Isolation and Purification of Proteins

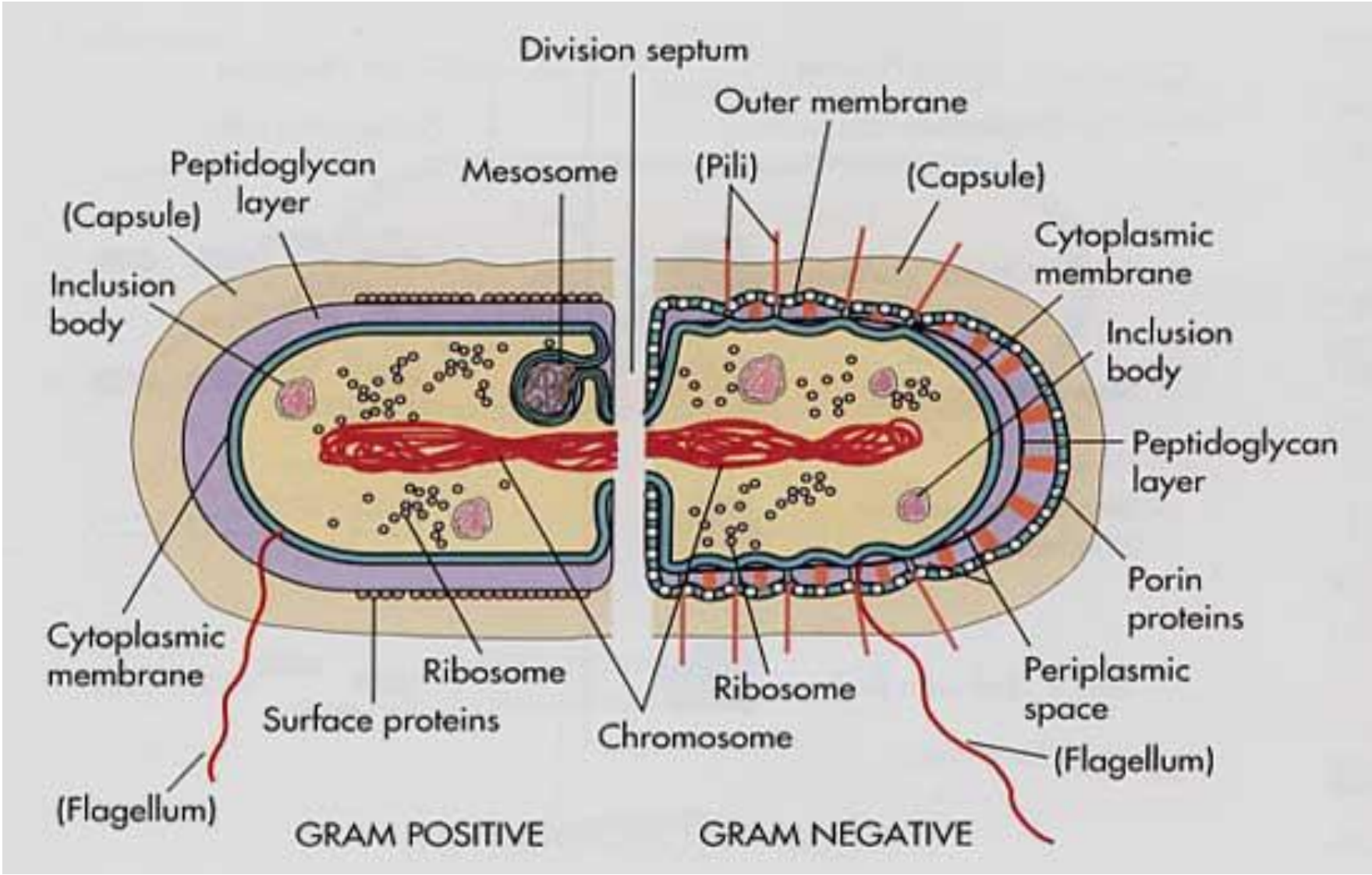
Dr. Aws Alshamsan
Department of Pharmaceutics
Office: AA87
Tel: 4677363
aalshamsan@ksu.edu.sa

Objectives of this Lecture

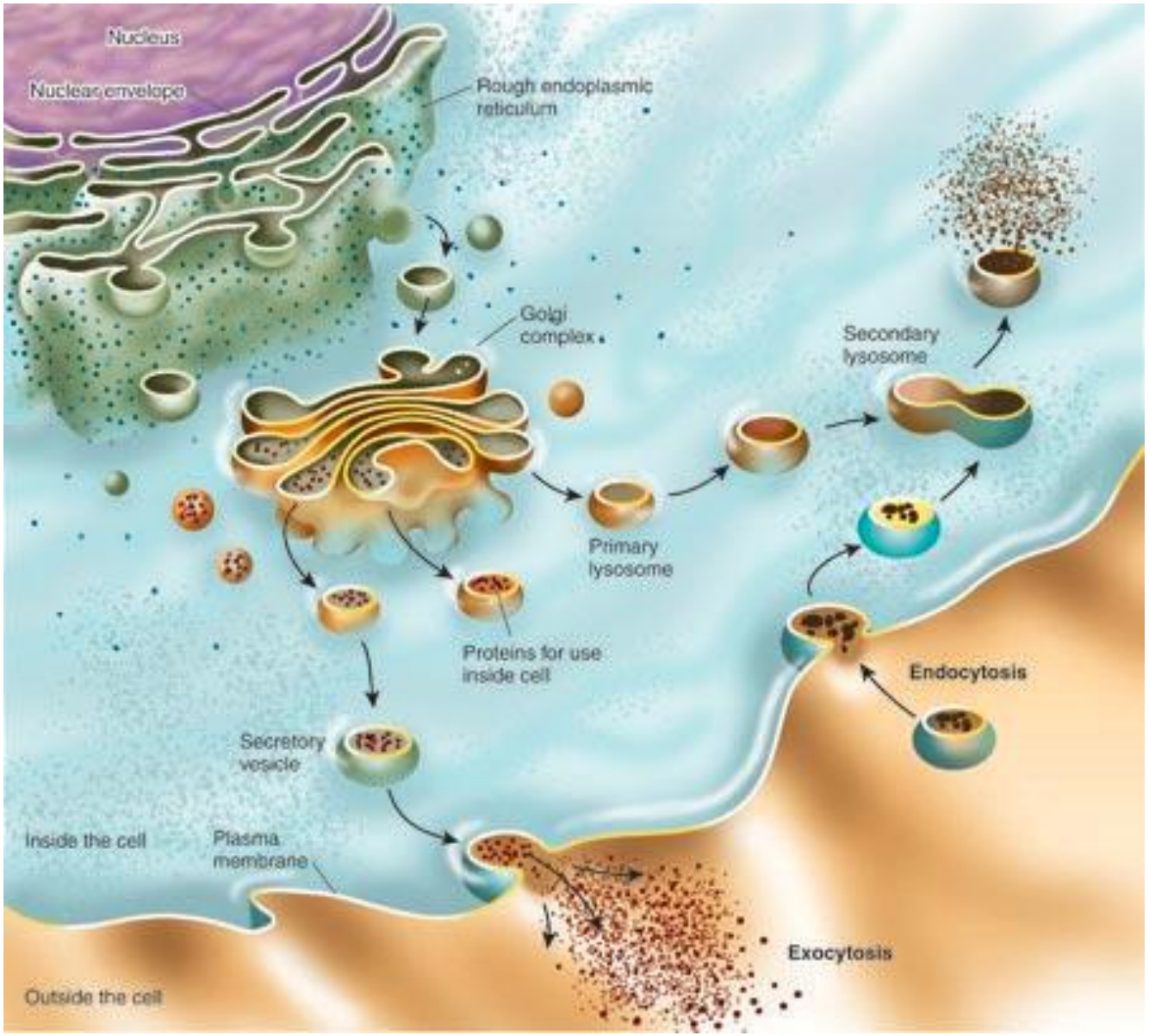
By the end of this lecture you will be able to:

1. Describe most common methods of protein isolation and purification
2. Compare between different methods of protein purification
3. Construct a purification algorithm based on your knowledge in protein purification

Intracellular and Extracellular



Intracellular and Extracellular

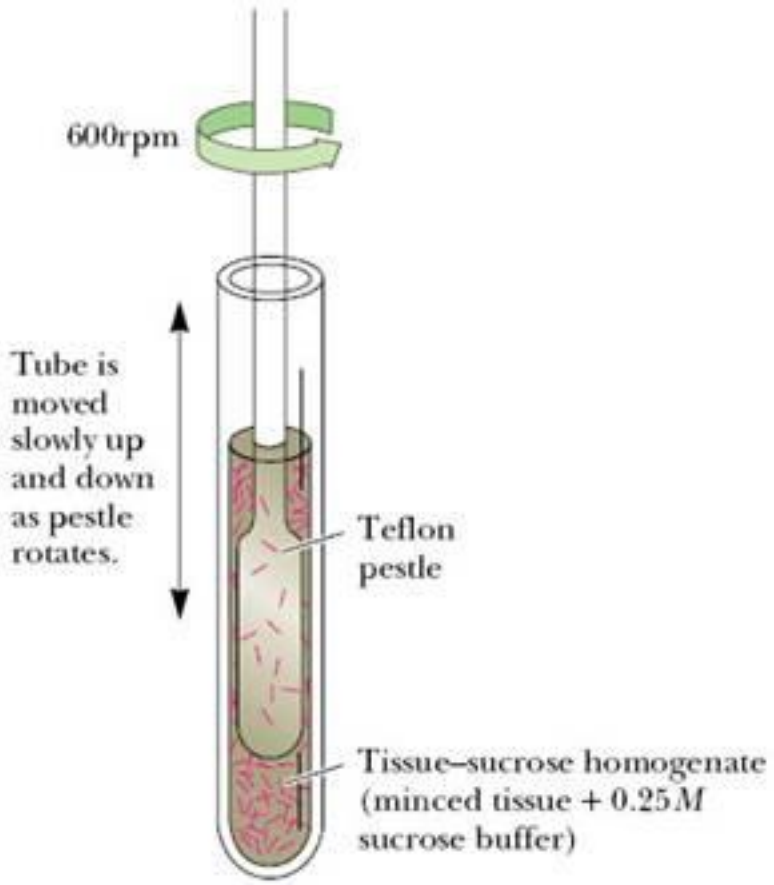


Isolation of Extracellular Proteins

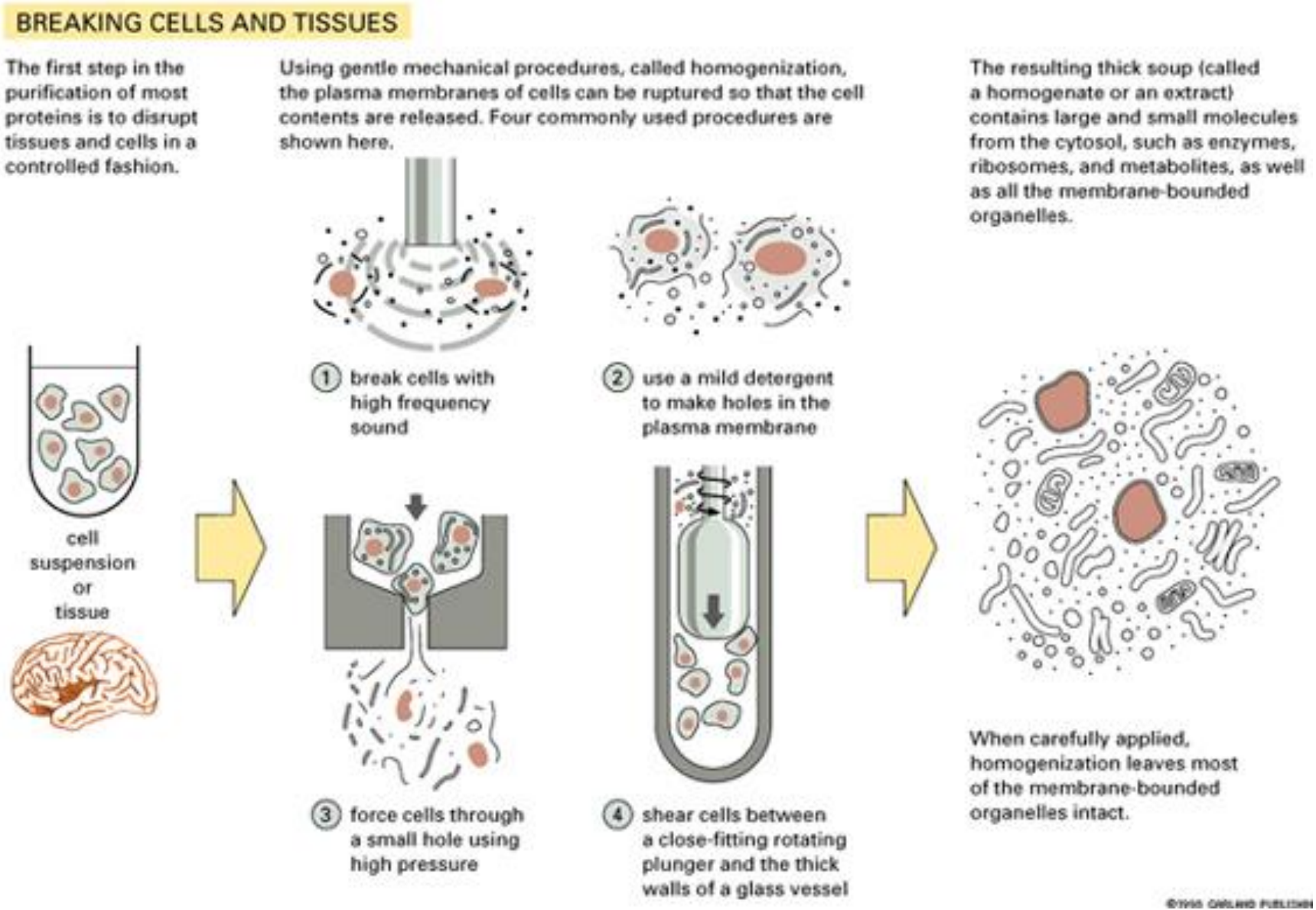
- No need for cell disruption
- Secreted soluble proteins can be collected in the cell supernatant after centrifugation
- Membrane-bound proteins might be released from the cell simply using detergents

Isolation of Intracellular Proteins

- Needs cell disruption:
  - Detergents lysis
  - Enzymatic lysis
  - Osmotic lysis
  - Freeze-thaw cycles
  - Ultrasonication
  - Homogenization



Isolation of Intracellular Proteins

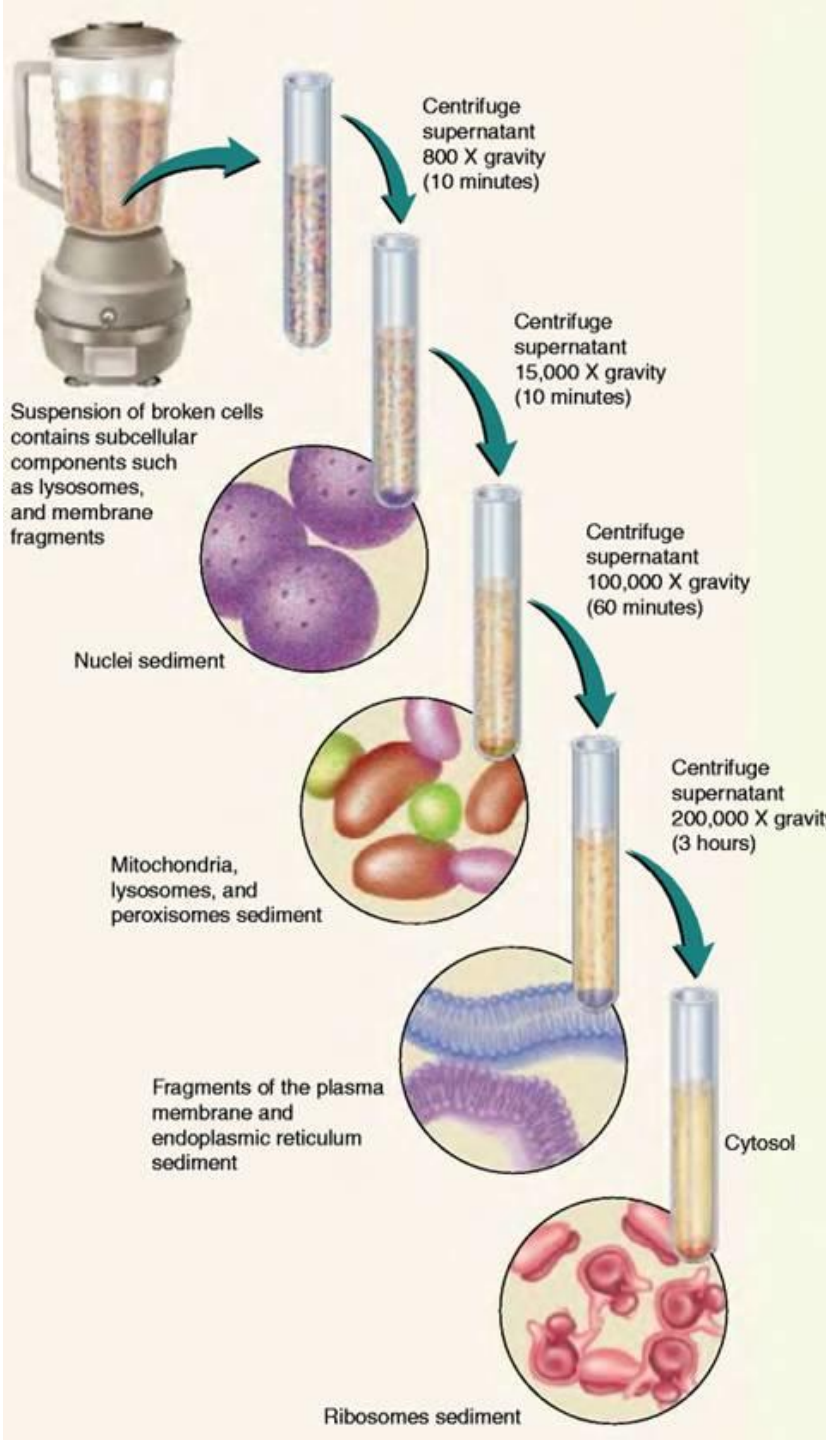


Purification of Proteins

- Differential centrifugation
- Differential salt precipitation
- Differential solvent precipitation
- Preparative electrophoresis
- Column chromatography

More than one approach may be required

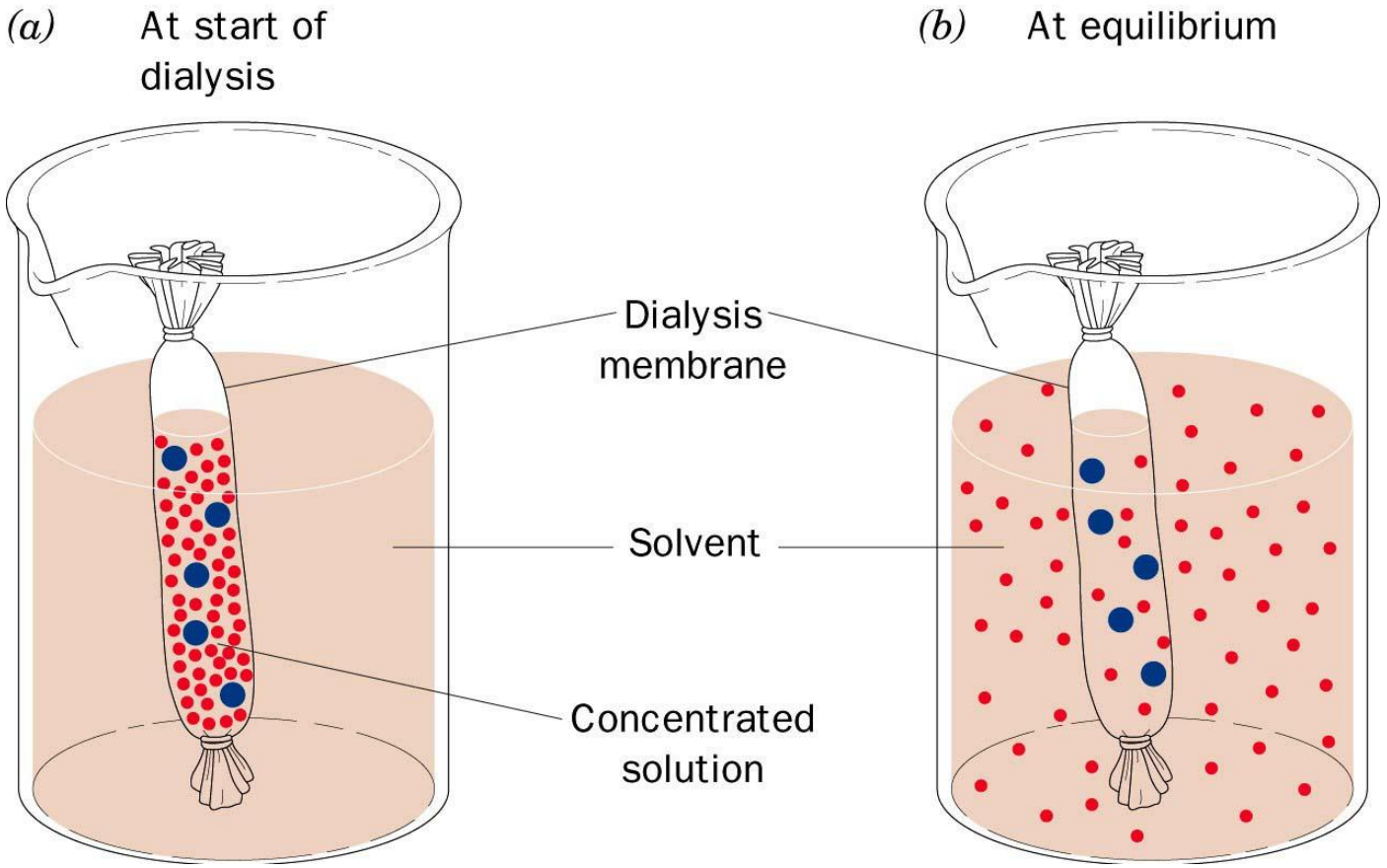
Differential Centrifugation



Differential Centrifugation

- **Separation of proteins (or any material) on the basis of their size, mass, and density**
- **It is a function of the size of the protein and the speed if centrifugation**
- **It is gives us a rough separation. It is basically fractioning!**

Dialysis


(a) At start of dialysis
(b) At equilibrium
Dialysis membrane
Solvent
Concentrated solution

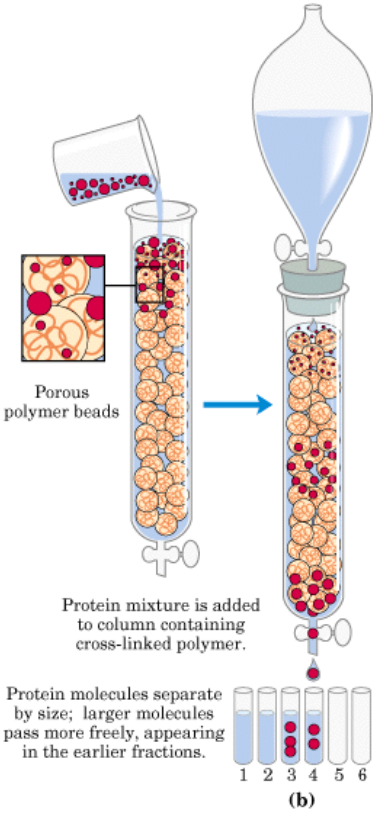
Dialysis

- **Based on osmotic pressure**
- **It allows you to get rid of most salt ions**
- **It is impossible to remove the salts completely**
  - ***Why?***

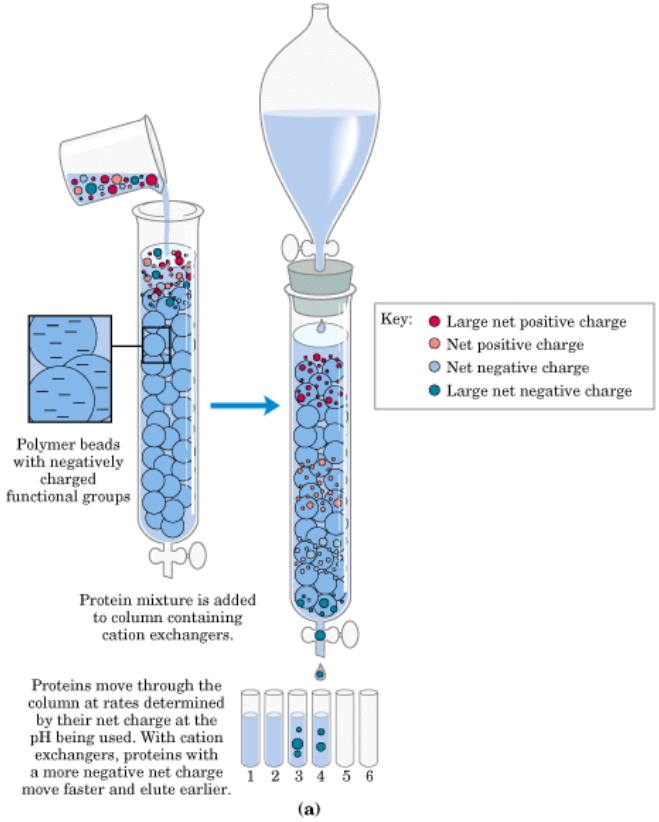
Column Chromatography

- Most common and best approach to purifying larger amounts of proteins
- Achieves Highest level of purity and largest amount
- Requires low effort
- Lowest likelihood to damage the protein product
- Standard method for pharmaceutical industry

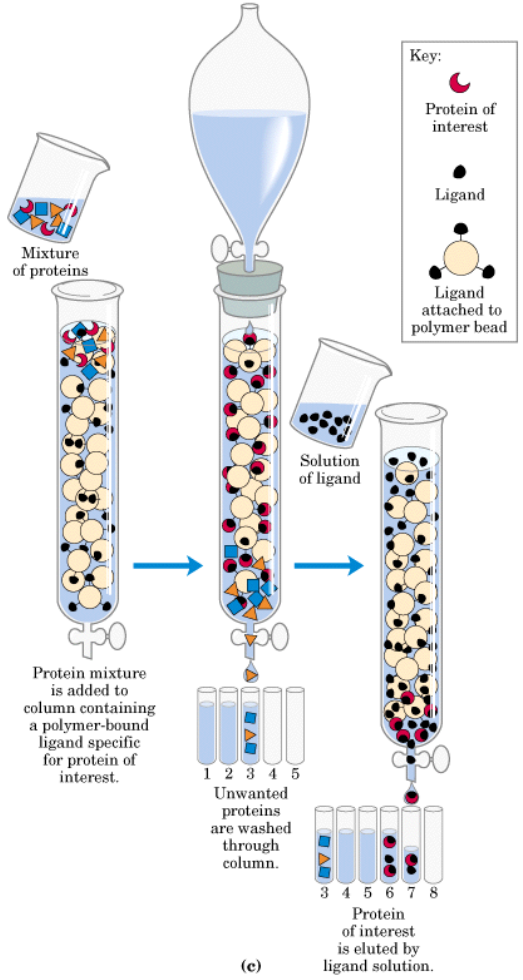
Gel Filtration



Ion Exchange

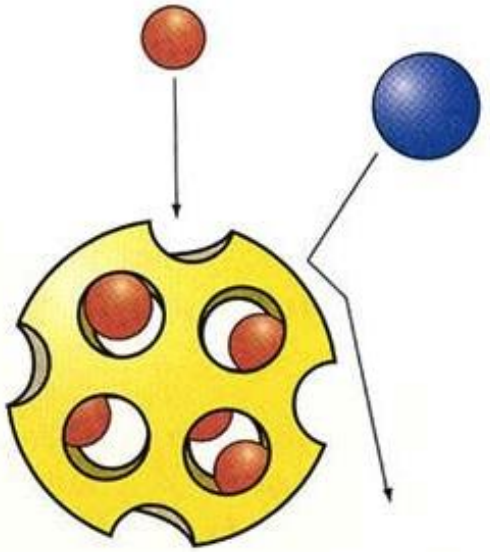


Affinity



Gel-Filtration Chromatography

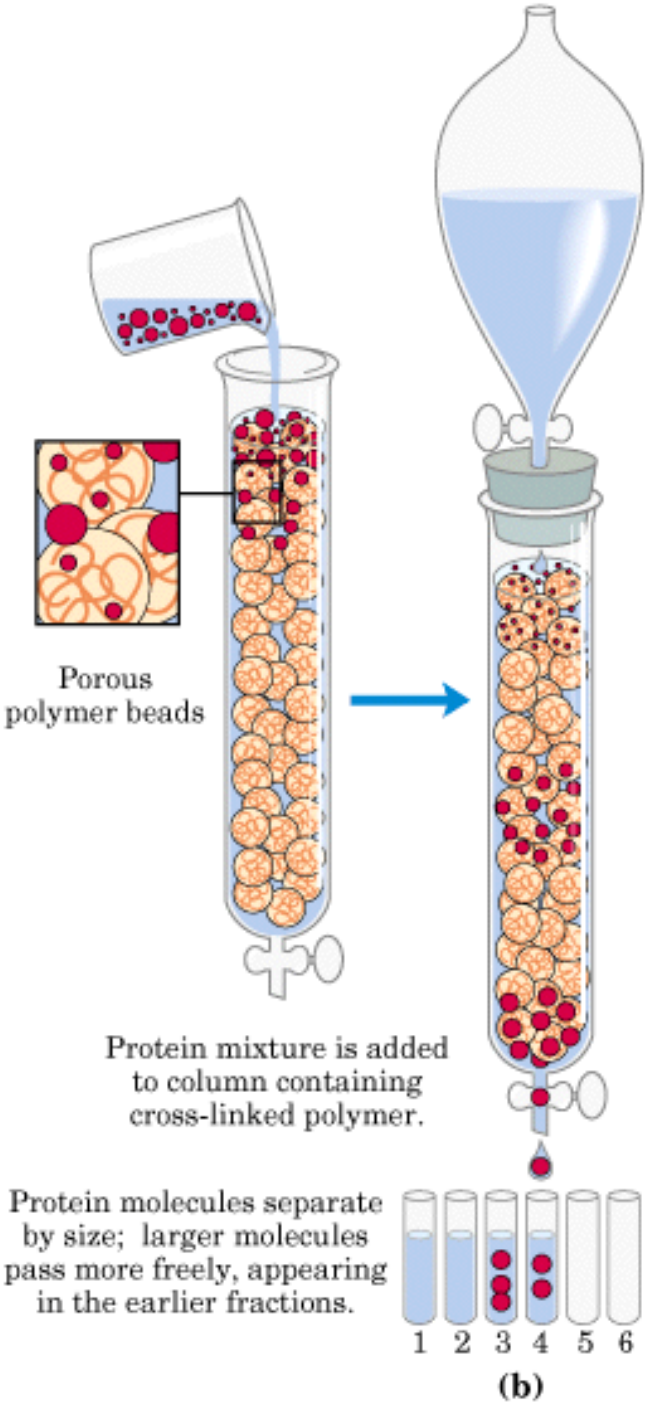
- AKA Size-Exclusion (or **molecular exclusion**) Chromatography
- Molecules are separated according to differences in their **size** as they pass through a hydrophilic polymer
- Polymer beads composed of cross-linked dextran (dextrose) which is highly and uniformly porous (like Swiss cheese)
- Large proteins come out first (can't fit in pores), small proteins come out last (get stuck in the pores)



Gel-Filtration Chromatography

You need to have a way to know where your protein is:

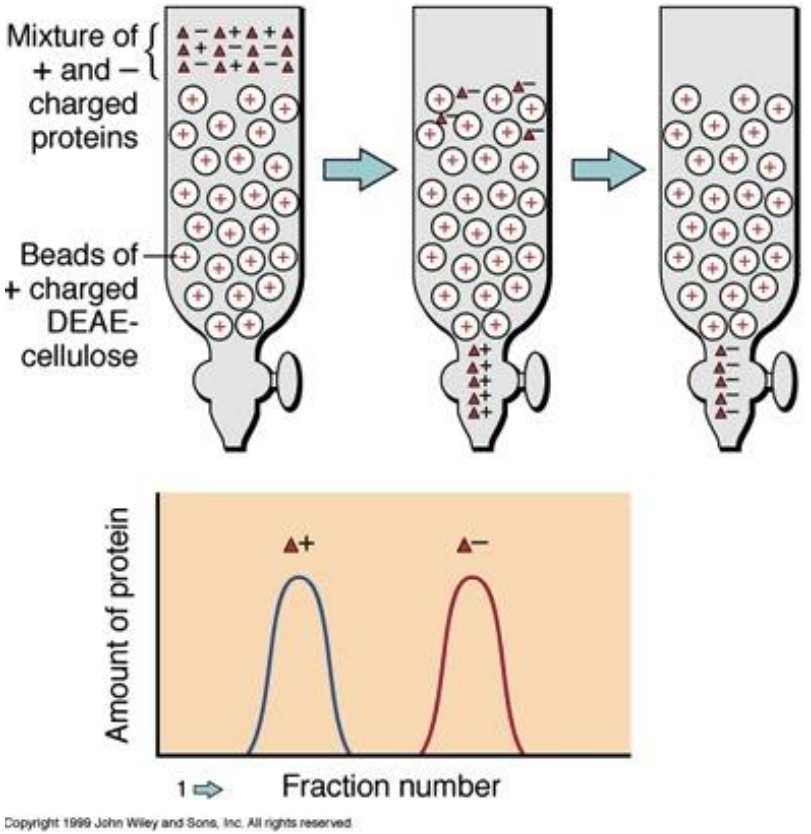
1. Molecular weight marker
2. Add a target to your protein e.g. if you want to purify an enzyme add a substrate to the tubes and see where does it appear



Ion-Exchange Chromatography

- Principle is to separate on basis of charge “adsorption”
- Highest resolving power
- Highest loading capacity
- Widespread applicability
- Most frequent chromatographic technique for protein purification

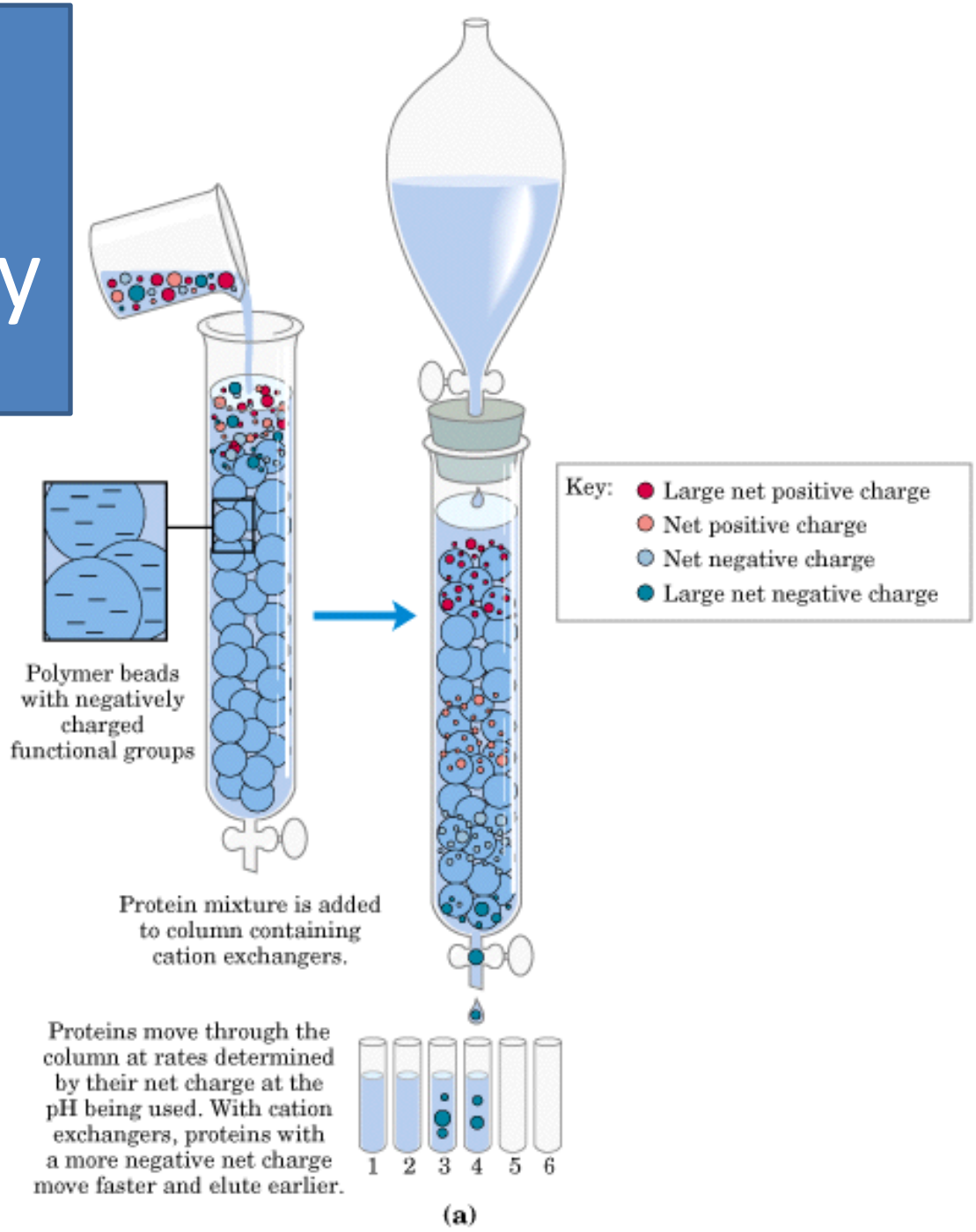
This column represents cation exchange chromatography



Ion-Exchange Chromatography

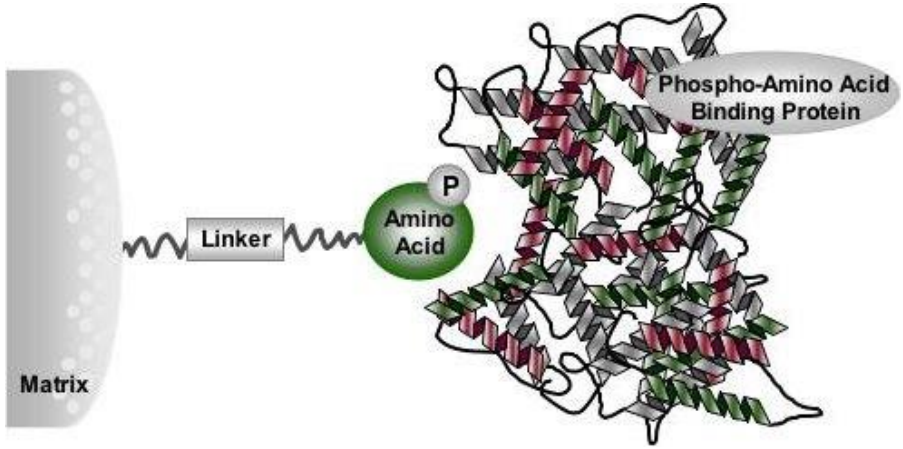
The ion exchange process is based on concentration of the counter ion

This column represents anion exchange chromatography



Affinity Chromatography

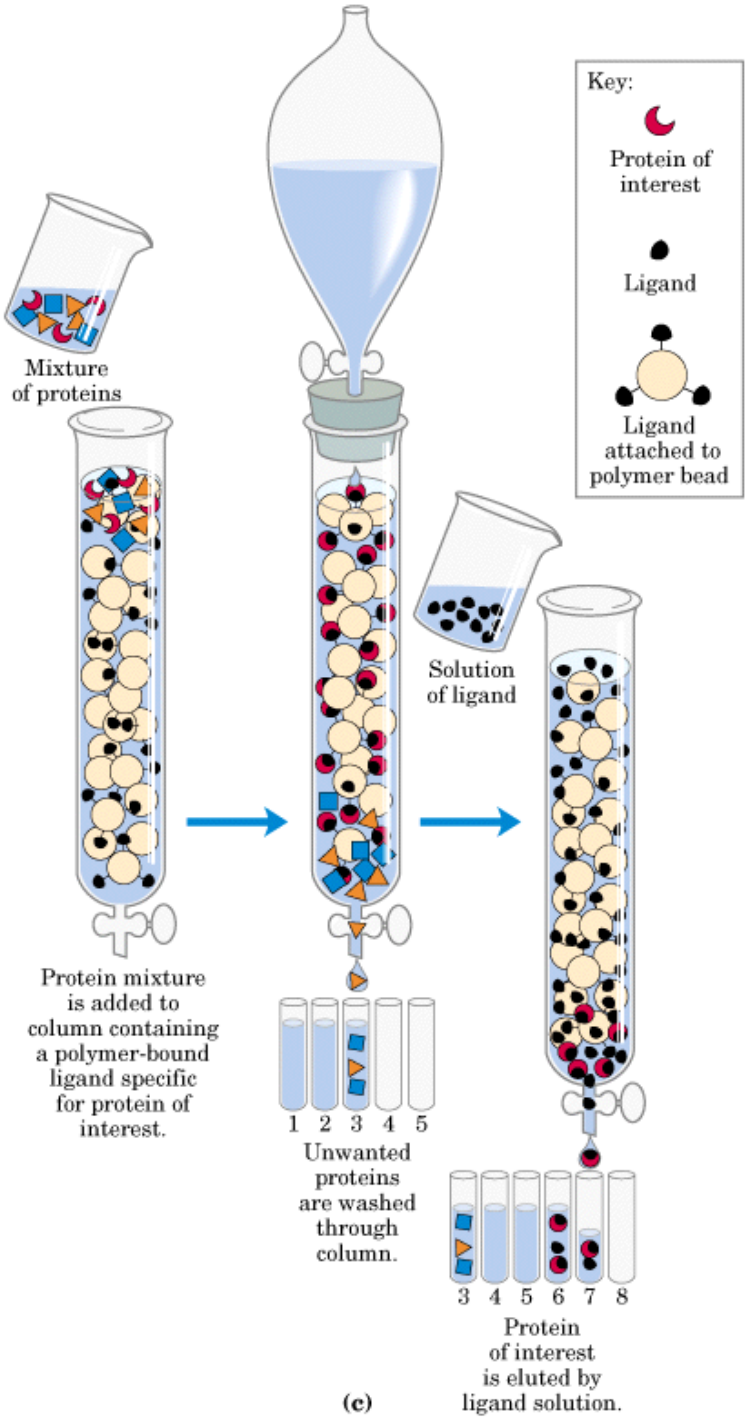
- Adsorptive separation in which the molecule to be purified specifically and reversibly binds (adsorbs) to a complementary binding substance (a ligand) immobilized on an insoluble support (a matrix or resin)
- Purification is 1000X or better from a single step (highest of all methods)
- Preferred method if possible



Affinity Chromatography

How can I get my protein out of the column?

The protein is not covalently bound to the ligand



Isoelectric Focusing

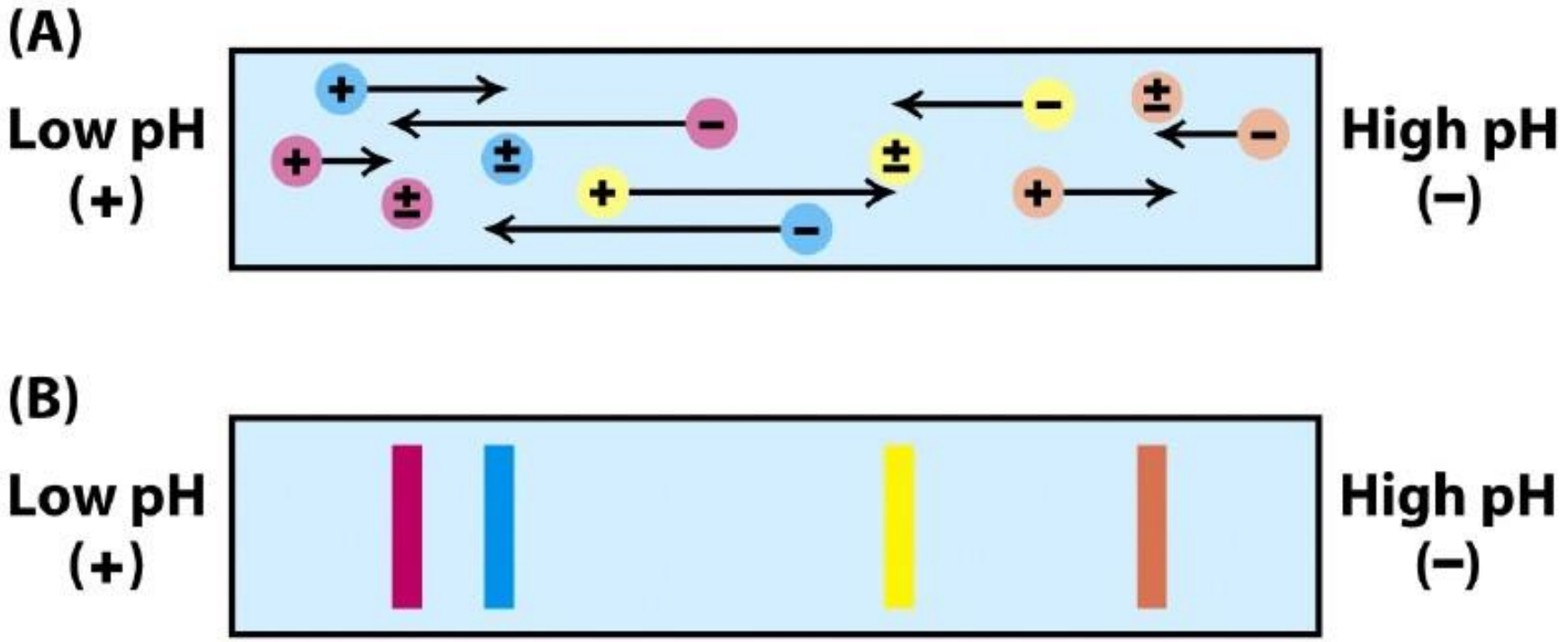


Figure 3-11
Biochemistry, Sixth Edition

Polyacrylamide Gel Electrophoresis

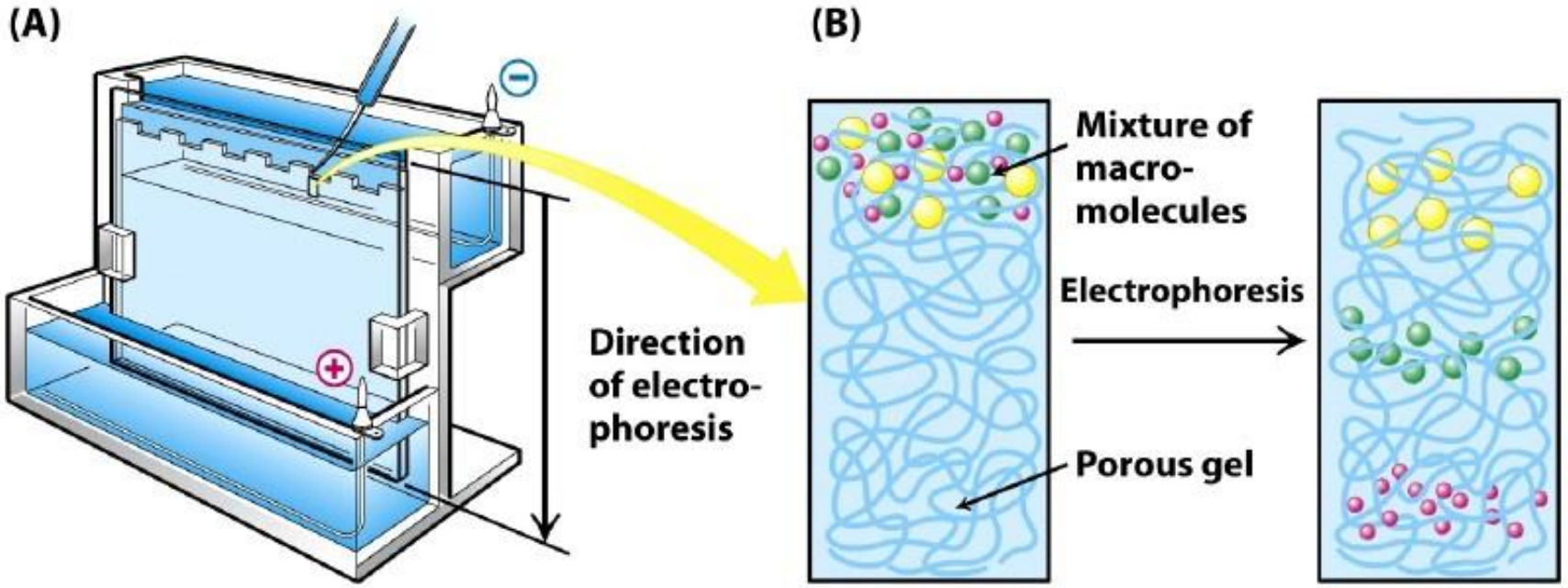
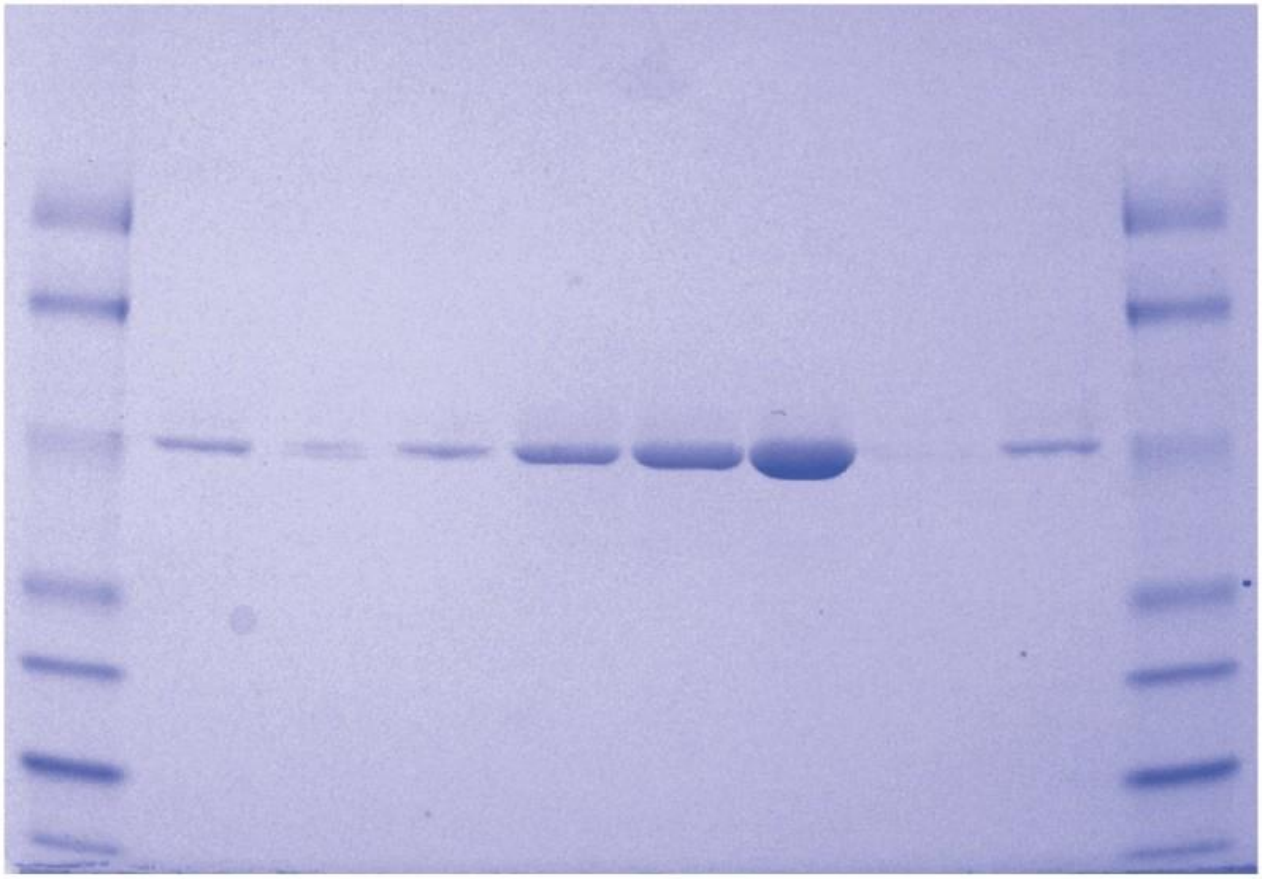


Figure 3-7
Biochemistry, Sixth Edition

Electrophoresis separates proteins based on their size using electrical current

Polyacrylamide Gel Electrophoresis



Largest

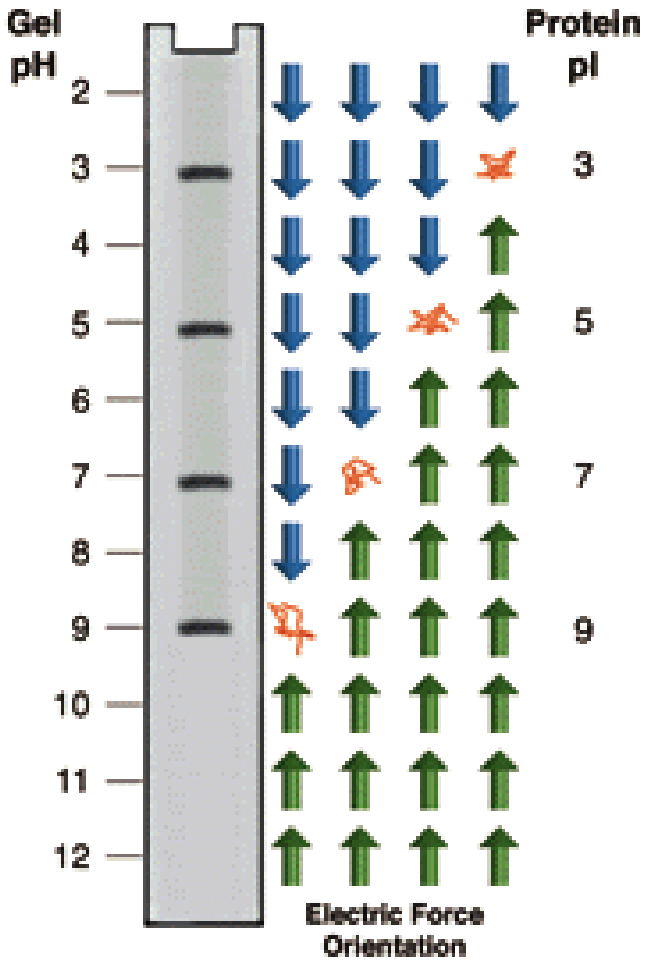
Smallest

Figure 3-9
Biochemistry, Sixth Edition

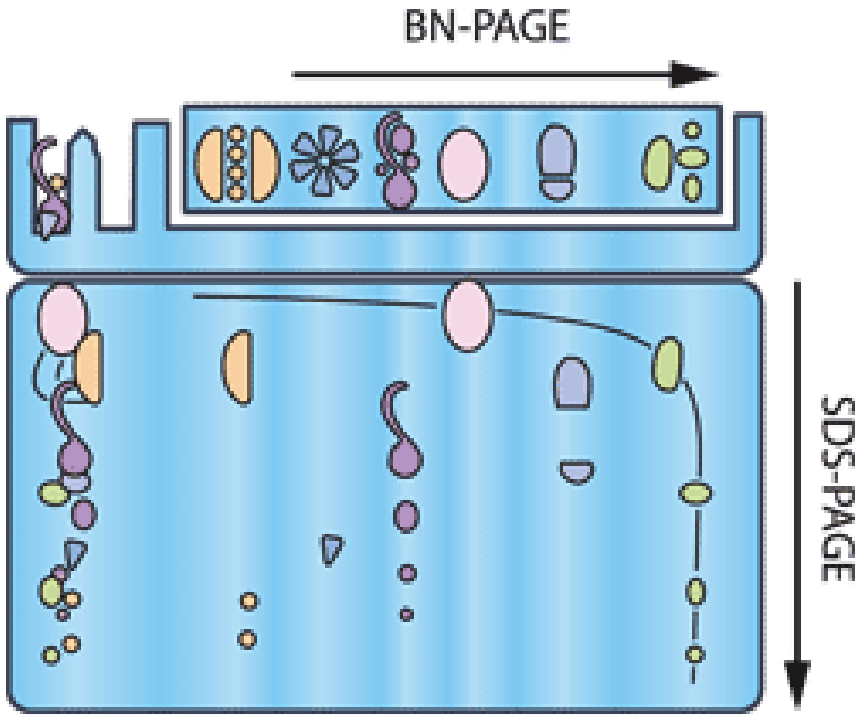
Preparative electrophoresis

2D Polyacrylamide Gel Electrophoresis (2D PAGE)

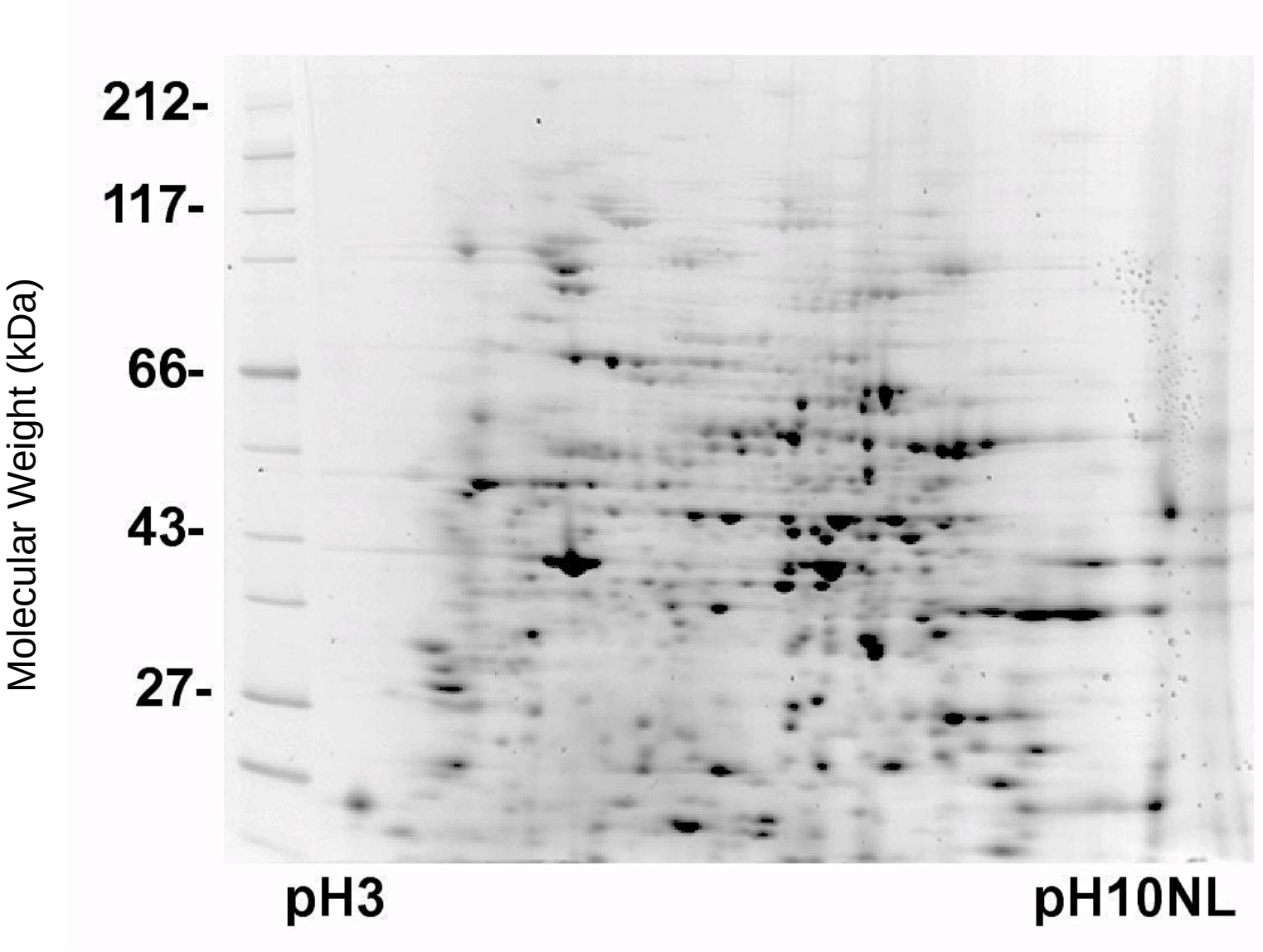
Step 1: Isoelectric Focusing (IEF)



Step 2: SDS-PAGE



2-D PAGE



Now you are able to:

- ✓ Describe most common methods of protein isolation and purification
- ✓ Compare between different methods of protein purification
- ✓ Construct a purification algorithm based on your knowledge in protein purification