Slide : Protein Purification and Characterization Techniques

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Sections : 1,2,3

■ Slide □ Sheet

Biochemistry

Mousa Suboh

Protein Purification and Characterization Techniques

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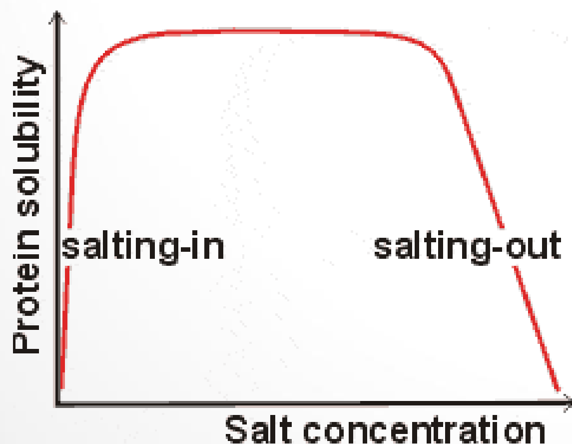
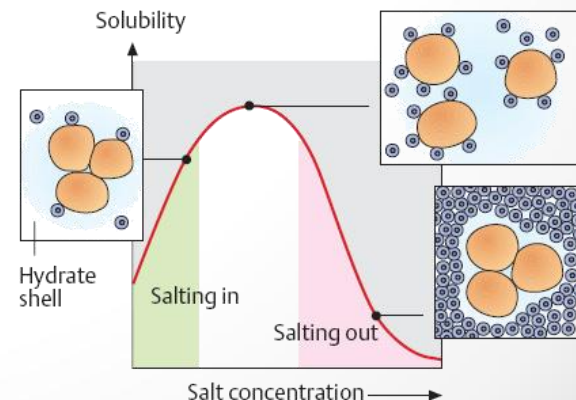
Extracting Pure Proteins from Cells

- 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

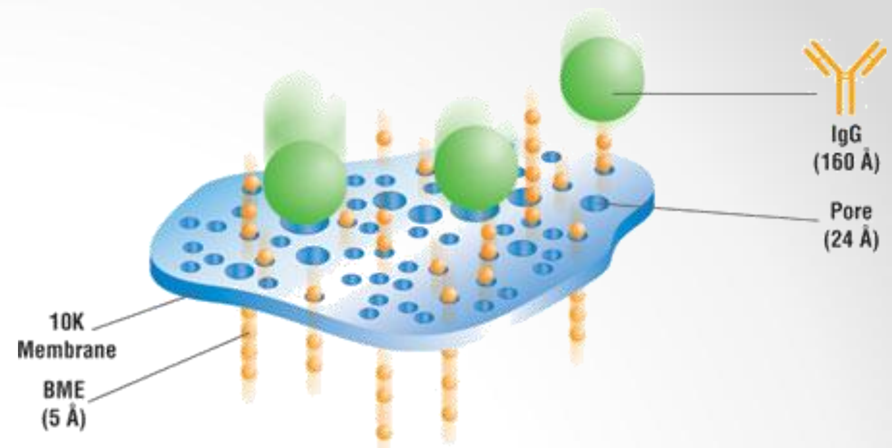
- Are proteins soluble? If yes, to which limit?
- Salt stabilizes the various charged groups on a protein molecule & enhance the polarity of water, thus attracting protein into the solution & enhancing the solubility of protein
- Ammonium sulfate is the most common reagent to use at this step
- This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

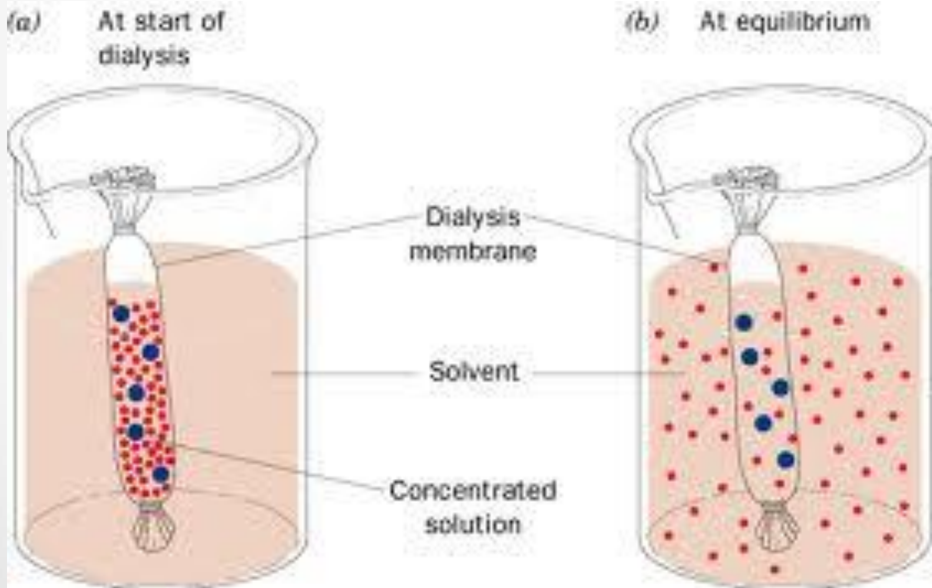




Dialysis

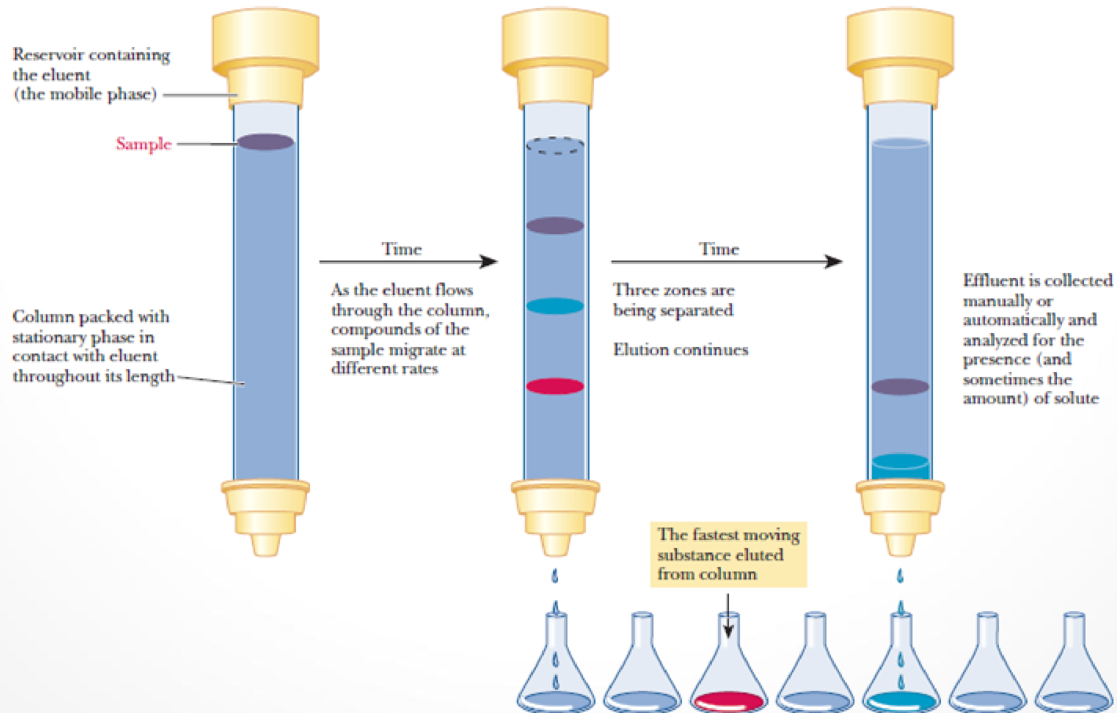
- Principle of diffusion
- Concept of MW cut-off
- Pure vs. crude





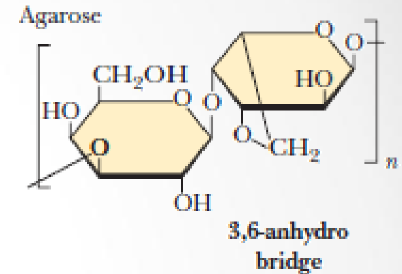
Column Chromatography

- 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?

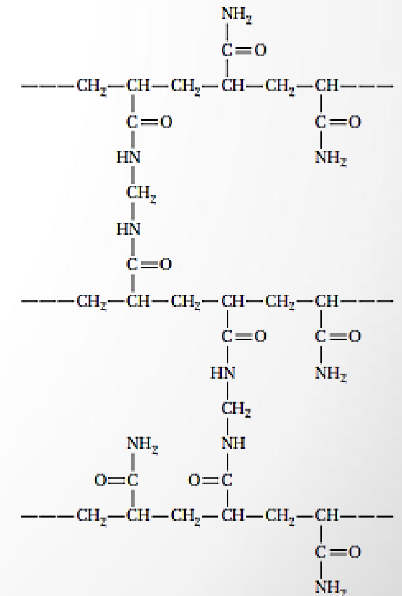


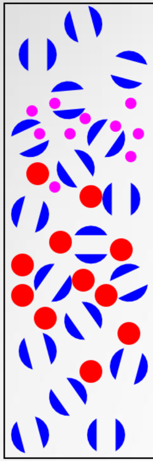
Size-exclusion chromatography Gel-filtration chromatography

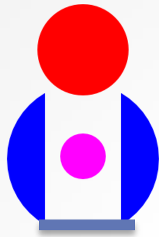
- Separation on the basis of size (MW)
- Stationary (cross-linked gel particles): consist of one of two kinds of polymers; the 1st is a carb. polymer (ex. **dextran** or **agarose);** often referred to by Sephadex & Sepharose The 2nd is based on **polyacrylamide**



- 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



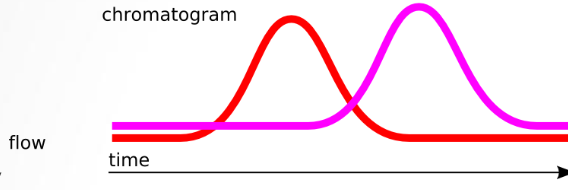




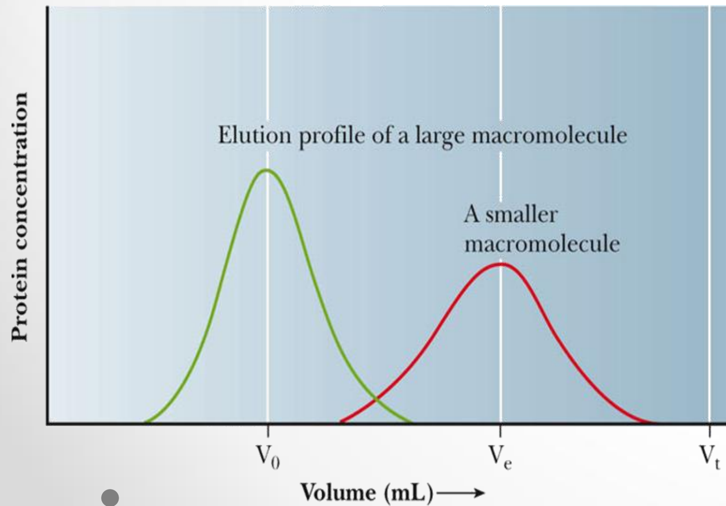
Large particles cannot enter gel and are excluded. They have less volume to traverse and elute sooner.

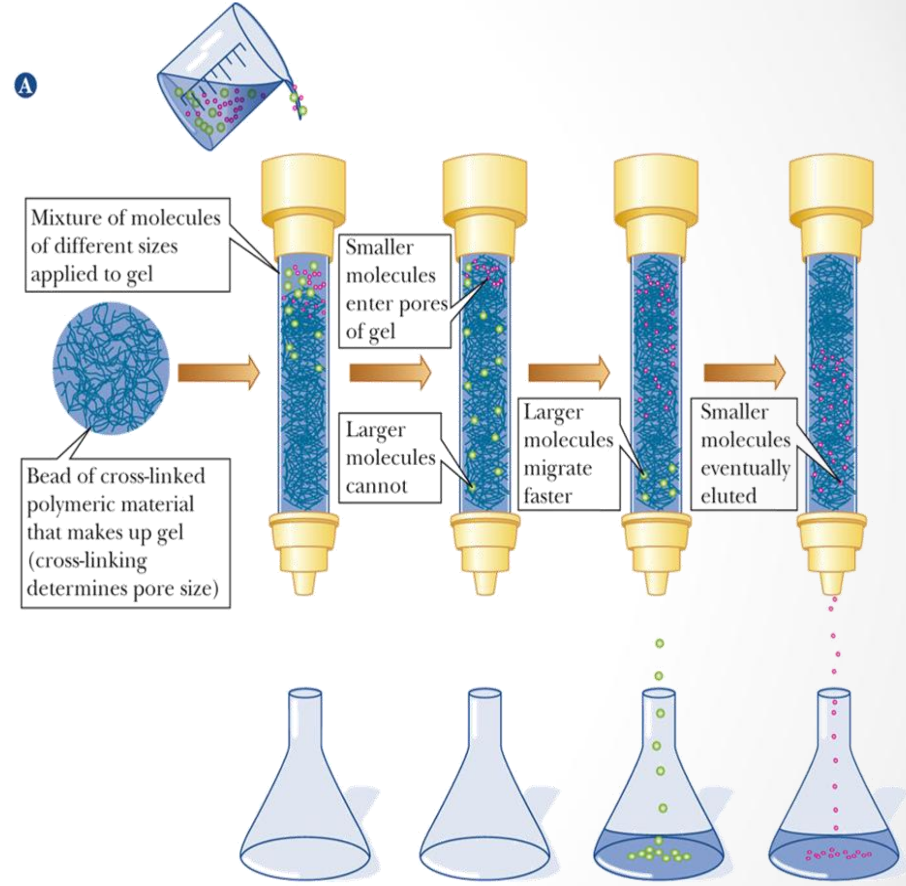
Small particles can enter gel and have more volume to traverse. They elute later.

flow



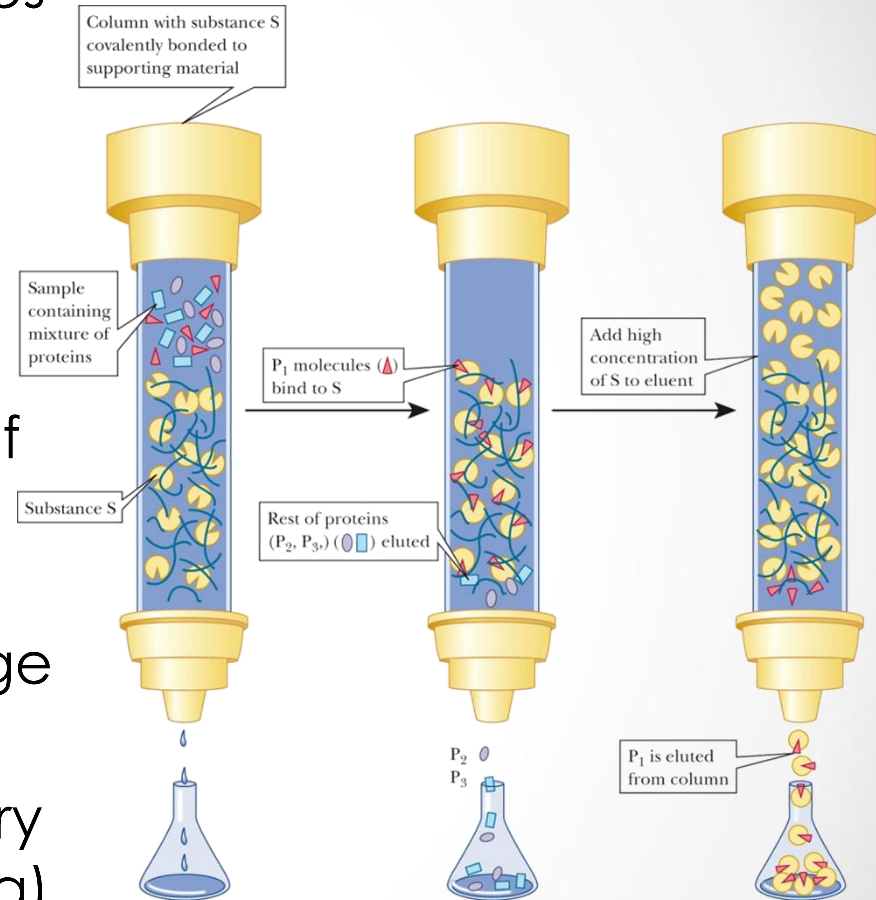
Molecular-sieve chromatography





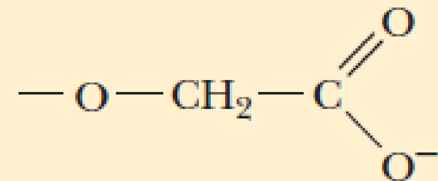
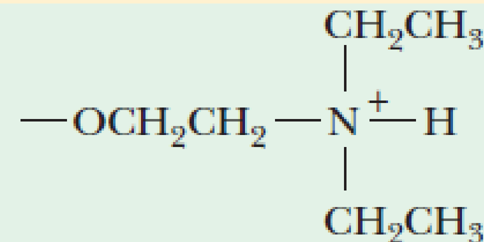
Affinity chromatography

- 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)



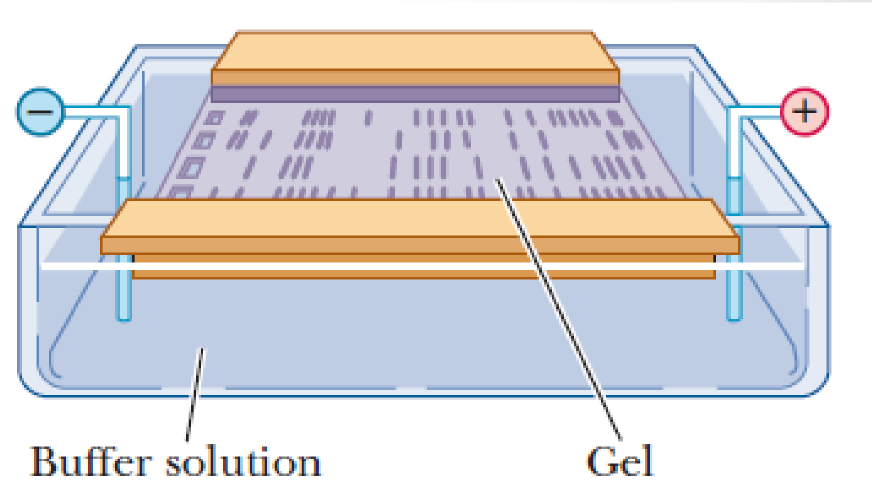
Ion-exchange chromatography

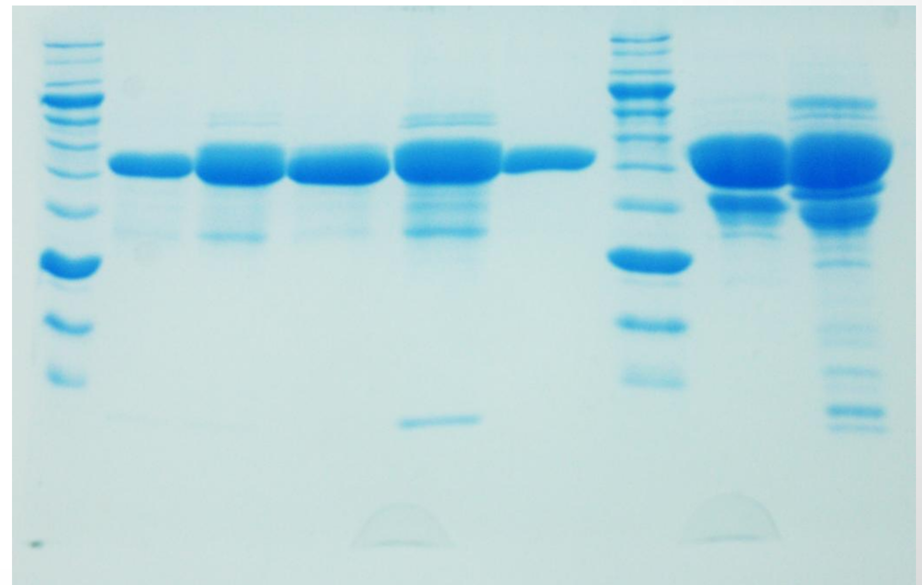
- Interaction based on net charge & is less specific
- Resin is either negatively charged (**cation exchanger**) **or** positively charged (**anion exchanger**)
- Buffer equilibration, exchange resin is bound to counter-ions. A cation-exchange resin is usually bound to Na+ or K+ ions, and an anion exchanger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

Weakly acidic: carboxymethyl (CM) cellulose	
Weakly basic: diethylaminoethyl (DEAE) cellulose	

Electrophoresis

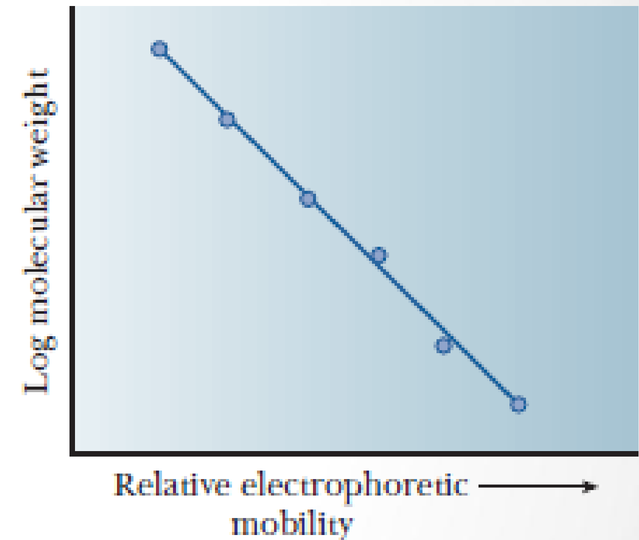
- Based on the motion of charged particles in an electric field
- Macromolecules have differing mobilities based on their charge, shape, and size
- The most common medium is a polymer of agarose or acrylamide





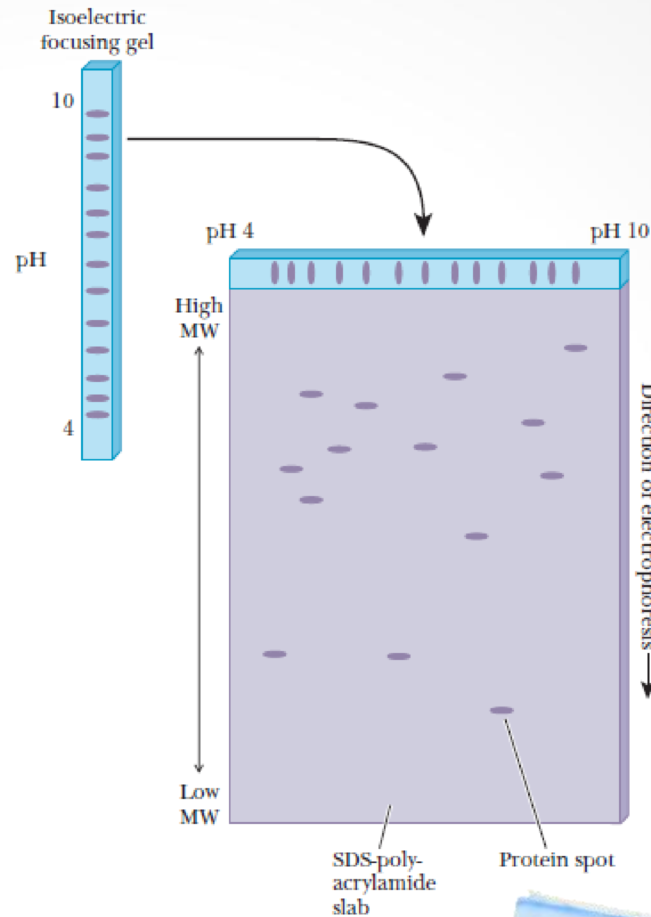
Agarose vs. PAGE

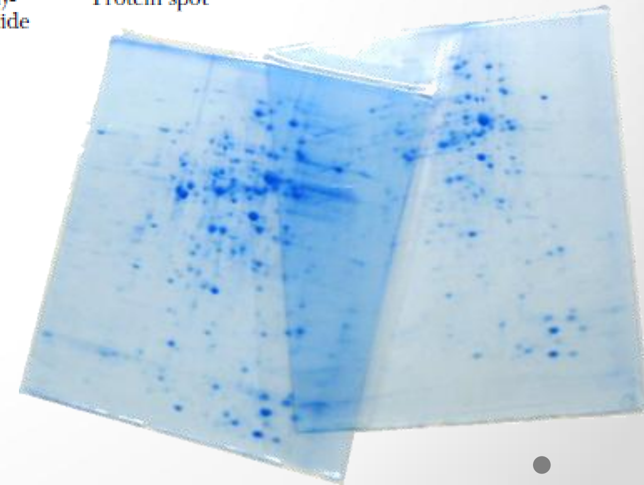
- Agarose (nucleic acids), PAGE (proteins)
- In PAGE: SDS or NO-SDS $\{CH_3(CH_2)_{10}CH_2OSO_3Na^+\}$
- SDS completely denatures proteins (multi-subunit proteins)
- Acrylamide offers higher resistance to large molecules
- Shape and charge are approximately the same (sizes is the determining factor)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



Isoelectric focusing

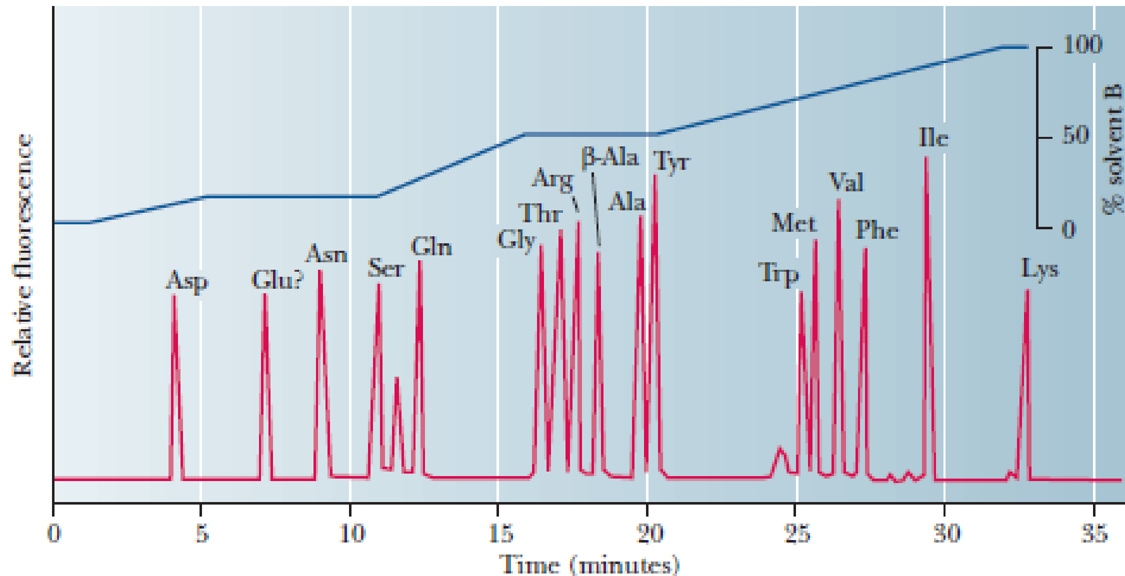
- Proteins have different isoelectric points
- Gel prepared with a pH gradient parallel to electric-field gradient
- Two-dimensional gel electrophoresis (2-D gels)

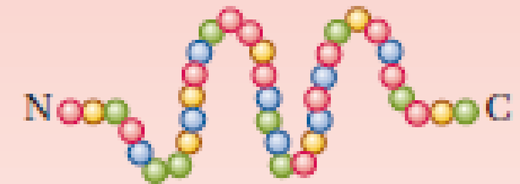




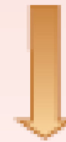
Protein sequencing - Edman Method

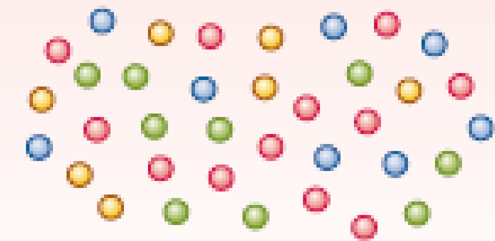
- Step 1: how much and which amino acids are involved
- **Hydrolysis** (heating + HCl) **& Separation** (ion-exchange chromatography or by **high performance liquid chromatography, HPLC)**





Hydrolyze to constituent amino acids





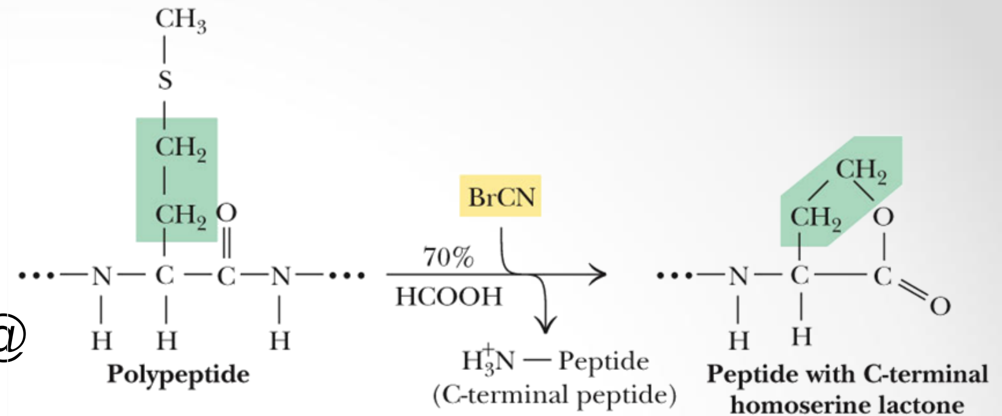
Separate and identify individual amino acids

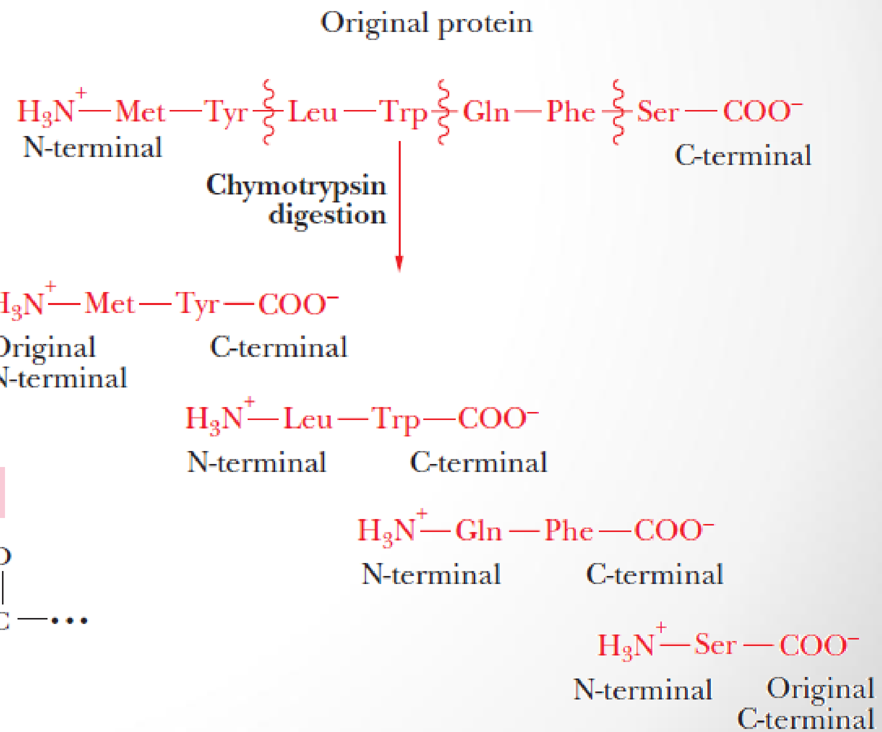
Protein sequencing - Edman Method

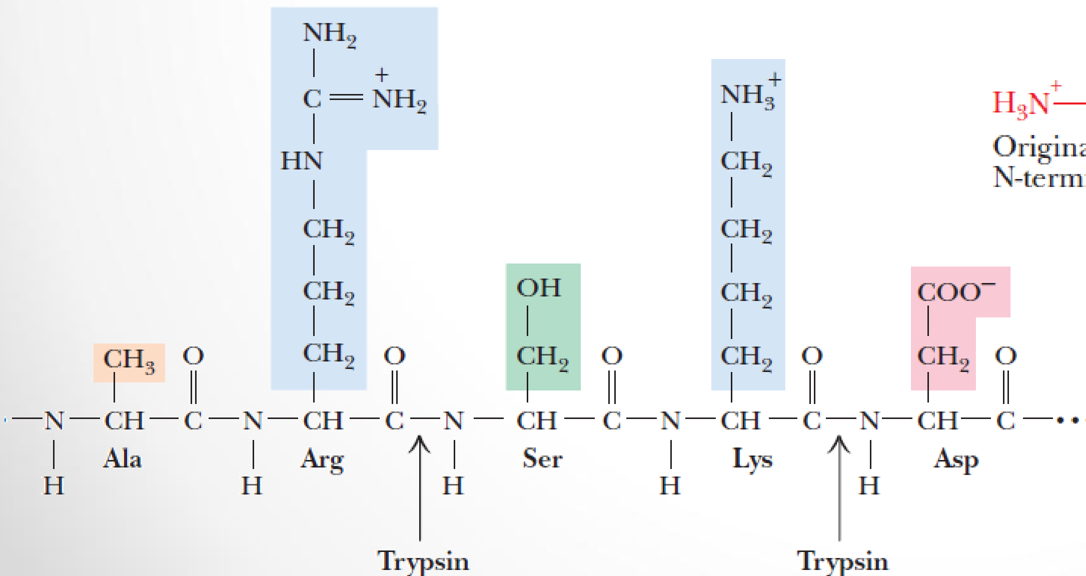
- Step 2: determining the identities of N-terminal and C-terminal ends of protein
- Necessary esp. to determine if the protein consists of one or two polypeptide chains
- Steps 3: cleavage into smaller fragments **(Edman degradation)**

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

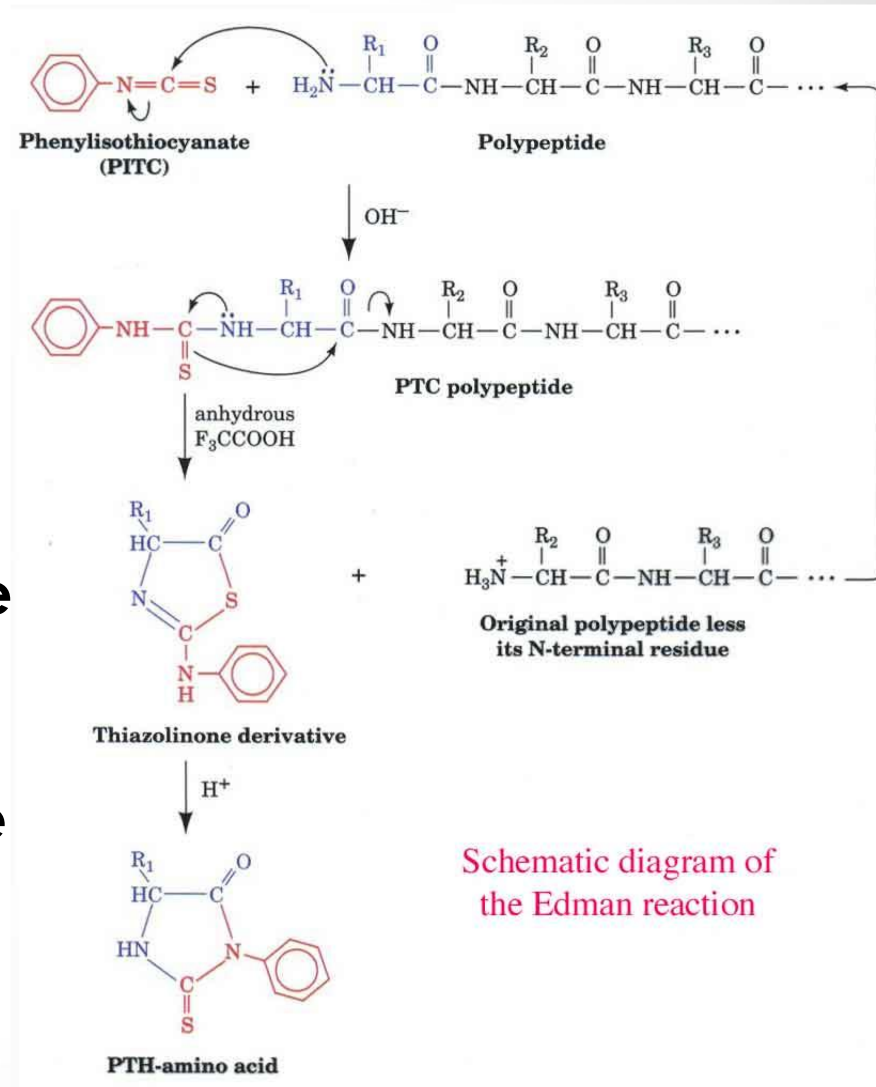






Technique

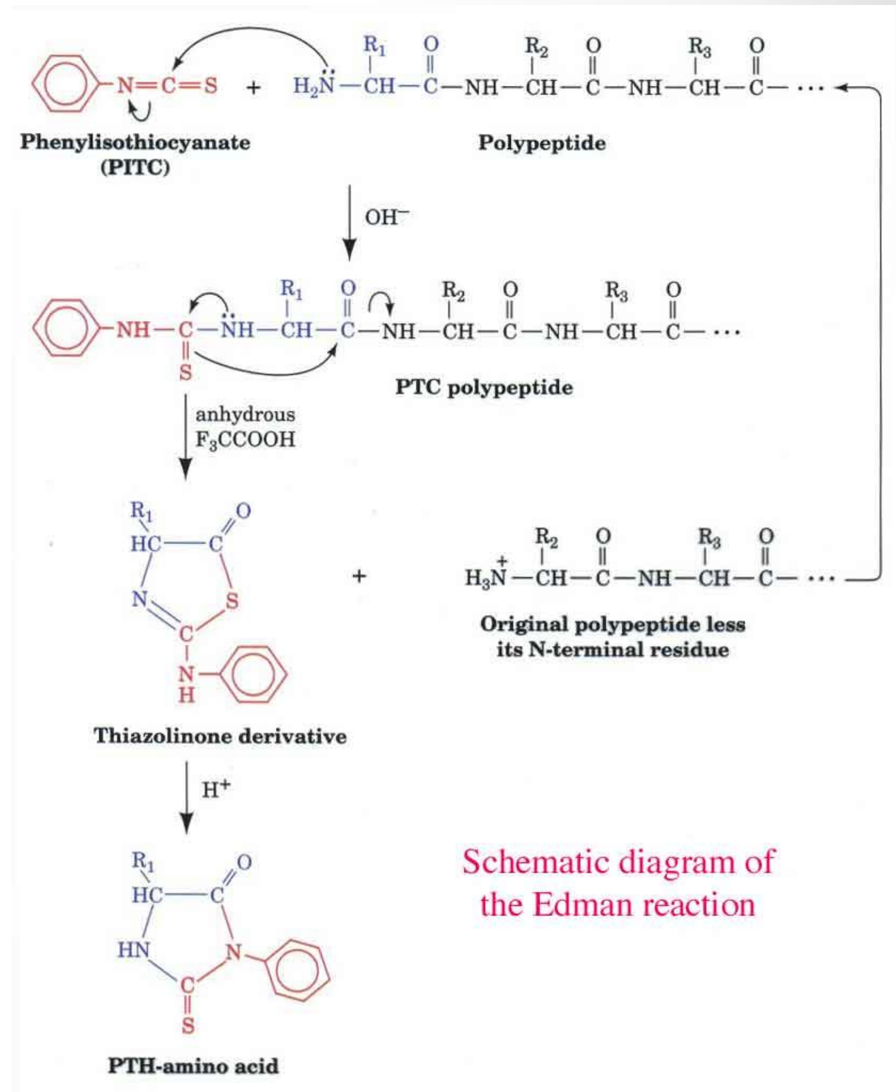
1) Peptides are immobilized onto polyvinylidene fluoride (PVDF) membrane
2) Under mildly alkaline conditions, phenylisothiocyanate (PITC) is reacted with an uncharged terminal group on the amino acid chain to form a phenylthiocarbamoyl derivative
3) Which is cleaved using Trifluoroacetic acid producing its anilinothiazolinone derivative (ATZ-amino acid). The next terminal amino acid is now exposed and ready for the same reactions to occur



Schematic diagram of the Edman reaction

Technique

4) A wash is performed to remove excess buffers and reagents and the ATZ amino acid is selectively extracted with ethyl acetate and converted to a more stable phenylthiohydantoin (PTH)-amino acid derivative
5) Identification of the PTH amino acid derivative (chromatography or electrophoresis)
6) The process can now be repeated



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