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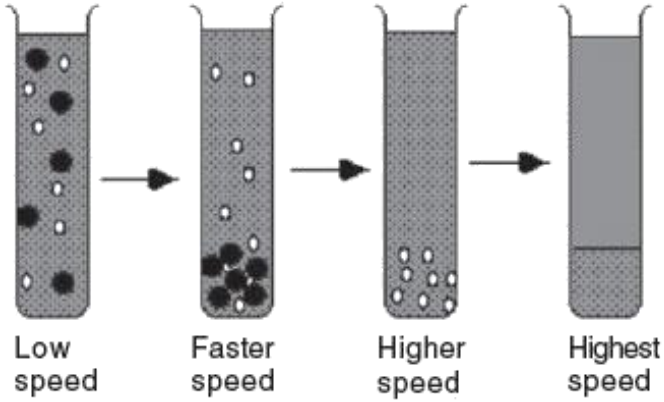
Protein Purification & Characterization Techniques

Extracting Pure Proteins from Cells

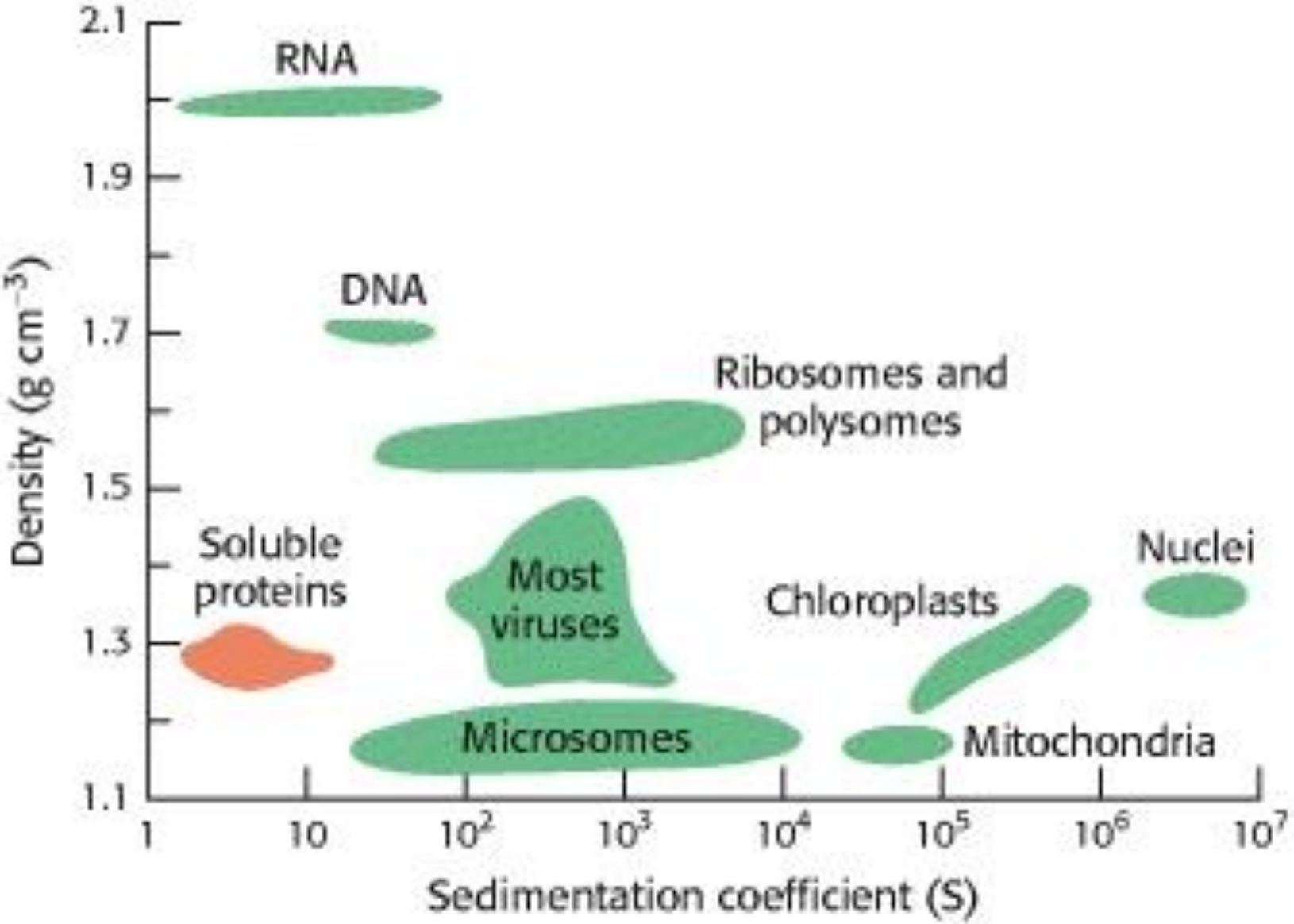
- Purification techniques focus mainly on size & charge
- The first step is **homogenization** (grinding, Potter–Elvejhem homogenizer, sonication, freezing and thawing, detergents)

- **Differential centrifugation**

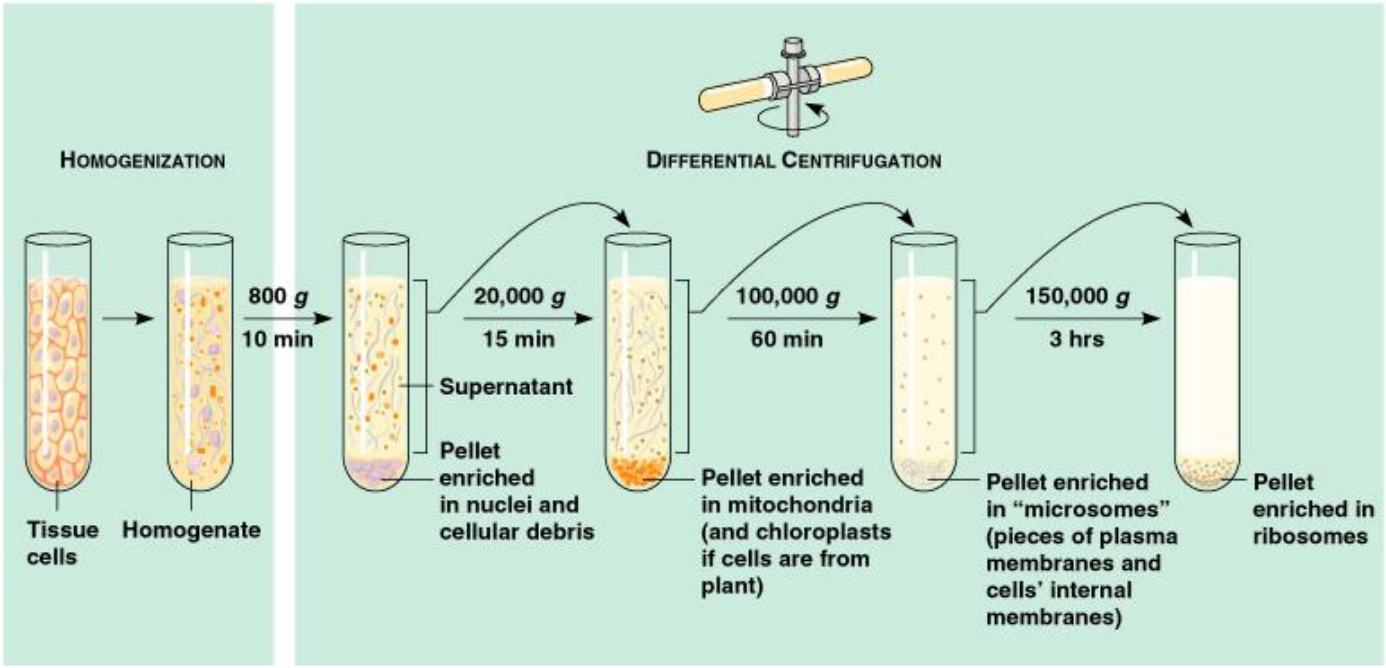


Sedimentation

- A particle sediments by centrifugation
- Sedimentation depends on its mass and shape
- The sedimentation of a particle is constant and can be defined as a sedimentation coefficient
  - Sedimentation coefficient = 10^{-13} s = 1 Svedberg
- The sedimentation of a particle depends on its
  - Mass (direct correlation)
  - Density (direct correlation)
  - Shape (inverse correlation)
  - The density of the solution (inverse correlation)


2.1
1.9
1.7
1.5
1.3
1.1
Density (g cm^{-3})
RNA
DNA
Ribosomes and polysomes
Soluble proteins
Most viruses
Nuclei
Chloroplasts
Microsomes
Mitochondria
1
10
10^2
10^3
10^4
10^5
10^6
10^7
Sedimentation coefficient (S)

Example: cell fractionation



(800 *g:* unbroken cells & nuclei; 20,000 *g:* mitochondria; 100,000 – 150,000 *g:* ribosomes and membrane fragments)

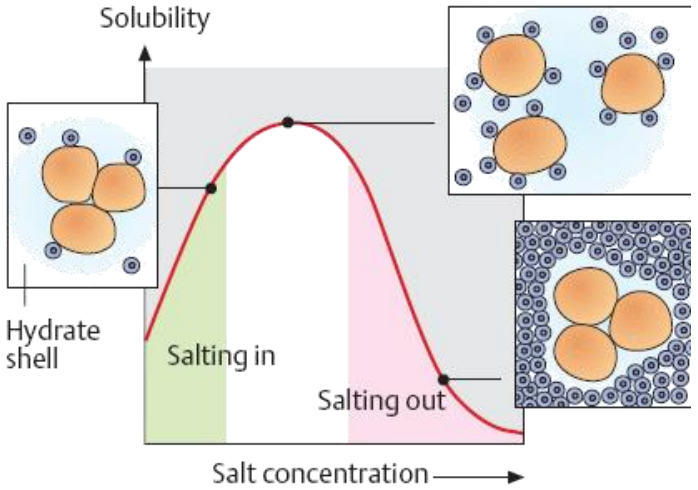
This treatment separates cell components on the basis of size. In general, the largest units experience the largest centrifugal force and move the most rapidly

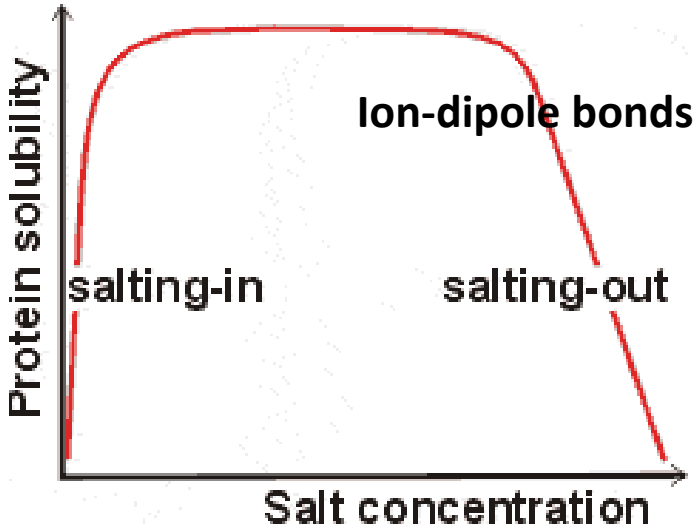
Basis of protein separation

- Proteins can be purified on the basis
  - Solubility
  - Size
  - Charge
  - Specific binding affinity

Salting in & out

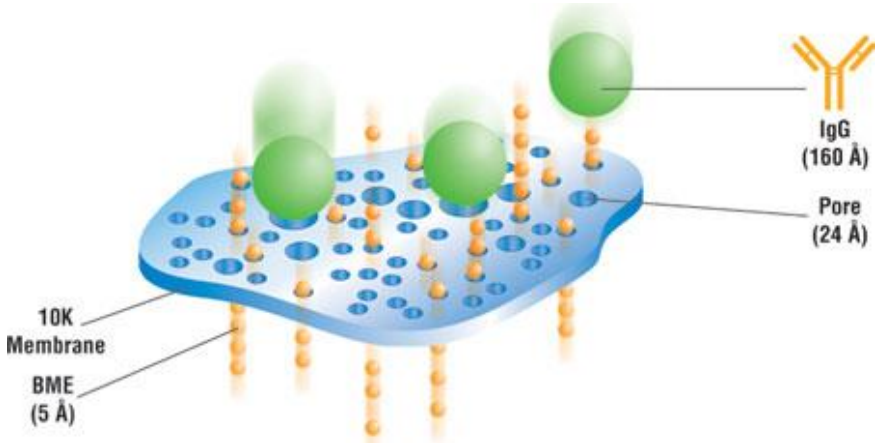
- **Are proteins soluble**? If yes, to which limit?
- Salt:
  - Stabilizes the various charged groups on a protein molecule
  - Enhances the polarity of water
  - Enhances solubility
- Ammonium sulfate (the most common)
- This technique is important but results are crude

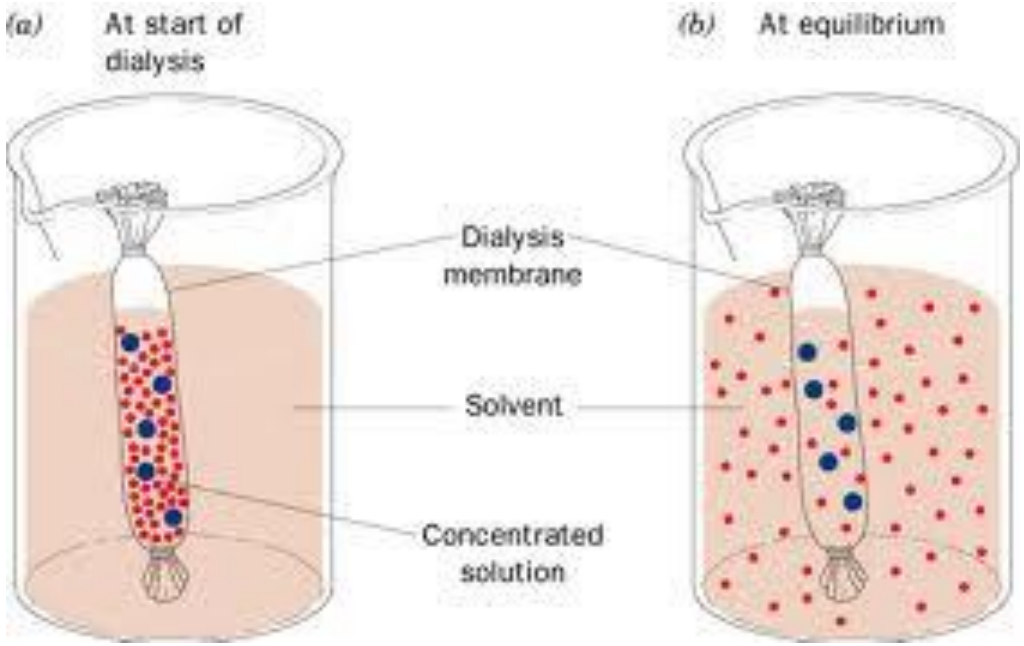


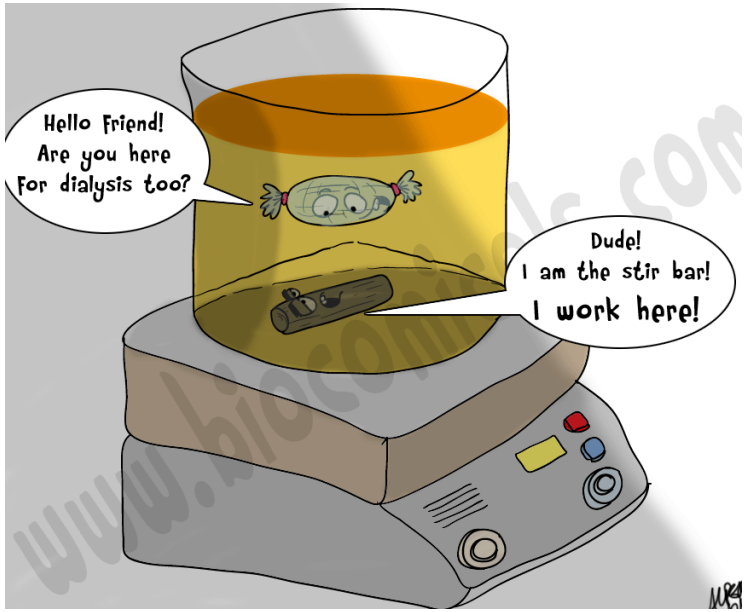


Dialysis

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

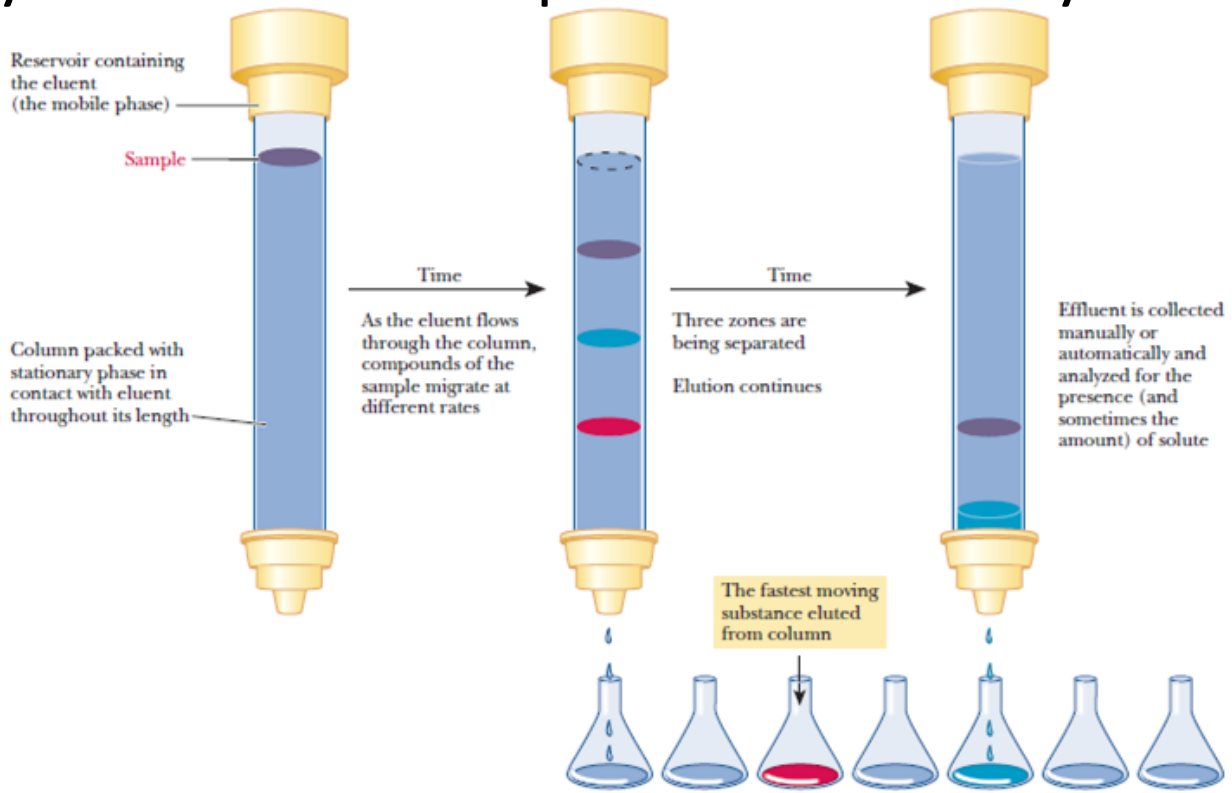




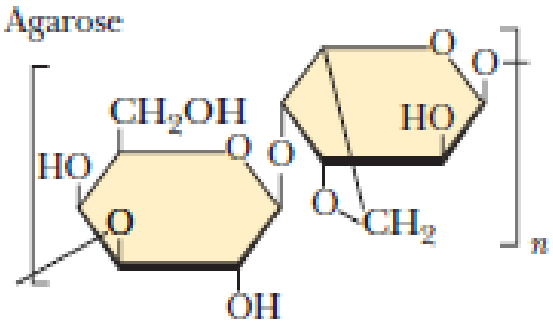


Column Chromatography

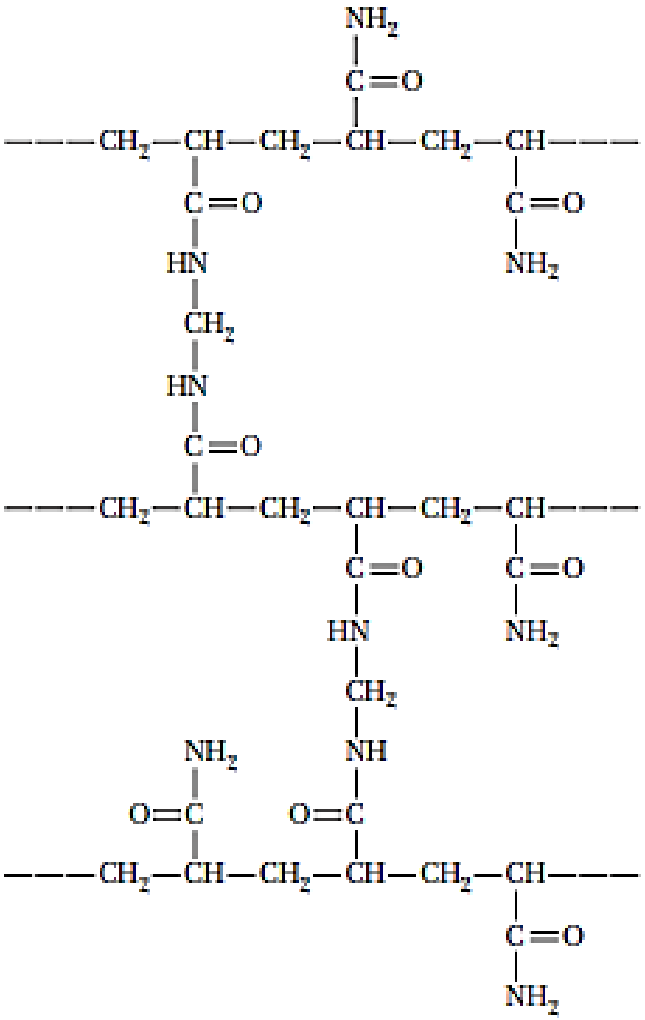
- Greek *chroma,* “color,” and *graphein,* “to write”
- Is it just for colourful proteins?
- Chromatography is based on two phases: stationary & mobile
  - What are the different kinds?



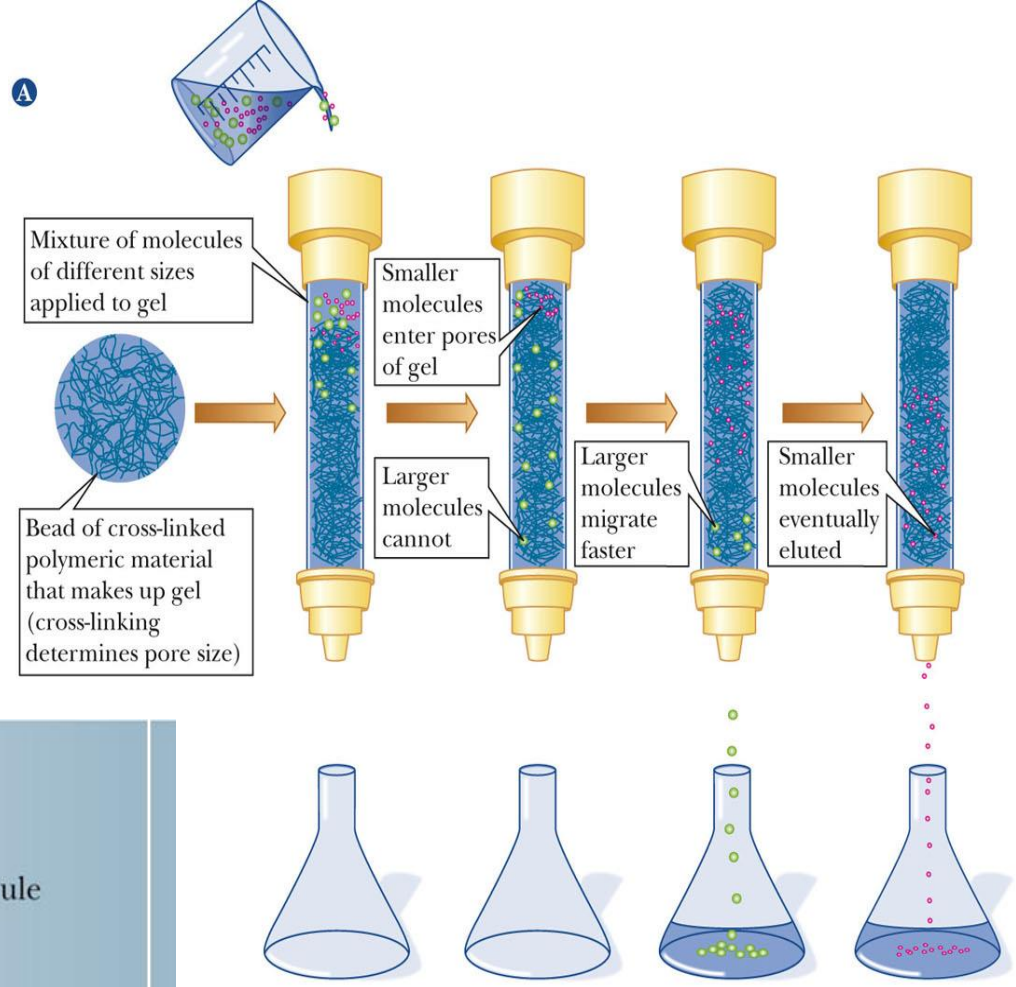
Size-exclusion chromatography
Gel-filtration chromatography

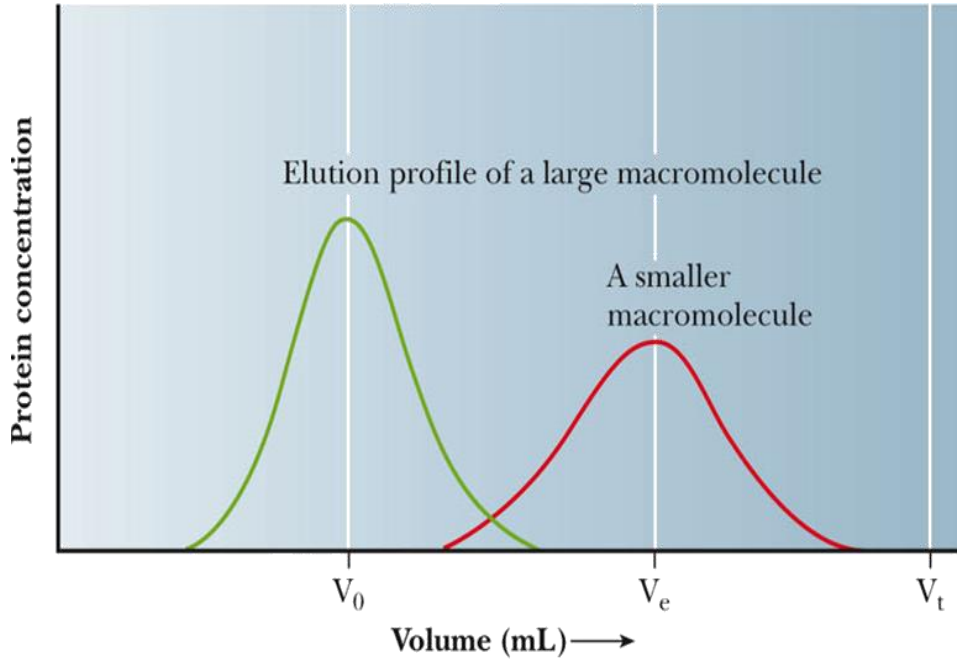


- Separation on the basis of size (MW)
- **Stationary** (cross-linked gel particles): consist of one of two kinds of polymers; the 1st is a carb. polymer (ex. **dextran** or **agarose);** The 2nd is based on **polyacrylamide**
- Extent of crosslinking & pore size (exclusion limit)
- Convenient & MW estimate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



Molecular-sieve chromatography

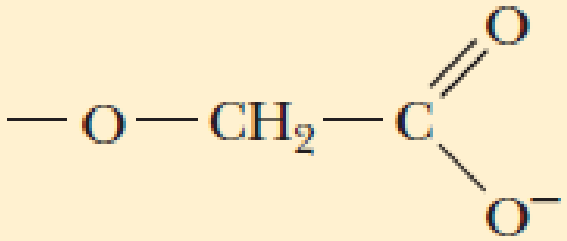




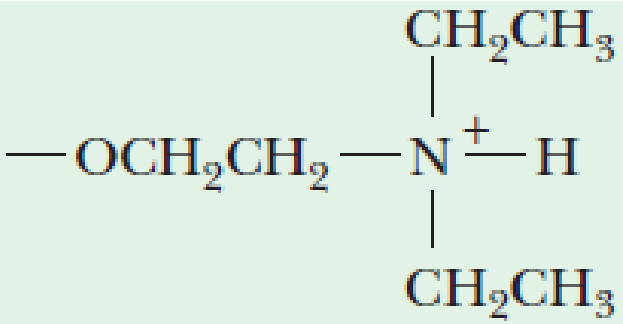
Ion-exchange chromatography

- Interaction based on net charge & is less specific
- Resin is either negatively charged (**cation exchanger**) **or** positively charged (**anion exchanger**)

Weakly acidic: carboxymethyl (CM) cellulose

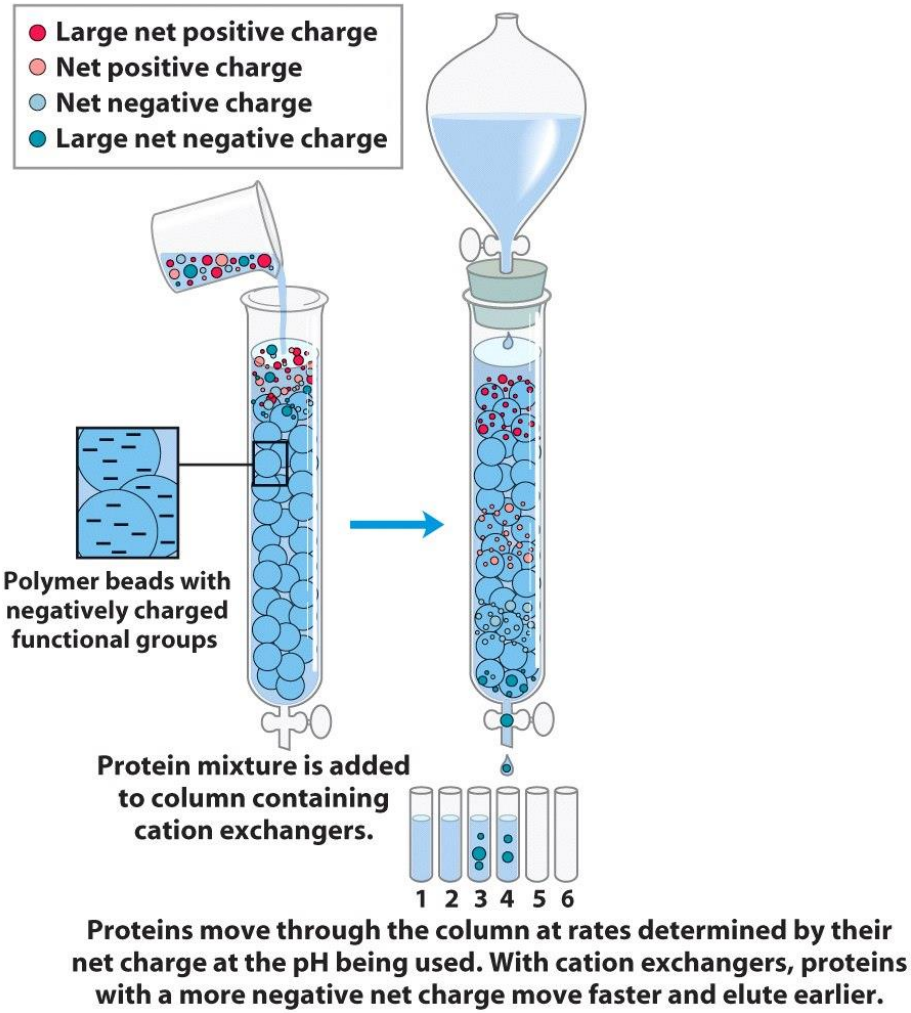


Weakly basic: diethylaminoethyl (DEAE) cellulose



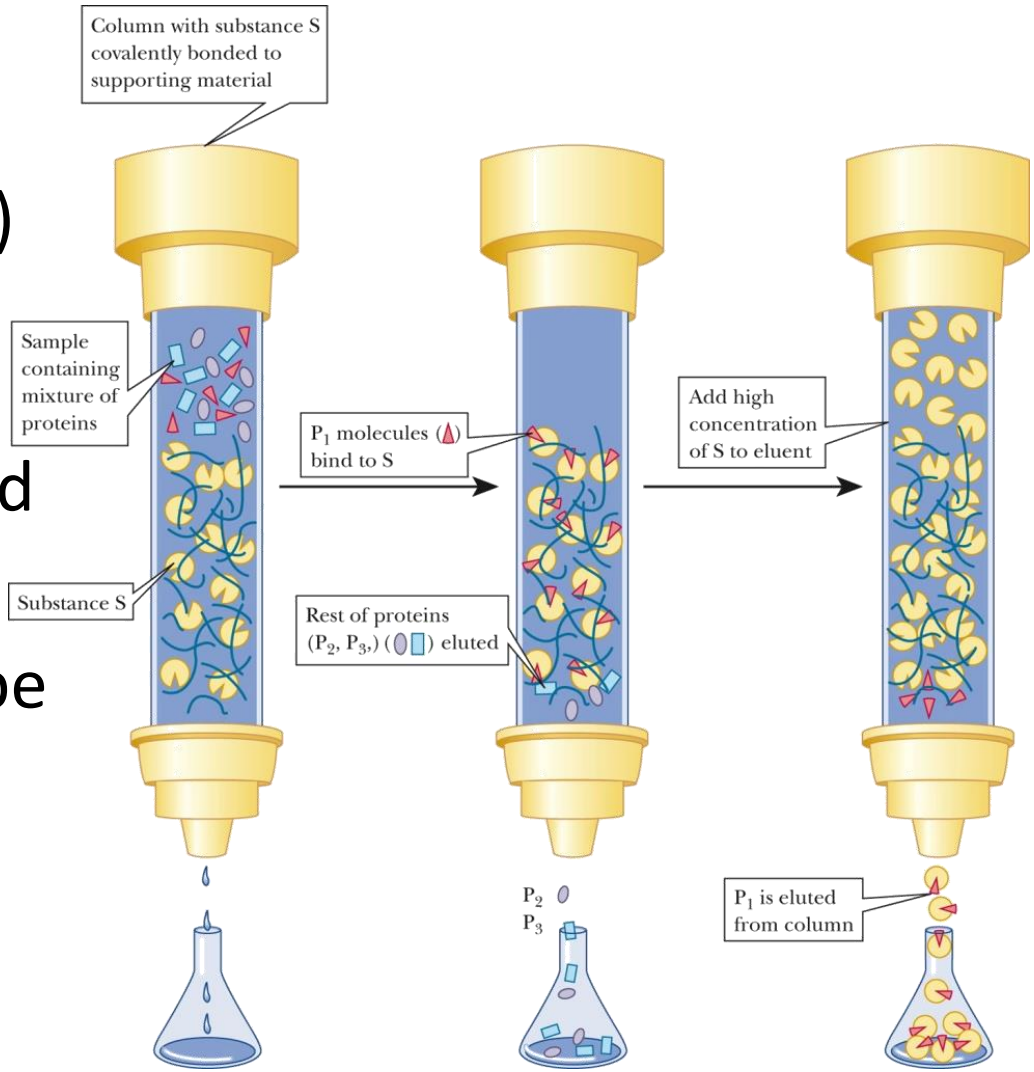
Ion-exchange chromatography

- 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)



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



Affinity chromatography

- 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)