Protein Purification

Gel filtration / Mass Exclusion chromatography: separate by size

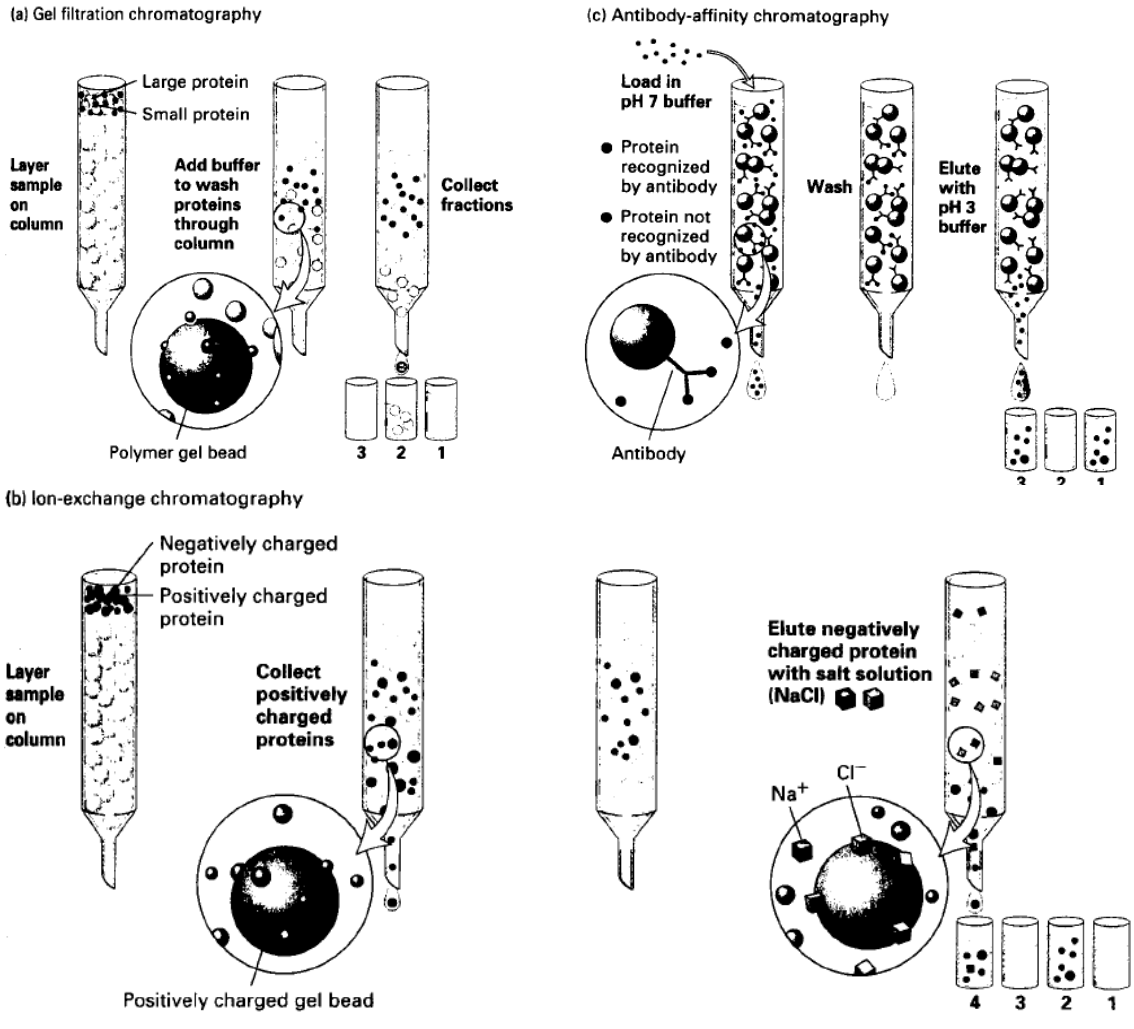
- Porous resins are added into column
- Molecules that are too big to enter the pores travel quickly through the column
- Smaller molecules that enter the pores travel slowly through the column, since they have a longer path to travel

Ion-exchange chromatography: separate by charge

- Charged resins are added into column
- Cation-exchange column to select for positively charged proteins
    - Negatively charged groups on resins attract positively charged proteins and slows down their movement through the column
- Anion-exchange column to select for negatively charged proteins
    - Positively charged groups on resins attract negatively charged proteins and slows down their movement through the column
- Wash column with increasing salt concentrations to elute protein

Affinity chromatography: separate by affinity to a particular ligand (substrate)

- Resin with a particular ligand attached are added into the column
- Proteins that have an affinity to the ligand bind to them, and thus stay in the column; other proteins simply travel through the column and come out first
- Wash column to disrupt the binding between the ligand and attached proteins and elute protein
    - Do so by adding salt, changing pH, changing solvent, adding free ligand, adding compounds that bind more strongly to the ligand, or more…



Protein Electrophoresis

Native PAGE: separate by size of native conformation

- PAGE = PolyAcrylamide Gel Electrophoresis
- Polyacrylamide/bisacrylamide (crosslinker) form a "web-like" net
- An electric field is placed across the gel; negatively charged proteins move to positive pole, and vice versa
- Smaller-sized, more tightly packed molecules are able to travel through the gel more quickly than larger-sized molecules
- "Native" because protein is in original, undisrupted conformation
- Agarose can be used in place of PAGE (although the resolution decreases)

SDS-PAGE: separate by size of denatured conformation (molecular weight)

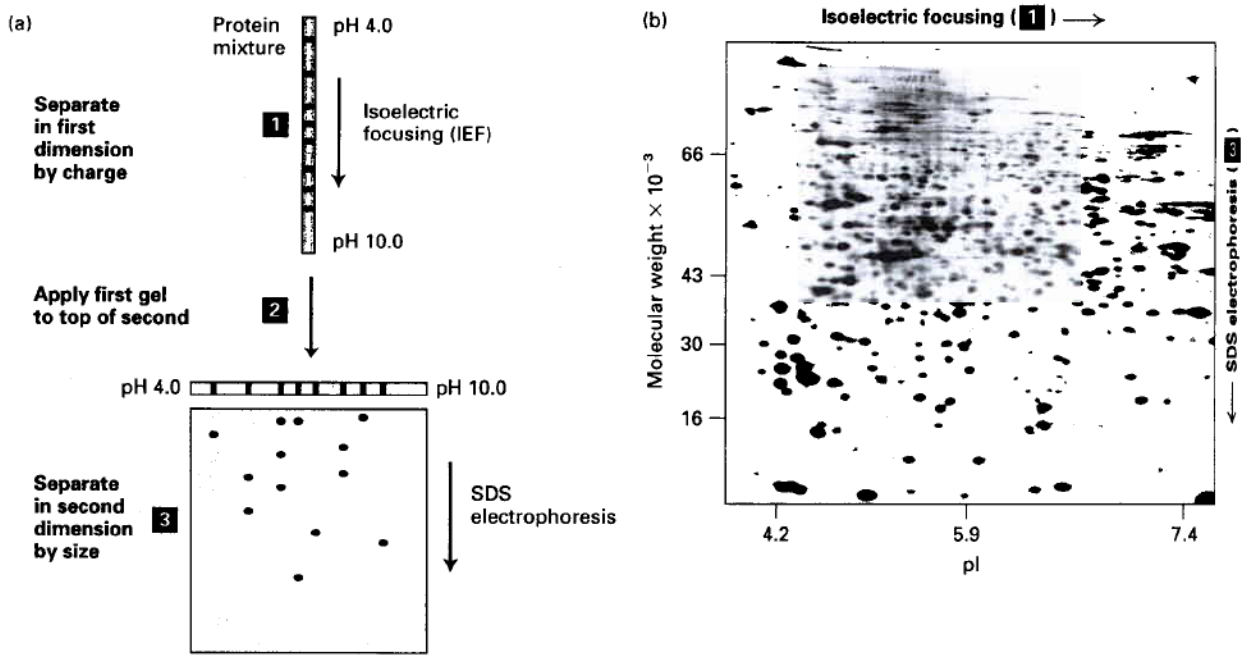
- Similar to above, expect SDS (sodium dodecyl sulfate) is added
- SDS is a detergent that denatures and negatively coats the protein at a constant ratio of 1 SDS/2 AA; thus, proteins have a charge proportional to their size
- β-mercaptoethanol is also added to reduce S-S bonds
- Distance protein travels is proportional to log of molecular weight

Isoelectric focusing (IEF): separate by pI

- A protein's pI is the pH at which the protein has no net charge.
- At a pH > pI, the protein has a negative charge
- At a pH < pI, the protein has a positive charge
- A pH gradient is made in the gel with ampholytes, a mixture of polyanionic and polycationic molecuels
- Proteins move in the gradient until its pI = pH

2D gel electrophoresis: separate by pI and molecular weight

- IEF gel is run first
- SDS-PAGE gel is run on second dimension



Images from Lodish textbook