Protein Purification and Characterization Techniques

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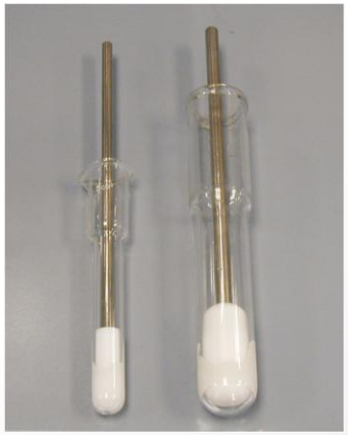
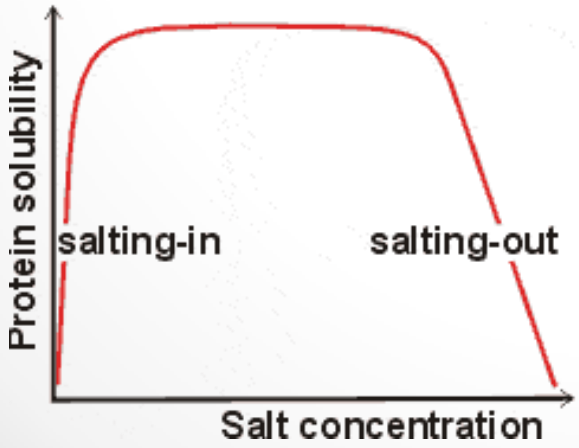
Extracting Pure Proteins from Cells

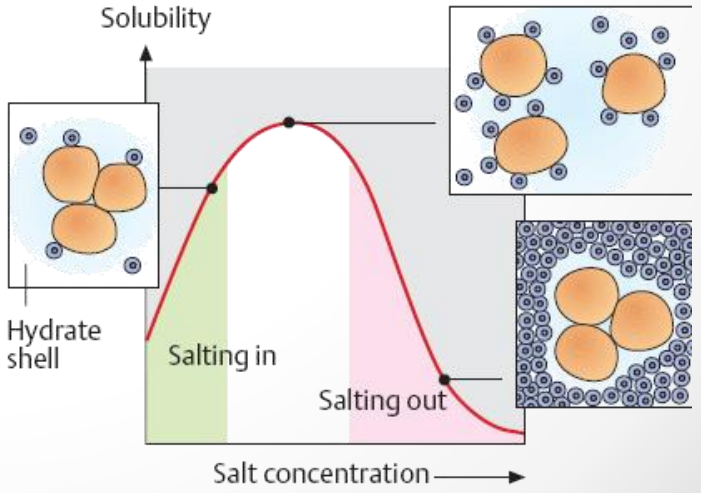
- Purification techniques focus mainly on size & charge
- The first step is **homogenization** (grinding, Potter–Elvejhem homogenizer, sonication, freezing and thawing, detergents)
- **Differential centrifugation** (600 *g*: unbroken cells & nuclei; 15,000 *g*: mitochondria; 100,000 *g*: ribosomes and membrane fragments)



Salting in & out

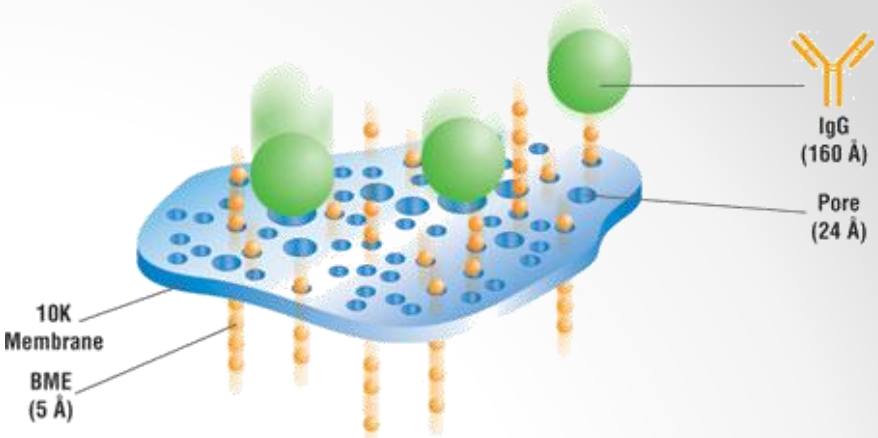
- Are proteins soluble? If yes, to which limit?
- Salt stabilizes the various charged groups on a protein molecule and enhance the polarity of water, thus attracting protein into the solution and enhancing the solubility of the protein
- Ammonium sulfate is the most common reagent to use at this step
- This technique is important but results are crude

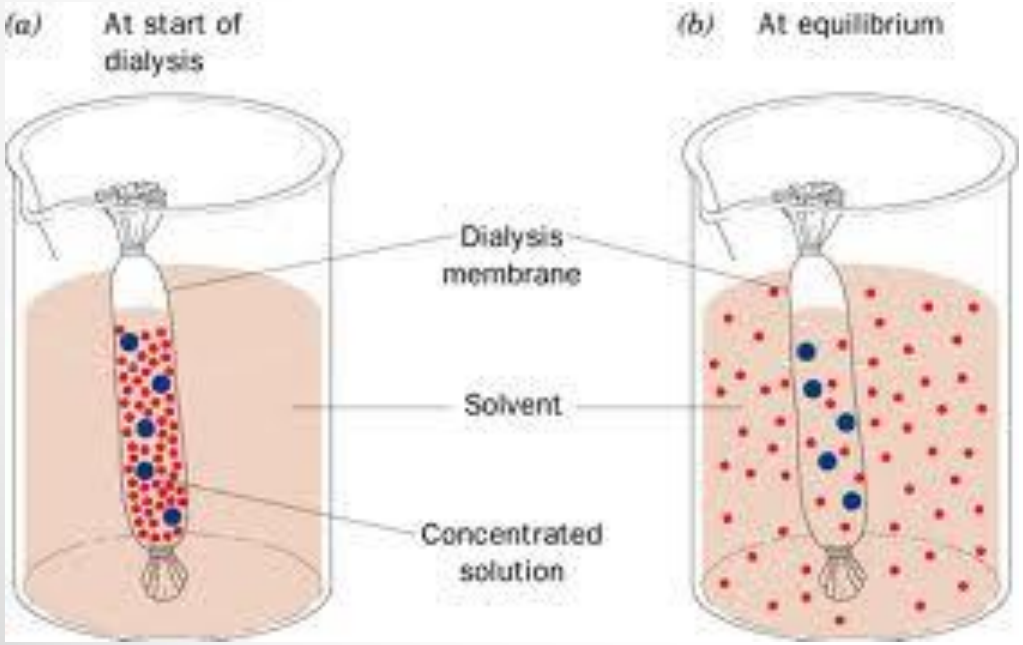


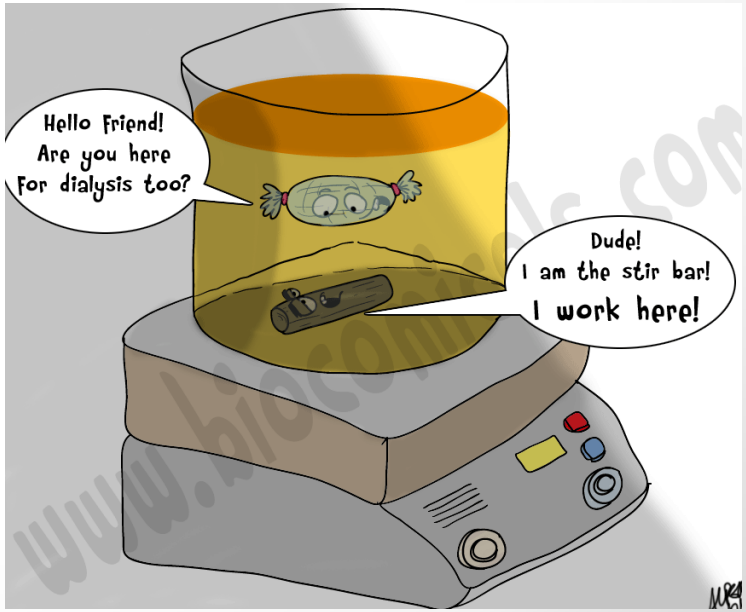


Dialysis

- Principle of diffusion
- Concept of MW cut-off
- Pure vs. crude

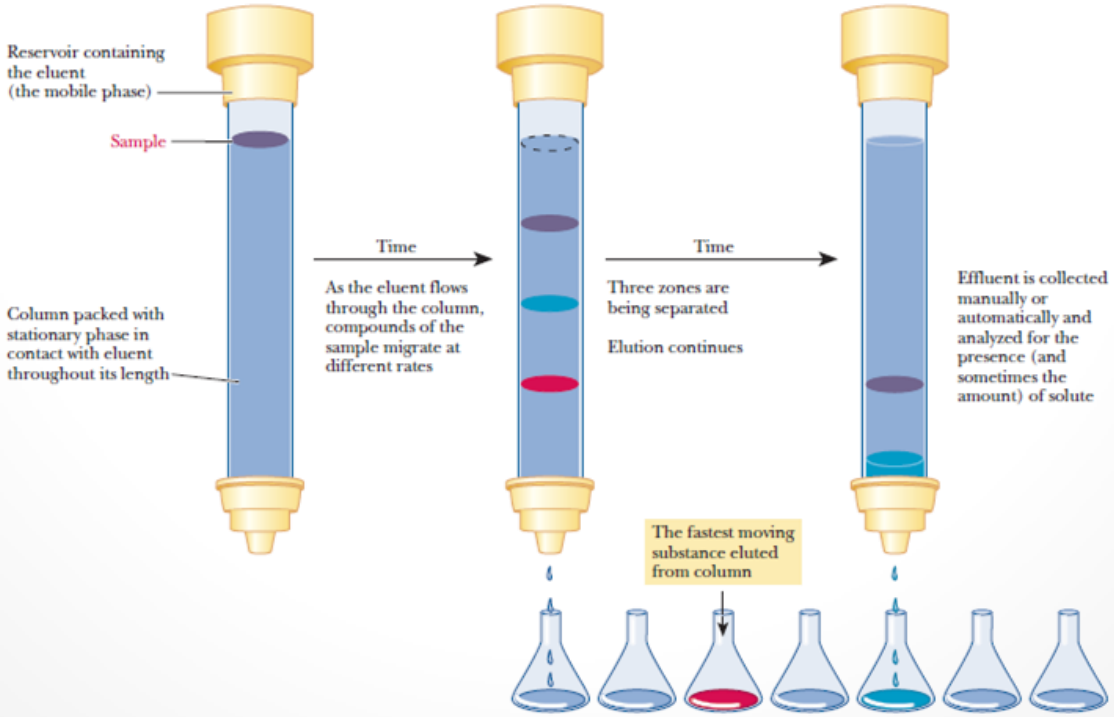






Column Chromatography

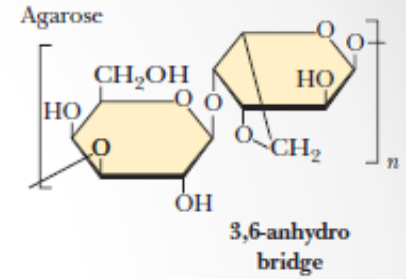
- Greek *chroma,* "color," and *graphein,* "to write"
- Is it just for colourful proteins?
- Chromatography is based on two phases: stationary & mobile
- What are the different kinds?

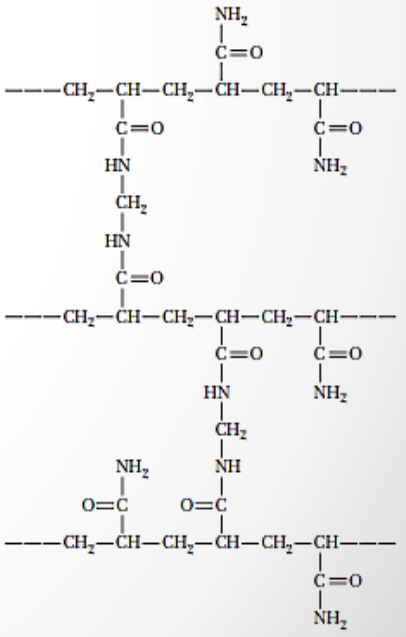


Size-exclusion chromatography

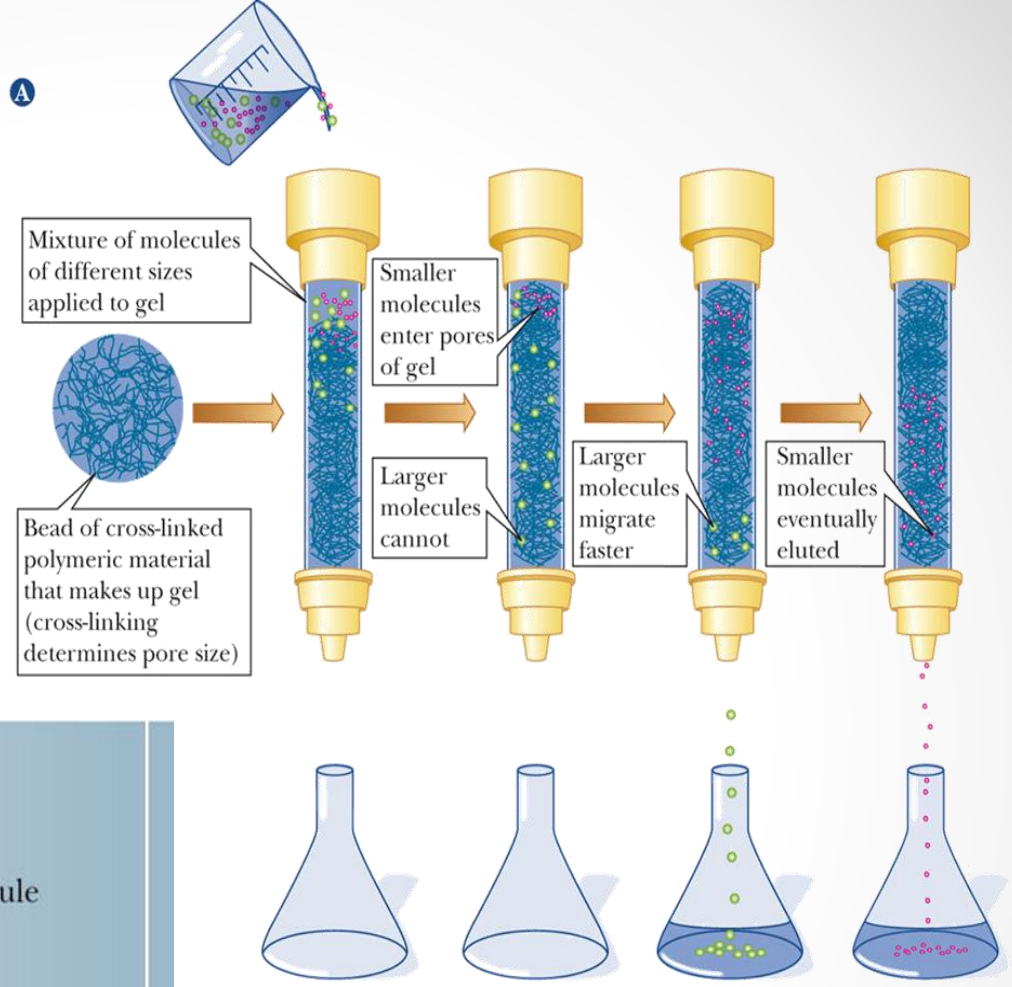
Gel-filtration chromatography

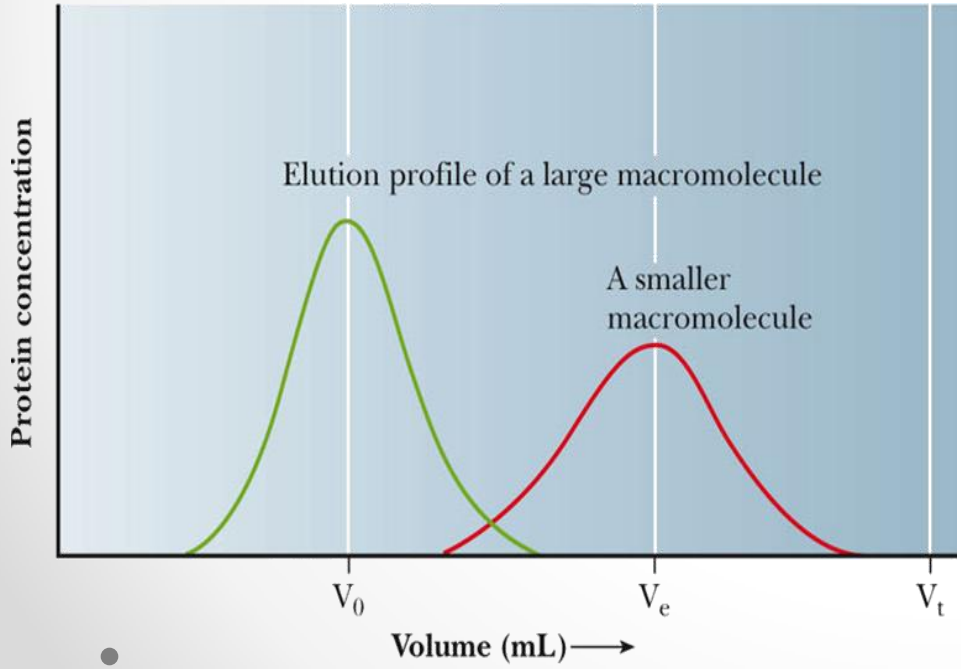
- Separation on the basis of size (MW)
- Stationary (cross-linked gel particles): consist of one of two kinds of polymers; the 1st is a carb. polymer (ex. **dextran** or **agarose);** often referred to by Sephadex and Sepharose. The 2nd is based on **polyacrylamide** (Bio-Gel)
- Extent of crosslinking & pore size (exclusion limit)
- Convenient & MW estimate
- Each gel has range of sizes that separate linearly with the log of the molecular weight





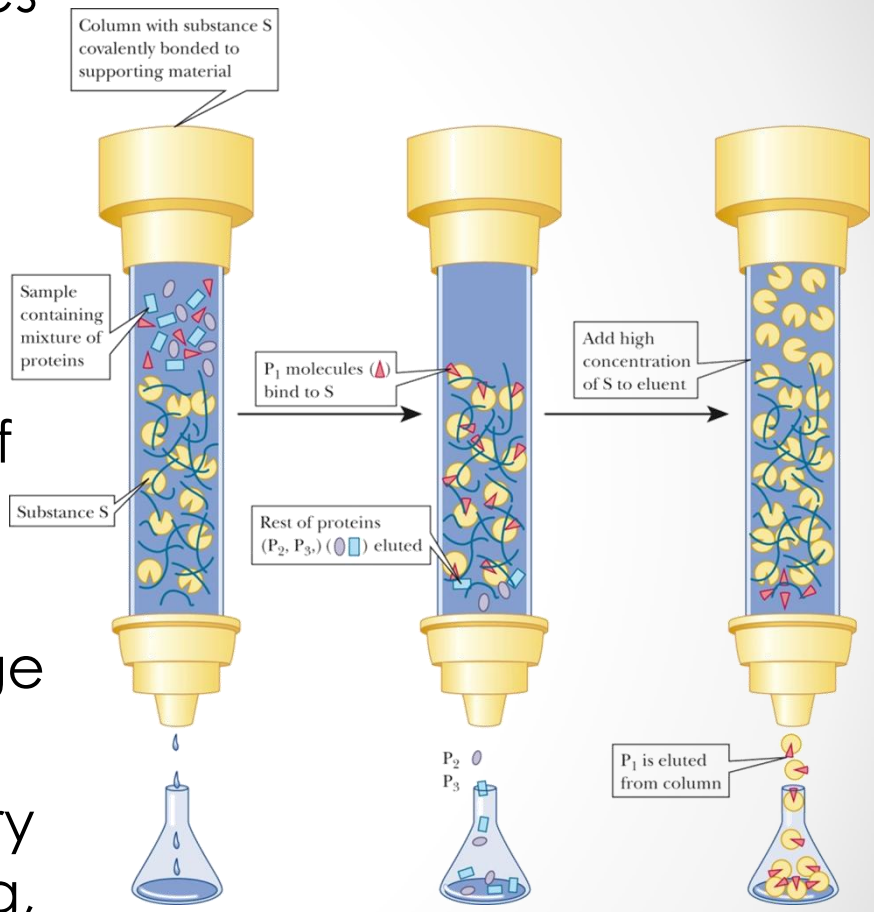
Molecular-sieve chromatography





Affinity chromatography

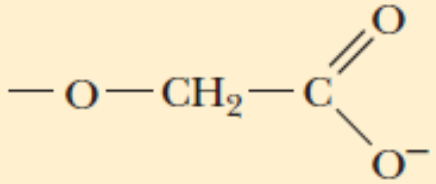
- It has specific binding properties
- The polymer (stationary) is covalently linked to a *ligand* that binds specifically to the desired protein
- The bound protein can be eluted by adding high conc. of the soluble ligand
- Protein–ligand interaction can also be disrupted with a change in pH or ionic strength
- Convenient & products are very pure (Antigen-antibody, His-tag, GST-Tag)



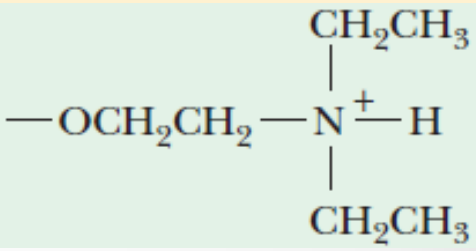
Ion-exchange chromatography

- Interaction based on net charge & is less specific
- Resin is either negatively charged (**cation exchanger**) **or** positively charged (**anion exchanger**)
- Buffer equilibration, exchange resin is bound to counter-ions. A cation-exchange resin is usually bound to Na+ or K+ ions, and an anion exchanger is usually bound to Cl^- ions
- Proteins mixture loading
- Elution (pH change or higher salt concentration)

Weakly acidic: carboxymethyl (CM) cellulose

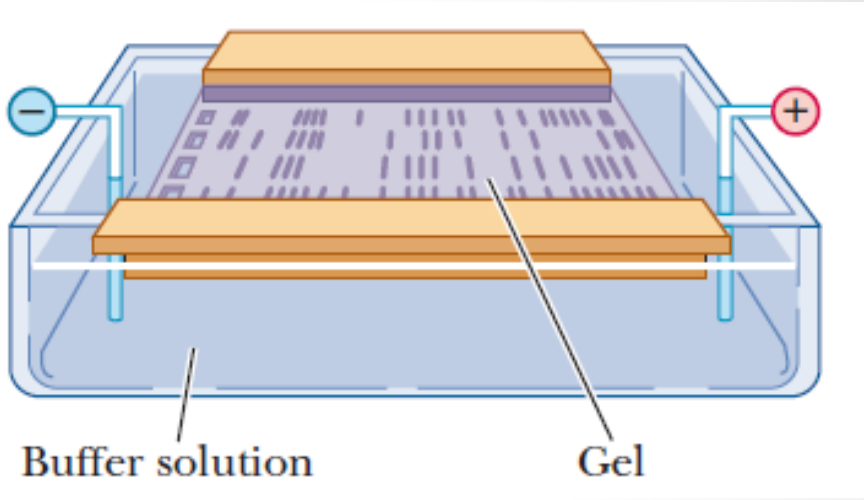


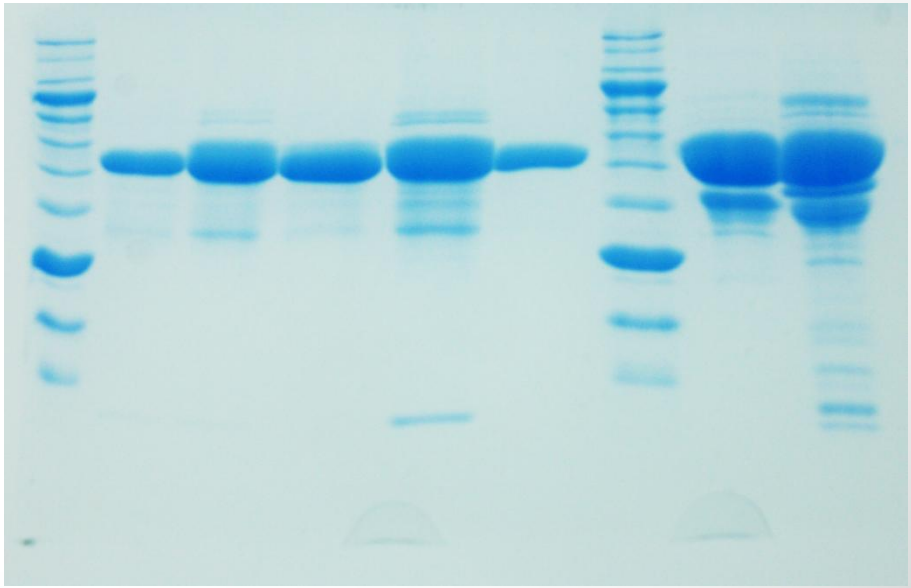
Weakly basic: diethylaminoethyl (DEAE) cellulose



Electrophoresis

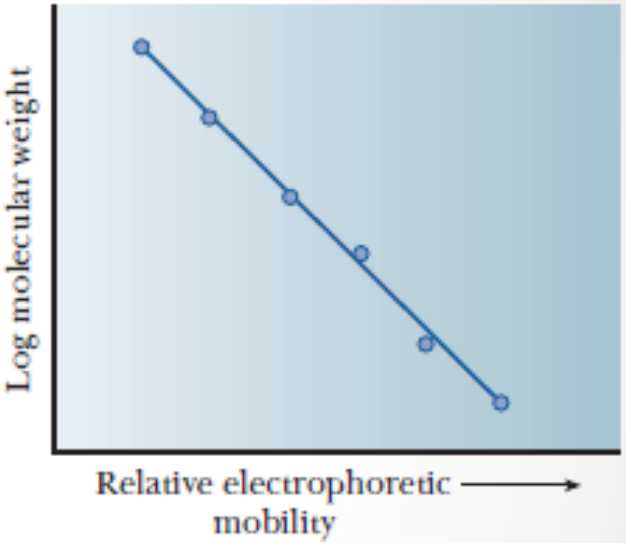
- Based on the motion of charged particles in an electric field
- Macromolecules have differing mobilities based on their charge, shape, and size
- The most common medium is a polymer of agarose or acrylamide





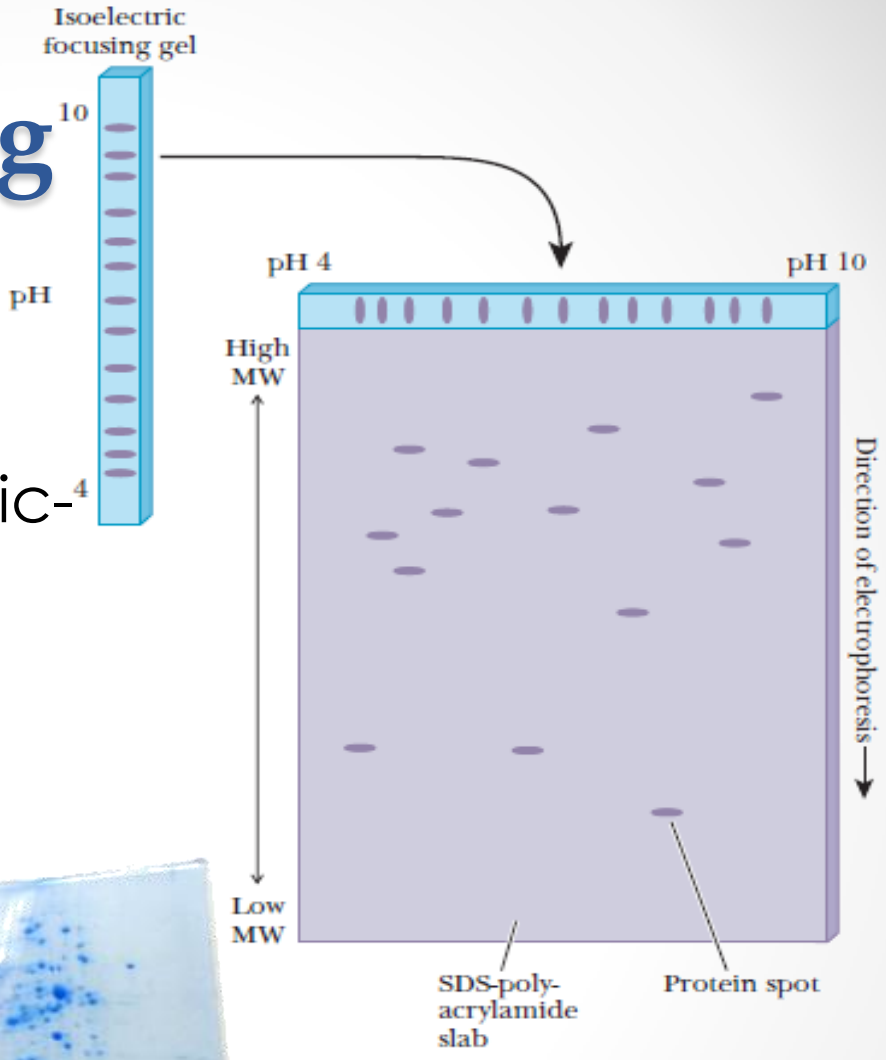
Agarose vs. PAGE

- Agarose (nucleic acids), PAGE (proteins)
- In PAGE: SDS or NO-SDS $\{CH_3(CH_2)_{10}CH_2OSO_3Na^+\}$
- SDS completely denatures proteins (multi-subunit proteins)
- Acrylamide offers higher resistance to large molecules
- Shape and charge are approximately the same (sizes is the determining factor)
- Acrylamide without the SDS (**native gel):** study proteins in their native conformation (mobility is not an indication of size)

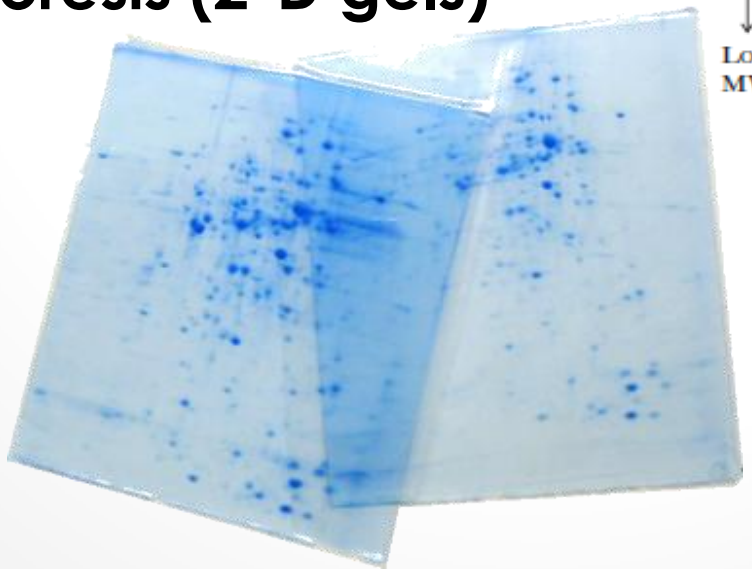


Isoelectric focusing

- Proteins have different isoelectric points
- Gel prepared with a pH gradient parallel to electric-field gradient

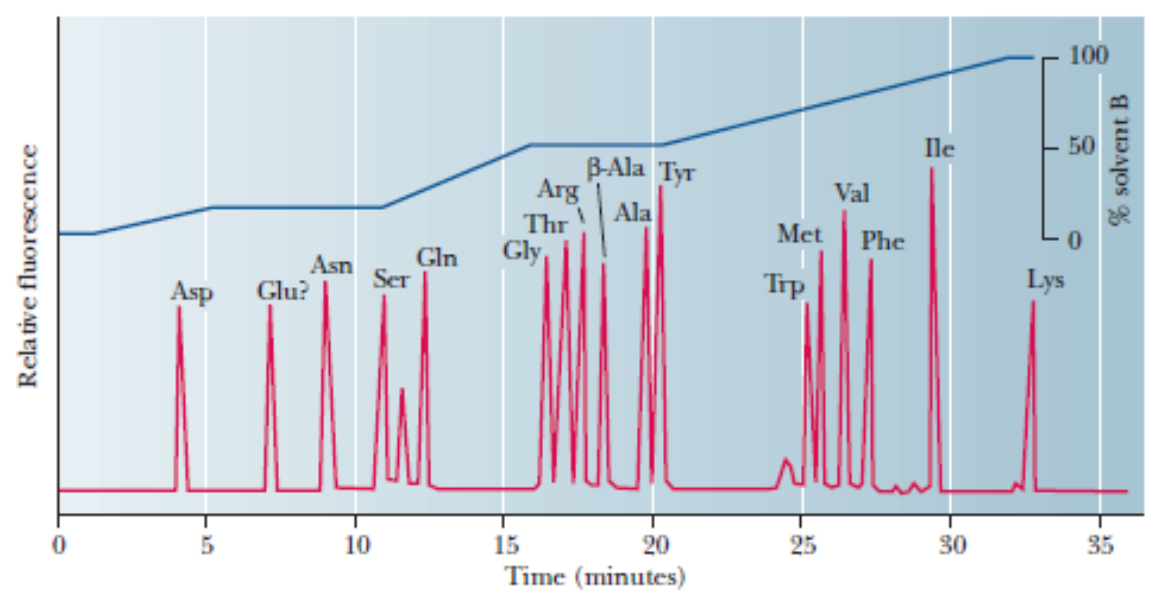


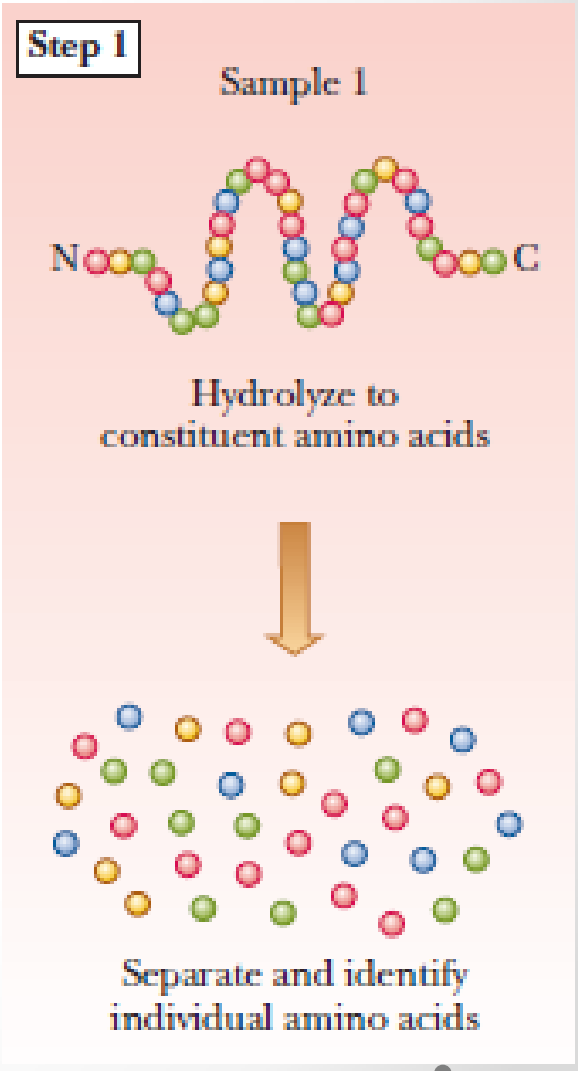
- **Two-dimensional gel electrophoresis (2-D gels)**



Protein sequencing - Edman Method

- Step 1: how much and which amino acids are involved
- **Hydrolysis** (heating + HCl) **& Separation** (ion-exchange chromatography or by **high performance liquid chromatography, HPLC)**



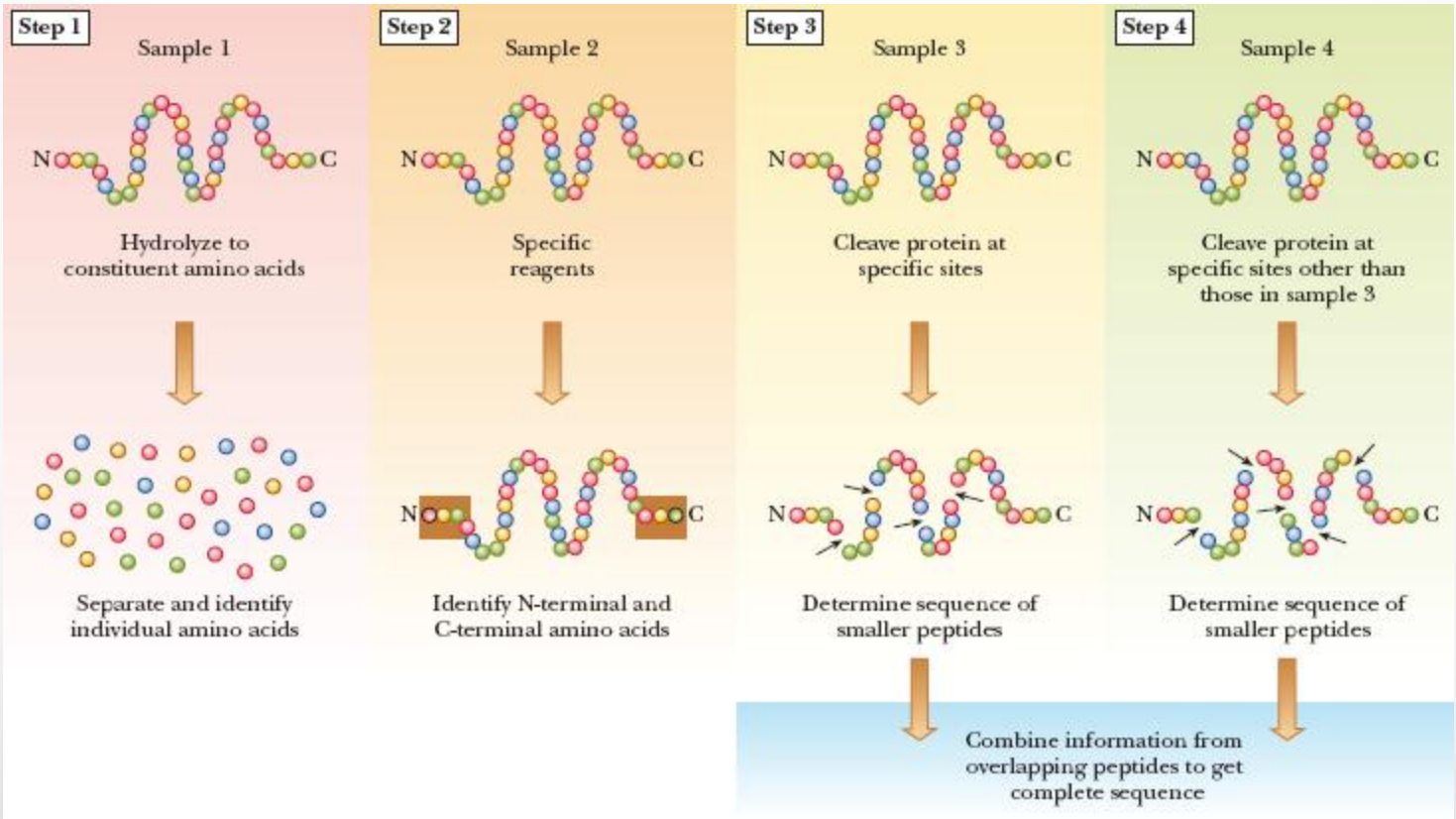


Protein sequencing - Edman Method

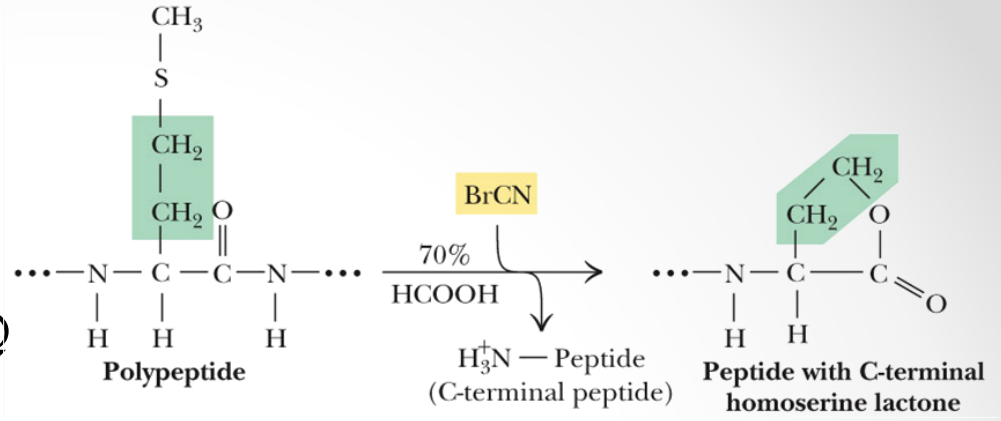
- Step 2: determining the identities of N- & C- termini ends of protein
- Necessary to determine if the protein has of 1 or 2 polypeptide chains
- Steps 3: cleavage into smaller fragments **(Edman degradation)**

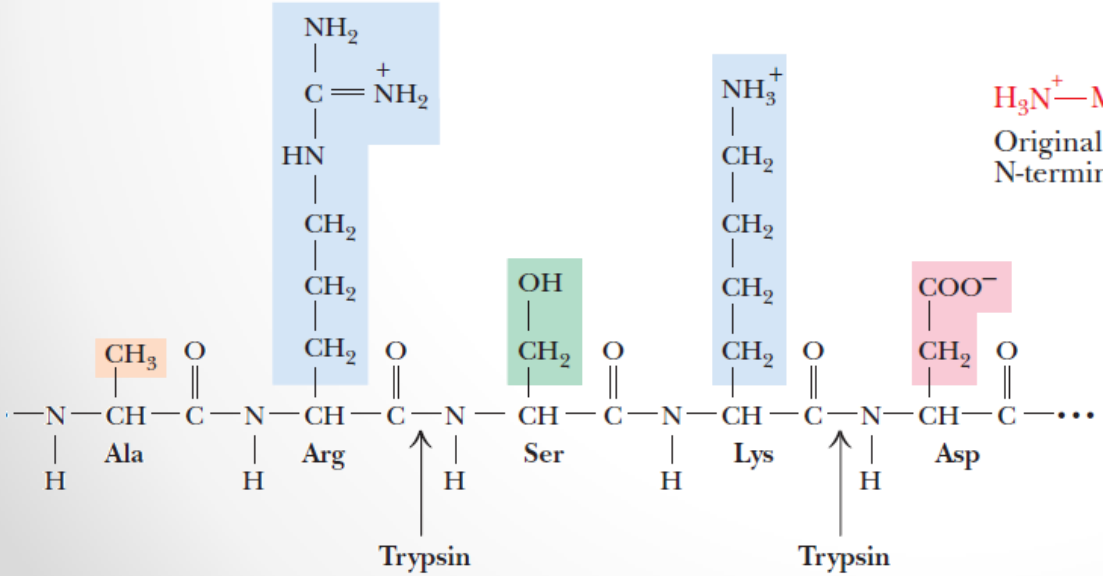
1) **Enzymes-** Trypsin, Chymotrypsin

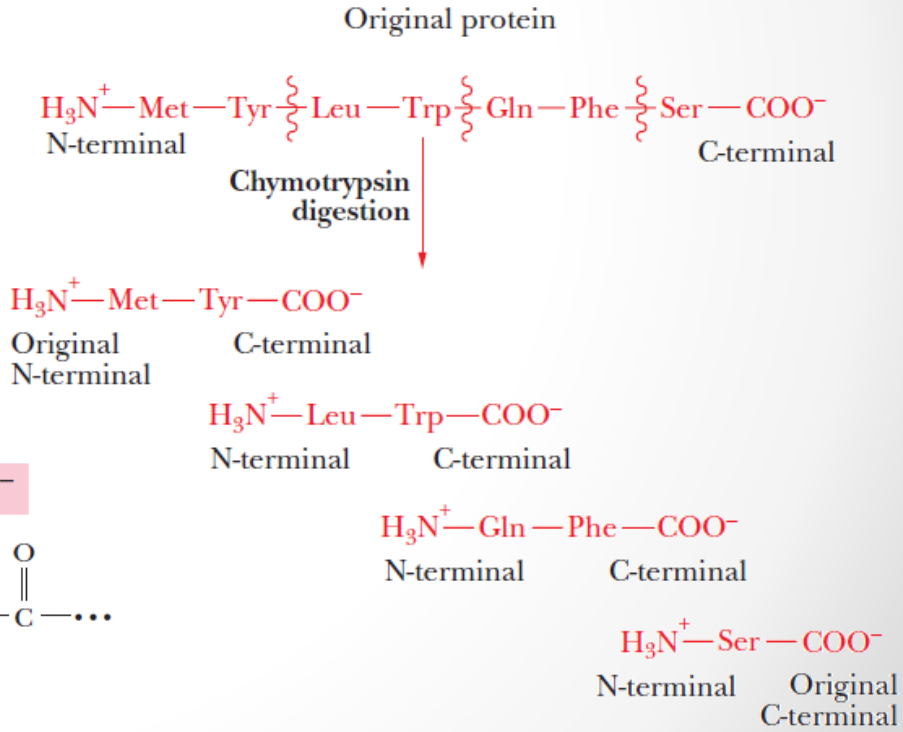
2) **Chemical reagents-** Cyanogen bromide CNBr



- **Trypsin: Cleaves @ C-terminal of (+) charged side chains**
- **Chymotrypsin: Cleaves @ C-terminal of aromatics**
- **CNBr: Cleaves @ C-terminal of INTERNAL methionines**





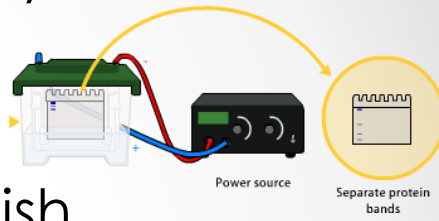


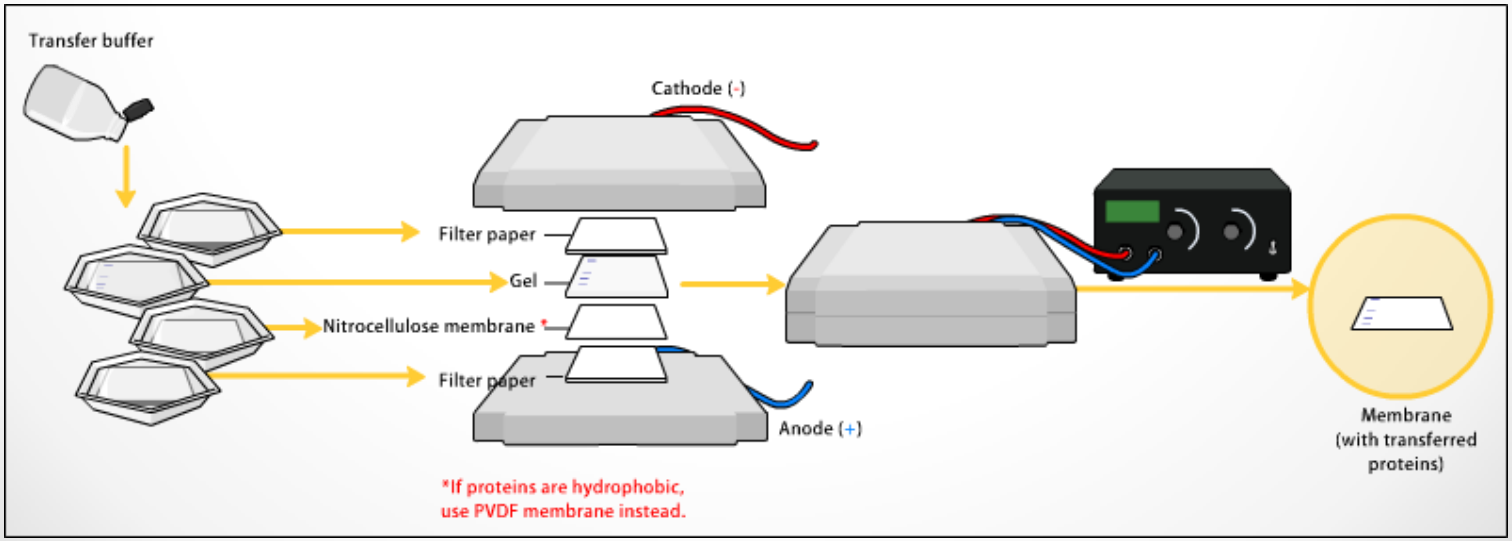
Protein sequencing – prediction from DNA & RNA

- If the sequence of the gene is known, this is very easy
- If the sequence of the gene is unknown (newly isolated proteins)? Sequence a short segment, complementary RNA, isolate mRNA, PCR, gene sequencing

Immunoassays – Western blot "Protein Immunoblot"

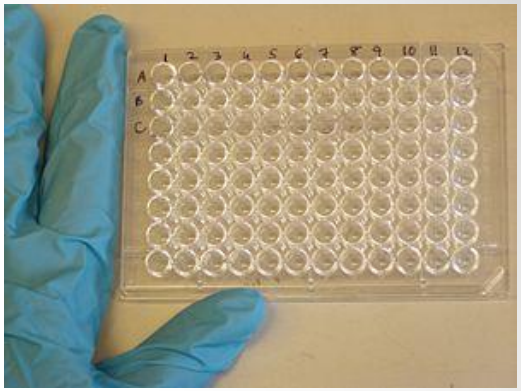
- From gel to a membrane (nitrocellulose or polyvinylidene difluoride, PVDF)
- Detection:
- Colorimetric: enzymes bound to 2nd Ab (horseradish peroxidase)
- Chemiluminescent: reporter 2nd Ab (CCD cameras)
- Radioactive detection: X-rays
- Fluorescent detection: fluorescently labeled probe

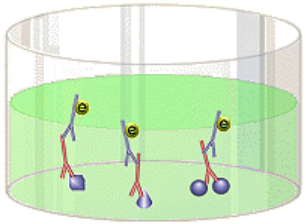




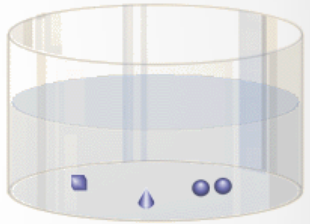
Immunoassays - ELISA

- Enzyme-Linked Immunosorbent Assay
- Detect & quantify substances (peptides, proteins, antibodies & hormones)
- Usually done in 96-well polystyrene plates (passively bind antibodies and proteins)
- Apllication:
- Screening (HIV, Hepatitis B&C)
- Detecting food allergens, such as milk, peanuts, walnuts, almonds, and eggs
- Hormones (HCG, LH, TSH, T3, T4)





(Green, positive)



(No color, negative)