PROTEIN ELECTROPHORESIS

Protein electrophoresis is the movement of proteins within an electric field. Used to separate proteins for the purposes of analysis and purification.

Native PAGE- non denaturing gel. No denaturing and reducing agent are used. It was developed by Ornstein and Davis in 1964.

SDS PAGE- Sodium Dodecyl Sulfate (SDS) is added and reducing agents (beta mercaptoethanol) also used. It was developed by Laemmli in 1970. Therefore, known as Laemmli's protocol.

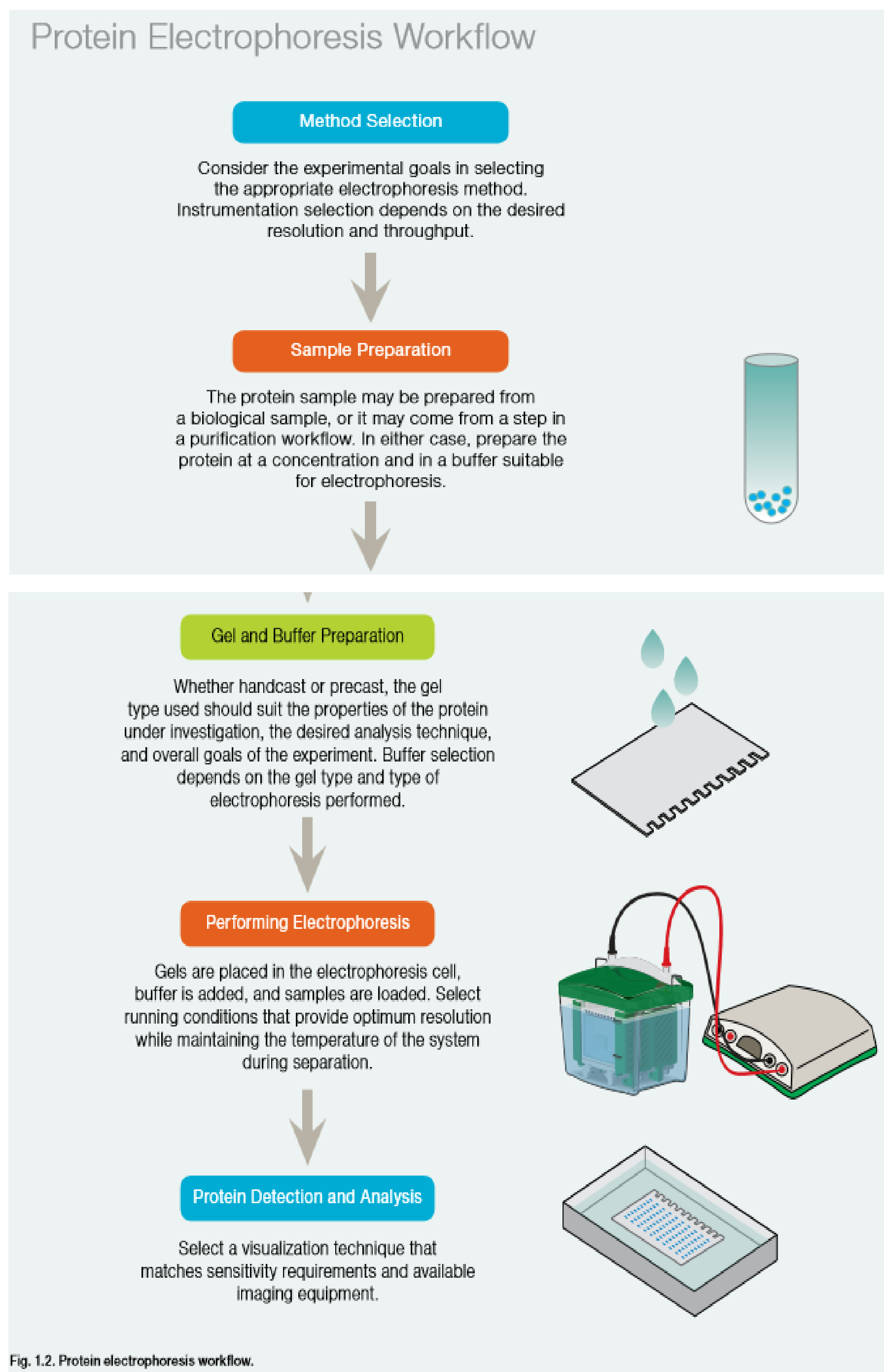


Fig. 1.2. Protein electrophoresis workflow.

Cross linked polyacrylamide gels are formed from the polymerization of acrylamide monomer in the presence of smaller amounts of N,N'-methylenebisacrylamide (also known as bis-acrylamide). Bis-acrylamide is two acrylamide molecules linked by a methylene group, and is used as a cross-linking agent.

Polymerizaytion is an example of free radical catalysis and is initiated by the addition of Ammonium Persulfate and the base N,N,N',N'-tetramethylenediamine (TEMED). TEMED catalyses the decomposition of the persulphate ion to give a free radical. Free radicals are highly reactive species due to an unpaired elctron. This free radical (R*) attacks acyrlamide monomer (M) generating RM*. This moiety will attack another M monomer and thereby chain continues.

All the solutions are degassed as oxygen mops up the free radicals and degassing also helps in prevention of air bubbles getting trapped in the gel.

Separating gel- to separate the proteins based on size.

Stacking gel- to concentrate the protein sample into a sharp band before it enters separating gel.

Samples to be run on gel on SDS PAGE are boiled in buffer containing SDS, β-mercaptoethanol, bromophenol blue and sucrose or glycerol.

SDS- binds and denatures the proteins. Proteins open in a rod shaped structure. On average, one SDS molecule binds every 2 amino acids.

β-mercaptoethanol- reduces any disulfide bridges which are holding proteins tertiary structure.

Bromophenol blue- ionisable tracking dye.

Sucrose or glycerol- provides density to the sample to settle in the well.

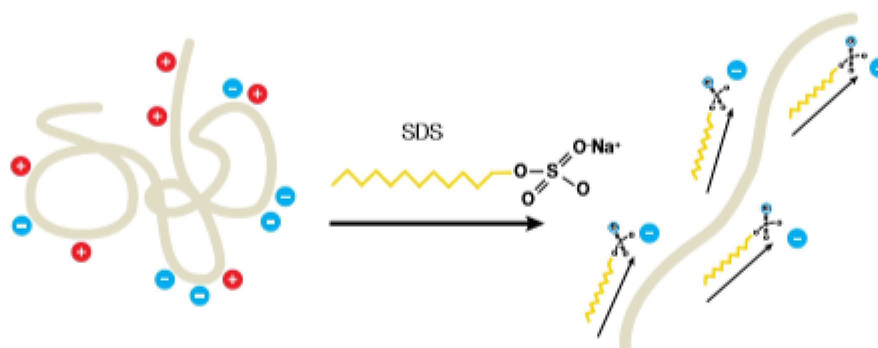
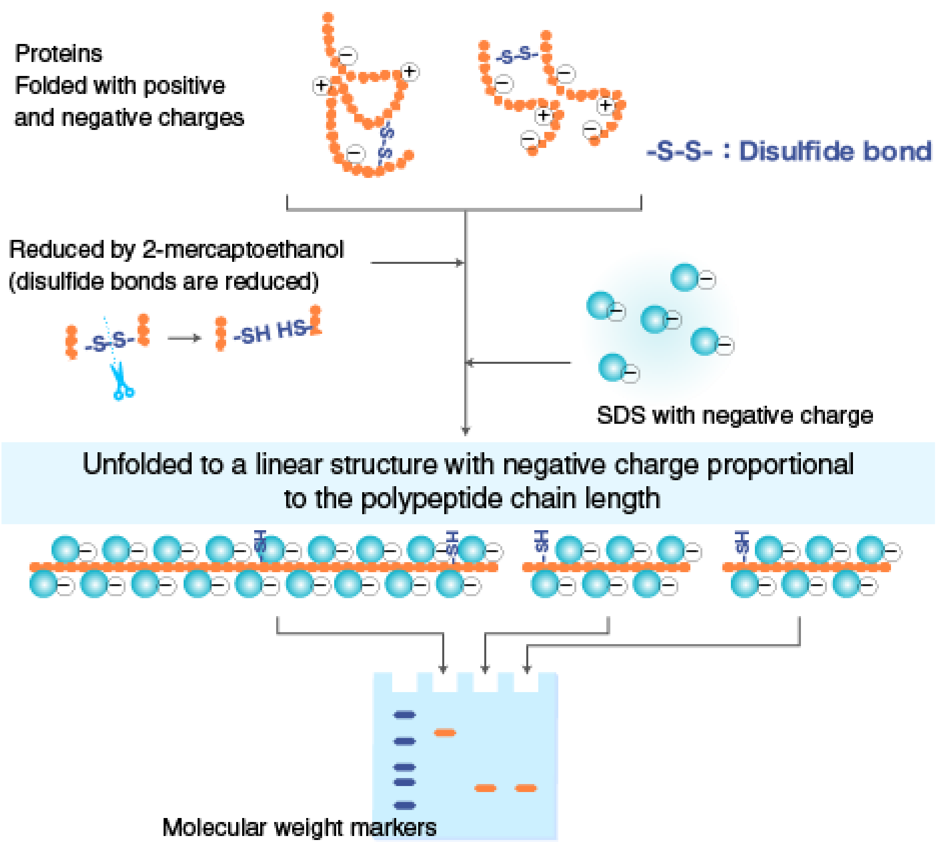


Figure: Effect of SDS on the conformation and charge of a protein.



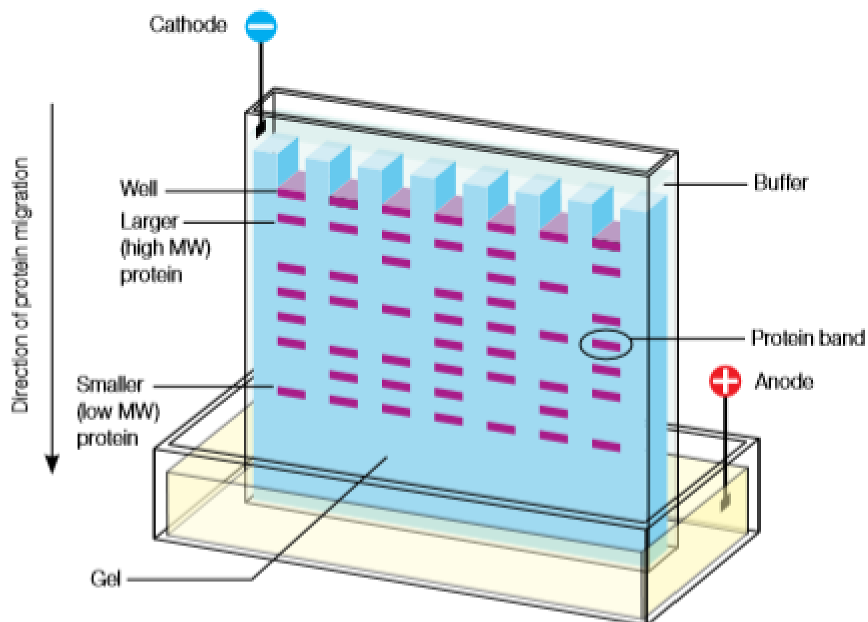


Figure: Schematic of electrophoretic protein separation in a polyacrylamide gel. MW-Molecular Weight.

Stacking and separating gels differ by different pore size (4-6 % and 10-20 % respectively), ionic strength and pH values (pH 6.8 or pH 8.8). The electrolyte most frequently used is an SDS-containing Tris-glycine-chloride buffer system. At neutral pH, glycine predominantly forms the zwitterionic form, at high pH the glycine lose positive charges and become predominantly anionic. In the stacking gel, the smaller, negatively charged chloride ions migrate in front of the proteins and the slightly larger, negatively and partially positively charged glycinate ions migrate behind the proteins. Whereas in the comparatively basic separating gel both ions migrate in front of the proteins. The pH gradient between the stacking and separation gel buffers leads to a stacking effect at the border of the stacking gel which concentrates the protein into a sharp band. This is achieved by utilising differences in ionic strength and pH between the electrophoresis buffer and the stacking gel buffer and involves a phenomenon known as Isotachophoresis.

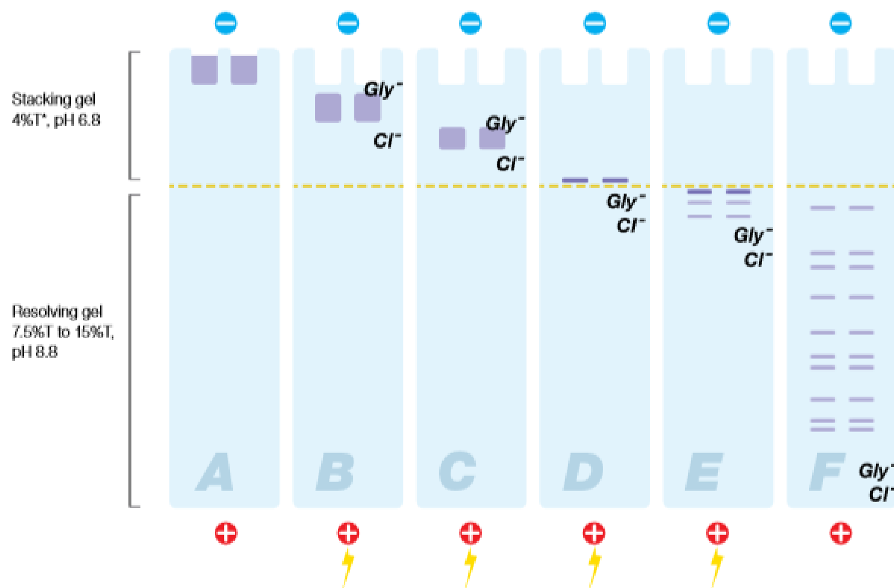


Fig. 2.2. Migration of proteins and buffer ions in a denaturing discontinuous PAGE system. A, Denatured sample proteins are loaded into the wells; **B,** Voltage is applied and the samples move into the gel. The chloride ions already present in the gel (leading ions) run faster than the SDS-bound proteins and form an ion front. The glycinate ions (trailing ions) flow in from the running buffer and form a front behind the proteins; **C,** A voltage gradient is created between the chloride and glycinate ions, which sandwich the proteins in between them; **D,** The proteins are stacked between the chloride and glycinate ion fronts. At the interface between the stacking and resolving gels, the percentage of acrylamide increases and the pore size decreases. Movement of the proteins into the resolving gel is met with increased resistance; **E,** The smaller pore size resolving gel begins to separate the proteins based on molecular weight only, since the charge-to-mass ratio is equal in all the proteins of the sample; **F,** The individual proteins are separated into band patterns ordered according to their molecular weights.

What are the Similarities Between SDS PAGE and Native PAGE?

- Both SDS PAGE and Native PAGE systems use polyacrylamide gel as the matrix of the gel.
- Both are used for separation and identification of proteins.
- Both use electrophoretic mobility to separate the compounds.
- The visualizing of the gel can be done by staining methods in both techniques.

Difference Between SDS PAGE and Native PAGE

SDS PAGE vs Native PAGE	
SDS PAGE or Sodium-dodecyl sulfate PAGE separates proteins based on their molecular weight, and it uses a denaturing gel.	Native PAGE uses non-denaturing gels and separates proteins based on their size, charge and the shape (3D conformation).
Type of Gel	
A denaturing gel is used in SDS-PAGE.	A non – denaturing gel is used in the native PAGE.
Presence of SDS	
SDS is present as a detergent to impart a negative charge on the sample in SDS PAGE.	SDS is not present in the native PAGE.
Separation Basis	
Separation of proteins depends on the molecular weight of the protein in SDS PAGE.	Separation depends on the size and shape of the protein molecule in the native PAGE.
Stability of the Protein	
Stability of the protein is low in SDS PAGE.	Stability of protein is high in the native PAGE.
Recovery of the Original Protein	
Not possible as it is denatured in SDS PAGE.	Possible on the native PAGE.
Reducing agent used in the loading dye	
β-mercaptoethanol is present.	β-mercaptoethanol is Absent.

References:

- **Principles and Techniques of Biochemistry and Molecular Biology by Wilson and Walker**
- **Bio Rad pdf sent on WhatsApp group**
- https://ruo.mbl.co.jp/bio/e/support/method/sds-page.html

HPLC

HPLC

- Liquid chromatography (mobile phase is liquid)
- Mobile phase is allowed to drip under high pressure (upto 400 at
∴ High performance & high speed.
- Stationary phase < Solid / Liquid

HPLC < Adsorptn – (if stationary phase solid) / Partition – (if stationary phase is liquid) / IEC / SEC

Variants of Partition Chroma.
In normal phase, stationary phase is polar, mobile phase is non-polar.
In Reverse phase, stationary phase is non-polar, mobile phase is polar.
(most commonly used forms of HPLC).

Gradient controller — Pump system — Sample introduction — column (resin-silica) — Data output / Detector

Pump system → (to provide const reproducible flow of solvent through the column)

Column → (Stainless steel or glass-Teflon tubing)
Analytical column - 2.1, 3.2 or 4.5mm in dia
Preparative column - upto 30mm
Lengths 5 to 100cm but 10 to 20cm columns are common.

- very small size particles used in column as packing material which gives a much greater surface area for interactions b/n the stationary phase & the molecules flowing past it.
- This allows a much better separation of the components of the mixture.

2 adv
- small particle size. ∴ greater surface area
- Better improvement of elution rates by applyg high pressure.

Links: first two are quite informative

https://www.youtube.com/watch?v=IUwRWn9pEdg

https://www.youtube.com/watch?v=MLoitPJQH3g

https://www.youtube.com/watch?v=kz_egMtdnL4