Different applications of protein electrophorasis

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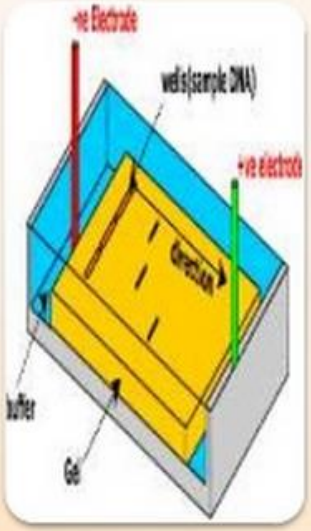
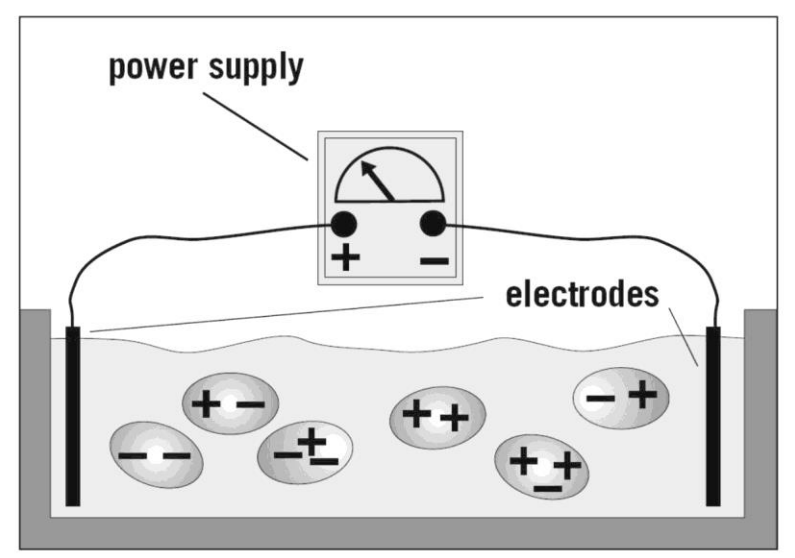
Meaning of electrophoresis



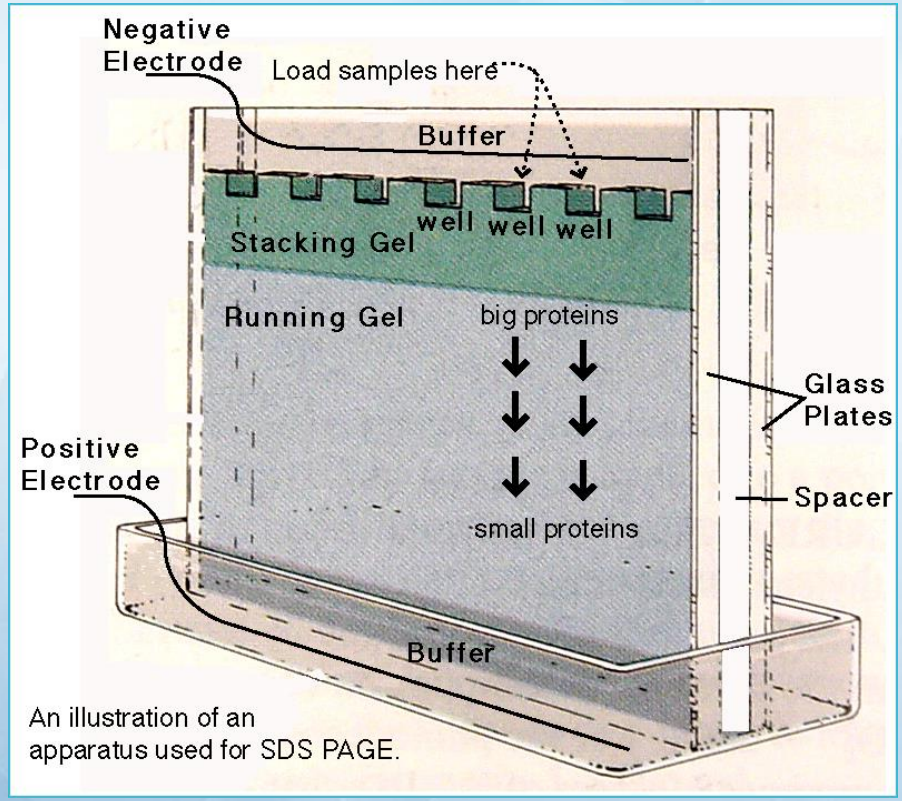
electrophoresis

- The term ‘electrophoresis’ was coined from the Greek word ‘*phoresis*’, which means ‘being carried’.
- Electrophorosis literally means ‘to carry with electricity’.

Principle

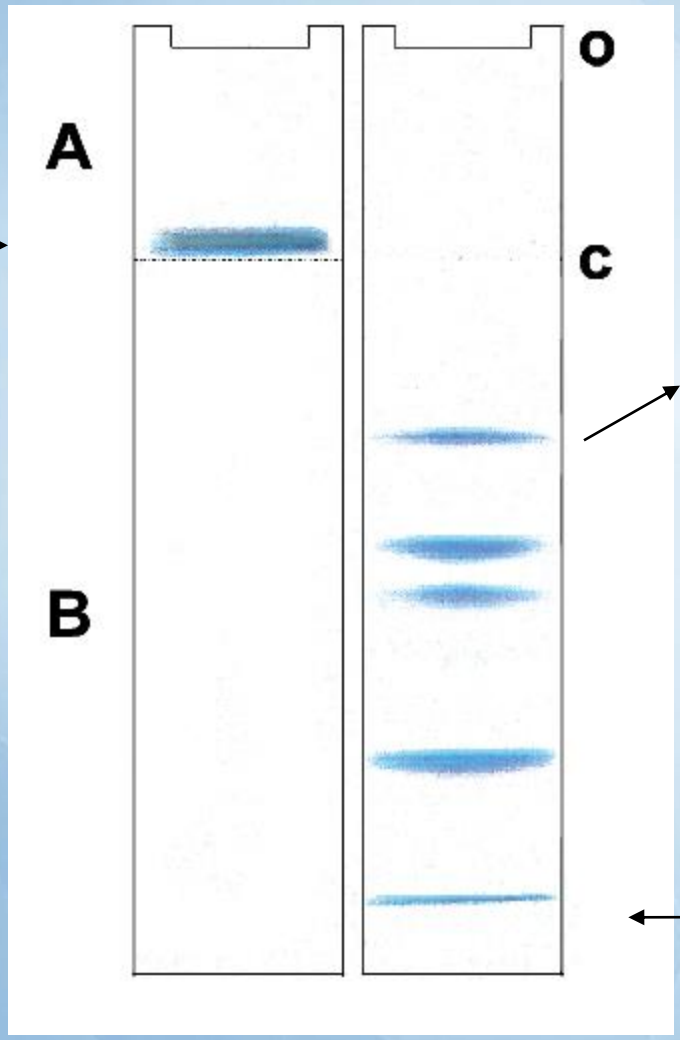


Proteins move in the electric field. Their relative speed depends on the charge, size, and shape of the protein



An illustration of an apparatus used for SDS PAGE.

Movement of Proteins on an SDS Gel



Protein Migration

-

Stacking of proteins at top of gel at start

Highest Molecular Wt. protein

Distribution of proteins in a charged field

+

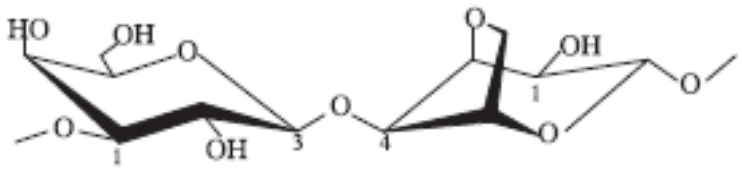
Low weight molecular dye

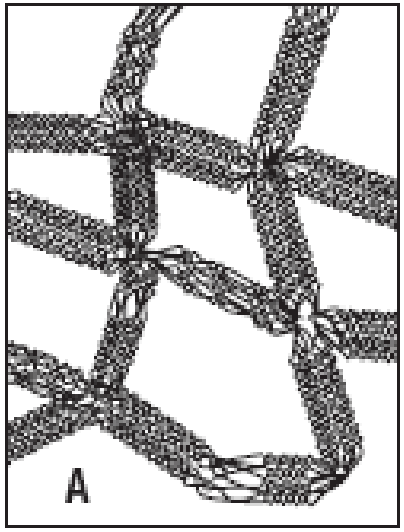
Gels

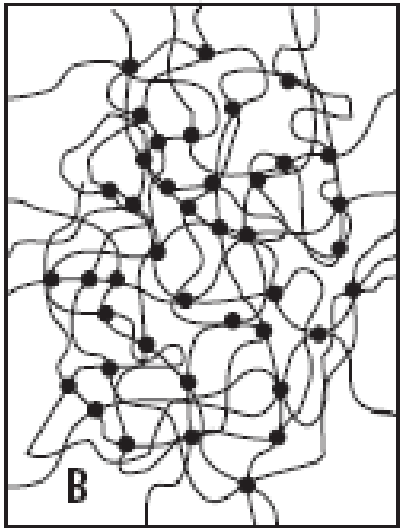
acrylamide

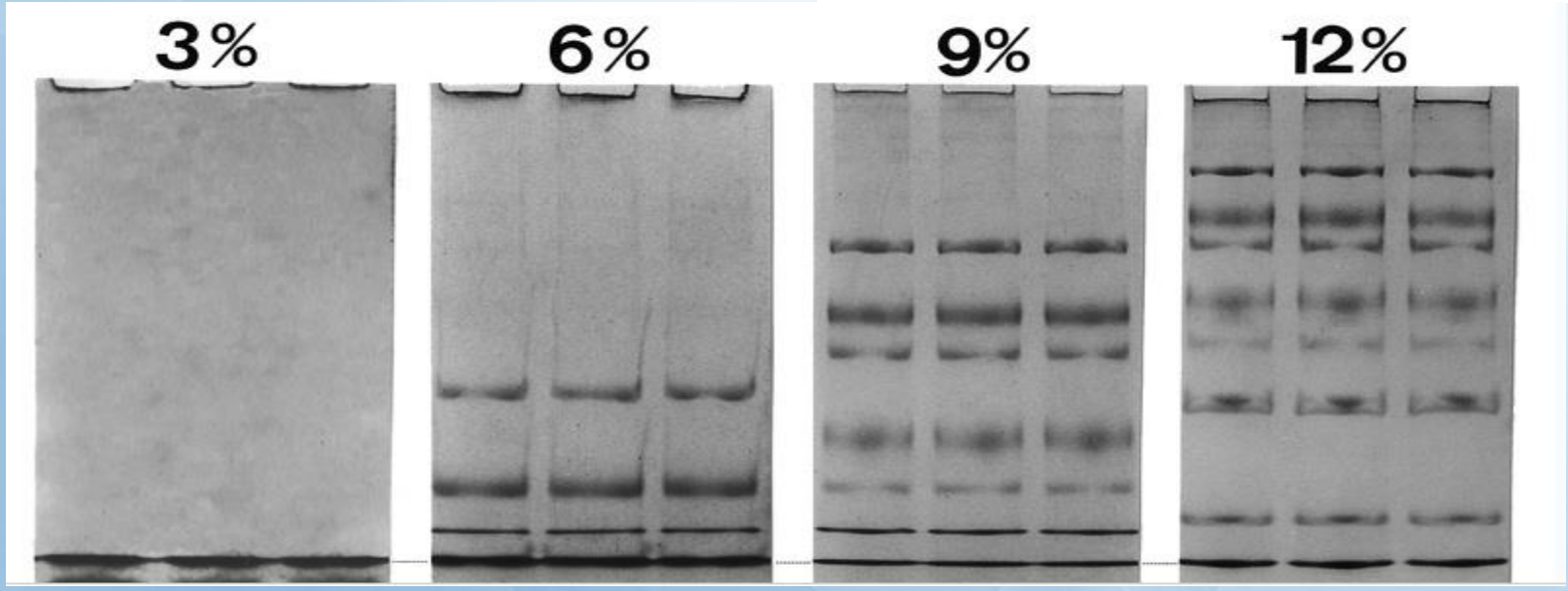
$$H_2C = CH\text{-}\overset{\overset{O}{\|}}{C}\text{-}NH_2$$

agarose









Recommended acrylamide concentration for protein electrophoresis

Separation size range (Kd)	% Acrylamide
~24-205	7.5%
~10-205	10%
~10-100	12%
~14-66*	12.5%
~14-45*	15%

* *The larger proteins fail to move significantly into the gel*

Protein visualization on gels

Common stains:

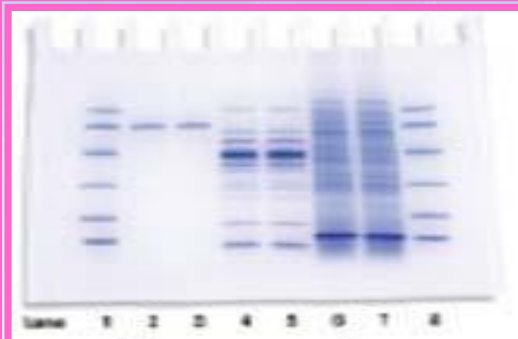
- **Coomassie Blue** in a fixative solution. Stain from a few hours to overnight. Destaining 4-12 hrs.

  * It provides a reasonably permanent record

- **Silver stain.** complex process, excellent, long-lasting record, sensitive.

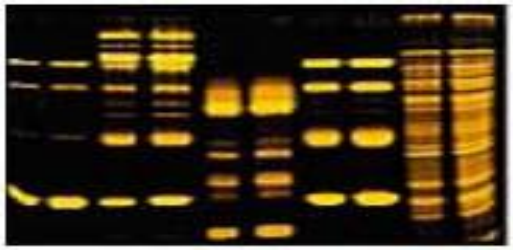
- **SYPRO (fluorescent)** staining is similar to Coomassie Blue in complexity, except the Destaining takes about 30 min.

  * It fades with time after a few hours



Coomassie

Silver

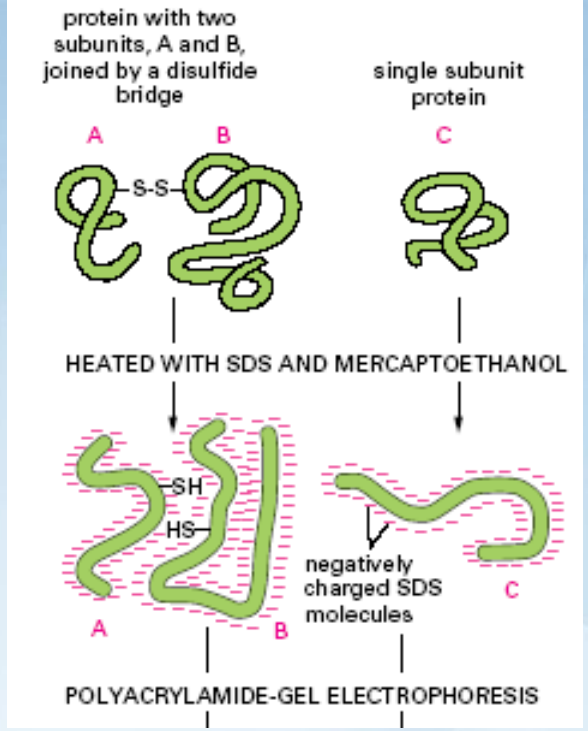


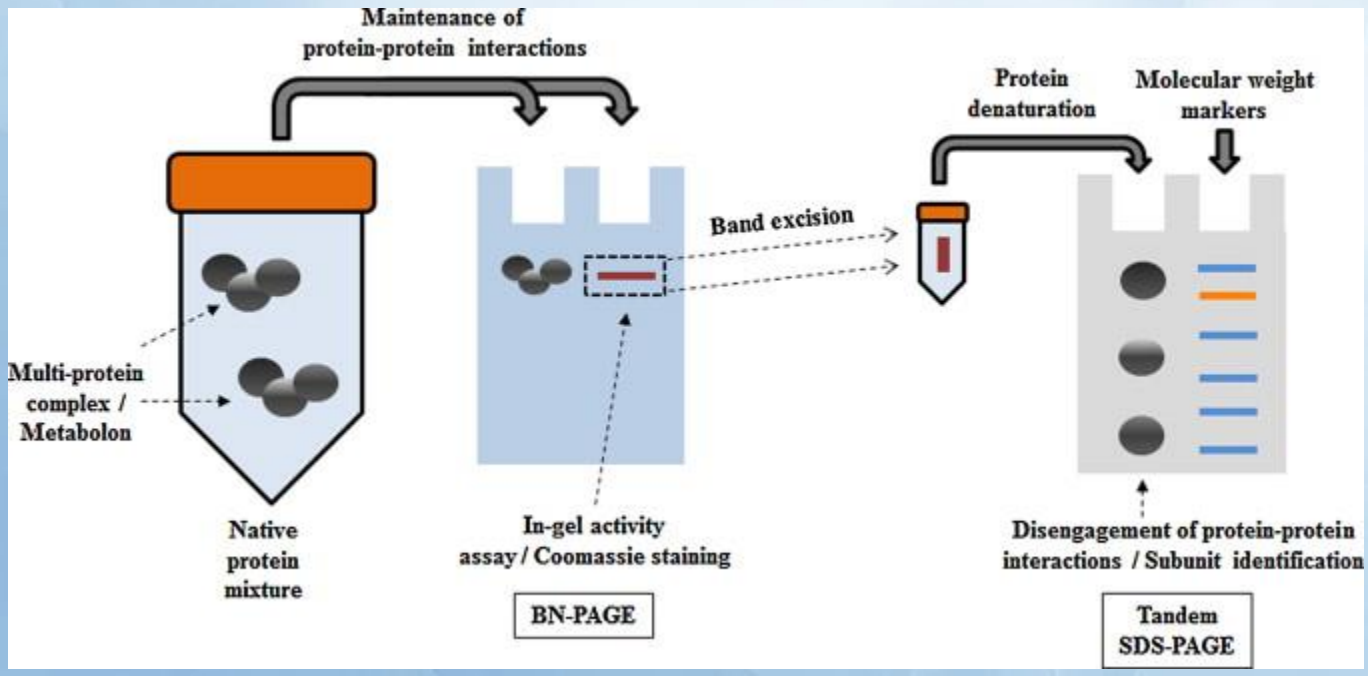
SYPRO orange

1. Native PAGE
2. Native Gradient PAGE
3. SDS PAGE
4. SDS Gradient PAGE
5. IEF
6. 2D PAGE
7. Western Blot

Native PAGE

Non-denatured protein

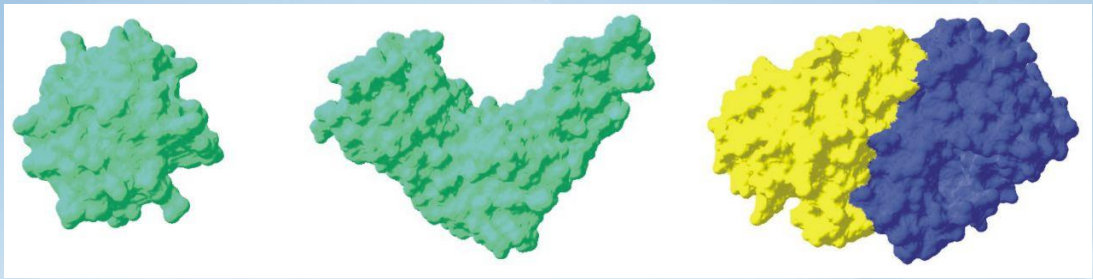


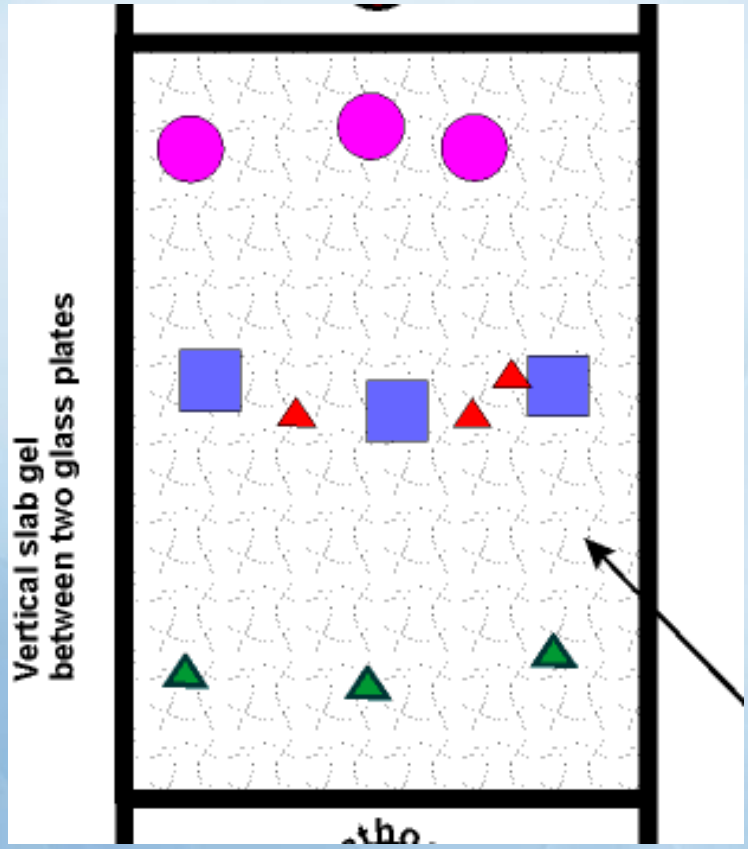


Native PAGE

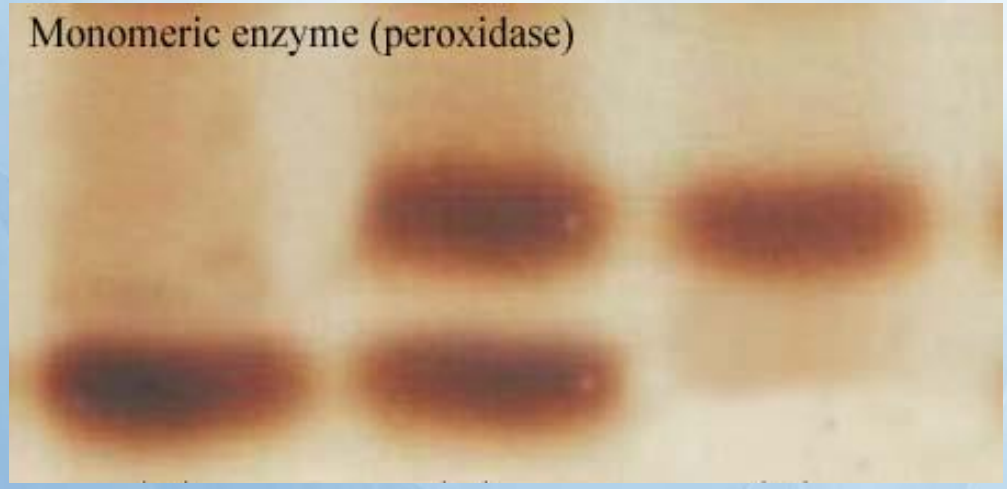
Separates by

- charge
- size
- shape

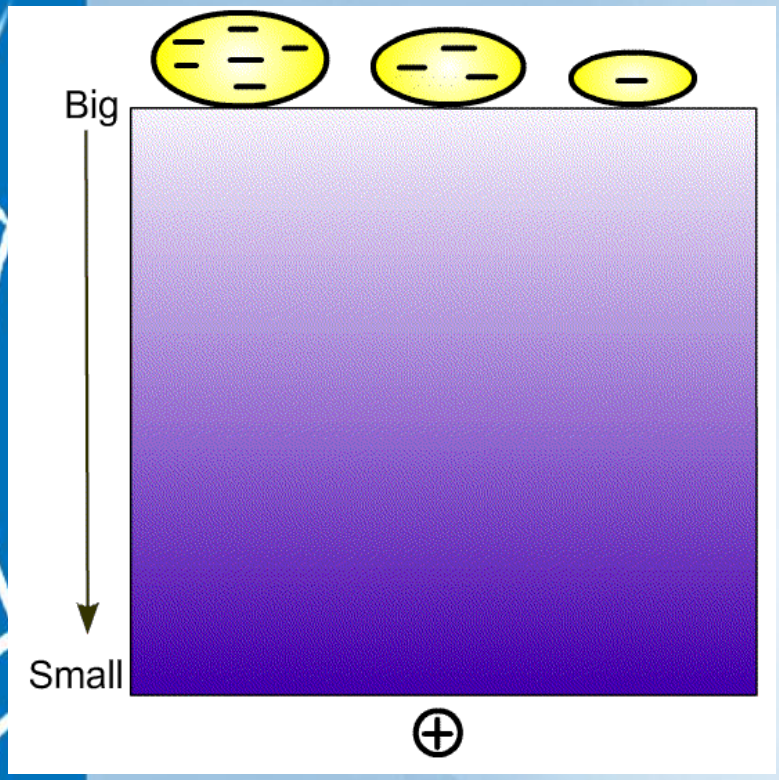


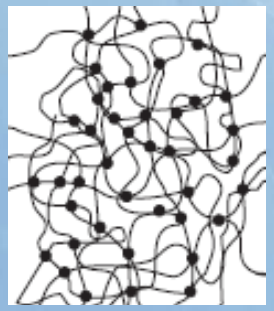


- reaction with specific activity stains (depending on enzyme).
  - substrates + cofactors + stain + buffer
- colored bands such as Est, Prx, Mdh …
- Colorless bands (white bands on a dark background, negatively stained) such as SOD.



Native gradient PAGE

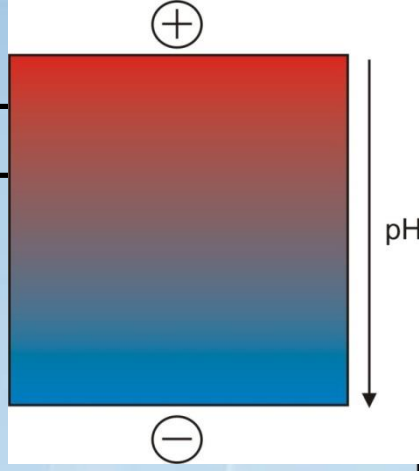




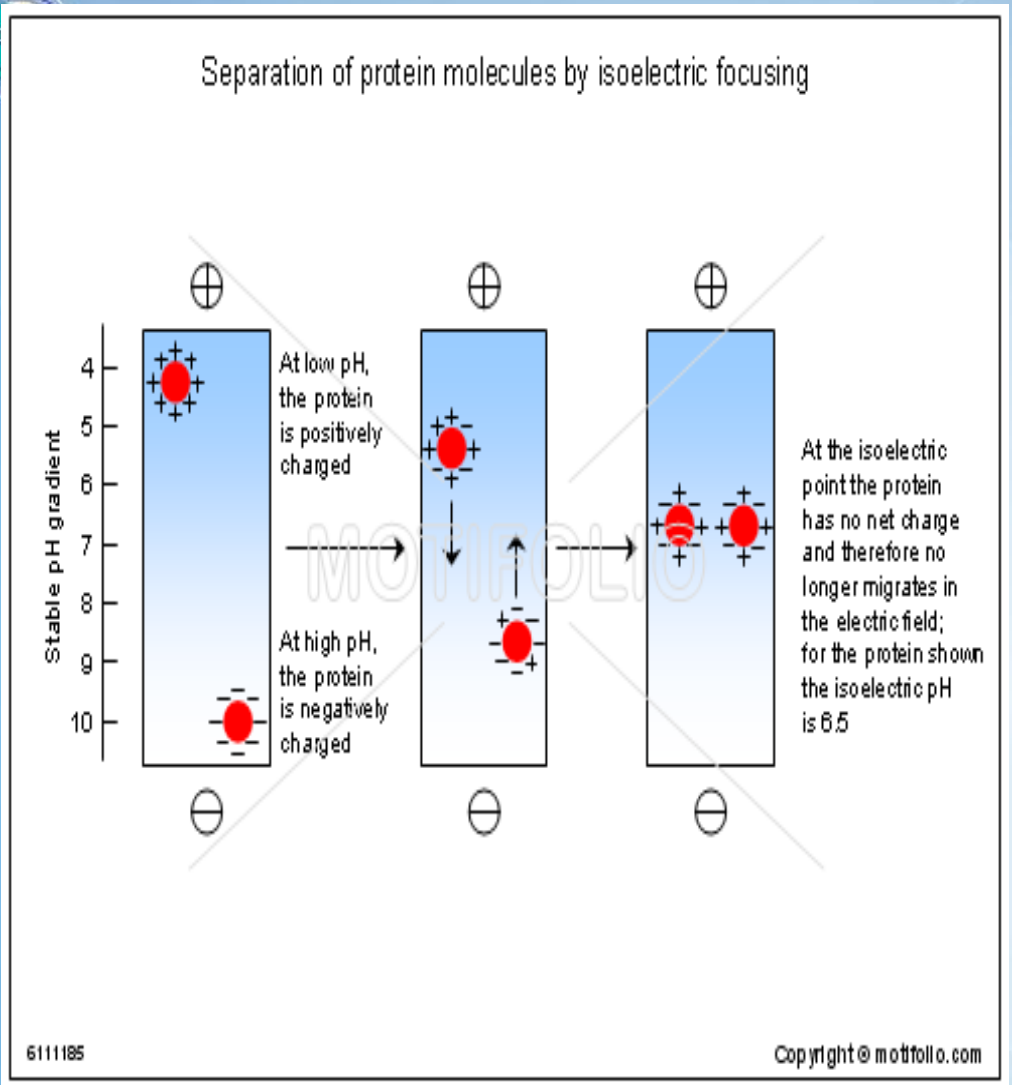
Separate native proteins by size – proteins stop moving when they reach a sertain gel density (but this may take a very long time ...)

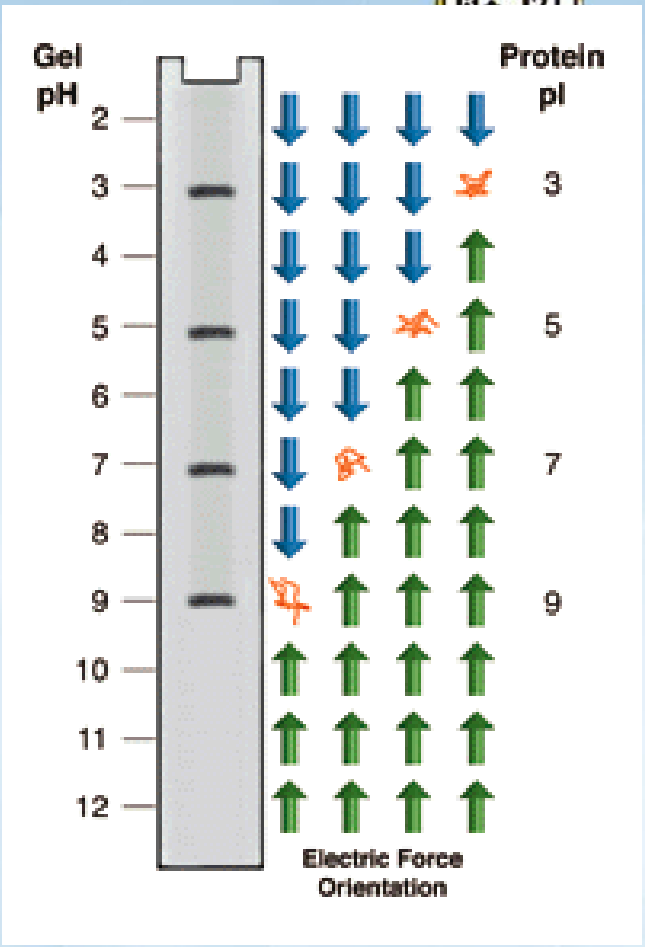
A great technique to study protien oligomerization!

What is Isolectric focusing?



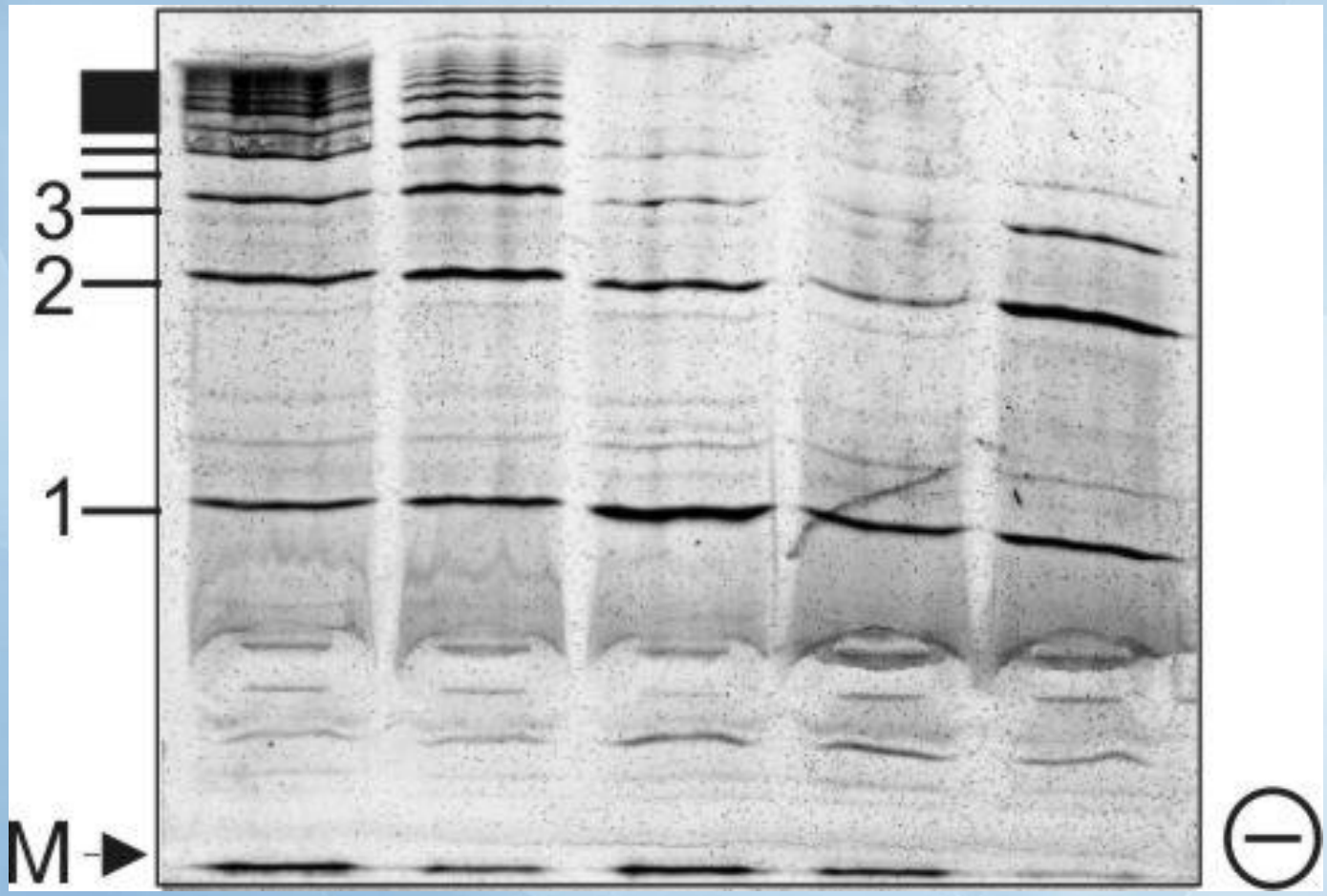
- Gel is prepared with pH gradient
- Separates proteins by their isoelectric points (pI)
- Each protein has own pI = pH at which the protein has equal amount of positive and negative charges (the net charge is zero)
- Charge on the protein changes as it migrates across pH
- When it gets to pI, has no charge and stops





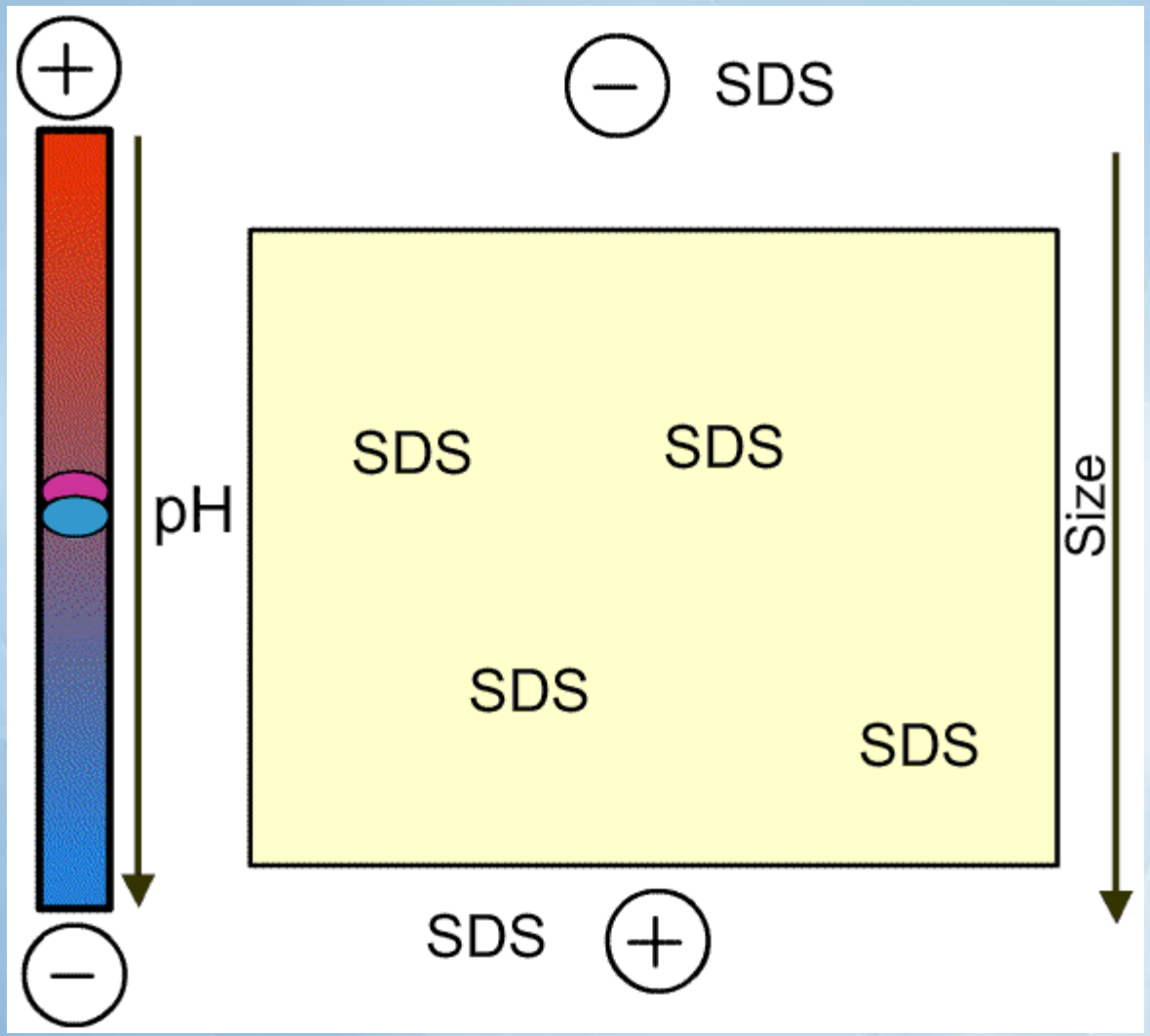
- PI of proteins can be theoretically predicted. Therefore, IEF can also be used for protein identification.

IEF example

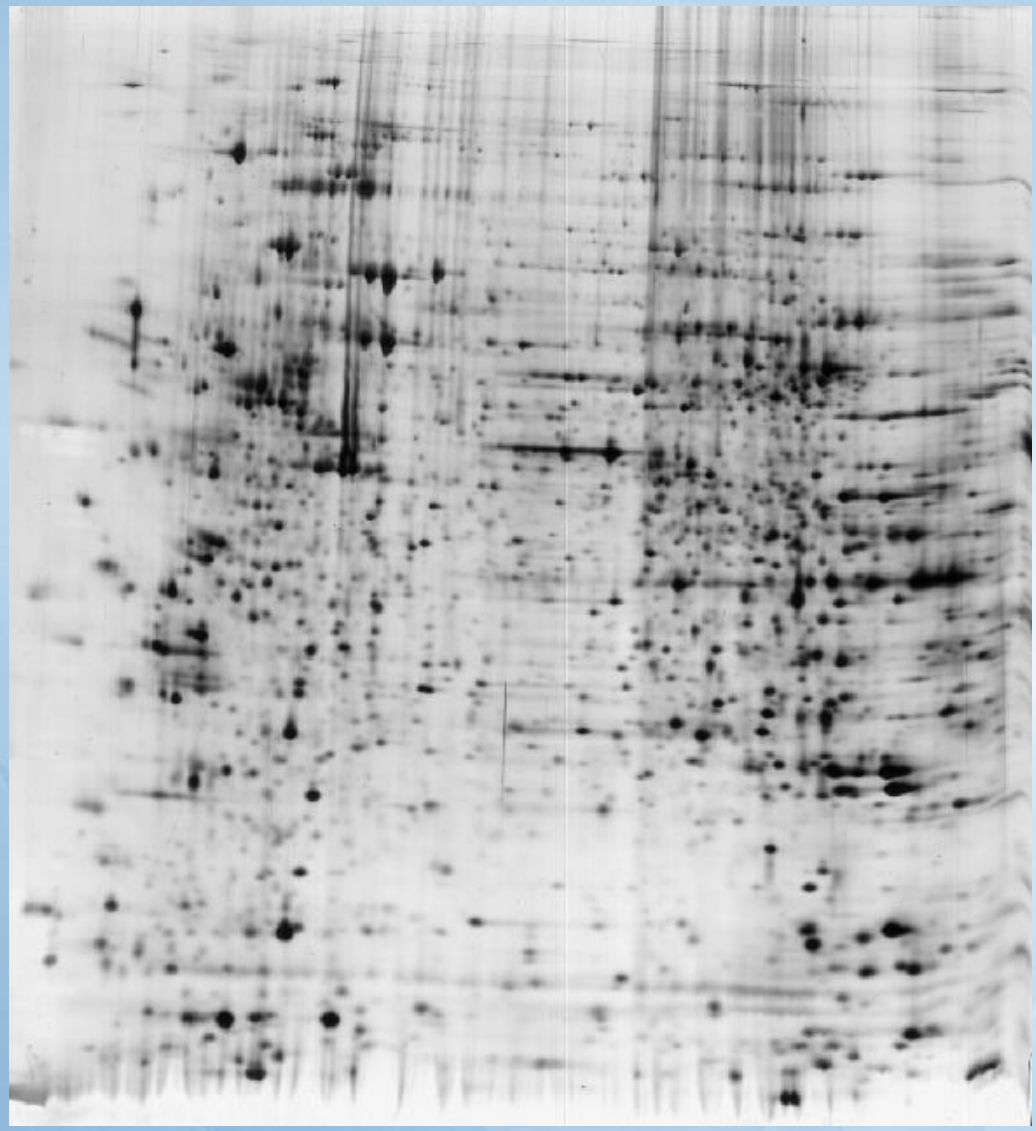


IEF 4-6.5 pH gradient

2D PAGE



2D PAGE



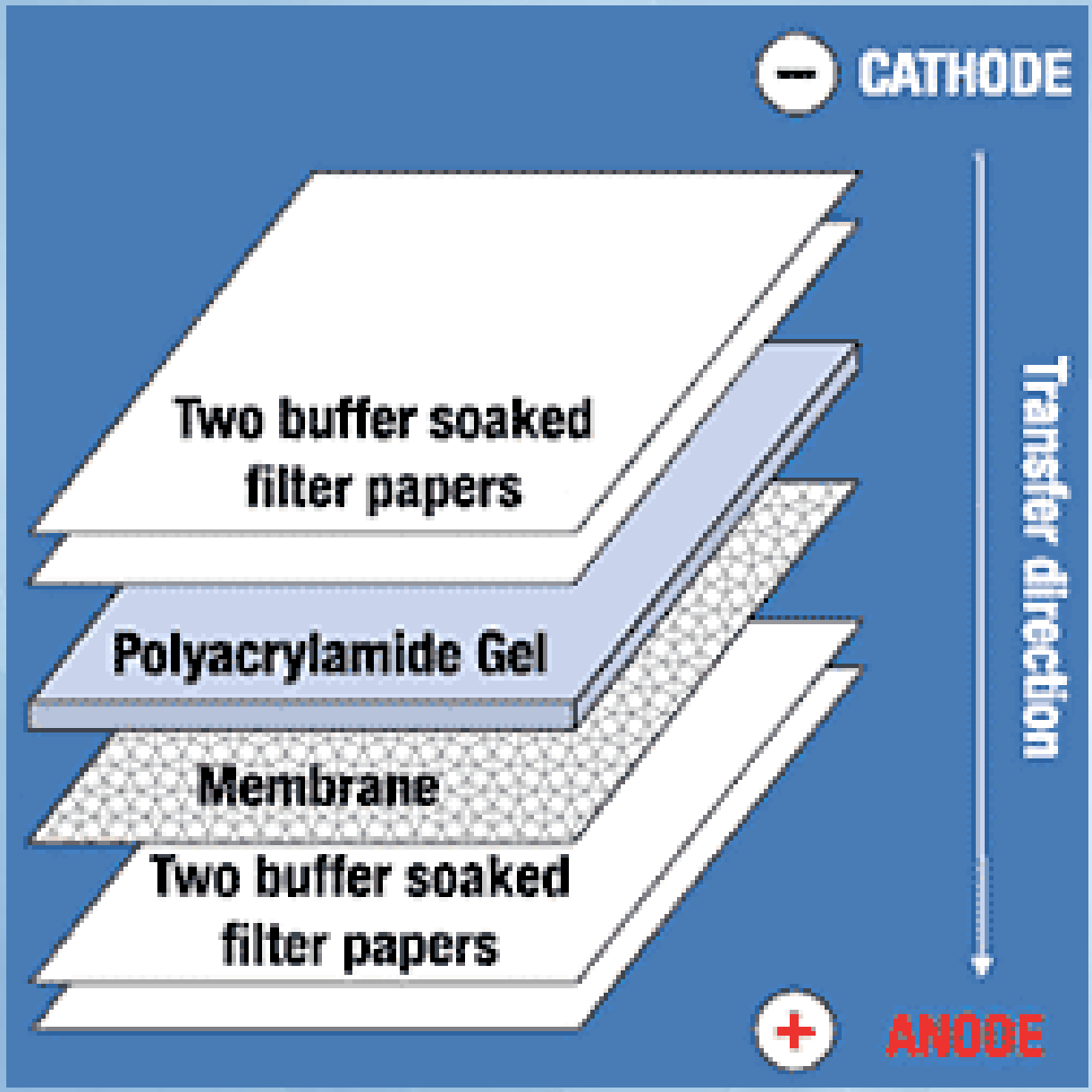
Western Blot Analysis

- Identifies protein through antibody interaction
- Run proteins on denatured gel (SDS-PAGE)
- Transfer (blot) proteins onto membrane
- Probe the membrane with primary antibody
- Add secondary antibody (this antibody is linked to an enzyme)
- Substrate is added and color appears

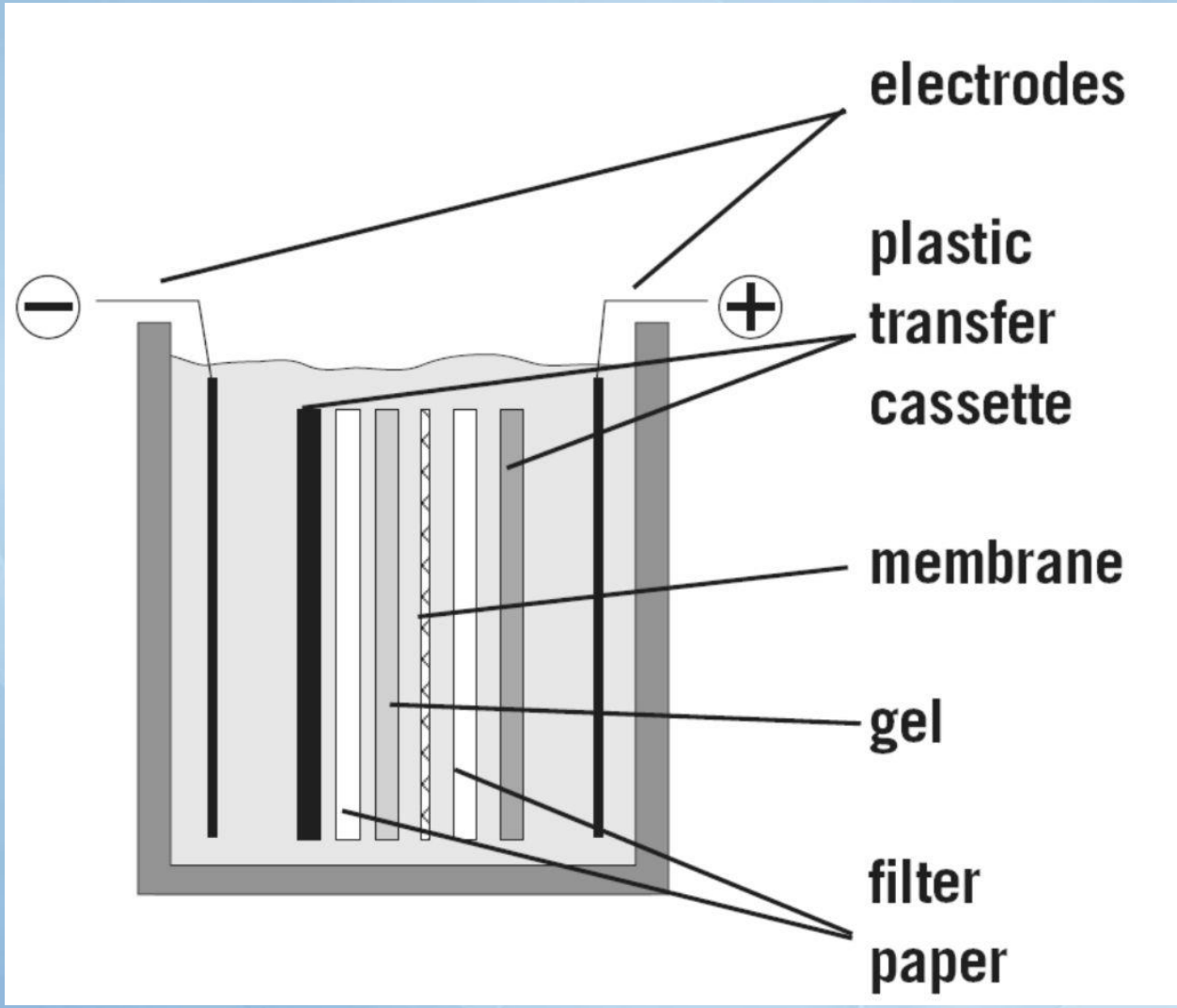
WB: 4 steps

1. Separation of proteins using SDS PAGE
2. Transfer of the proteins onto e.g. a nitrocellulose membrane (blotting)
3. Immune reactions
4. Visualization

Transfer of proteins to the membrane

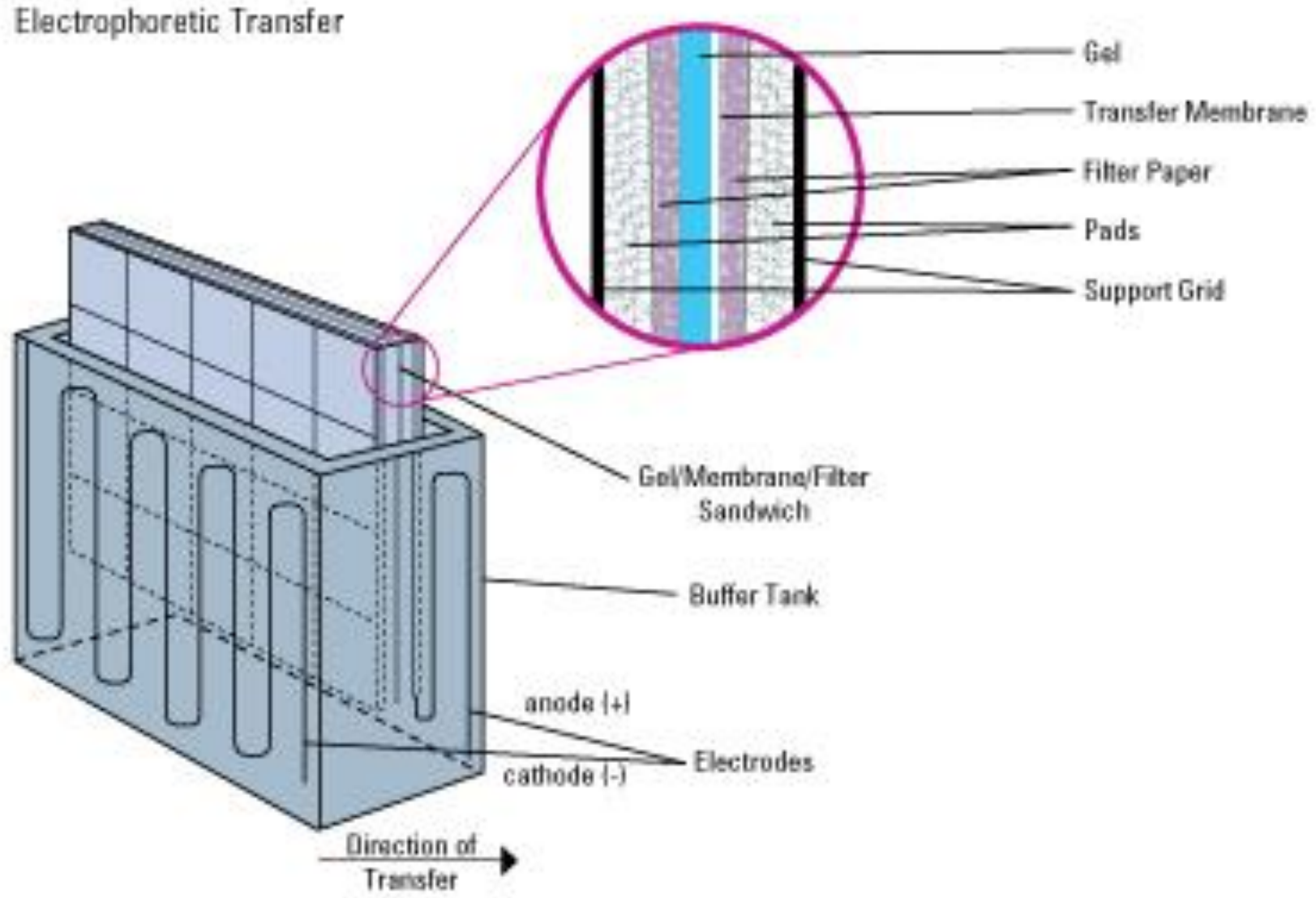


WB, Step 2: Blotting

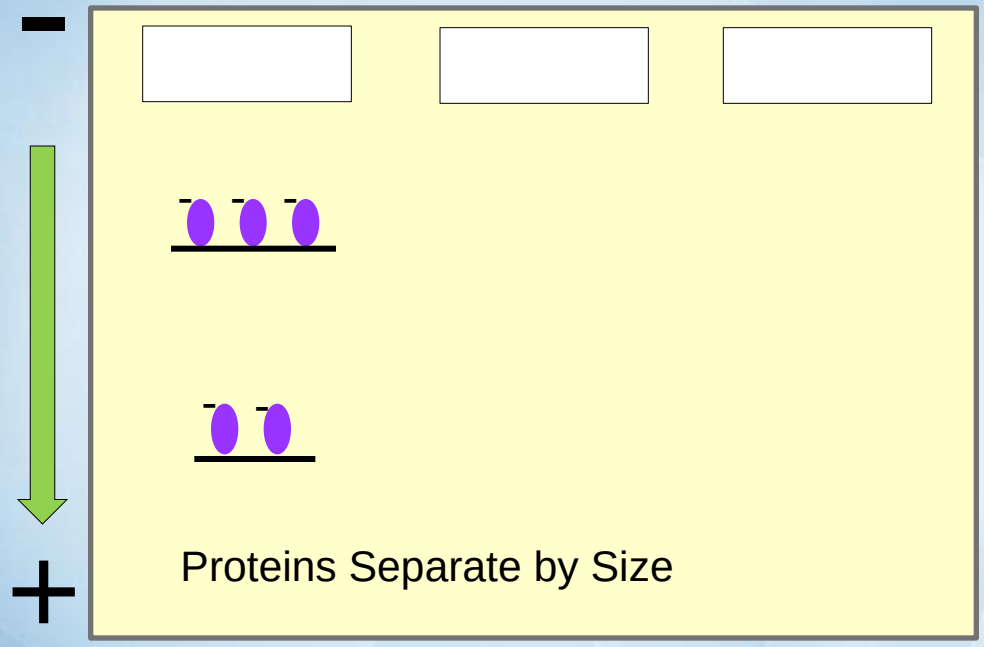


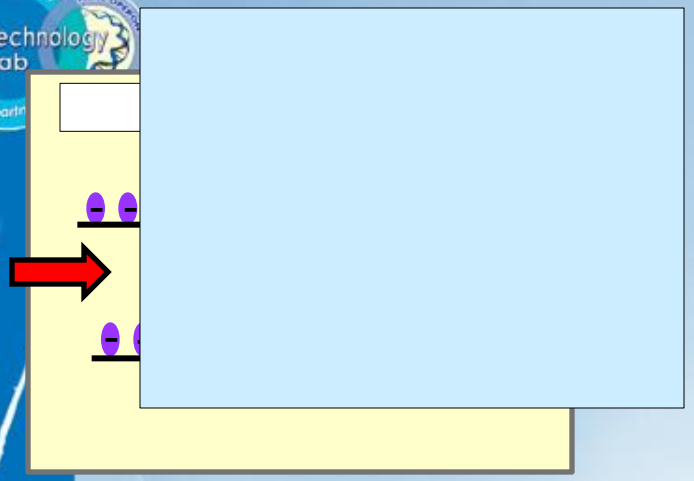

Buffer tank lid
Blue cooling unit
Gel holder cassette
and foam pads
Electrode
assembly

Western blotting-wet transfer apparatus

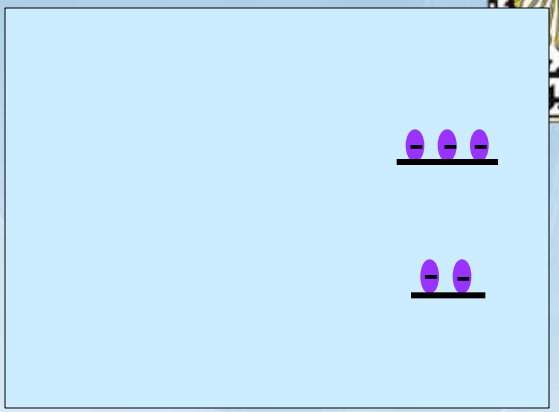


SDS-PAGE Western Blot Method

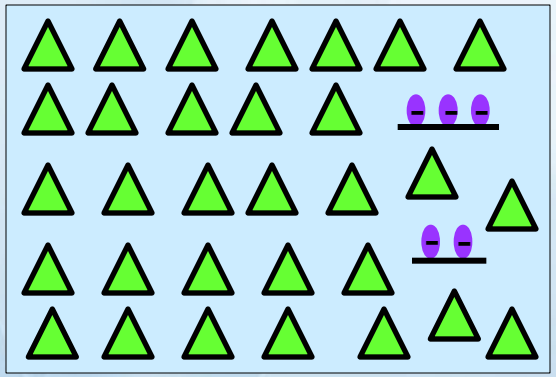




Transfer or Blot Protein from Gel to Nitrocellulose and/or PVDF Membrane

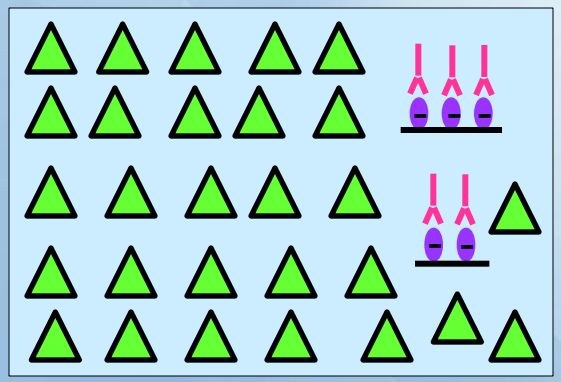


Block Membrane with Non-Specific Proteins

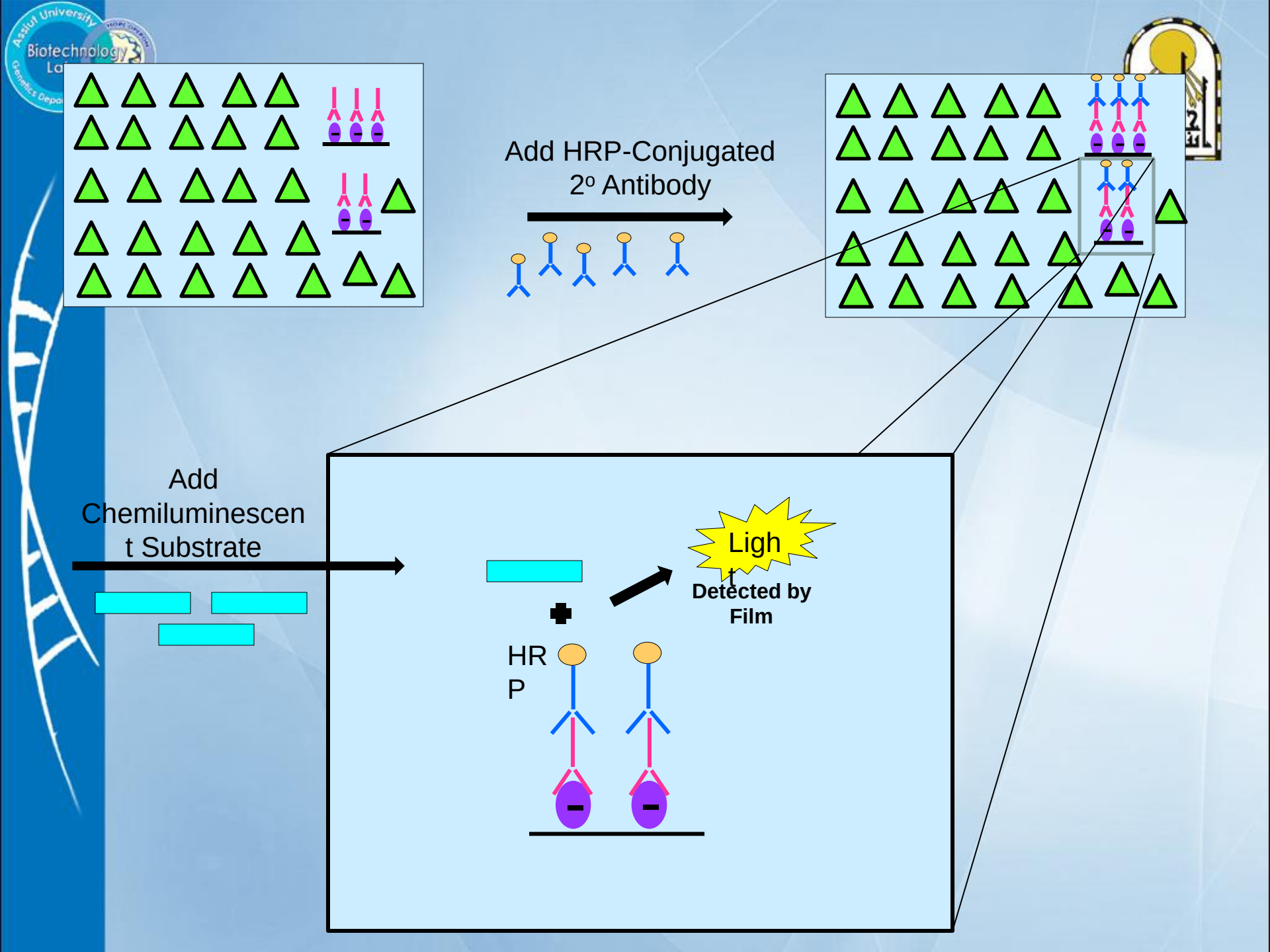


Non-Specific Proteins Bind to Unbound Regions of Membrane

Incubate Membrane with 1º Antibody



1º Antibody Binds Antigen (i.e. Protein of Interest)


Add HRP-Conjugated
2º Antibody
Add
Chemiluminescen
t Substrate
HR
P
Ligh
t
Detected by
Film