Sodium DodecylSulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE)

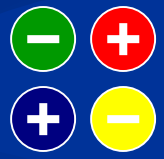
BBT 314 EXP 4

Experimental Goals

- To understand the principle of SDS-PAGE
- To become familiar with the SDS-PAGE setup

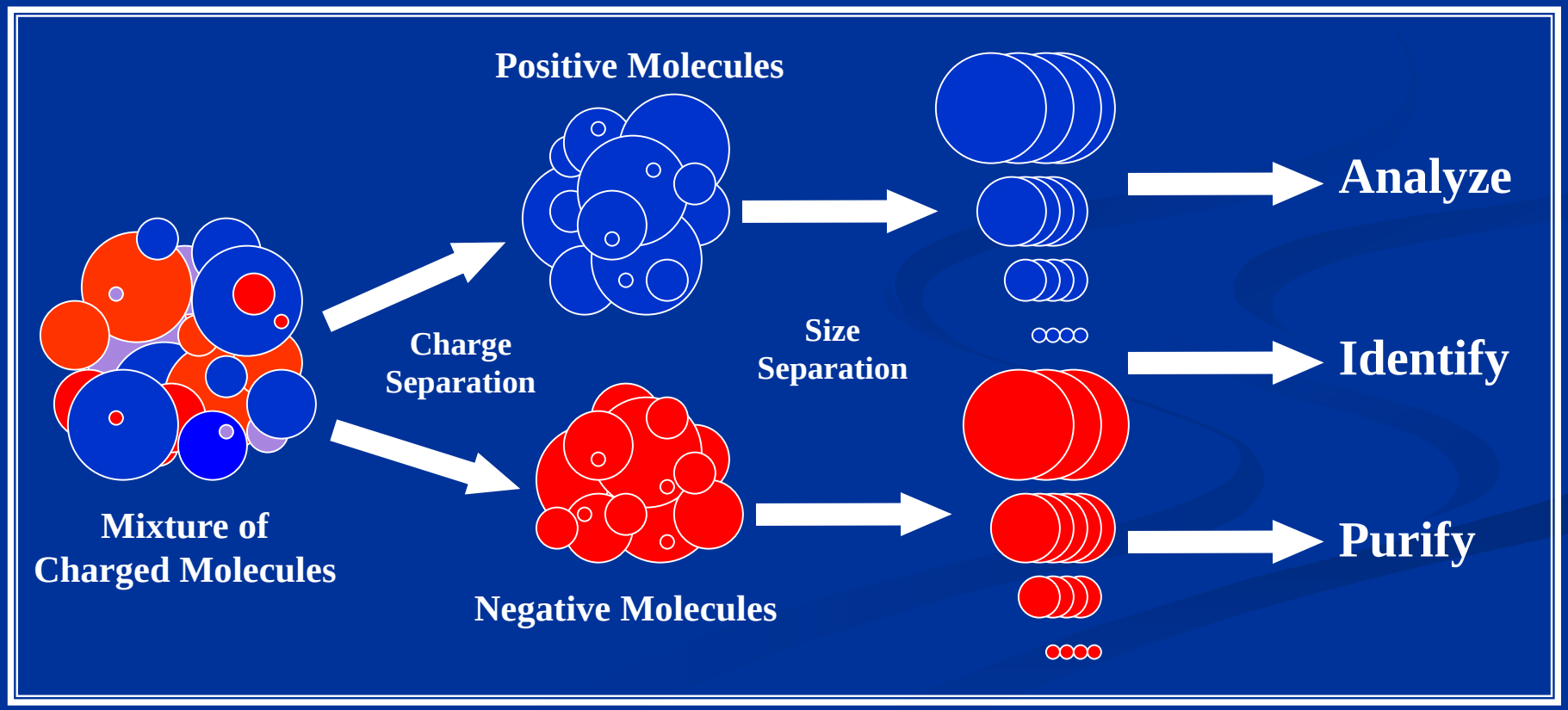
What is Electrophoresis?

Electrophoresis is a laboratory technique for separating molecules based on their charge



Separation of a Mixture of Charged Molecules

Charged molecules are separated based on their electrical charge and size within a matrix



Purpose

- SDS- PAGE is probably the most common analytical technique used to separate and characterize proteins.
- Can even separate proteins that are insoluble in water.
- Provides information about the molecular weight.
- Even allow to analyze the subunit composition of protein.

The gel (matrix)

- The gel (matrix) itself is composed of either agarose or polyacrylamide.

- Polyacrylamide is a cross-linked polymer of acrylamide.
  - Acrylamide is a potent neurotoxin and should be handled with care!

Polyacrylamide gels

- Have smaller pores than agarose, therefore high degree of resolving power.
- Can separate DNA fragments which range in size from 10-500 bp.
- DNA fragments which differ in size by one nucleotide can be separated from each other.
- Polyacrylamide gel electrophoresis is also used to separate protein molecules.

Protein Electrophoresis

- **Separate proteins based on**
  - Size (Molecular Weight - MW)
- **Allows us to**
  - characterize
  - quantify
  - determine purity of sample
  - compare proteins from different sources
- And it is a step in Western blot

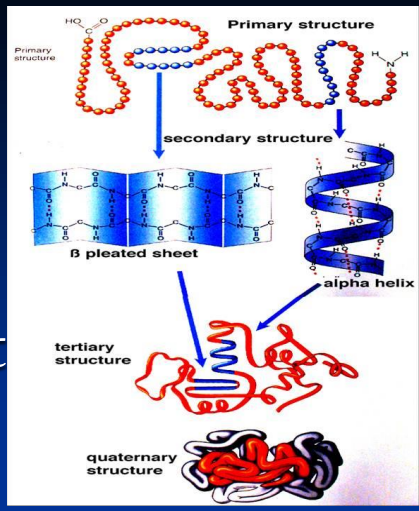
Protein Electrophoresis

- Proteins, unlike DNA, do not have a constant size to charge ratio
  - In an electric field, some will move to the positive and some to the negative pole, and some will not move because they are neutral

SDS-PAGE

- **SDS-PAGE** (sodium dodecylsulphate-polyacrylamide gel electrophoresis)
- The purpose of this method is to separate proteins according to their size, and no other physical feature
- In order to understand how this works, we have to understand the two halves of the name: **SDS** and **PAGE**

Sodium Dodecylsulphate



- Since we are trying to separate many different protein molecules of a variety of shapes and sizes,
  - we first want to get them to be linear
  - no longer have any secondary, tertiary or quaternary structure (i.e. we want them to have the same linear shape).
- Not only the mass but also the shape of an object will determine how well it can move through and environment.
- So we need a way to convert all proteins to the same shape - we use SDS.

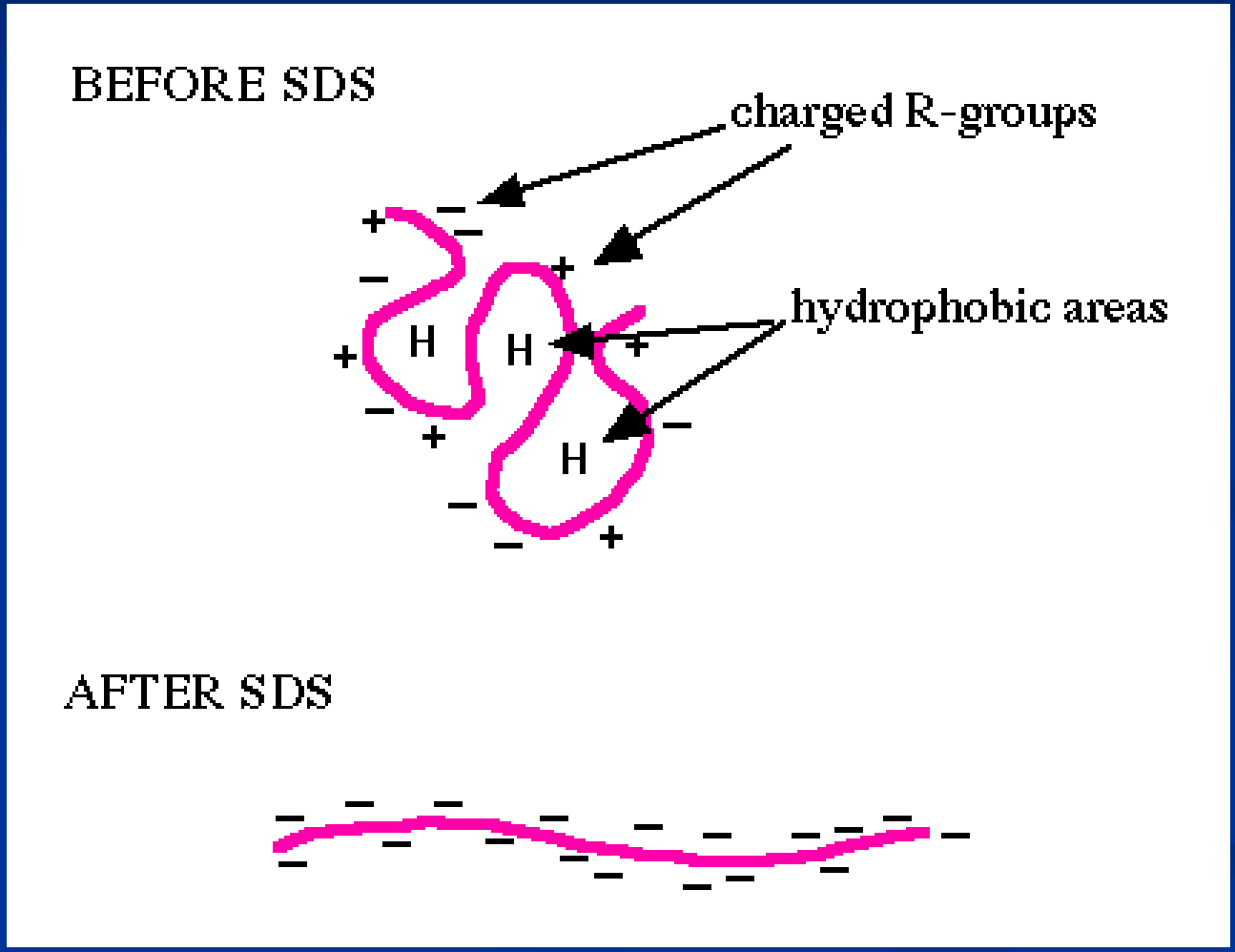
Sodium Dodecylsulphate

- SDS (sodium dodecyl sulfate) is a detergent that can dissolve hydrophobic molecules but also has a negative charge (sulfate) attached to it.
- If SDS is added to proteins, they will be soluablized by the detergent, plus all the proteins will be covered with many negative charges.

Sodium Dodecylsulphate

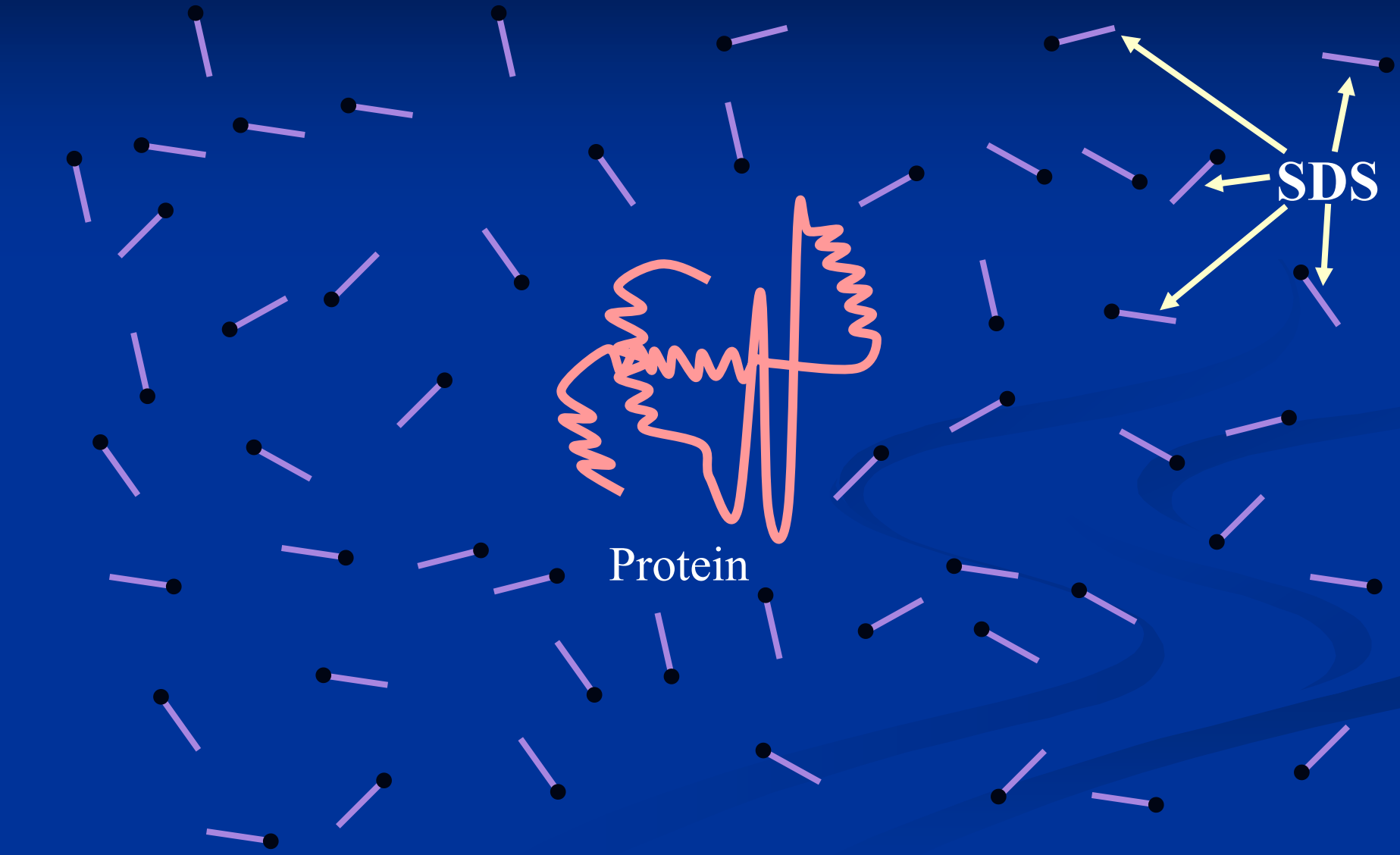
- A sample of protein, often freshly isolated and unpurified, is boiled in the detergent sodium dodecyl sulfate and beta-mercaptoethanol
  - The mercaptoethanol reduces disulfide bonds
  - The detergent disrupts secondary and tertiary structure
- The end result has two important features:
  1. all proteins contain only primary structure and
  2. all proteins have a large negative charge which means they will all migrate towards the positive pole when placed in an electric field.
- They migrate through a gel towards the positive pole at a rate proportional to their linear size
  - Molecular weights with respect to size markers may then be determined

Sodium Dodecylsulphate



Now we are ready to focus on the second half - PAGE.

SDS and Proteins



SDS and Proteins

- SDS nonpolar chains arrange themselves on proteins and destroy secondary tertiary and quarternary structrure

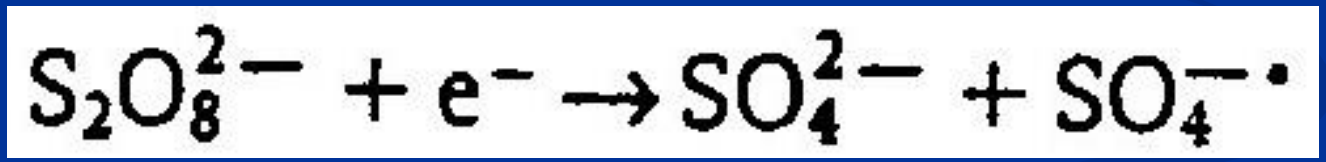
- So much SDS binds to proteins that the negative charge on the SDS drowns out any net charge on protein side chains
- In the presence of SDS all proteins have uniform shape and charge per unit length

Polyacrylamide Gel Electrophoresis (PAGE)

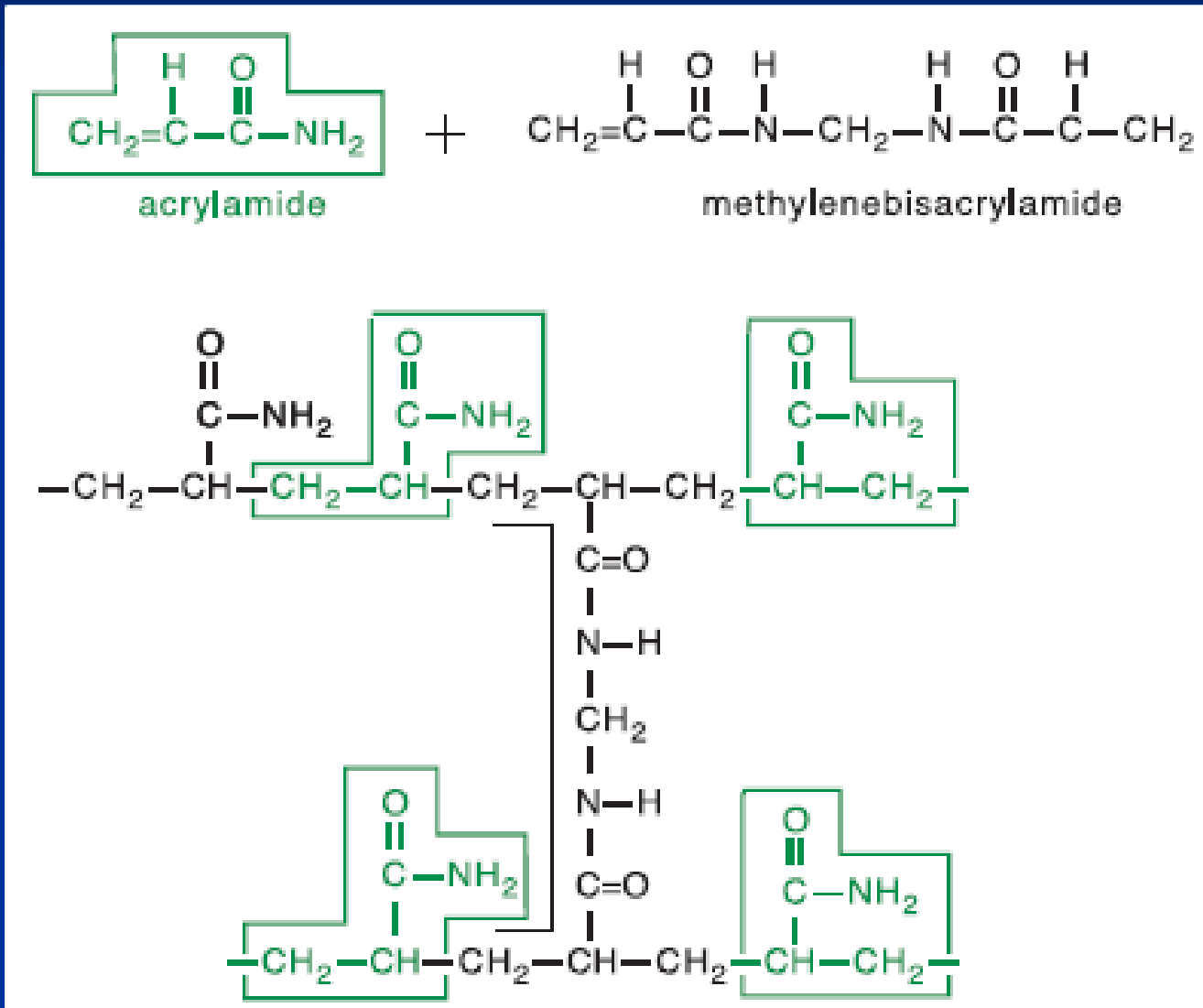
- PAGE is the preferred method for separation of proteins
- Gel prepared immediately before use by polymerization of acrylamide and N,N'-methylene bis acrylamide.
- Porosity controlled by proportions of the two components.

Catalyst of polymerization

- Polymerization of acrylamide is initiated by the addition of ammonium persulphate and the base *N,N,N',N'*-tetrametyhlenediamine (TEMED)
- TEMED catalyses the decomposition of the persulphate ion to give a free radical

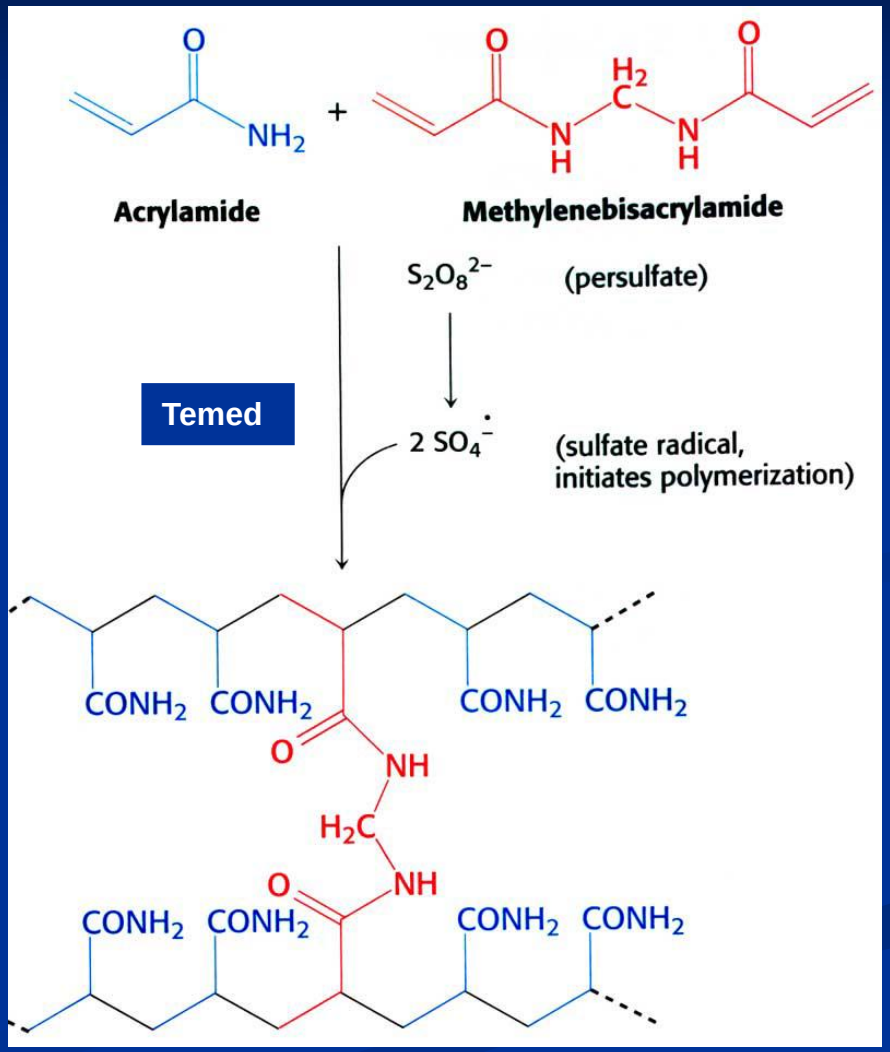


Polymerization of acrylamide



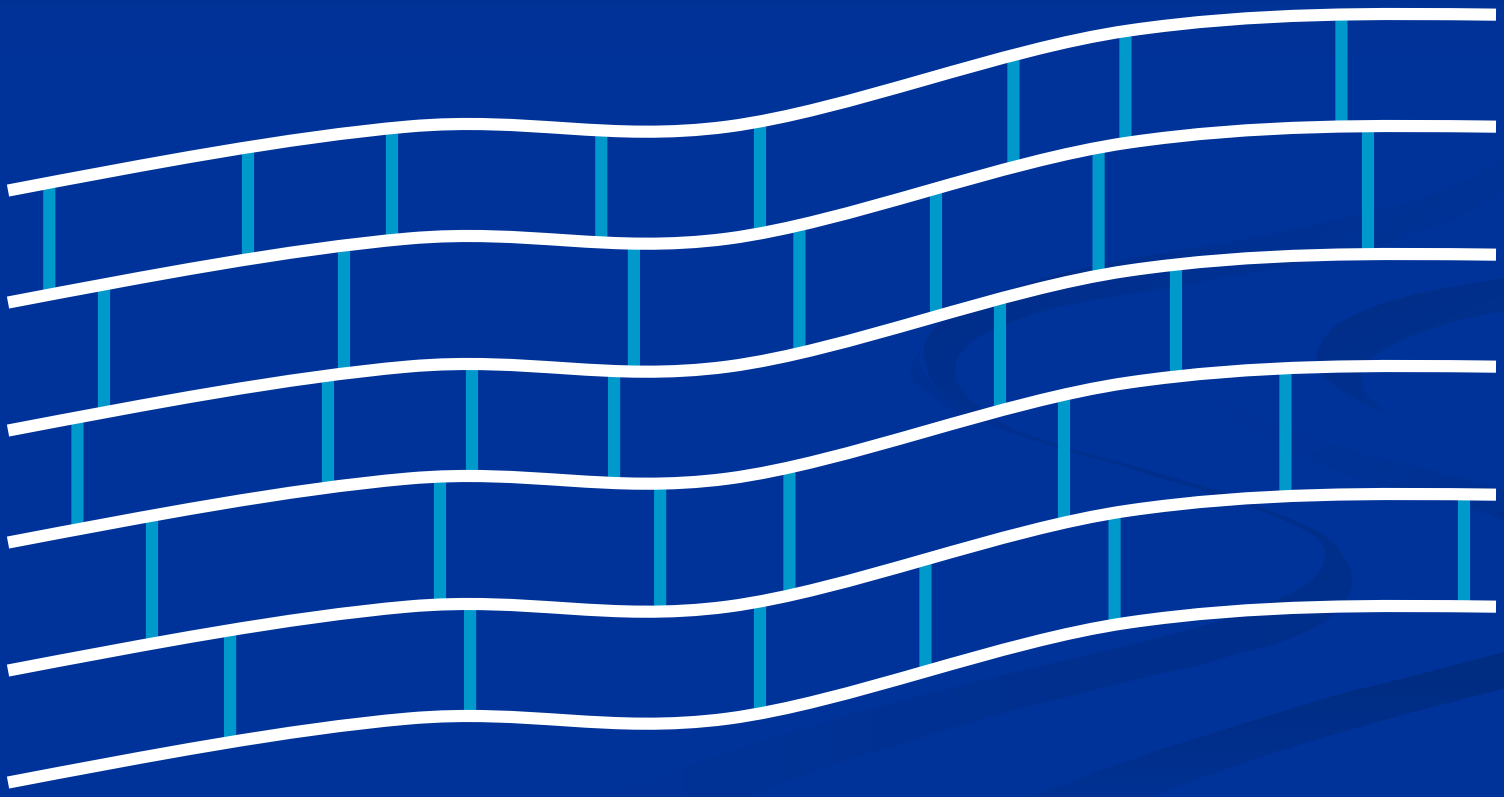
Polymerization of acrylamide

- Cross-linked polyacrylamide gels are formed from the polymerisation of acrylamide monomer in the presence of smaller amounts of *N,N'*-methylenebisacrylamide (bis-acrylamide)
- Bisacrylamide is the most frequently used cross linking agent for polyacrylamide gels



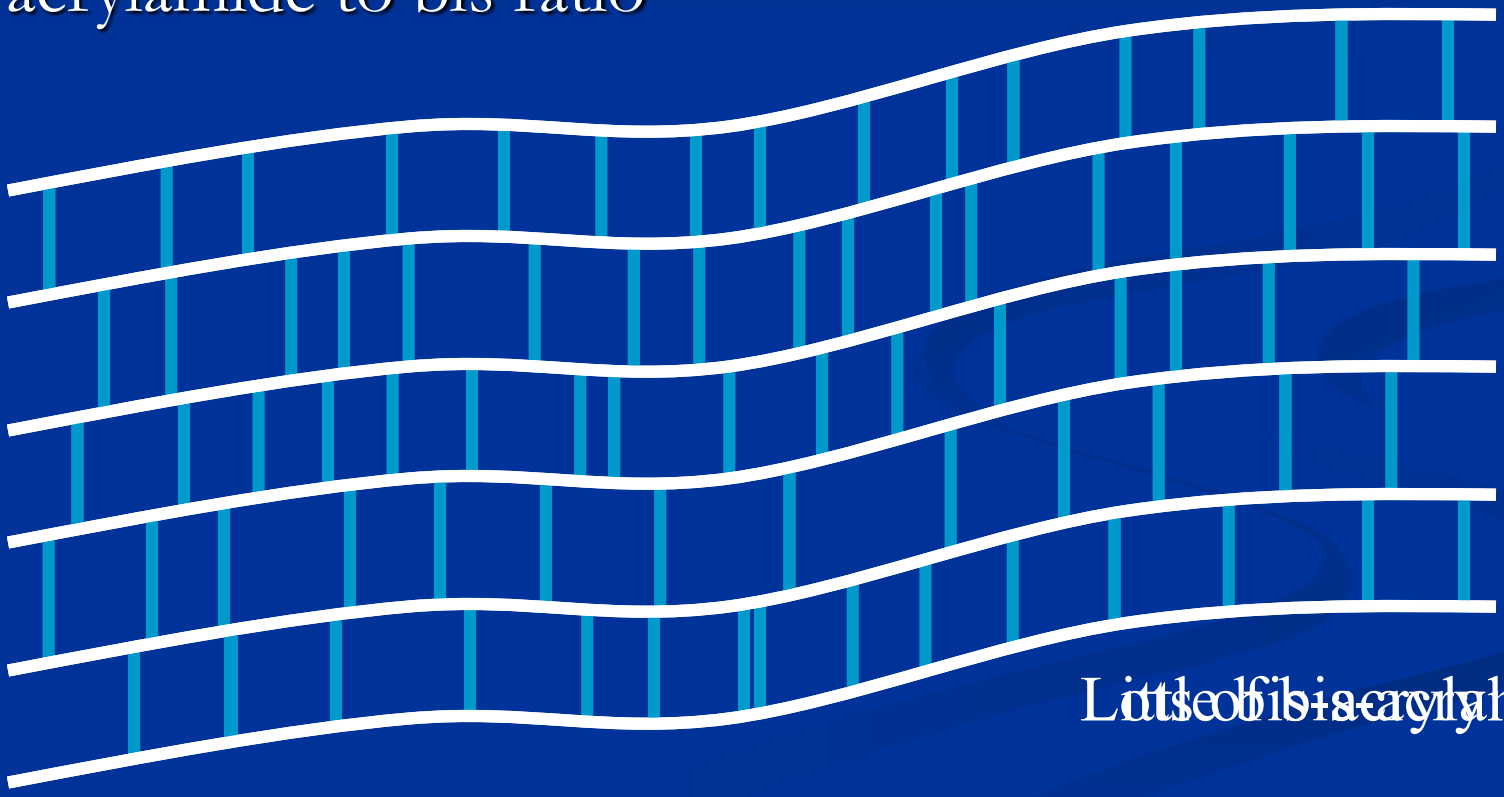
Polyacrylamide Gels

- Bis-Acrylamide polymerizes along with acrylamide forming cross-links between acrylamide chains

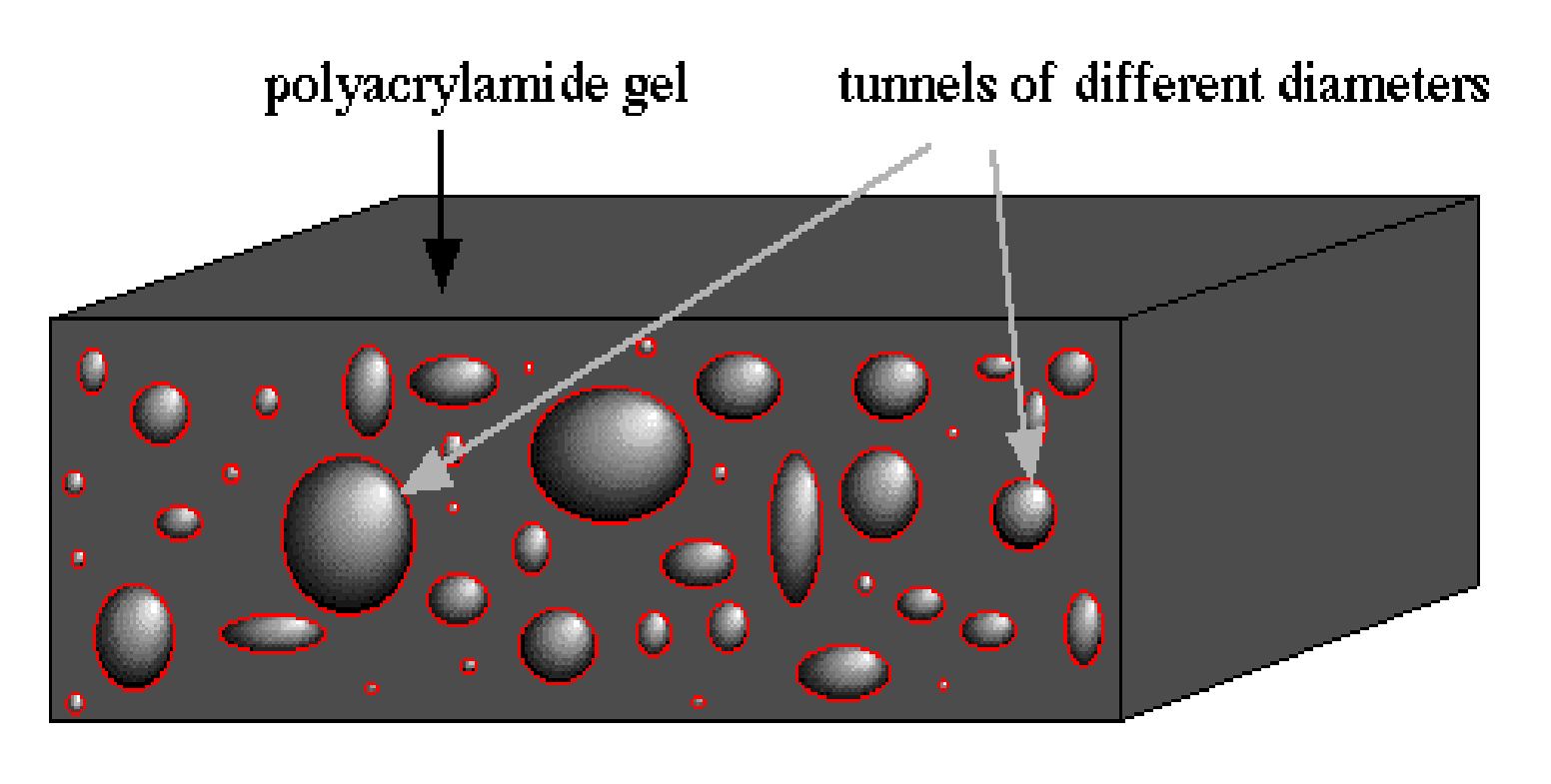


Polyacrylamide Gels

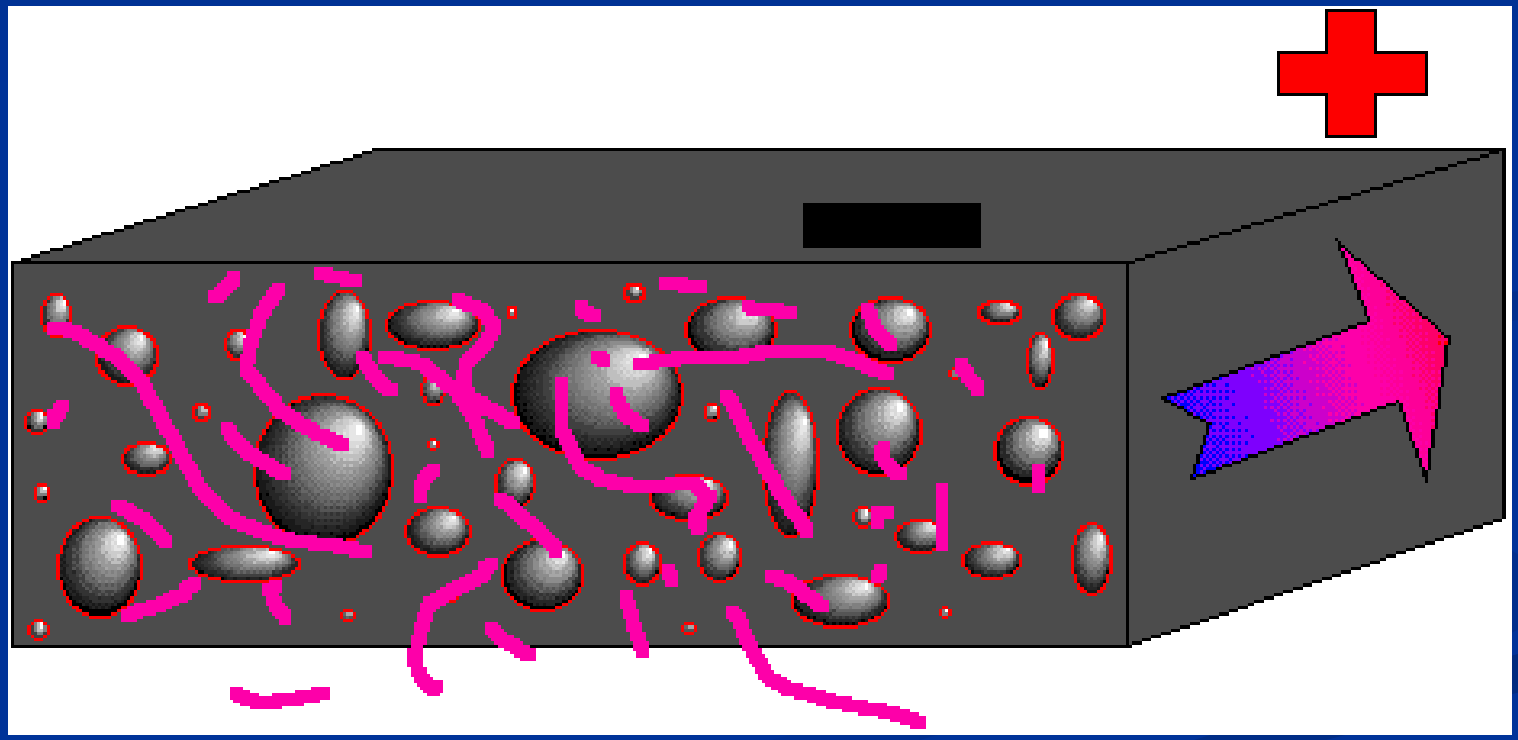
- Pore size in gels can be varied by varying the ratio of acrylamide to bis-acrylamide
- Protein separations typically use a 29:1 or 37.5:1 acrylamide to bis ratio



Side view

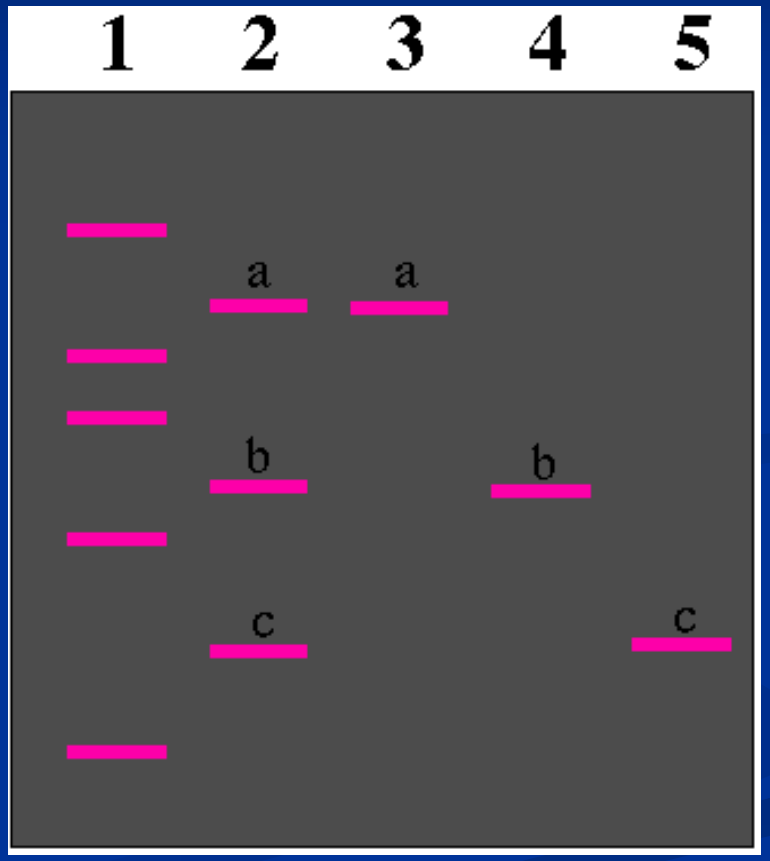


Movement of Proteins in Gel



Movement of Proteins in Gel

- smaller proteins will move through the gel faster while larger proteins move at a slower pace



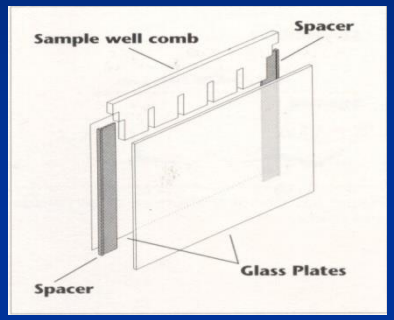
Components of the System

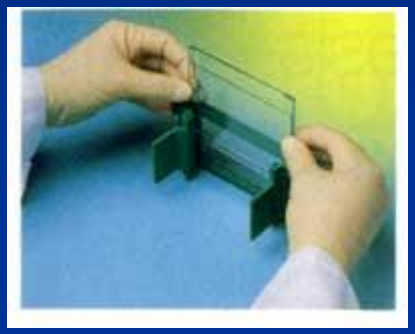
- DC Power Source, Reservoir/Tank, Glass Plates, Spacers, and Combs
- Support medium
  - Gel (Polyacrylamide)
- Buffer System
  - High Buffer Capacity
- Molecules to be separated
  - Proteins
  - Nucleic Acids

Vertical Gel Format: Polyacrylamide Gel Electrophoresis

Reservoir/Tank
Power Supply
Glass Plates, Spacers, and Combs

Step by Step Instructions on how to assemble the polyacrylamide gel apparatus



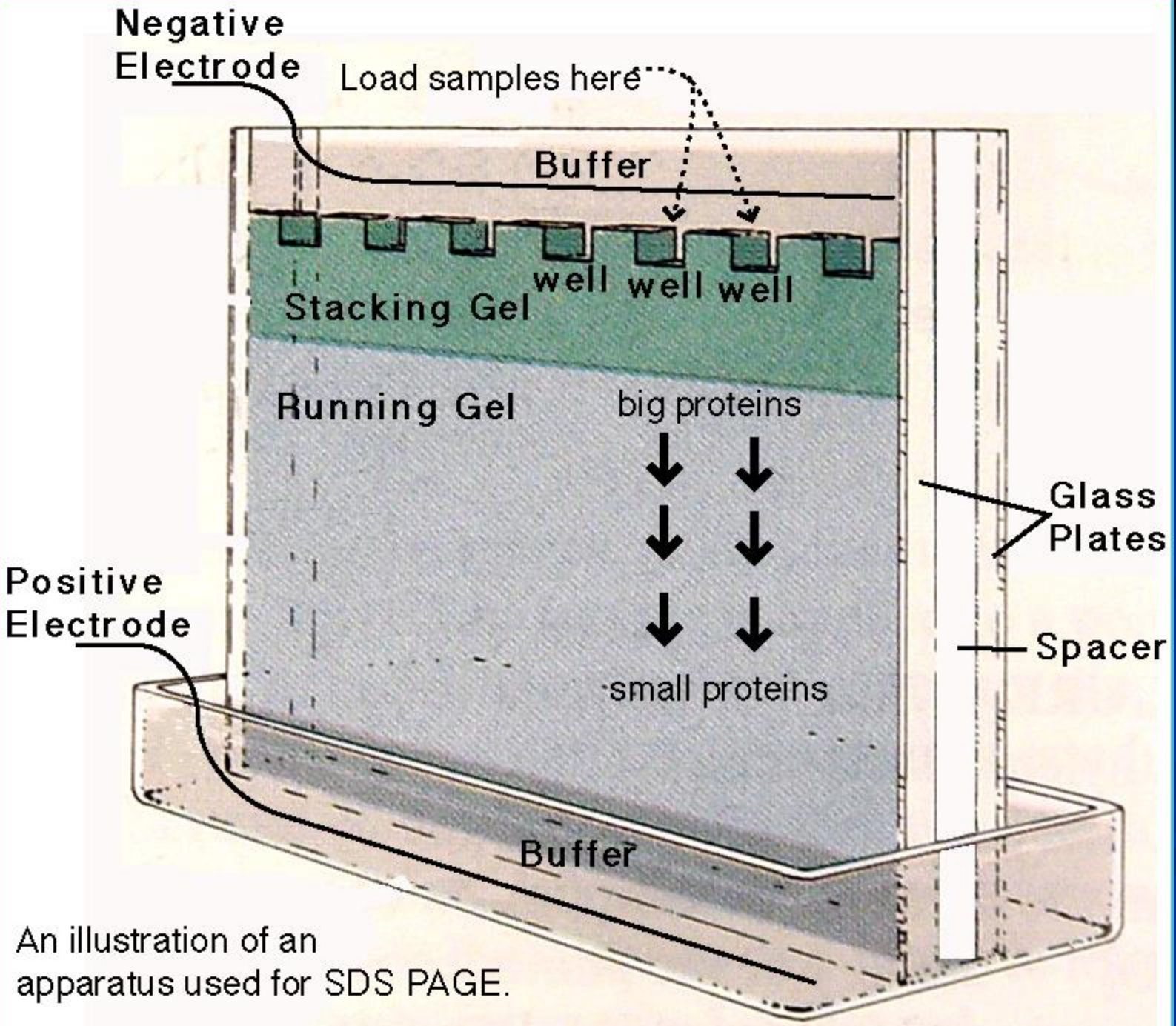


Procedure

- Prepare polyacrylamide gels
- Add diluted samples to the sample buffer
- Heat to 95°C for 4 minutes
- Load the samples onto polyacrylamide gel
- Run at 200 volts for 30-40 minutes
- Stain

Gel Preparation

Reagent	8% (Running Gel)	5% (Stacking Gel)
Acrylamide/ Bisacrylamide (40%) *	4.0 mls	2.5 mls
1 M Tris-HCl pH 8.8	7.5 mls	7.5 mls
water (distilled)	8.2 mls	9.7 mls
10% SDS	200 µl	200 µl
10% Ammonium Persulfate	100 µl	100 µl
TEMED (added last)	10 µl	10 µl
* = 19:1 w:w ratio of acrylamide to N,N'-methylene bis-acrylamide		



An illustration of an apparatus used for SDS PAGE.

Gel Preparation

- Mix ingredients **GENTLY!** in the order shown above, ensuring no air bubbles form.
- Pour into glass plate assembly CAREFULLY.
- **Overlay gel with isopropanol** to ensure a flat surface and to exclude air.
- Wash off isopropanol with water after gel has set (±15 min).

Sample Buffer

- **Tris** buffer to provide appropriate pH
- **SDS** (sodium dodecyl sulphate) detergent to dissolve proteins and give them a negative charge
- **Glycerol** to make samples sink into wells
- **Bromophenol Blue** dye to visualize samples

Heat to 95°C for 4 minutes

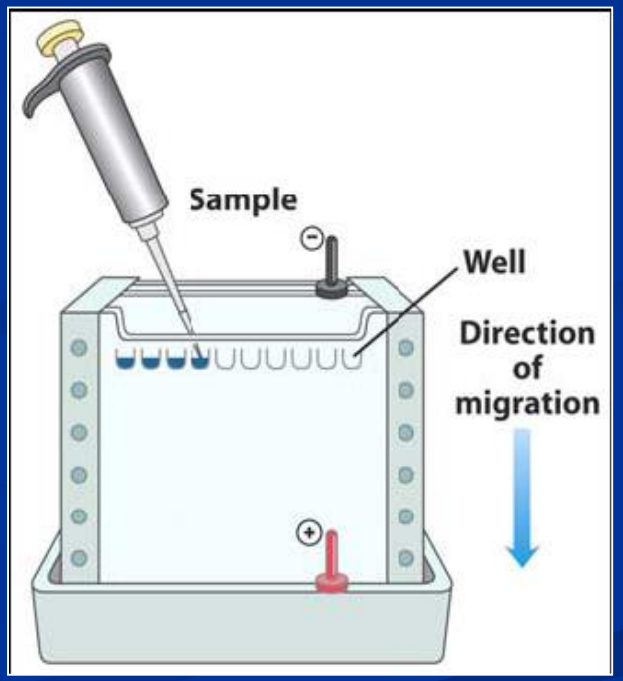
Loading Samples & Running the gel

- Run at 200 volts for 30-40 minutes

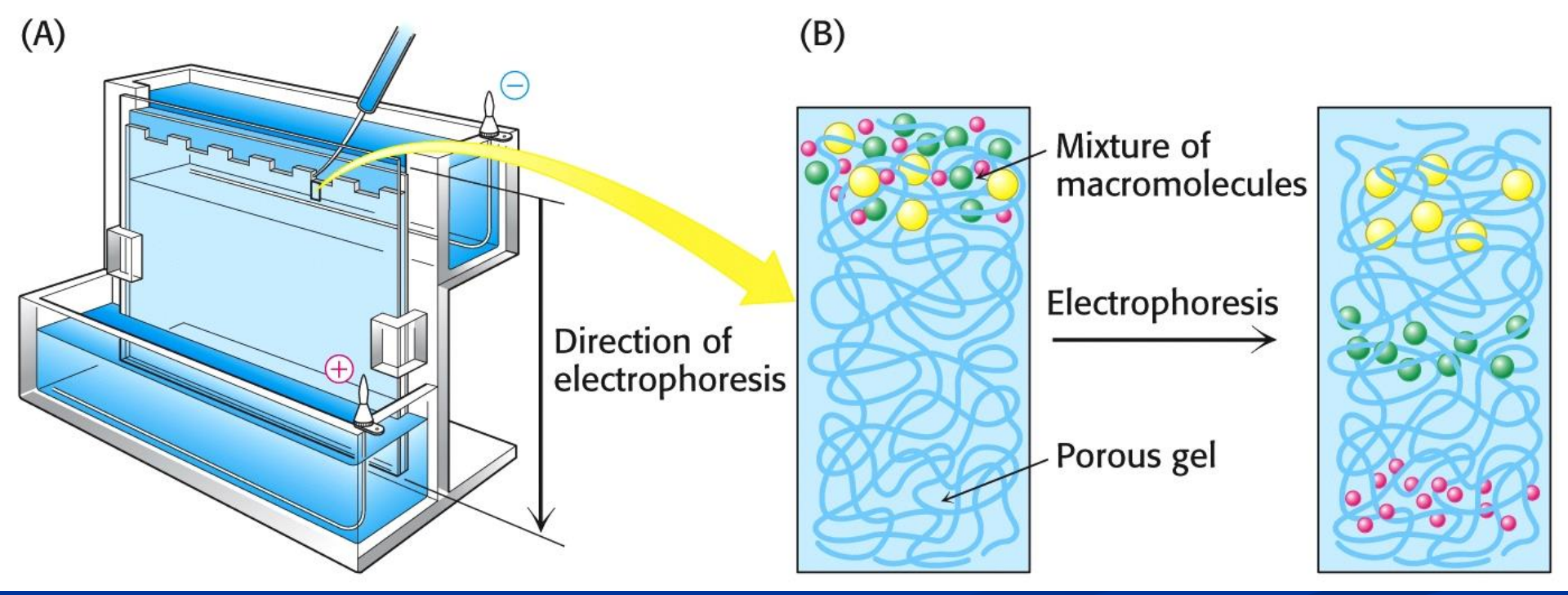
- **Running Buffer, pH 8.3**

  Tris Base 12.0 g
  Glycine 57.6 g
  SDS 4.0 g

  distilled water to 4 liter



SDS-PAGE


(A)
Direction of electrophoresis
(B)
Mixture of macromolecules
Electrophoresis
Porous gel

Staining Proteins in Gels

- Chemical stains detect proteins based on differential binding of the stain by the protein molecules and the gel matrix.
- They are nonspecific in action, detecting proteins without regard to their individual identities.
- The important characteristics for a useful stain are: low background, high sensitivity, large linear range and ease of use.

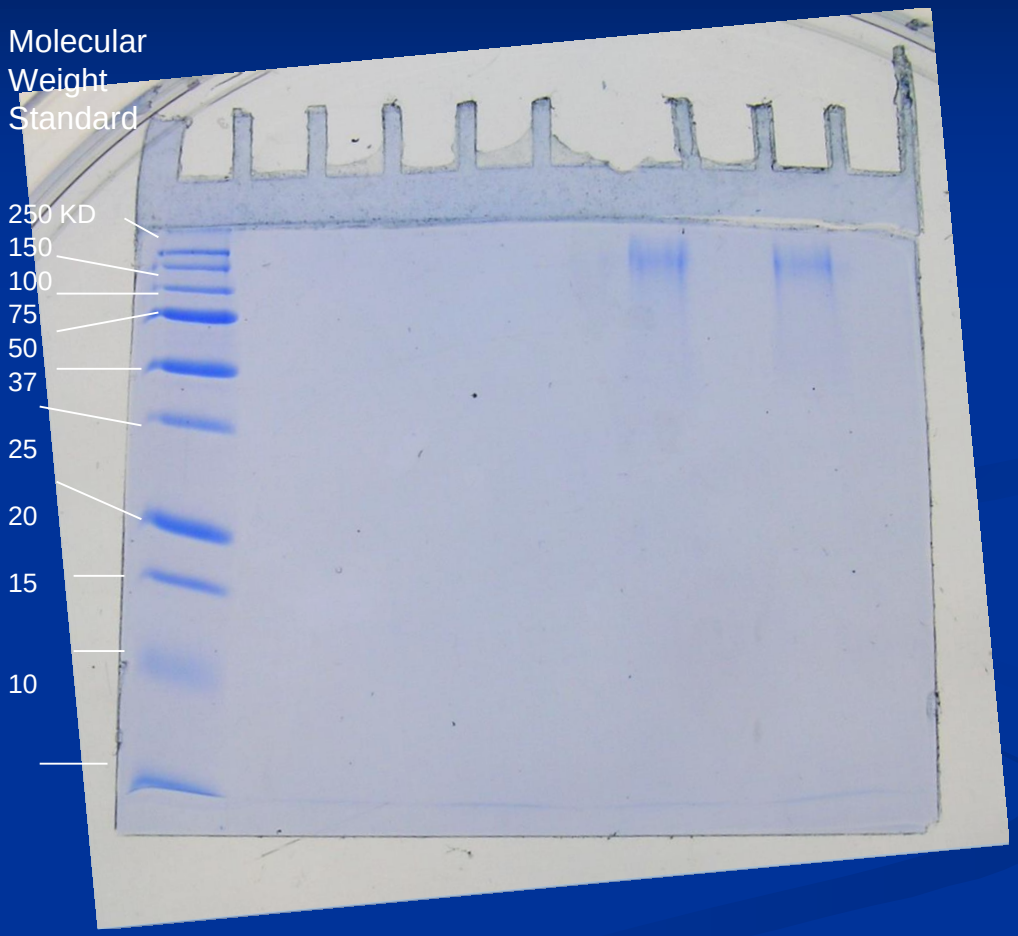
Staining Proteins in Gels

- **Coomassie Brilliant Blue**
  - The CBB staining can detect about 1 μg of protein in a normal band.
- **Silver Staining**
  - The silver stain system are about 100 times more sensitive, detecting about 10 ng of the protein.

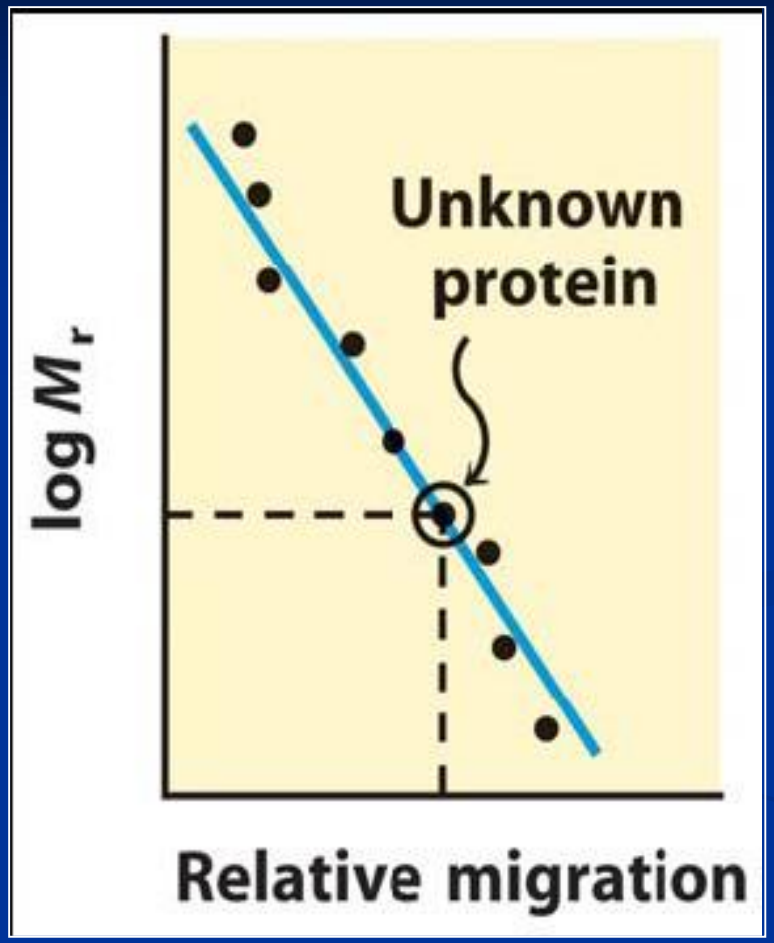
How to Quantify Proteins ?

- Densitometry

Molecular weight estimation by SDS-PAGE



Molecular weight estimation by SDS-PAGE



Calibration curve for molecular weight estimation.

Thank You!!!