Protein Purification and Characterization Techniques

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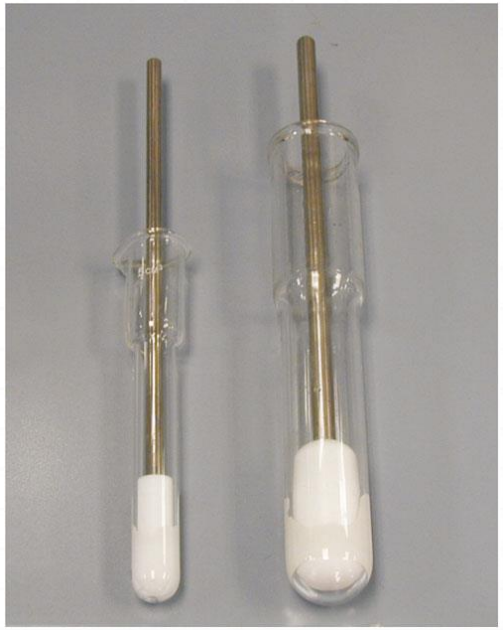
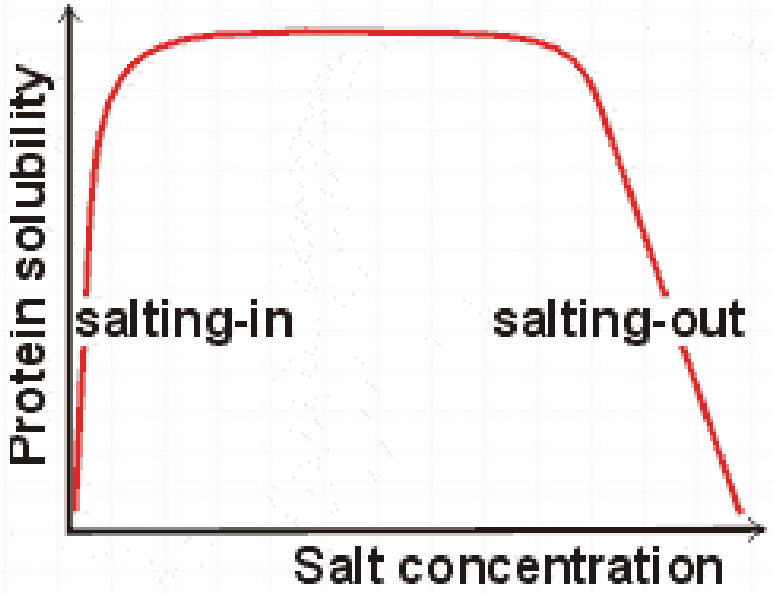
Extracting Pure Proteins from Cells

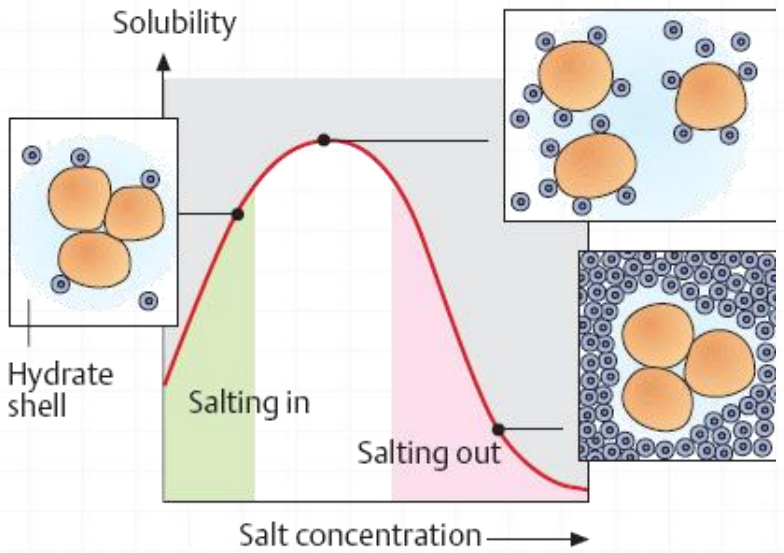
- Purification techniques focus mainly on size & charge
- The first step is **homogenization** (grinding, Potter–Elvejhem homogenizer, sonication, freezing and thawing, detergents)
- **Differential centrifugation (**600 *g:* unbroken cells & nuclei; 15,000 *g:* mitochondria; 100,000 *g:* ribosomes and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



Salting in & out

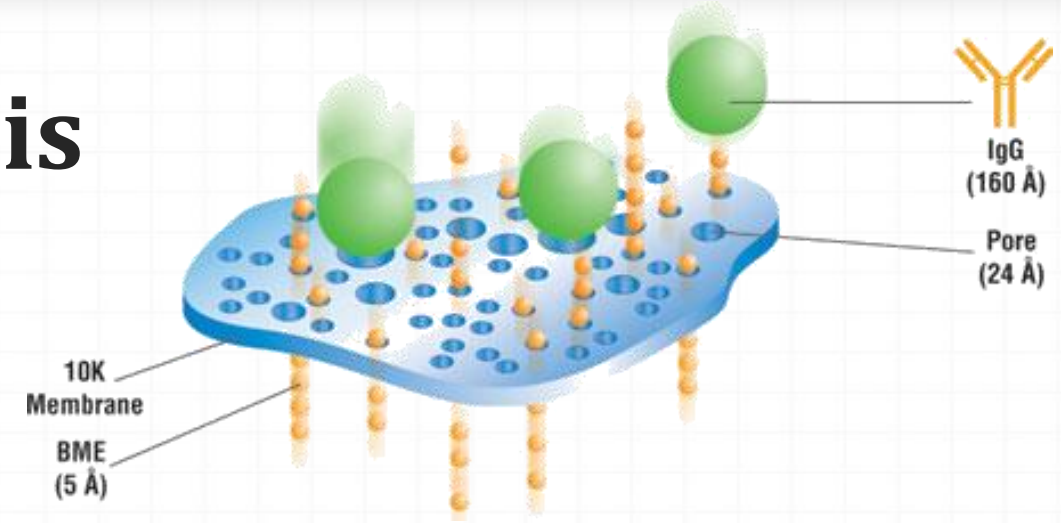
- Are proteins soluble? If yes, to which limit?
- Salt stabilizes the various charged groups on a protein molecule and enhance the polarity of water and increases the ionic strength, thus attracting protein into the solution and enhancing the solubility of protein
- Ammonium sulfate is the most common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

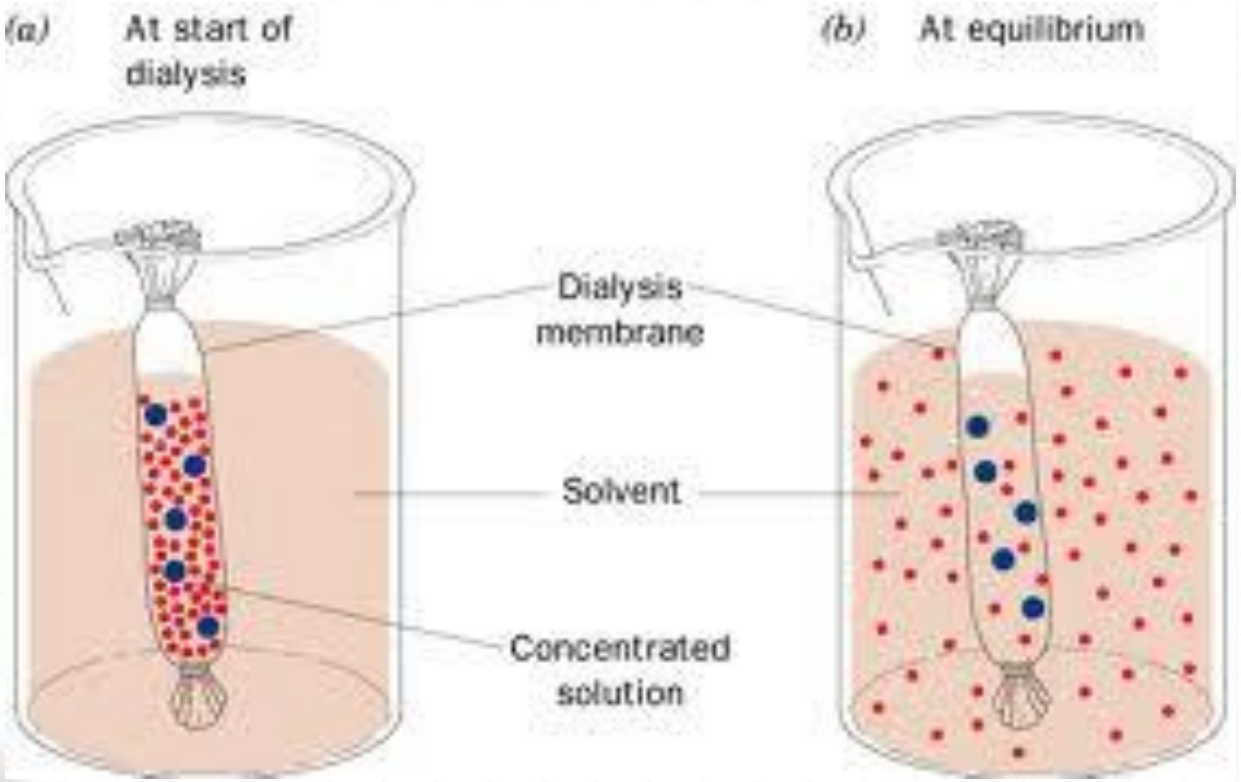




Dialysis

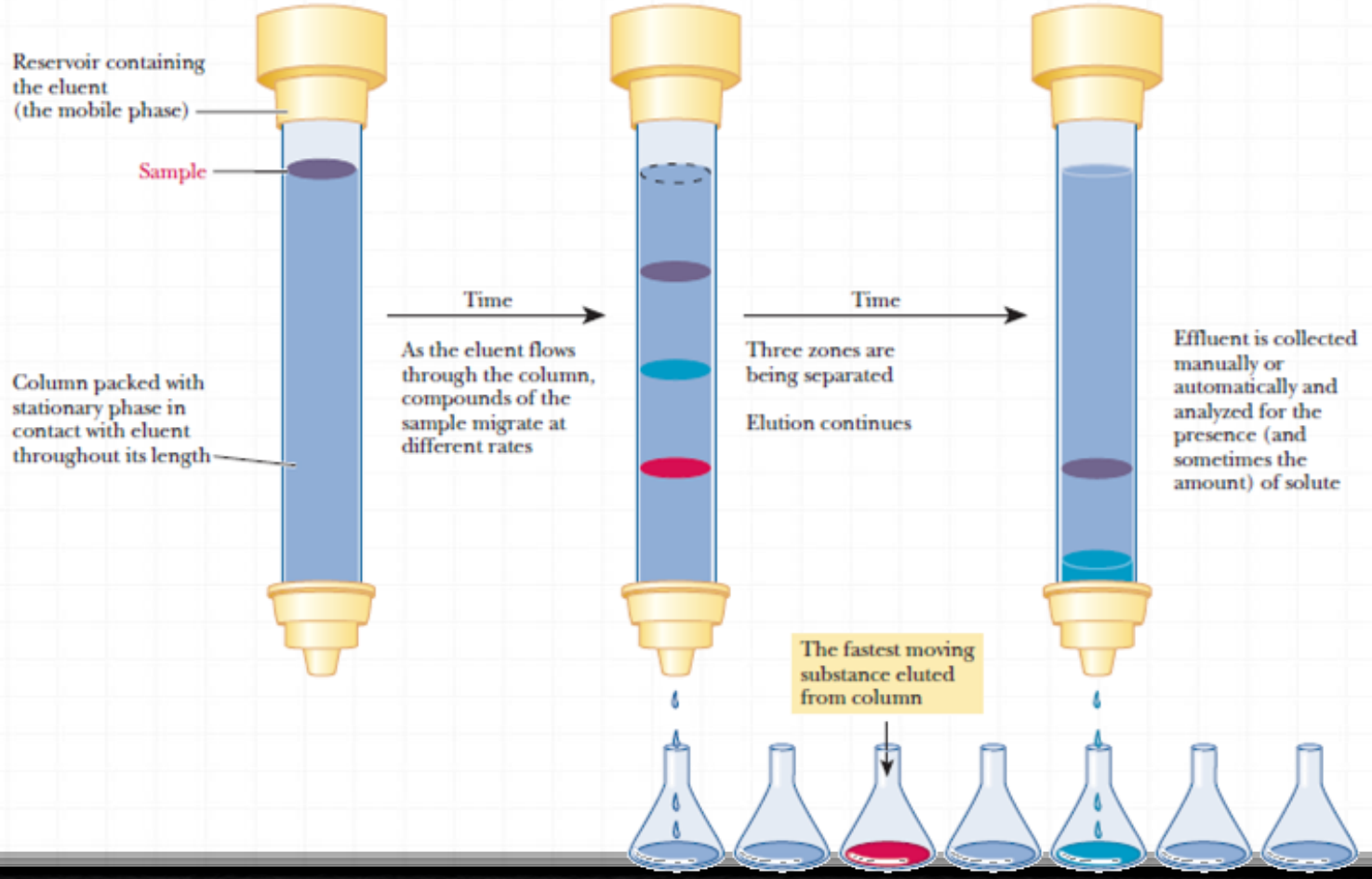
- Principle of diffusion
- Concept of MW cut-off
- Pure vs. crude



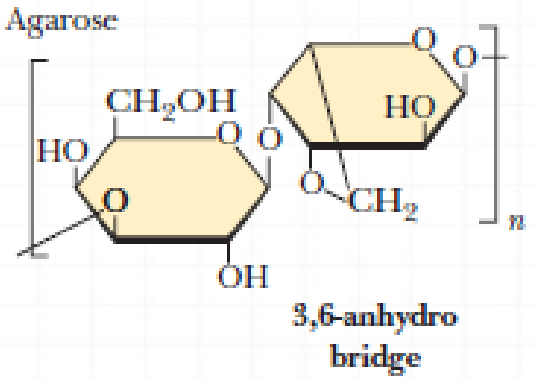


Column Chromatography

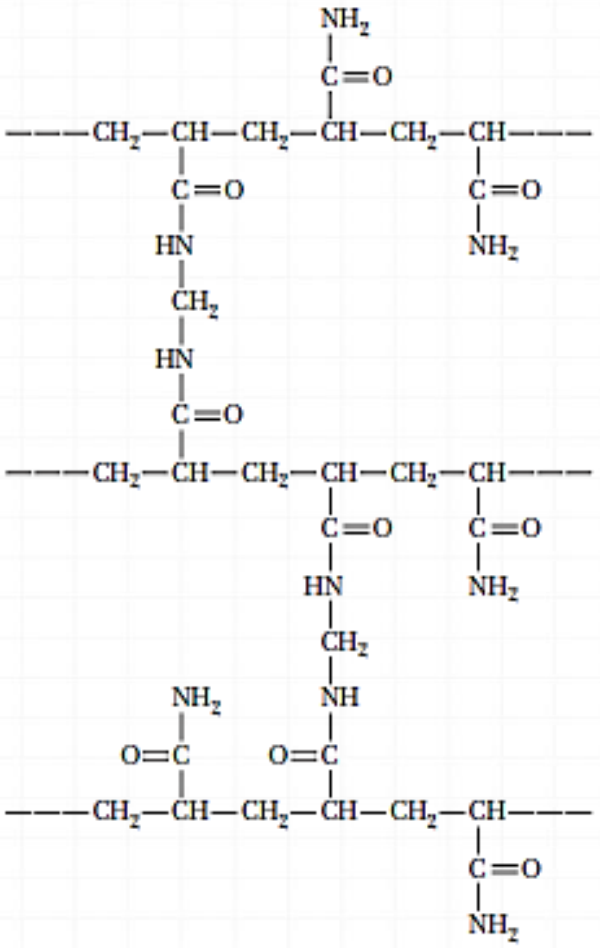
- Greek *chroma*, "color," and *graphein*, "to write"
- Is it just for colourful proteins?
- Chromatography is based on two phases: stationary & mobile
- Washing or Elution?
- What are the different kinds?



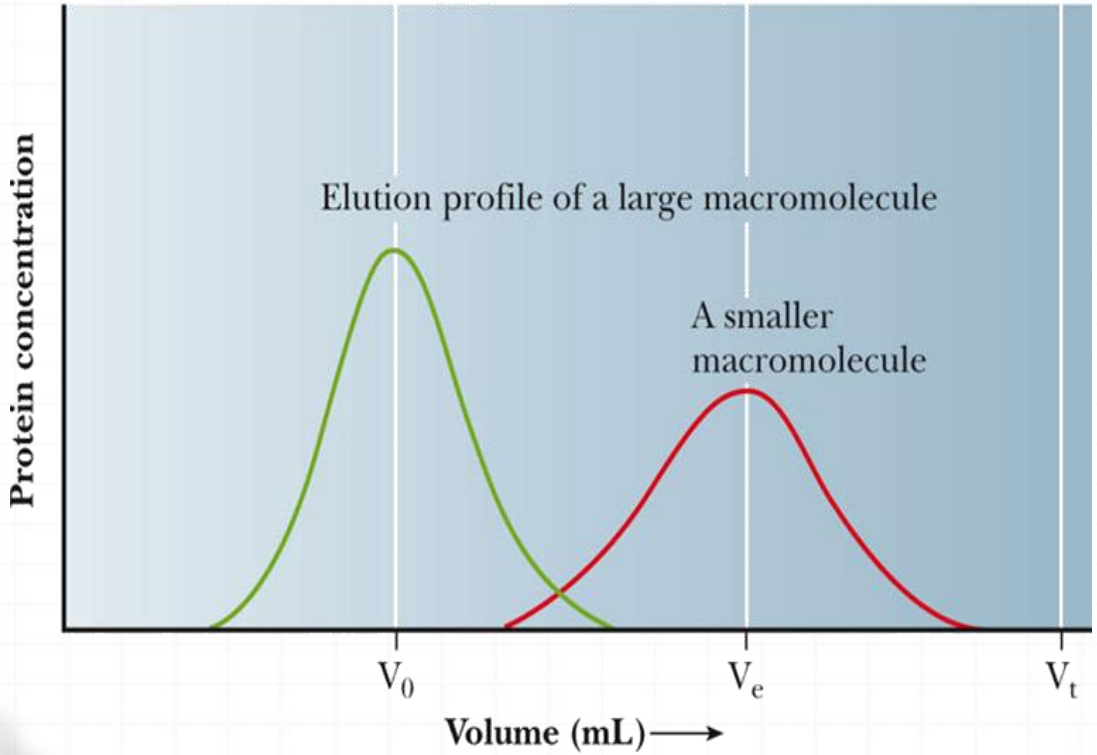
Size-exclusion chromatography
Gel-filtration chromatography

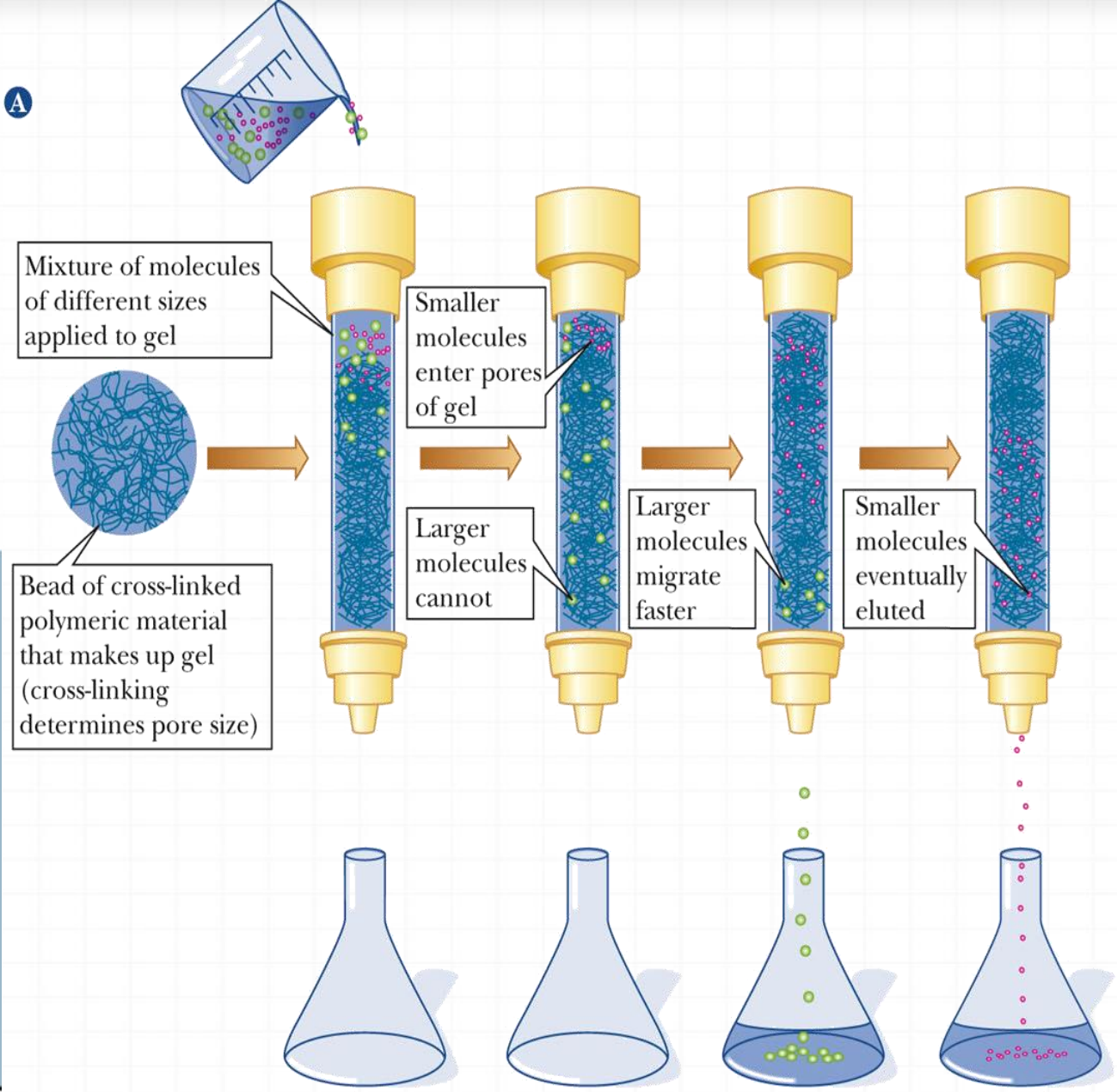


- Separation on the basis of size (MW)
- Stationary (cross-linked gel particles): consist of one of two kinds of polymers; the 1st is a carb. polymer (ex. **dextran** or **agarose);** often referred to by Sephadex and Sepharose. The 2nd is based on **polyacrylamide** (Bio-Gel)
- Extent of crosslinking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



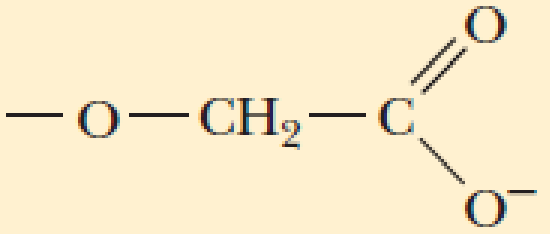
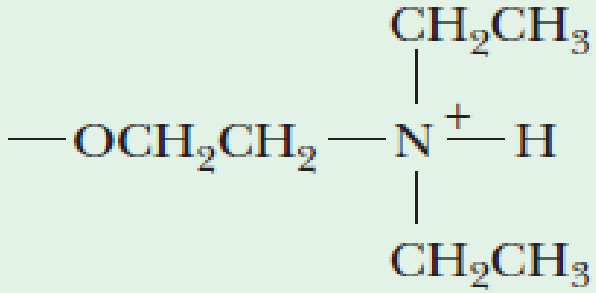
Molecular-sieve chromatography





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)

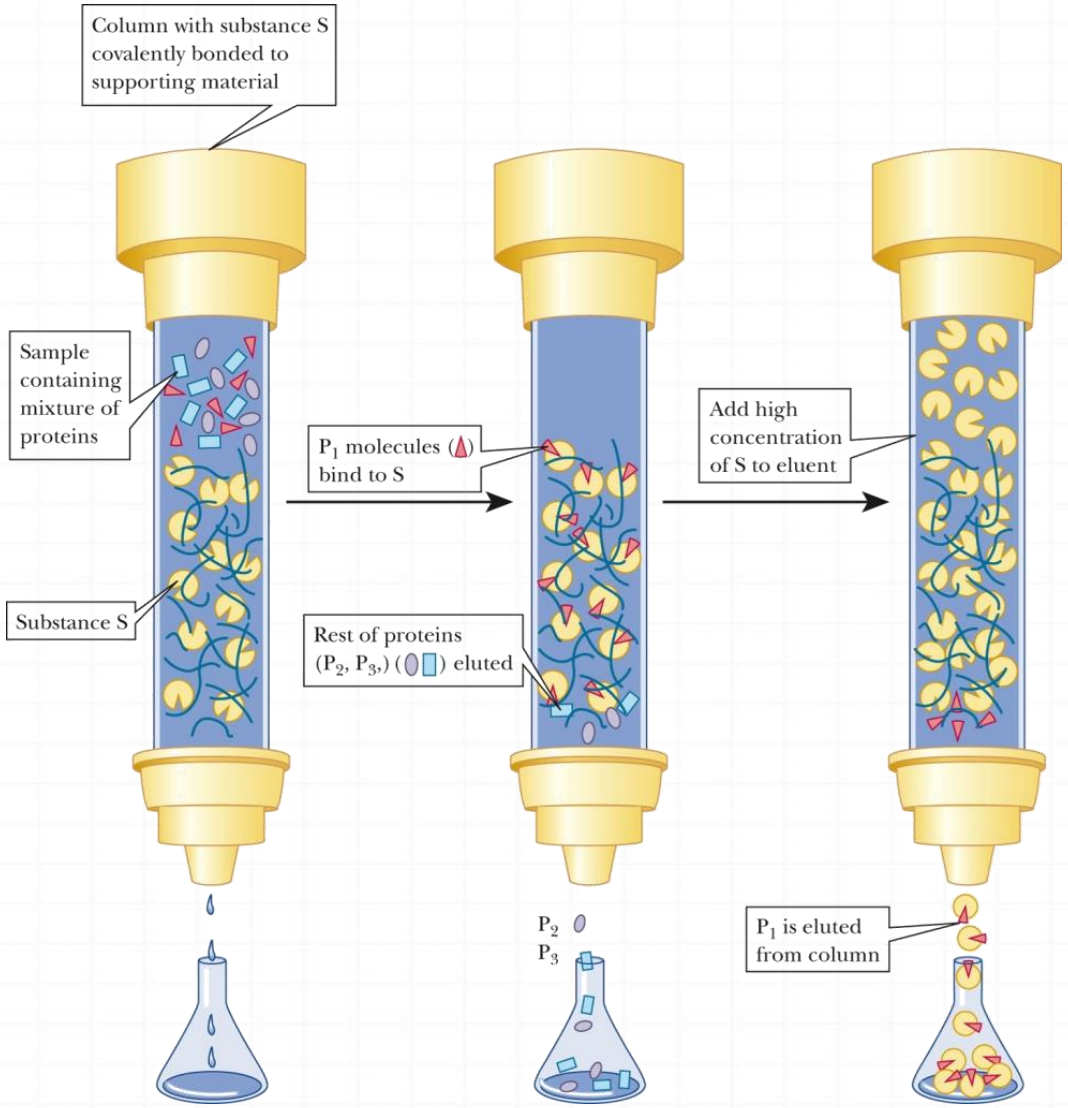
Weakly acidic: carboxymethyl (CM) cellulose	
Weakly basic: diethylaminoethyl (DEAE) cellulose	

Problem

- You have 5 different proteins (#1, #2, #3, #4, and #5), with different isoelectric points (pIs).
  - pI#5 = 2.3
  - pI#4 = 4.7
  - pI#1 = 7.2
  - pI#2 = 9.1
  - pI#3 = 12.1
- Starting the column at pH 6.5, the sample is added and then washed to remove unbound molecules. What is the order of protein elution in a
  - Cationic-exchange chromatography?
  - An anionic exchange chromatography?

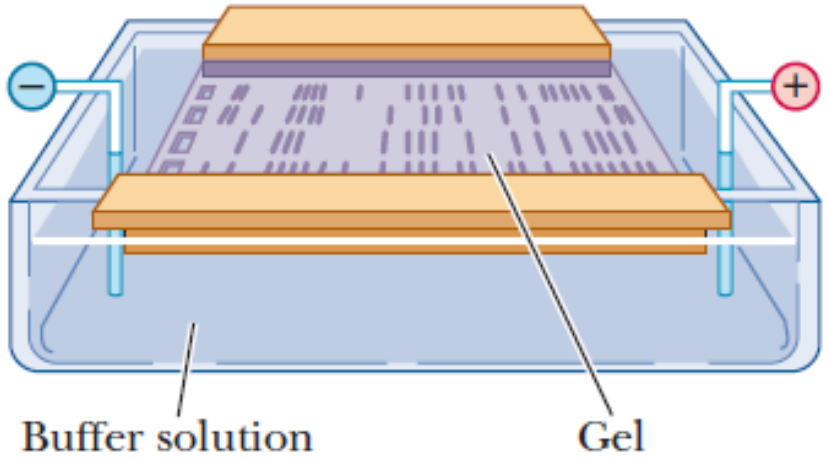
Affinity chromatography

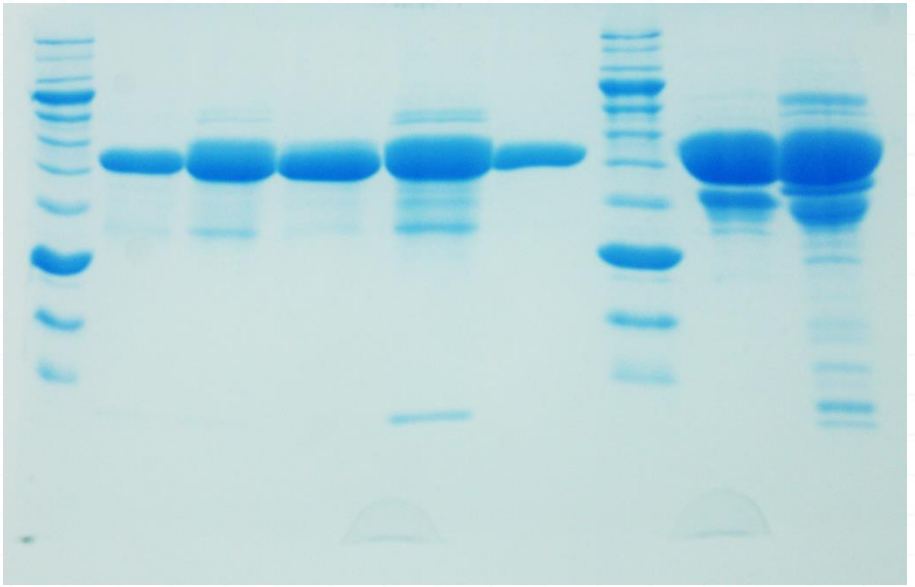
- It has specific binding properties
- The polymer (stationary) is covalently linked to a *ligand* that binds specifically to the desired protein
- The bound protein can be eluted by adding high conc. of the soluble ligand
- Protein–ligand interaction can also be disrupted with a change in pH or ionic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



Electrophoresis

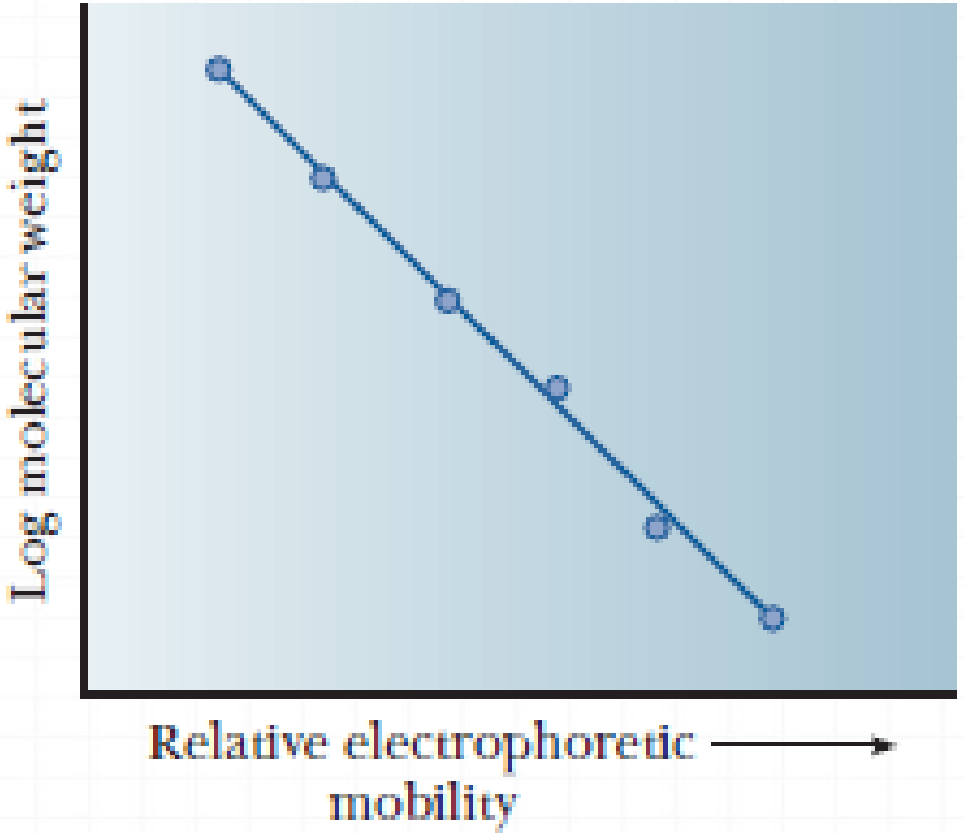
- Based on the motion of charged particles in an electric field
- Macromolecules have differing mobilities based on their charge, shape, and size
- The most common medium is a polymer of agarose or acrylamide





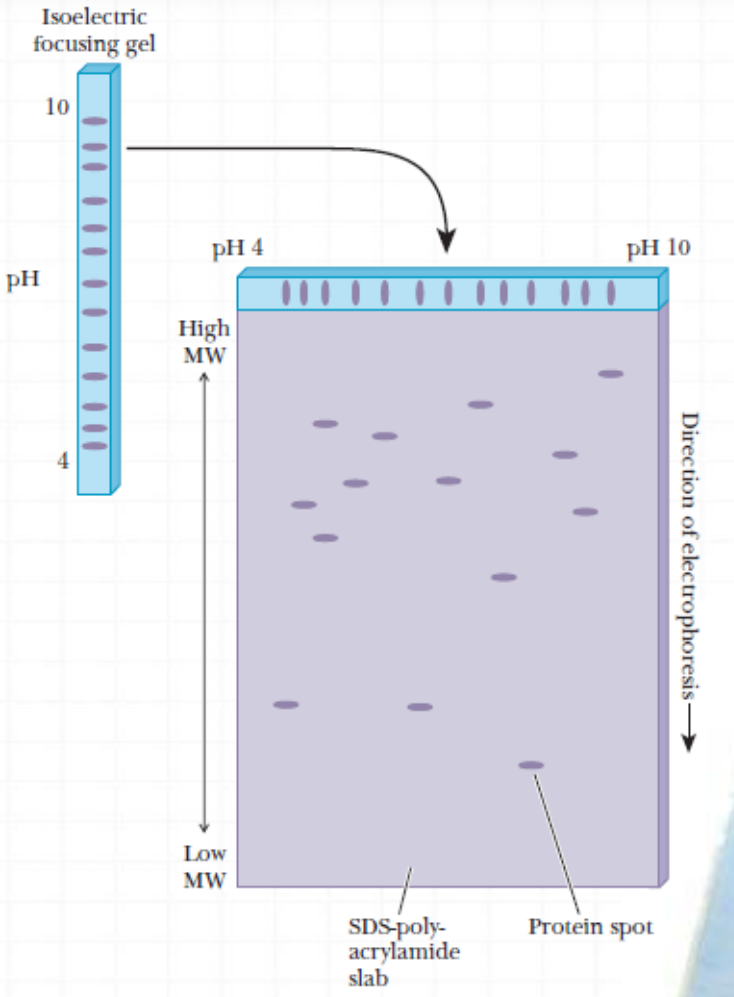
Agarose or PAGE?

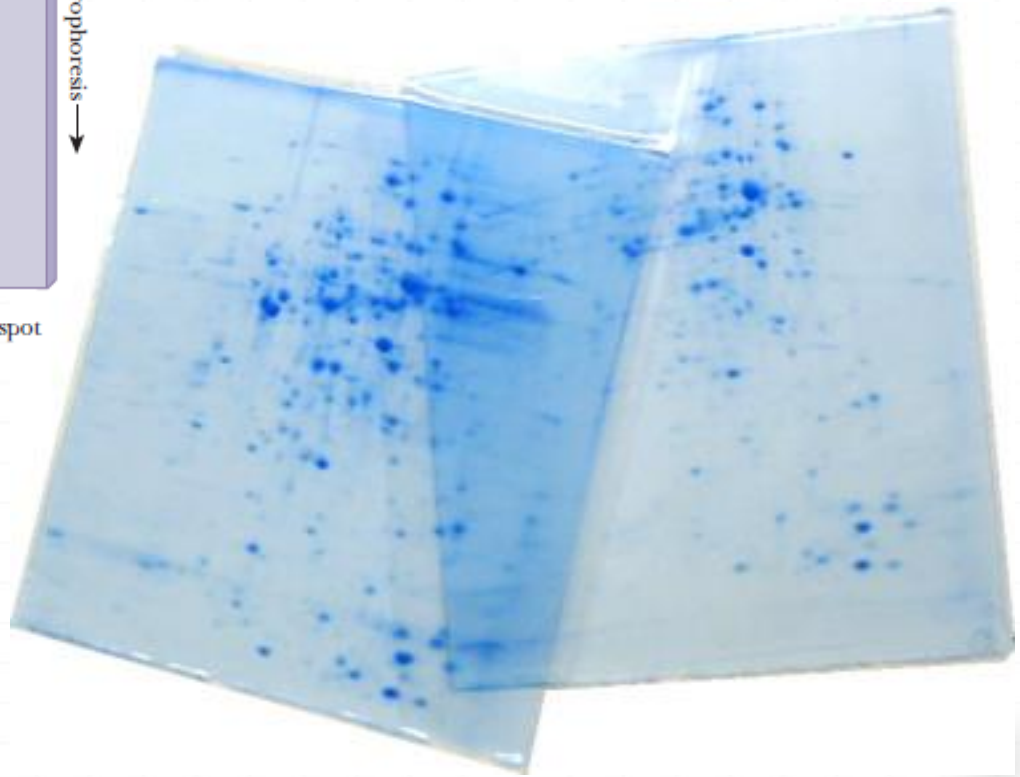
- Agarose (nucleic acids), PAGE (proteins)
- In PAGE: SDS or NO-SDS, detergent, $CH_3(CH_2)_{10}CH_2OSO_3Na^+$
- SDS completely denatures proteins (multi-subunit proteins)
- Acrylamide offers higher resistance to large molecules
- Shape and charge are approximately the same (sizes is the determining 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)





Questions

- Describe the protein's structure based on the following results of SDS-PAGE:

1. Under non-reducing condition, a protein exists as one 40-KDa band. Under reducing conditions, the protein exists as two 20-KDa bands.
2. Under non- reducing condition, a protein exists as two bands, 30 KDa and 20 KDa. Under reducing conditions, the protein also exists as two bands, 15 KDa and 10 KDa.
3. Under non- reducing condition, a protein exists as two bands, 40 KDa and 20 KDa. Under reducing conditions, the protein exists as one bands of 20 KDa.

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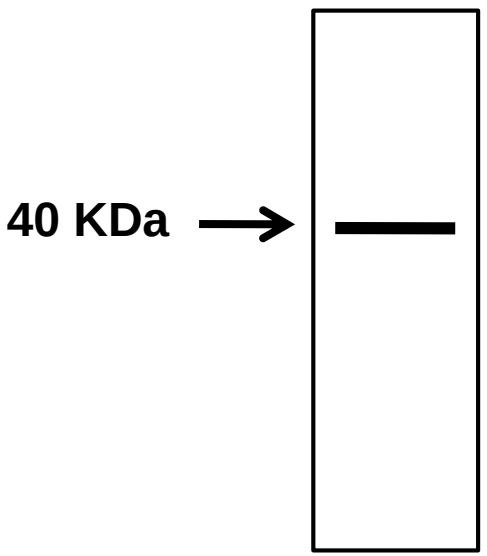
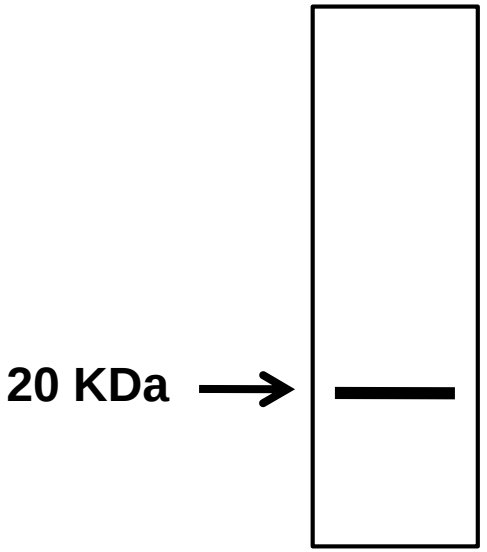
Non-reducing



Reducing



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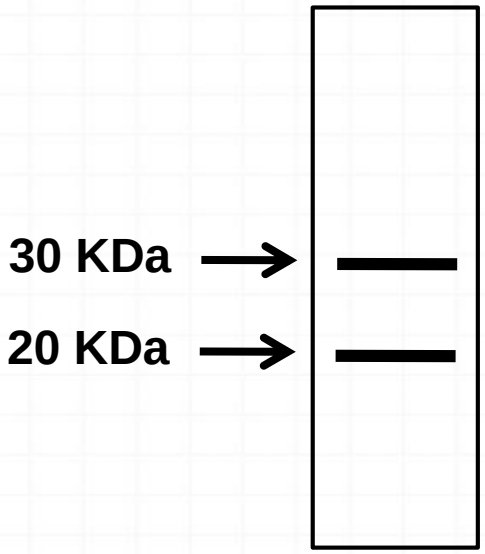
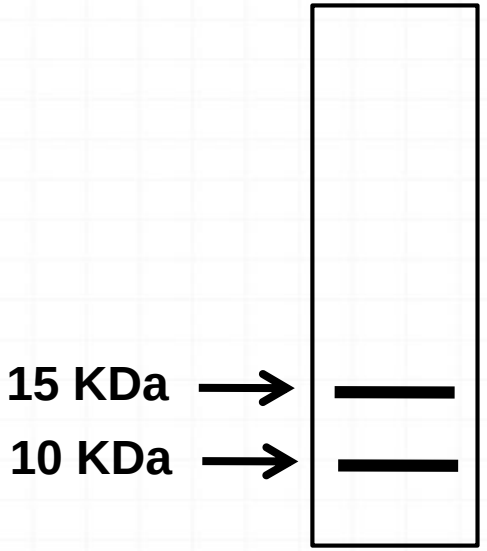
Non-reducing



Reducing



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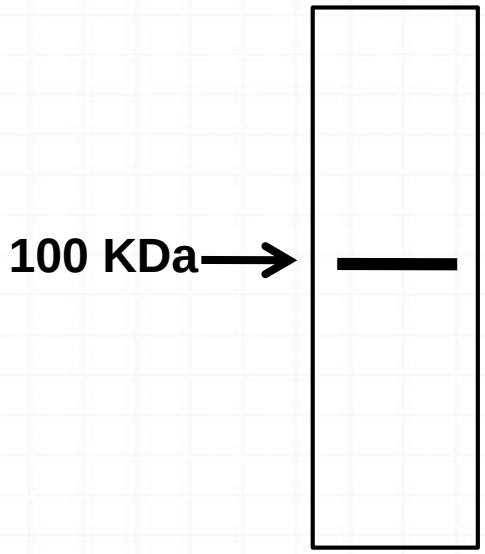
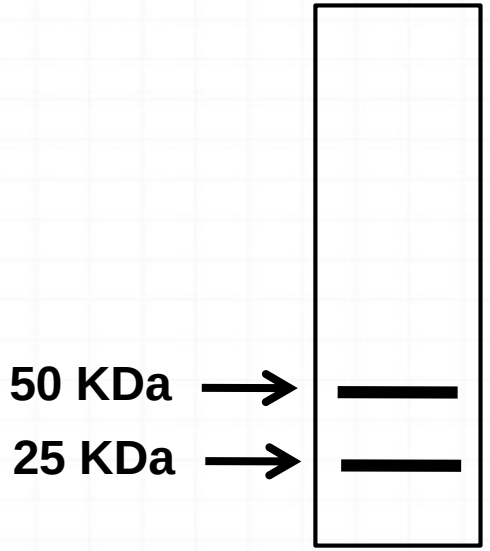


Reducing



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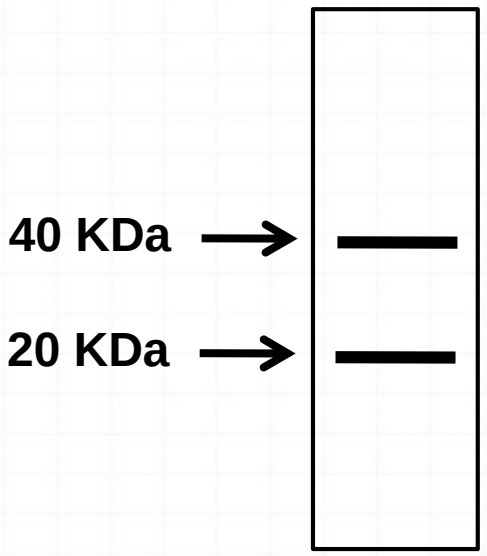
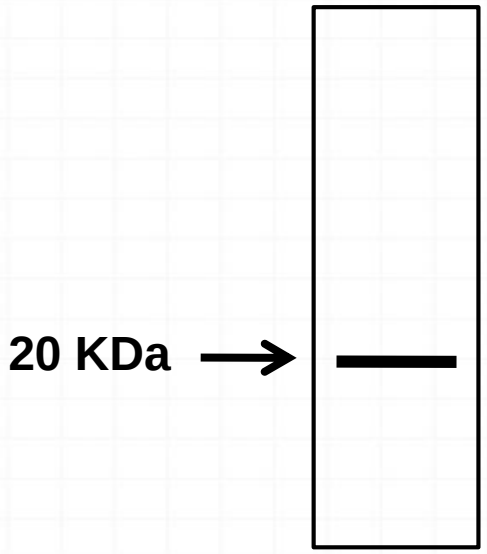
Non-reducing



Reducing



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