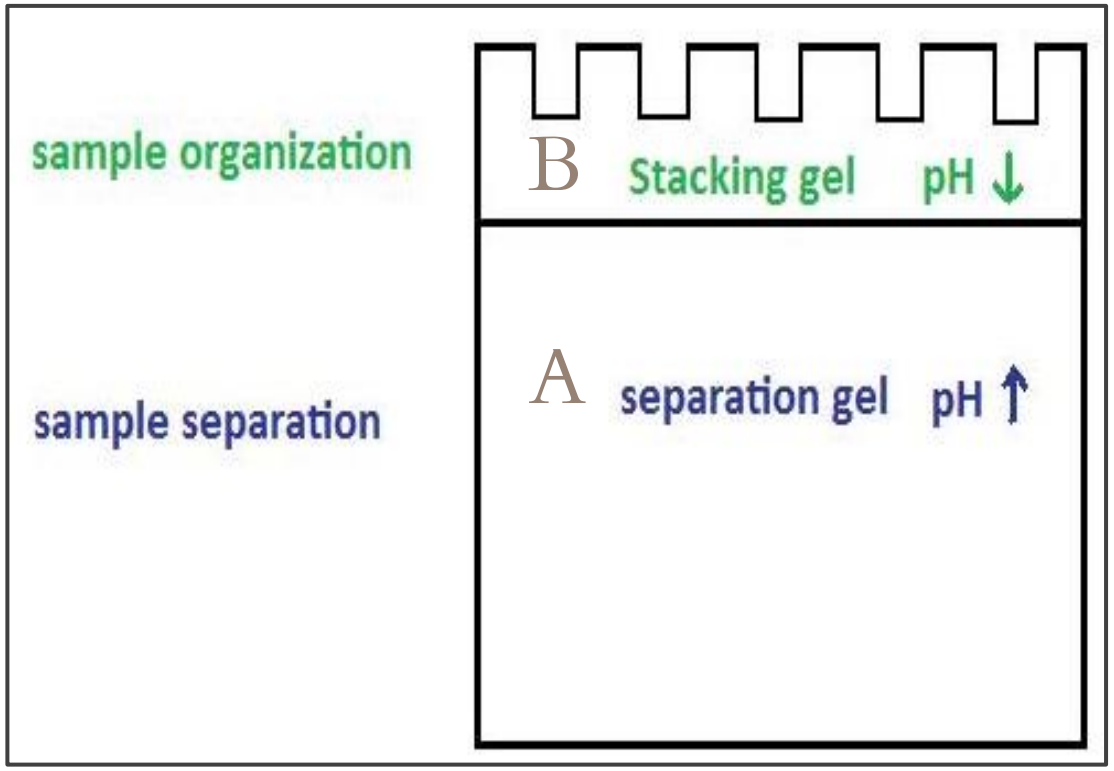
A. Separation gel:

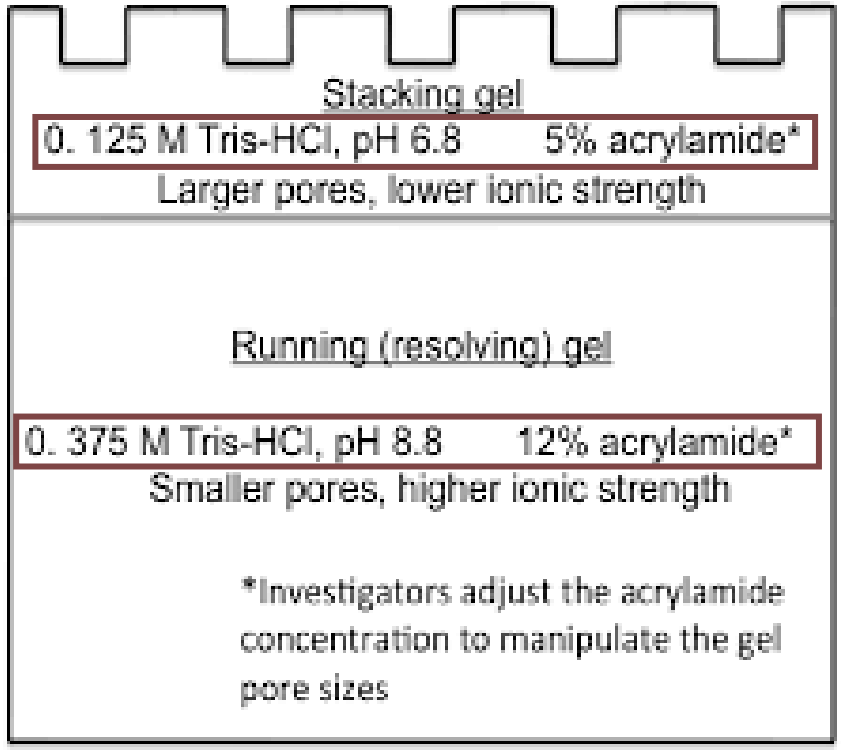
- **HIGH** concentration of the acrylamide stock and pH value.

B. Stacking gel:

- **LOW** concentration of the acrylamide stock and pH value.

The gel's two layers:





Discontinuous polyacrylamide gel

A. Separation Gel Contents:

- For 10 ml of separation gel:

Acrylamide%	6%	8%	10%	12%	15%
H_2O	5.2 ml	4.6 ml	3.8 ml	3.2 ml	2.2 ml
Acrylamide/Bis-acrylamide (30% / 0.8% w/v)	2 ml	2.6 ml	3.4 ml	4 ml	5 ml
1.5 M Tris (pH=8.8)	2.6 ml	2.6 ml	2.6 ml	2.6 ml	2.6 ml
10% w/v SDS	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
10% w/v Ammonium persulfate (APS)	100 µl	100 µl	100 µl	100 µl	100 µl
TEMED	10 µl	10 µl	10 µl	10 µl	10 µl

*Note: APS and TEMED must be added right before each use.

- The acrylamide percentage in SDS PAGE gel depends on the **size of the target protein in the sample.**

Acrylamide %	M.W. Range
7%	50 kDa - 500 kDa
10%	20 kDa - 300 kDa
12%	10 kDa - 200 kDa
15%	3 kDa - 100 kDa

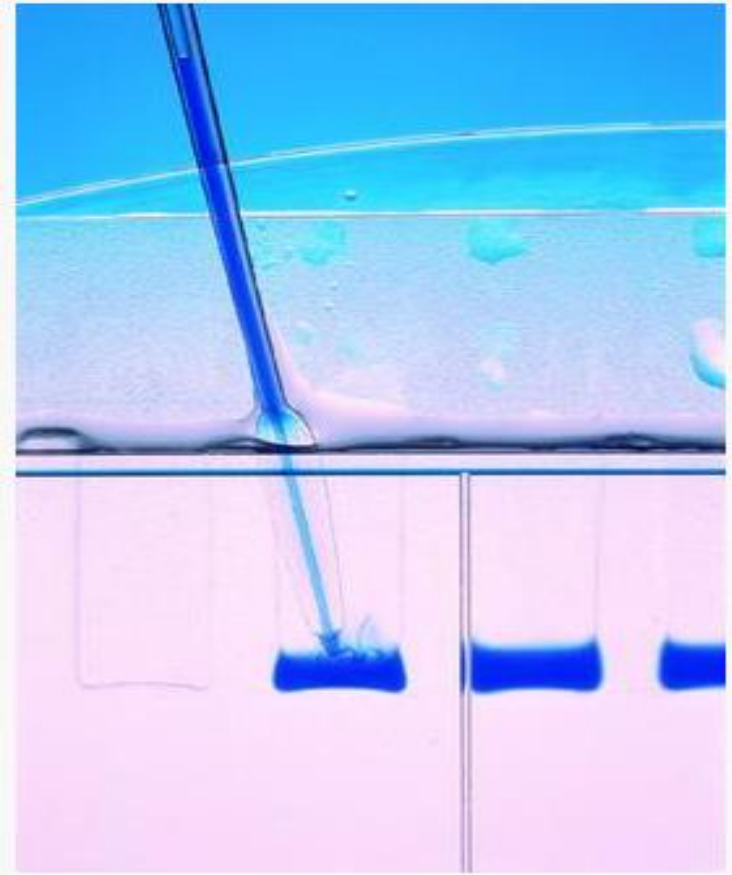
B. Stacking Gel Contents:

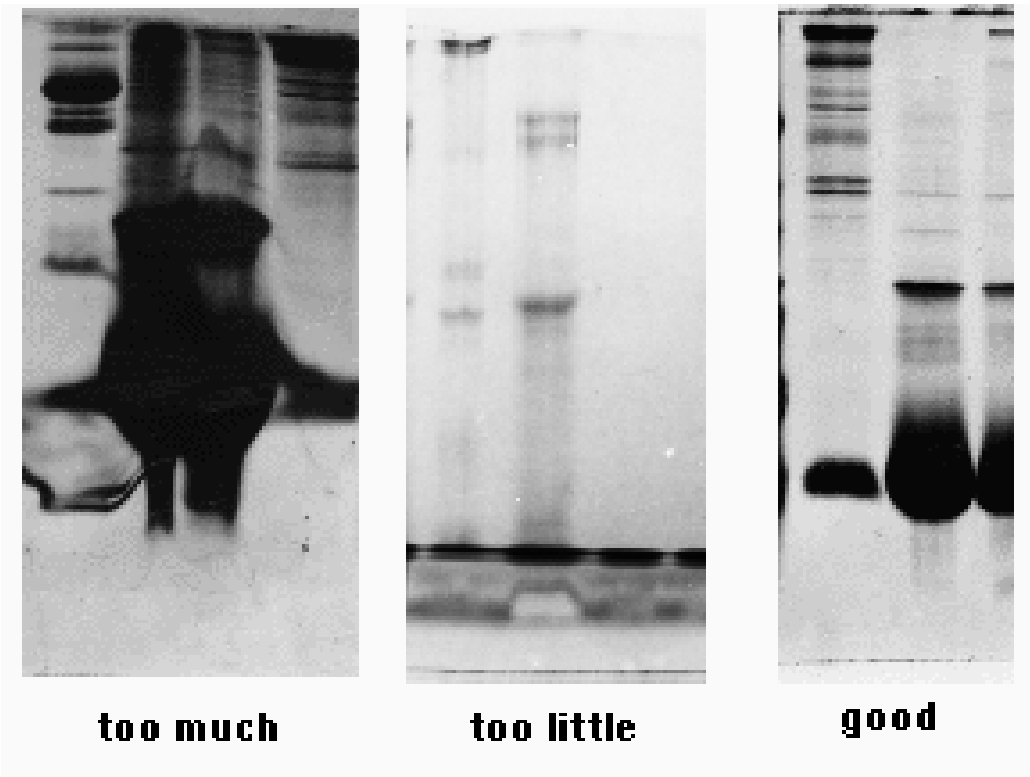
- For 5 ml of stacking gel:

	Volume
H_2O	2.975 ml
Acrylamide/Bis-acrylamide (30% / 0.8% w/v)	0.67 ml
0.5 M Tris-HCl (pH=6.8)	1.25 ml
10% w/v SDS	0.05 ml
10% w/v Ammonium persulfate (APS)	0.05 ml
TEMED	0.005 ml

The stacking gel has a lower concentration of acrylamide

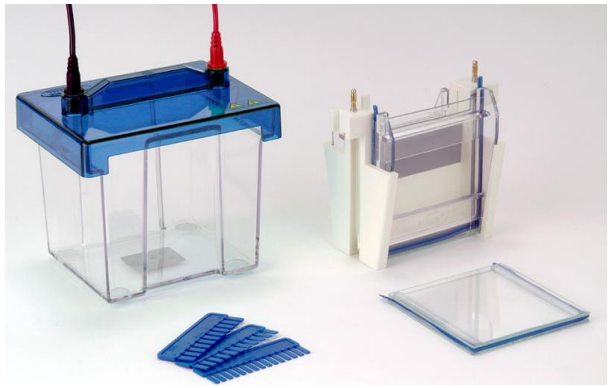
3. Sample Loading:





4. Running the gel using Running buffer (pH 8.3):

- It helps to deliver the electric current through the gel.
- It contains:
  - Tris-HCl.
  - Glycine.
  - SDS.

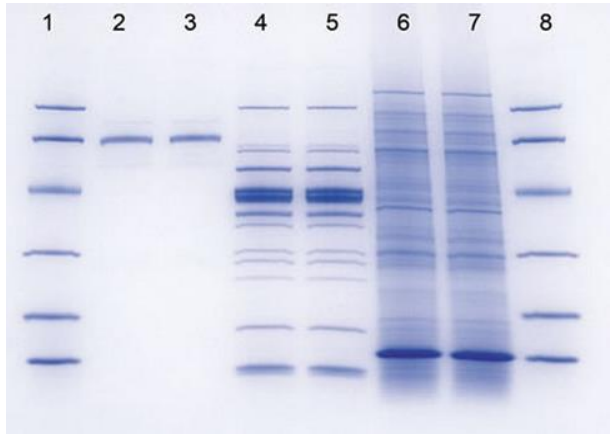


5. Stain the gel using staining buffer :

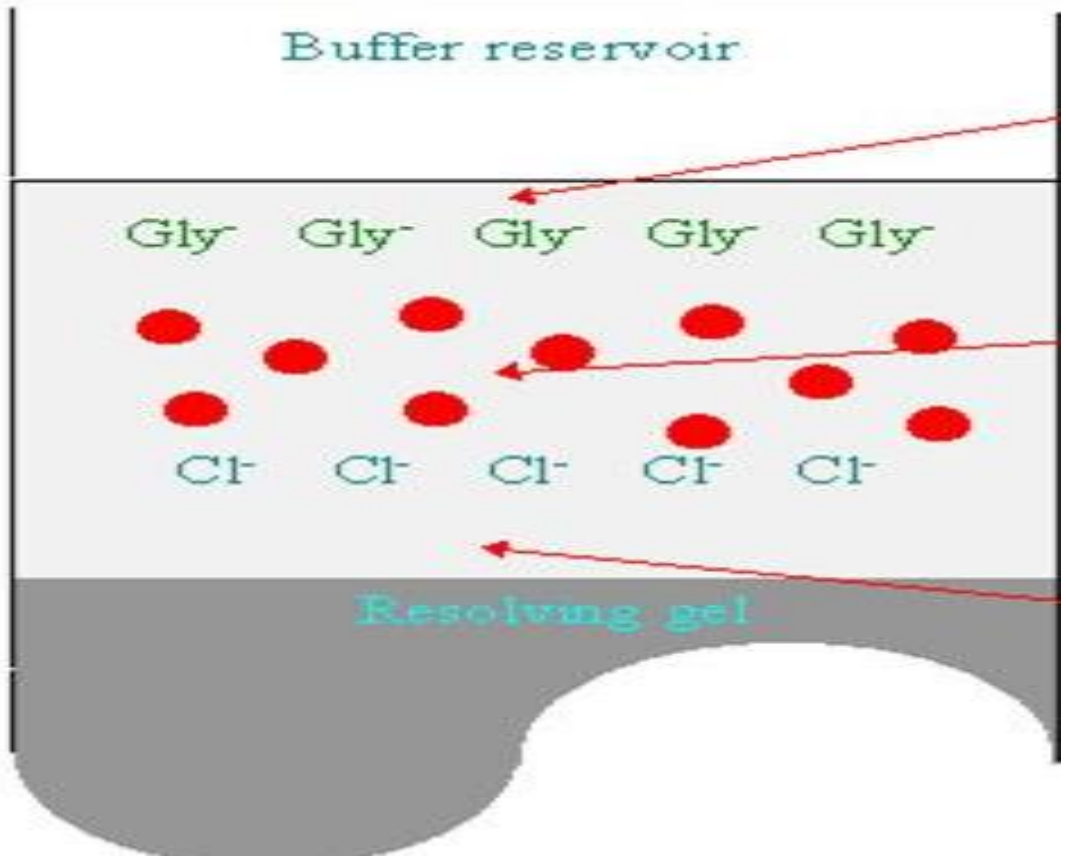
- It contains:
  - Glacial acetic acid
  - Methanol
  - **Coomassie brilliant blue 250-R.**

6. De-stain the gel using De-staining buffer:

- It contains:
  - Glacial acetic acid
  - Methanol

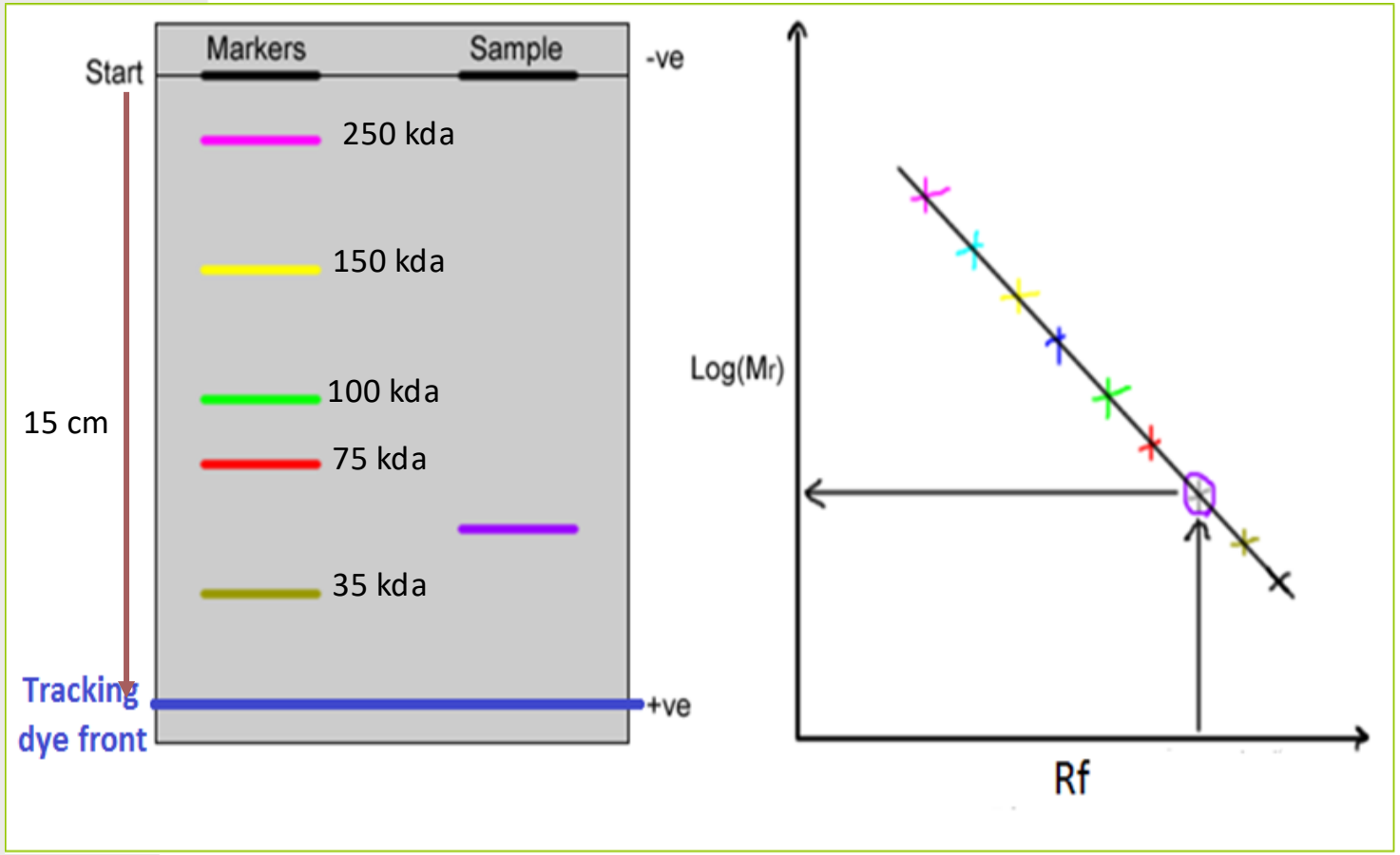


Proteins are sandwiched between the Cl^- ions and the glycine molecules.



Data Analysis

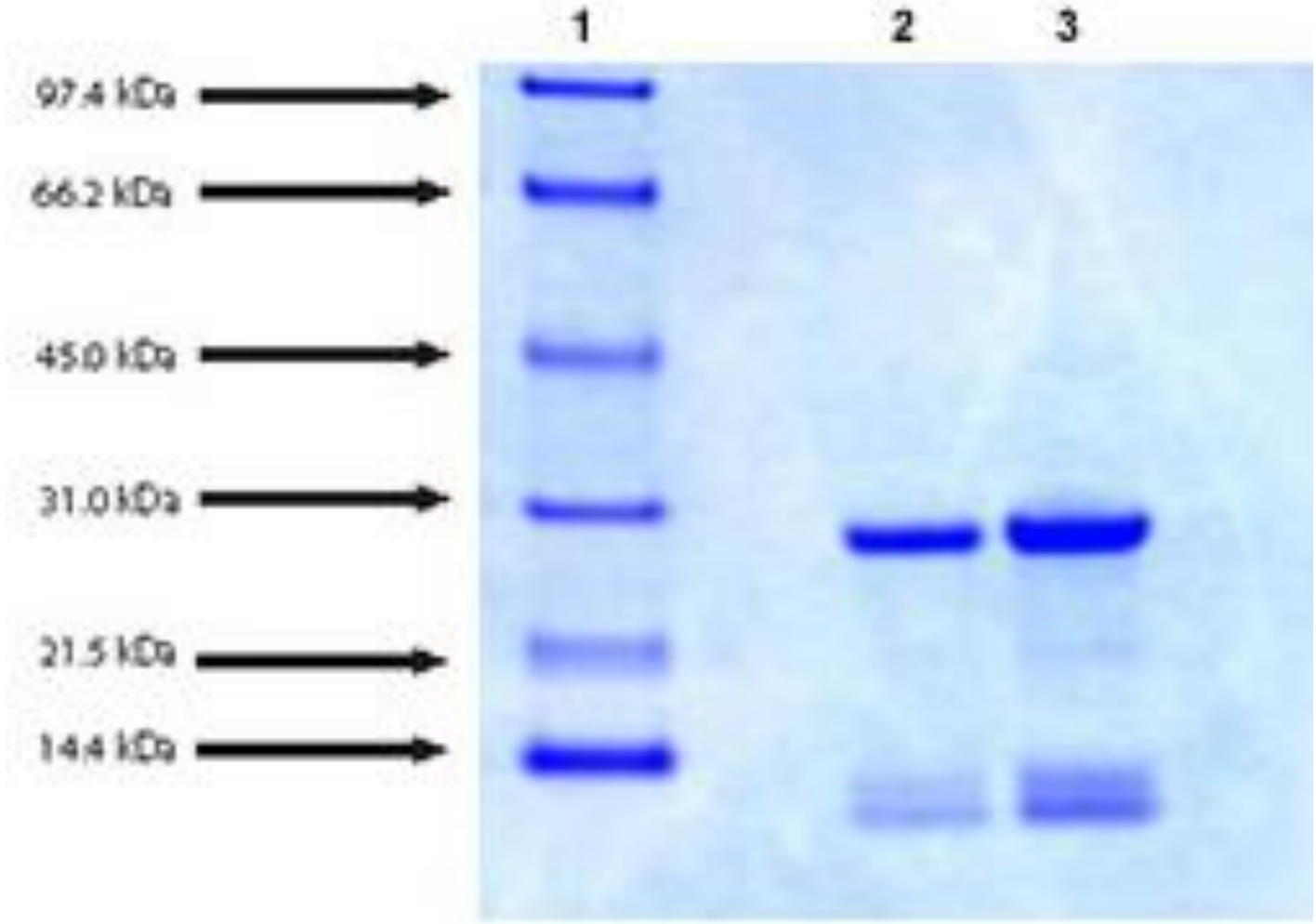
For Molecular Weight Determination:

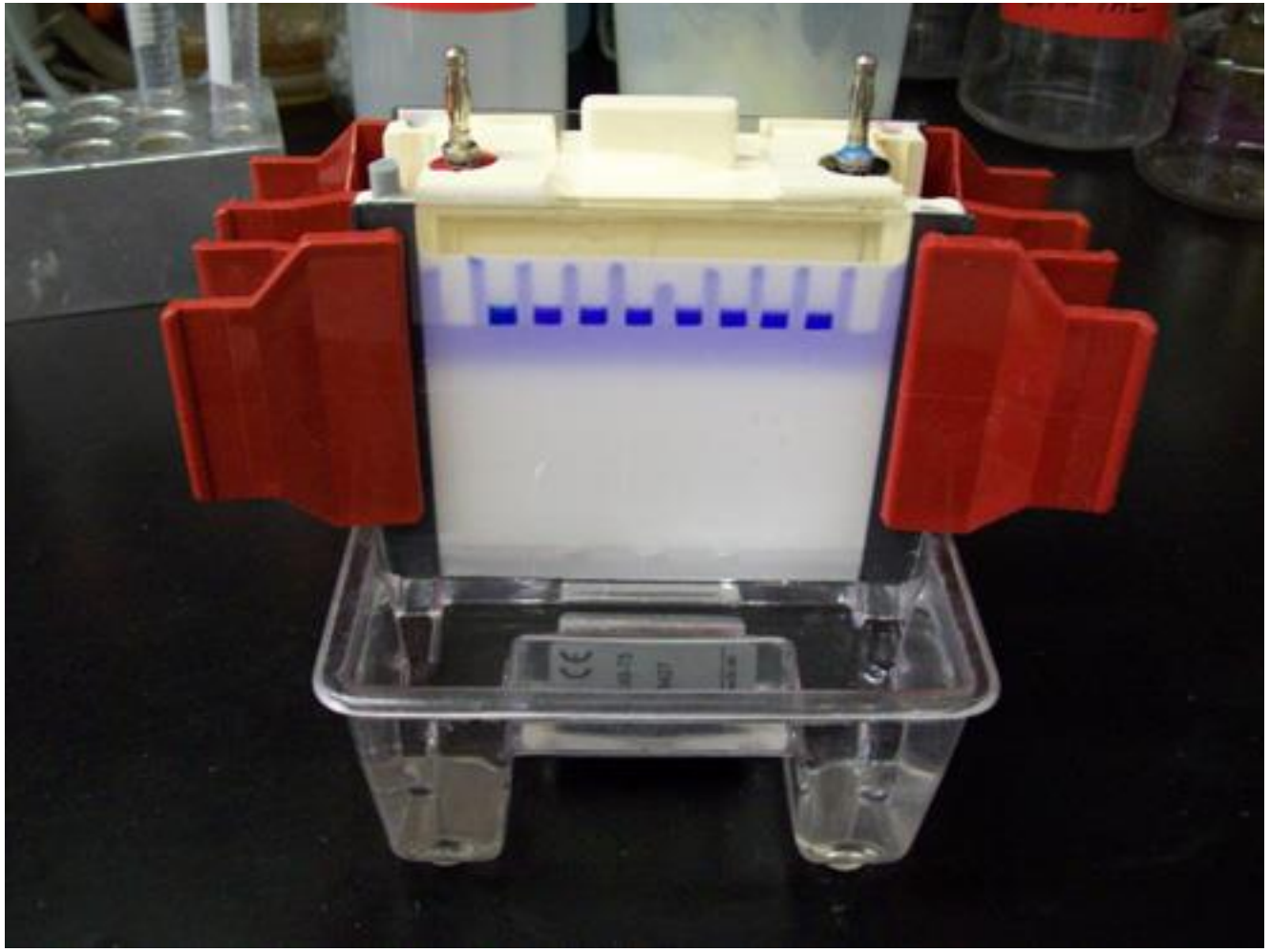


- R_f = Distance of migration of sample / Distance moved by tracking dye

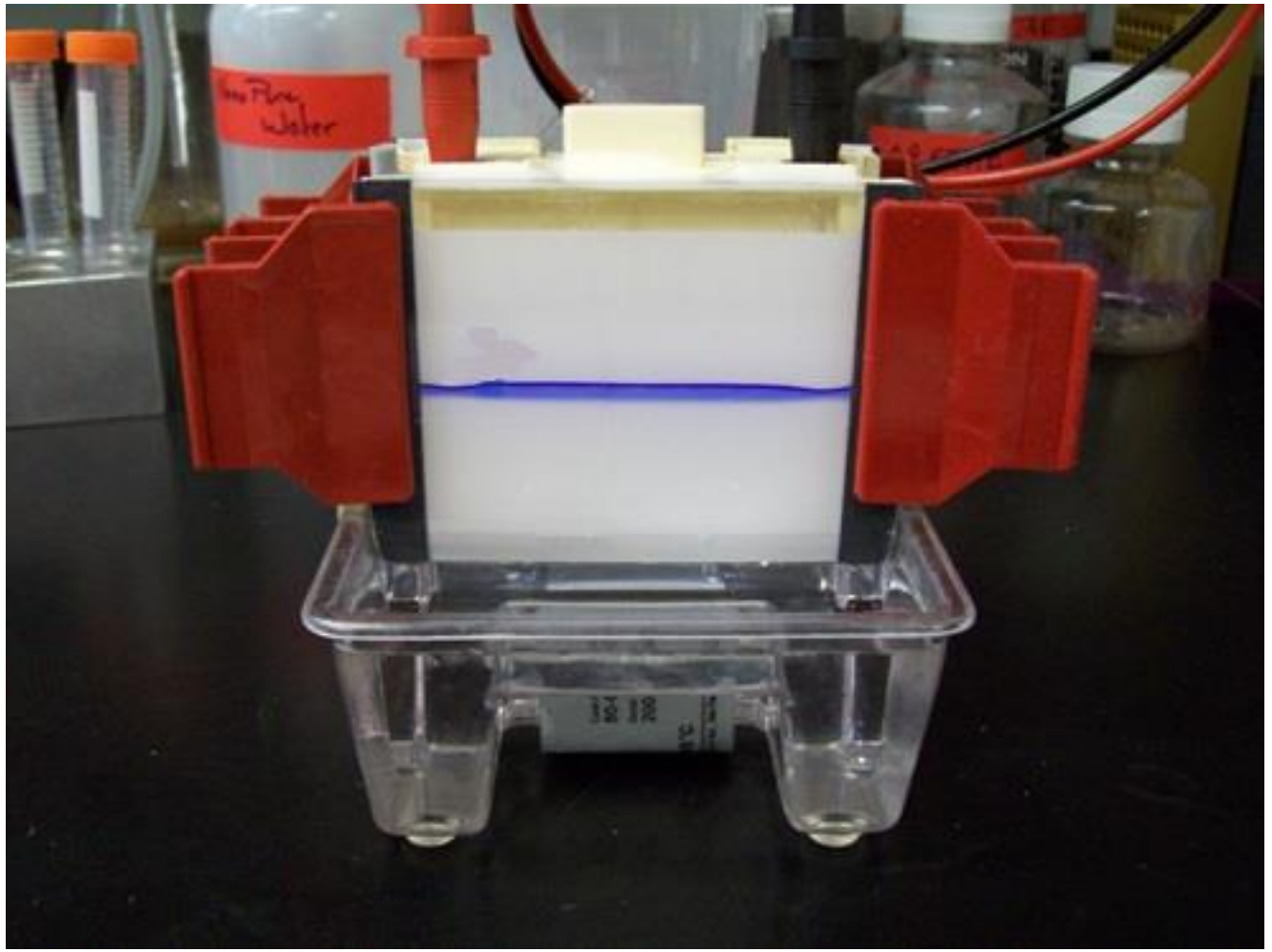
	Dye front (cm)	Distance (cm)	R_f	M.W (Da)	Log M.W (Da)
std_1	15 cm	1.5	0.1	250000	5.4
std_2		4	0.27	150000	5.18
std_3		5.5	0.37	100000	5
std_4		6.4	0.43	75000	4.88
std_5		12.3	0.82	35000	4.54
Un-known M.W sample		9.3	0.623	?	?

For Molecular Weight Determination cont':

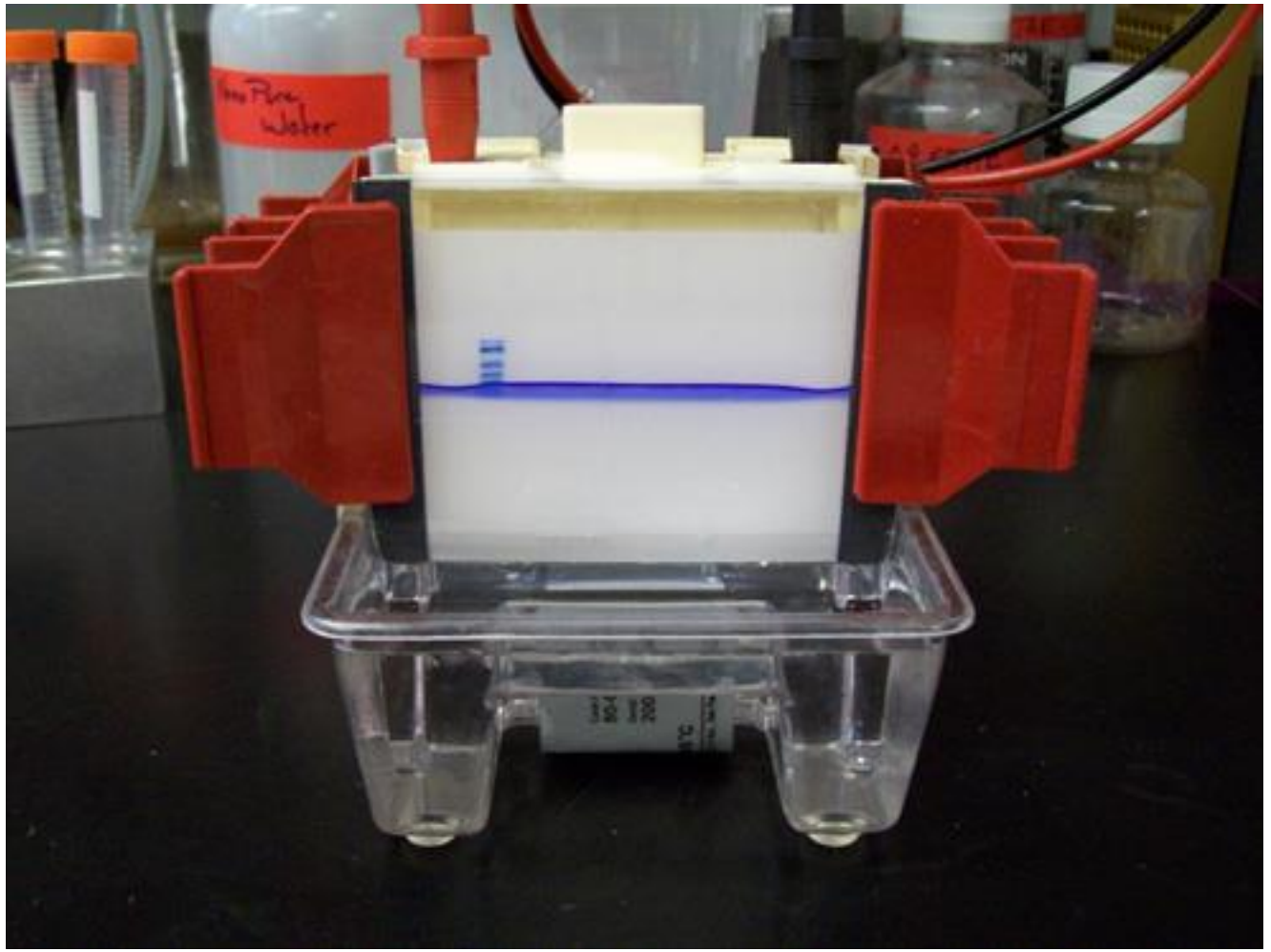




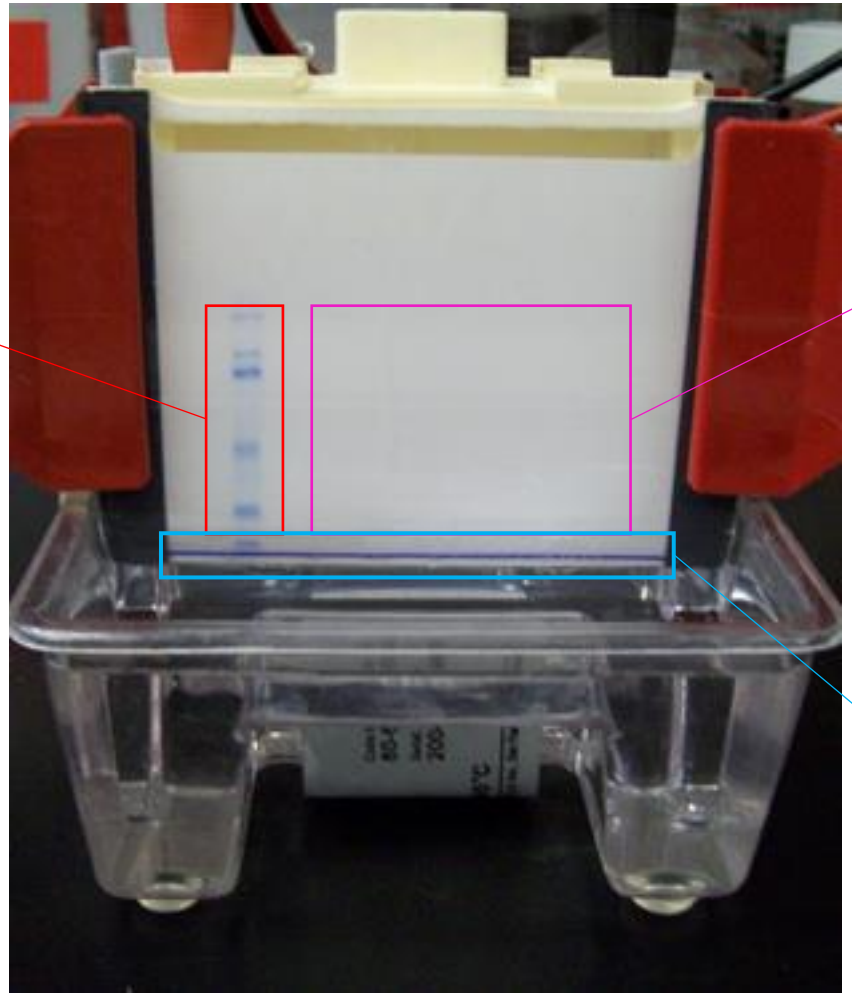
At beginning



After a while

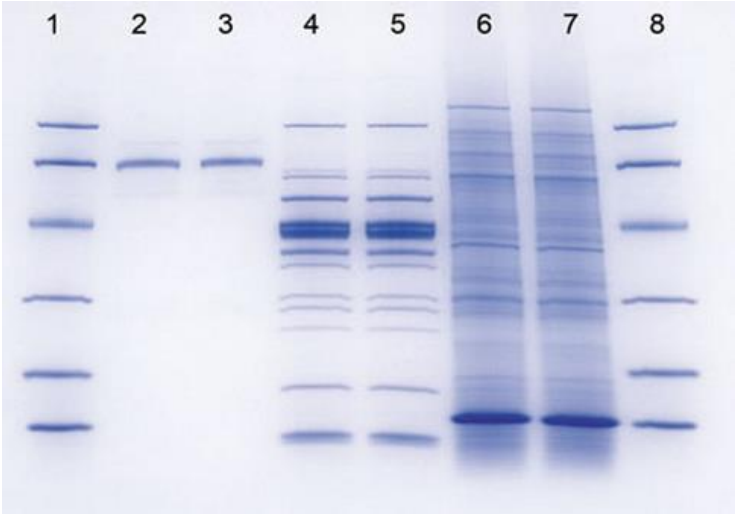


After more mins.

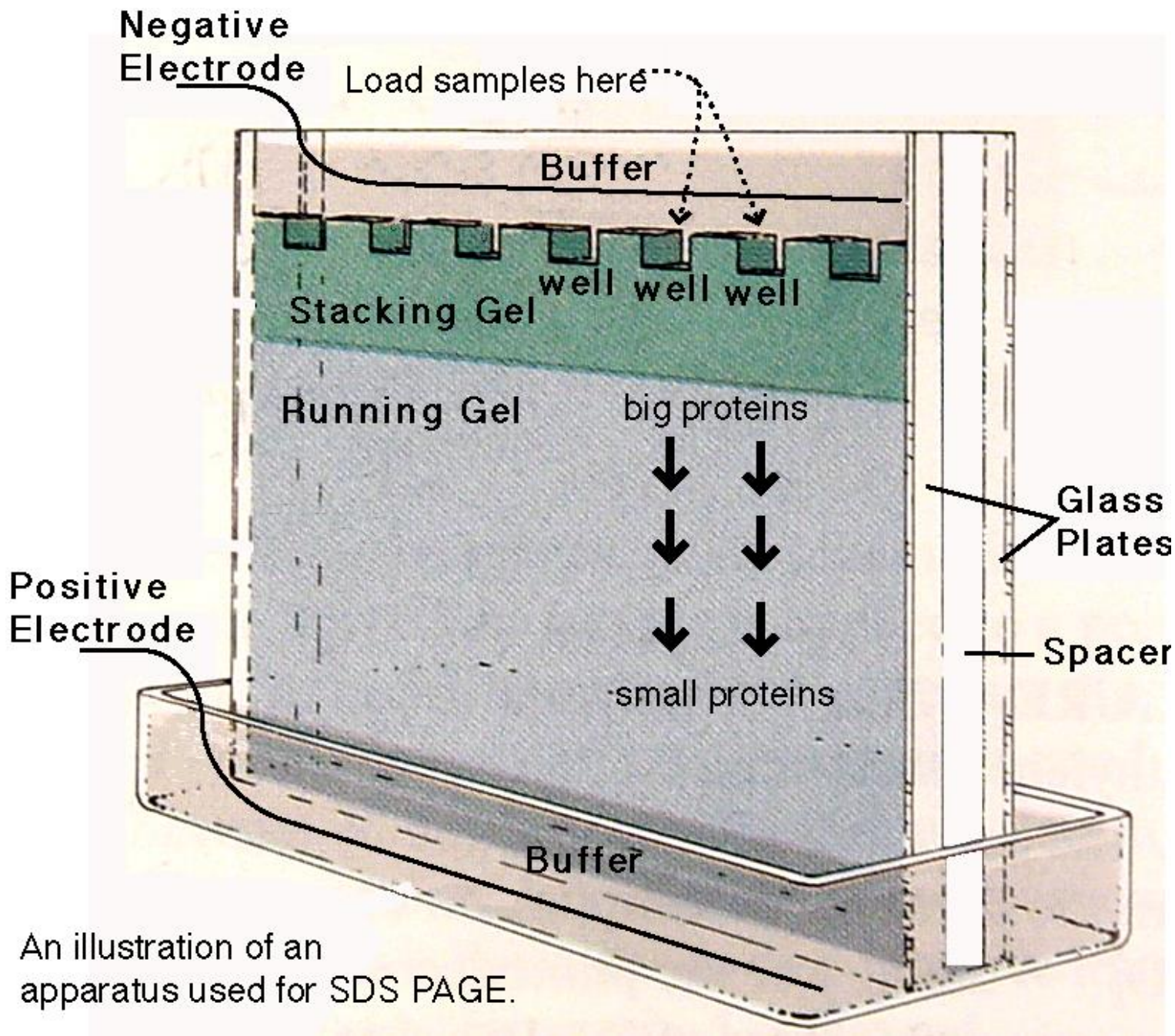


At the end

Staining



De Staining



An illustration of an apparatus used for SDS PAGE.

Supporting Materials:

- SDS PAGE principles – simple animated tutorial:

https://www.youtube.com/watch?v=3CrzY7jb9fQ

https://www.youtube.com/watch?v=i_6y6Z5UvwE