Protein analysis

Part I: Protein purification

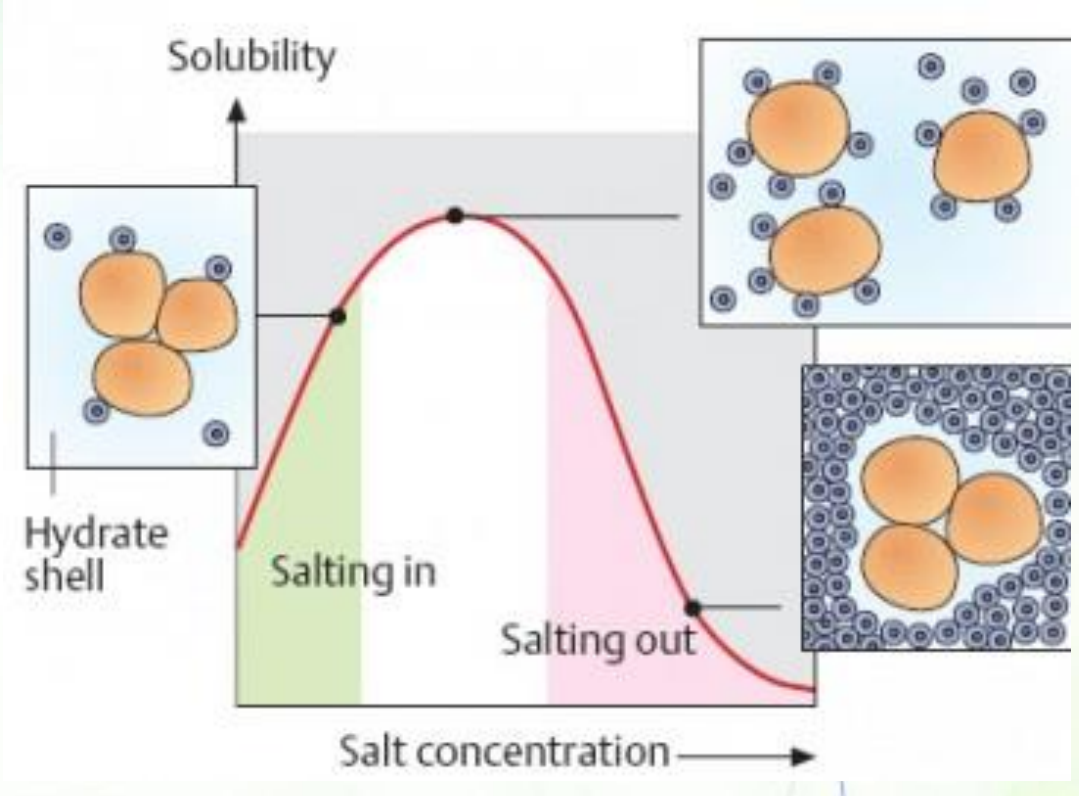
Summer 2023

Bases of protein separation

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

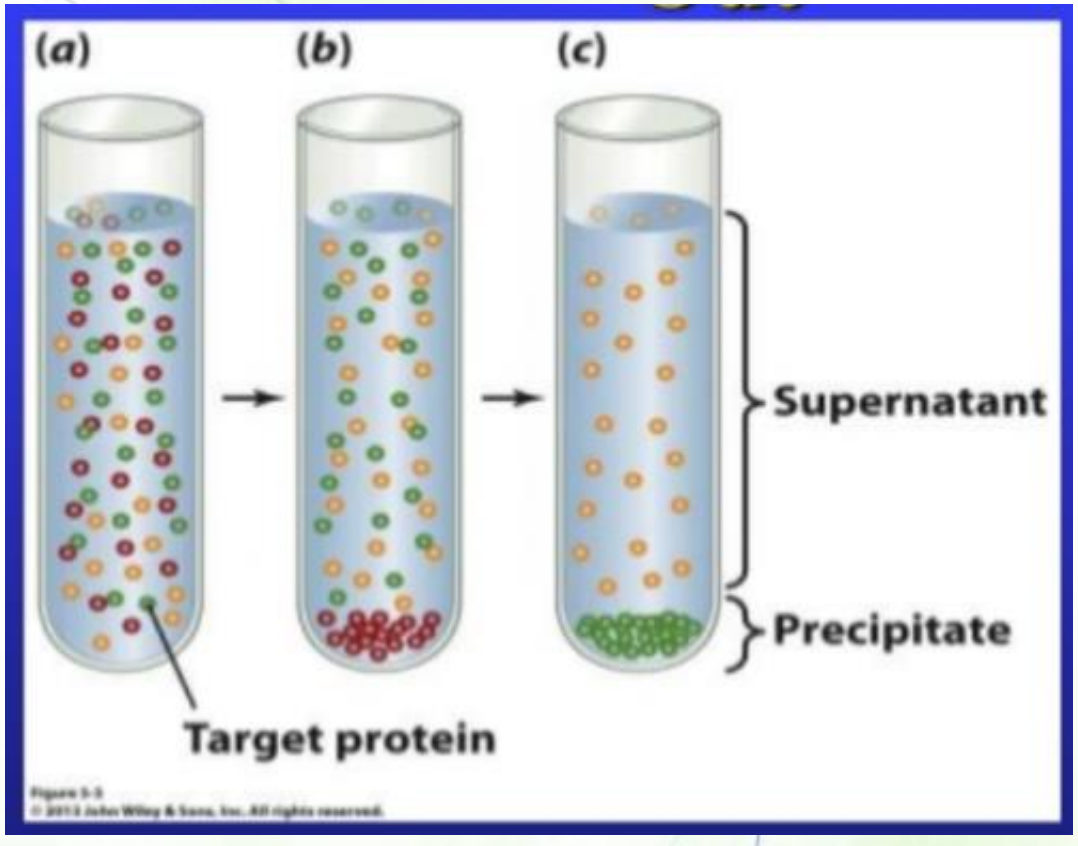
Salt fractionation (salting in...salting out)

- Proteins are poorly soluble in pure water, but their solubility increases as the ionic strength (a measure of the concentration of ions in that solution) increases.
  - salting in
- At very high concentrations of salt, most proteins become less soluble.
  - salting out
- Why?



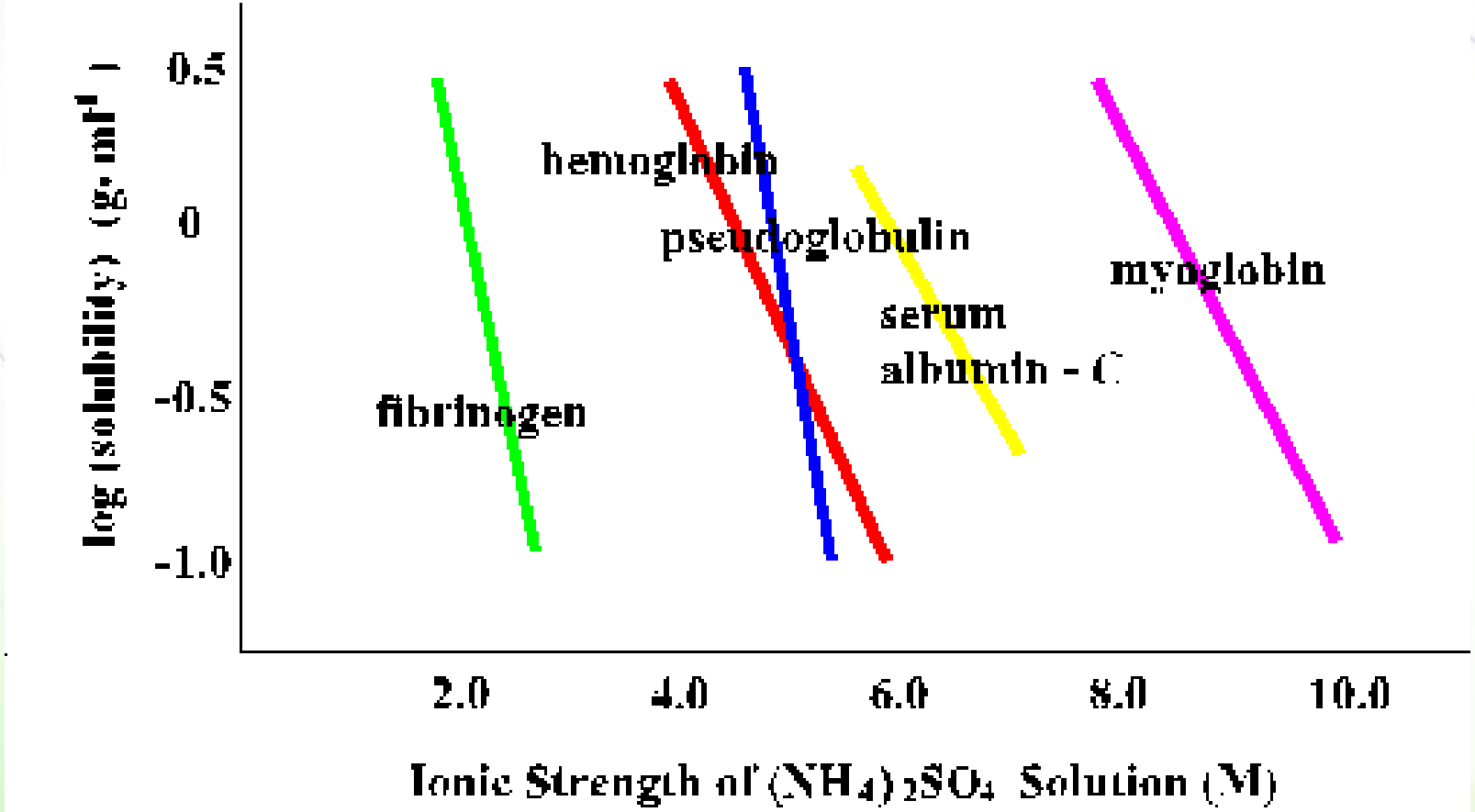
Why?

- When salt, like ammonium sulfate, is added to a protein solution, some of the water is taken away from the protein to make ion–dipole bonds with the ions.
- With less water available to hydrate the proteins, they begin to interact with each other through hydrophobic bonds.
- At a defined amount of salt, a precipitate that contains some proteins forms. These proteins can then be separated.
- When more salt is added, a different set of proteins, precipitates.
- And so on.

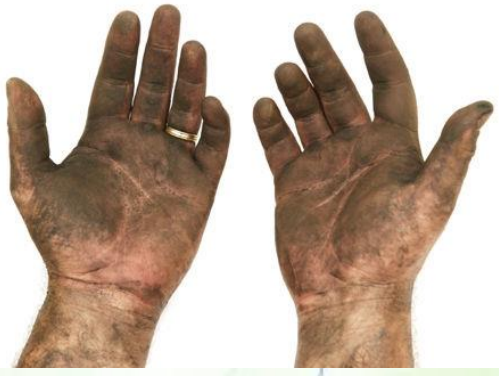


Uses of salting out

- Salting out can be used to fractionate proteins

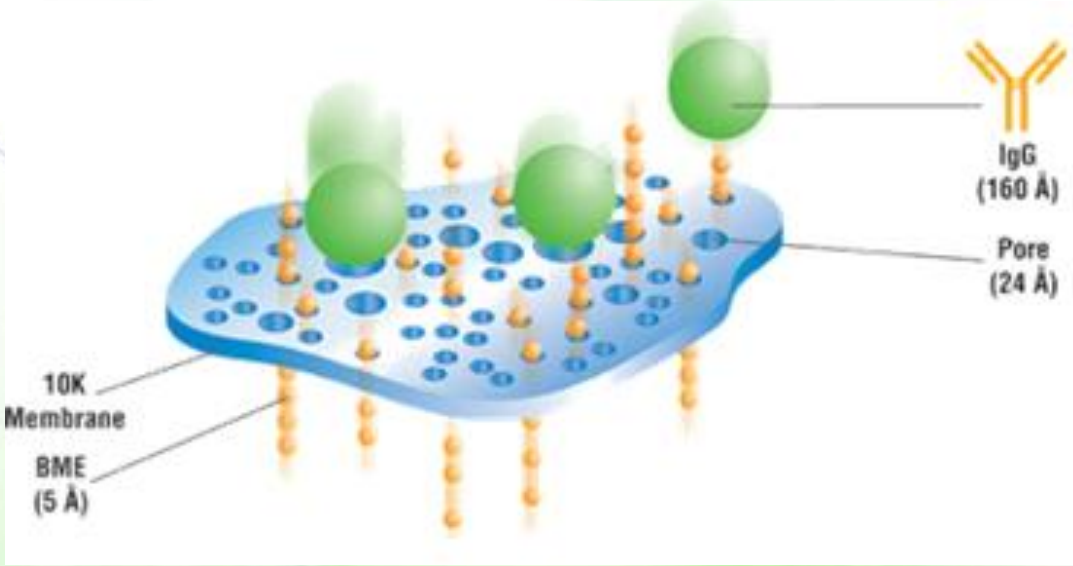


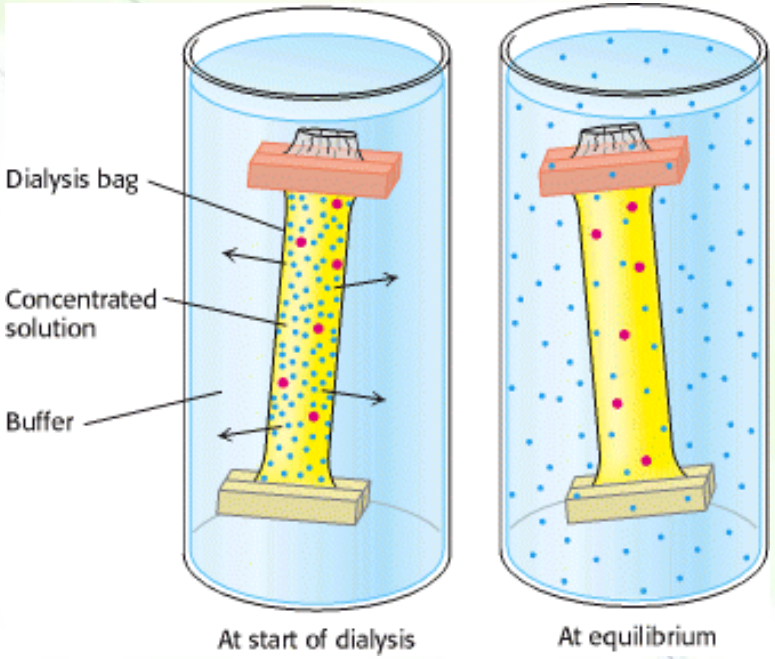
But it is dirty



Dialysis

- Proteins can be separated from small molecules by dialysis through a semi-permeable membrane.



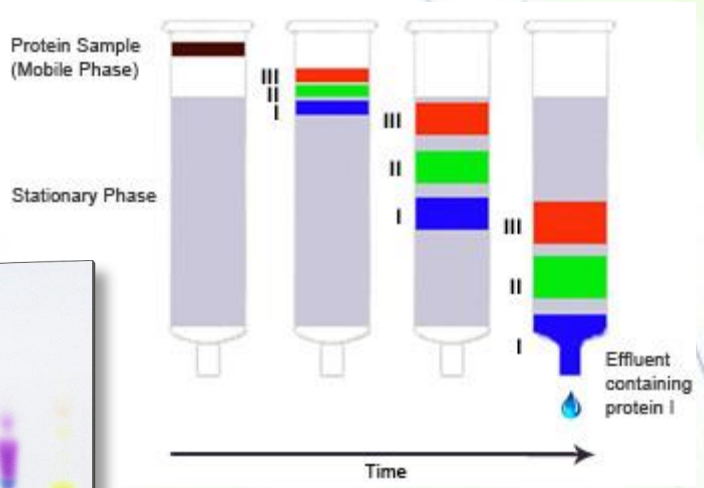


Disadvantage of dialysis

1. **Large number of larger proteins will still exist.**
2. **Smaller proteins of significance are lost.**

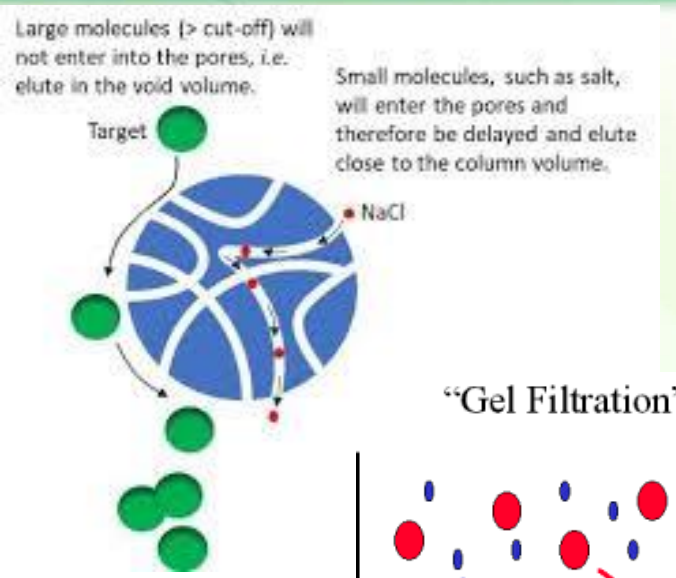
Chromatography techniques

- Separation of molecules present in a liquid or gaseous environment (mobile phase) via passing through a column (long tube) that contains an immobile phase (stationary phase).
- Types:
  - Gel filtration
  - Ion-exchange chromatography
  - Affinity chromatography

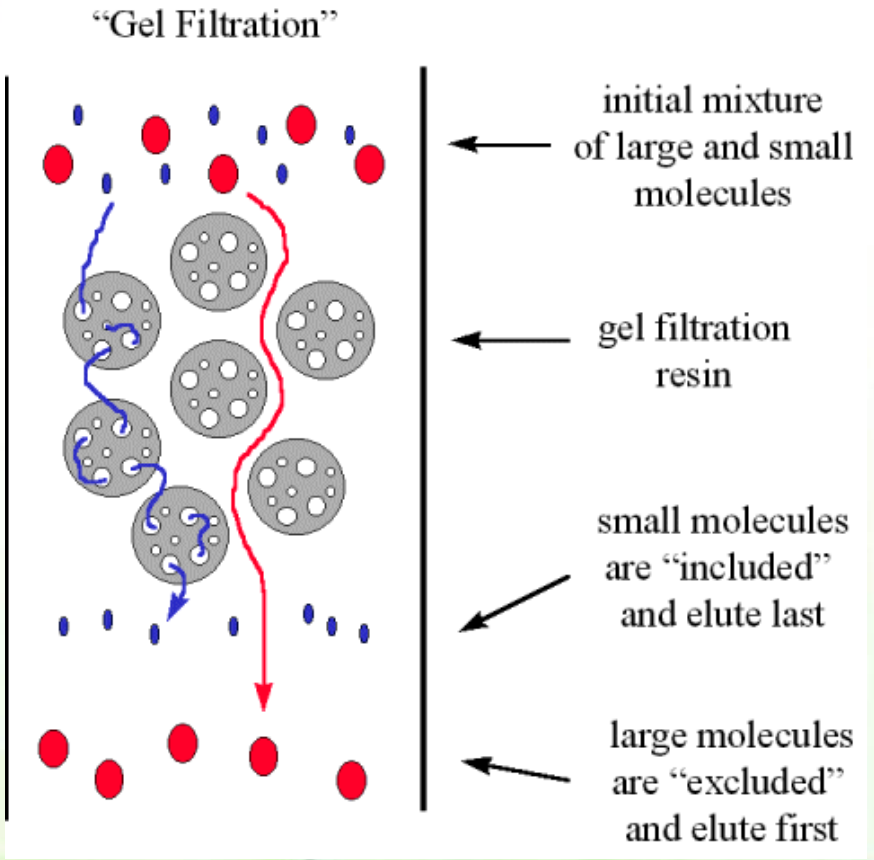


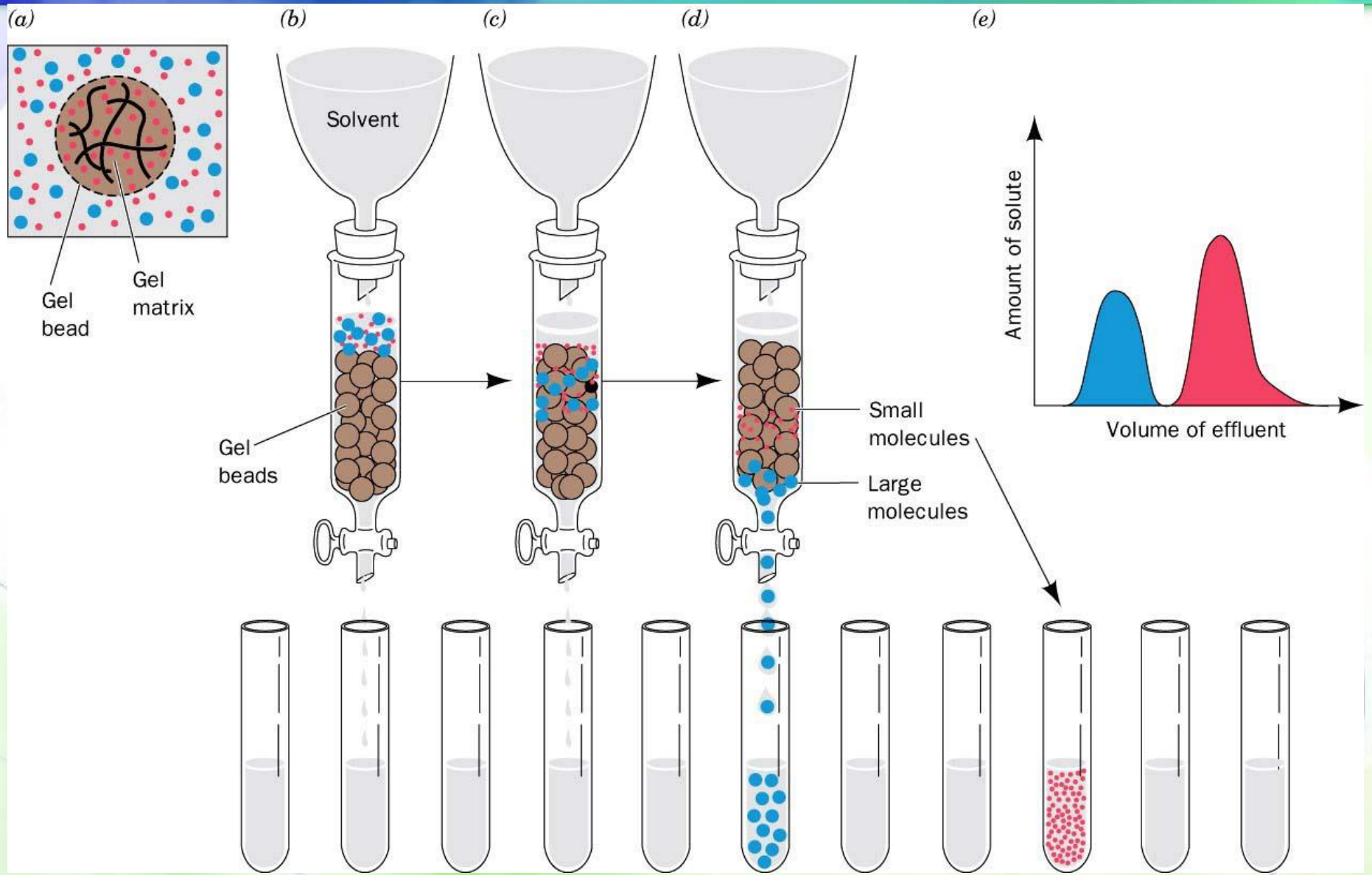
Gel-filtration chromatography

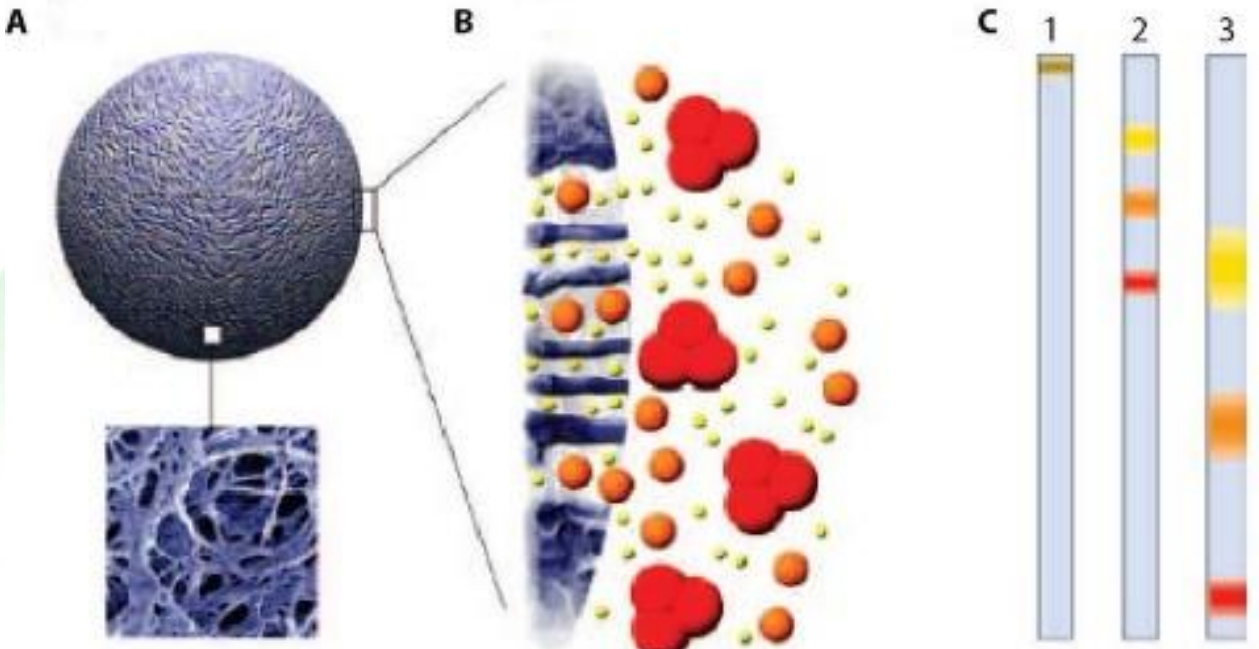
- Separations on the basis of size
  - Size-exclusion chromatography
  - Molecular sieve chromatography

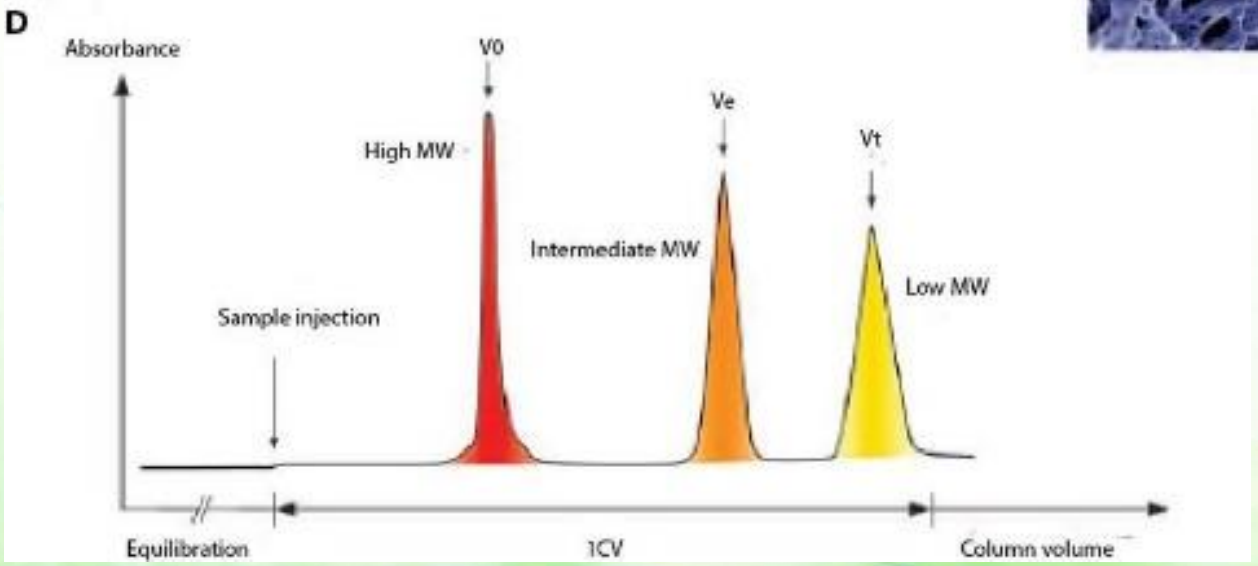


- The stationary phase: porous beads
- Large molecules flow more rapidly and come out first
- Smaller molecules take a longer time in the column and exit late



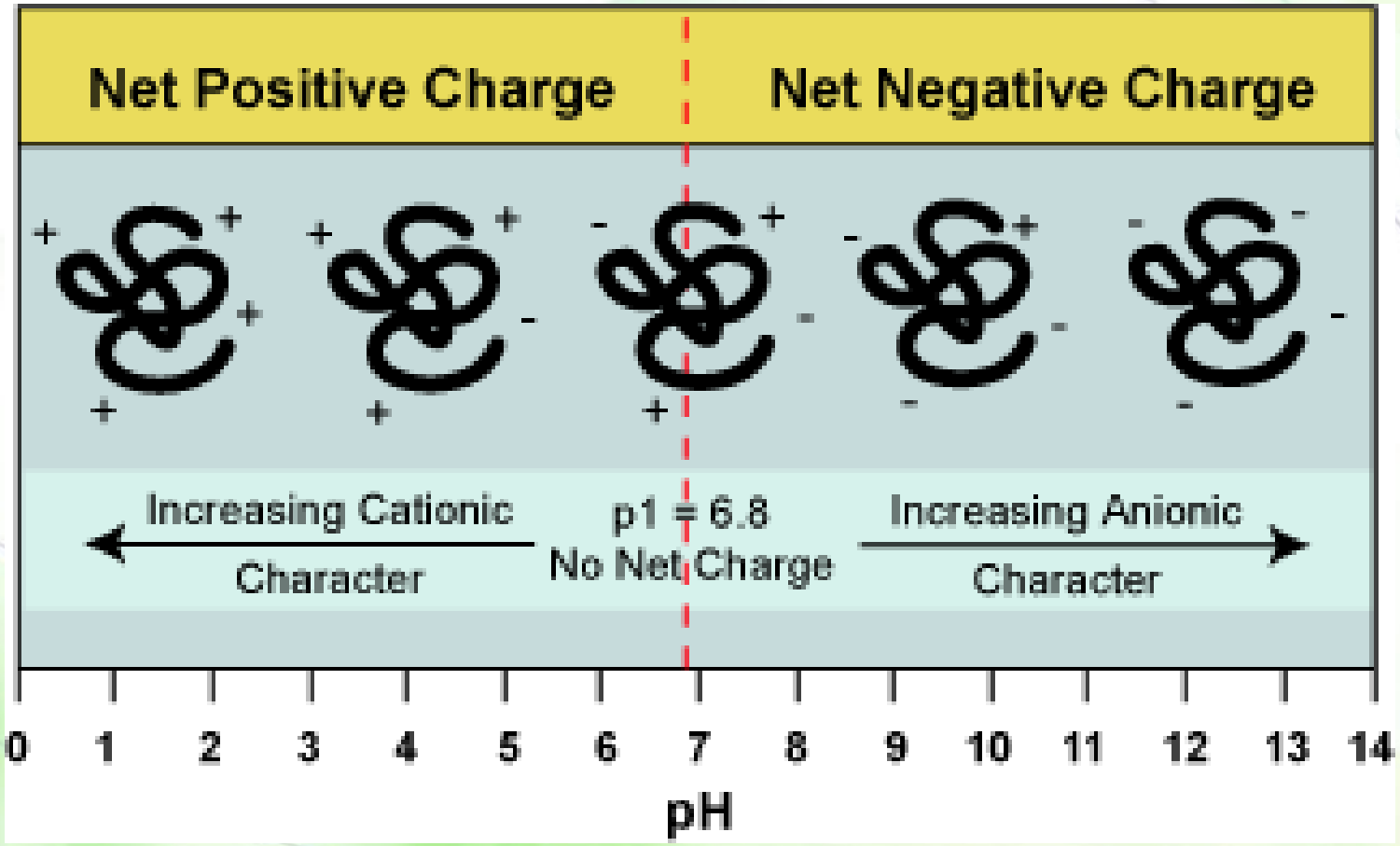

(a)
(b)
(c)
(d)
(e)
Gel bead
Gel matrix
Solvent
Gel beads
Small molecules
Large molecules
Amount of solute
Volume of effluent


A
B
C
1
2
3


D
Absorbance
V0
Ve
Vt
High MW
Intermediate MW
Low MW
Sample injection
Equilibration
1CV
Column volume

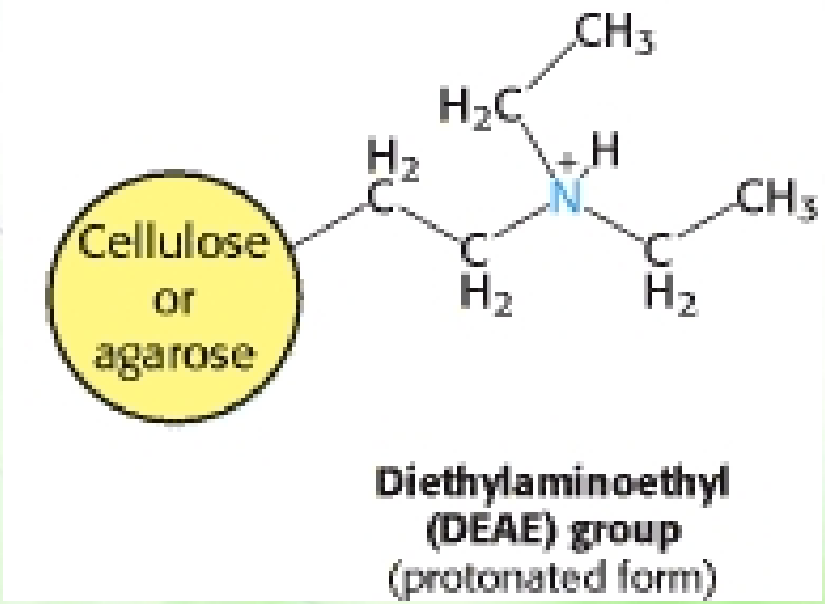
Ion-Exchange Chromatography

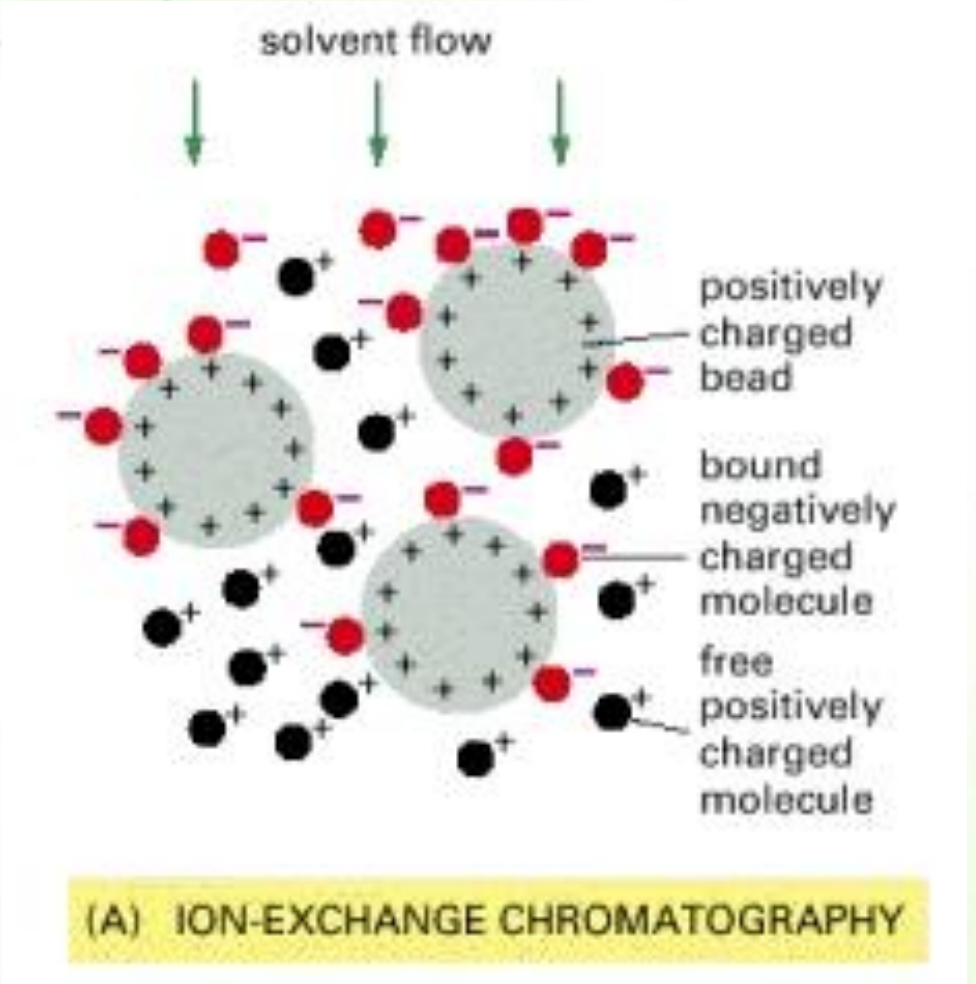
- Proteins have different isoelectric points (pI's) and net charges at various pH conditions.



Anionic-exchange chromatography

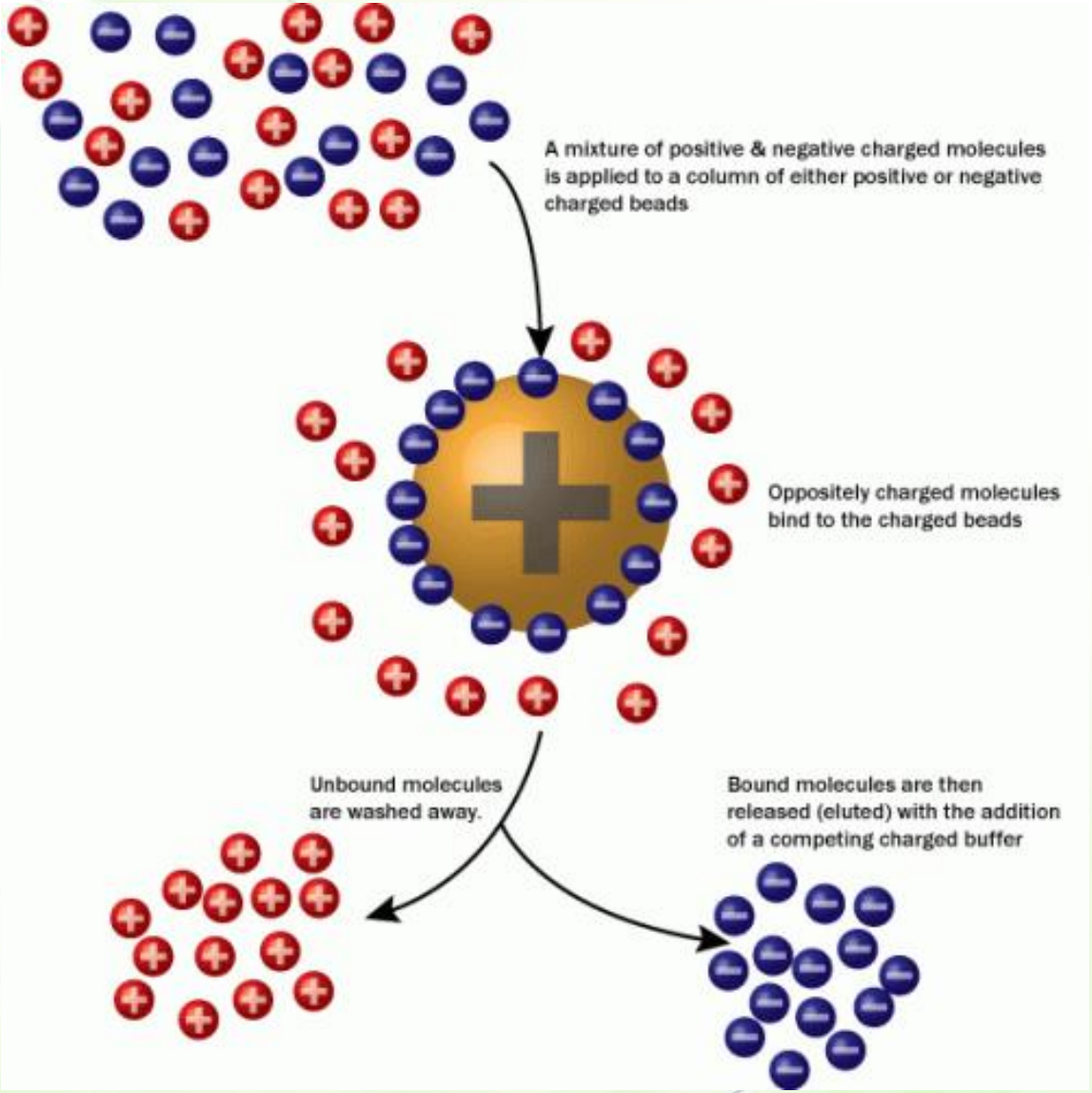
- The beads are positively-charged.
  - Diethylaminoethyl-cellulose column
- Negatively charged proteins are separated.





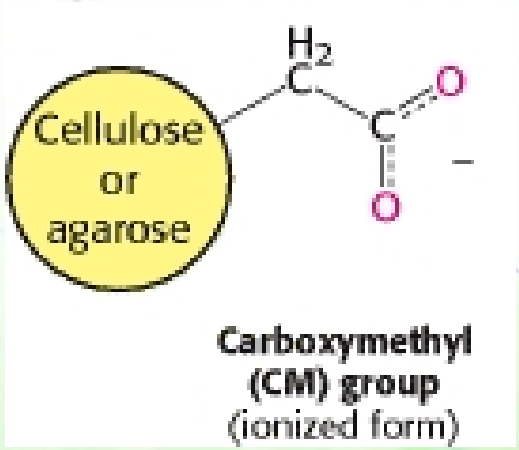
Elution

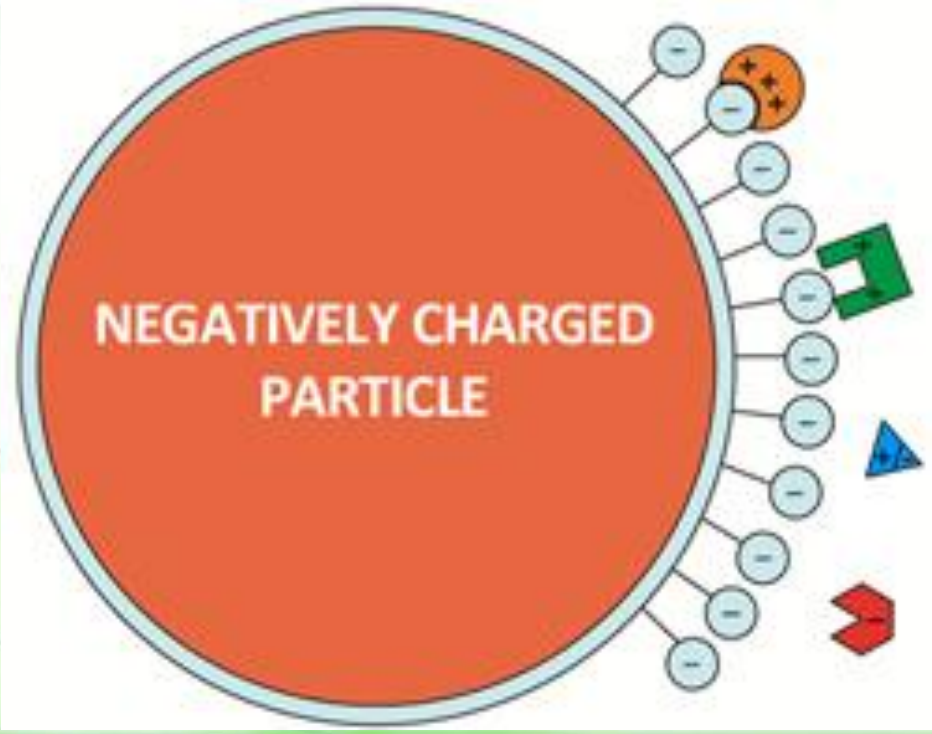
- A negatively-charged protein bound to such a column can then be eluted (released) by adding increasing concentrations of sodium chloride. Why?
  - Because chloride ions compete with negatively-charged groups on the protein for binding to the column.

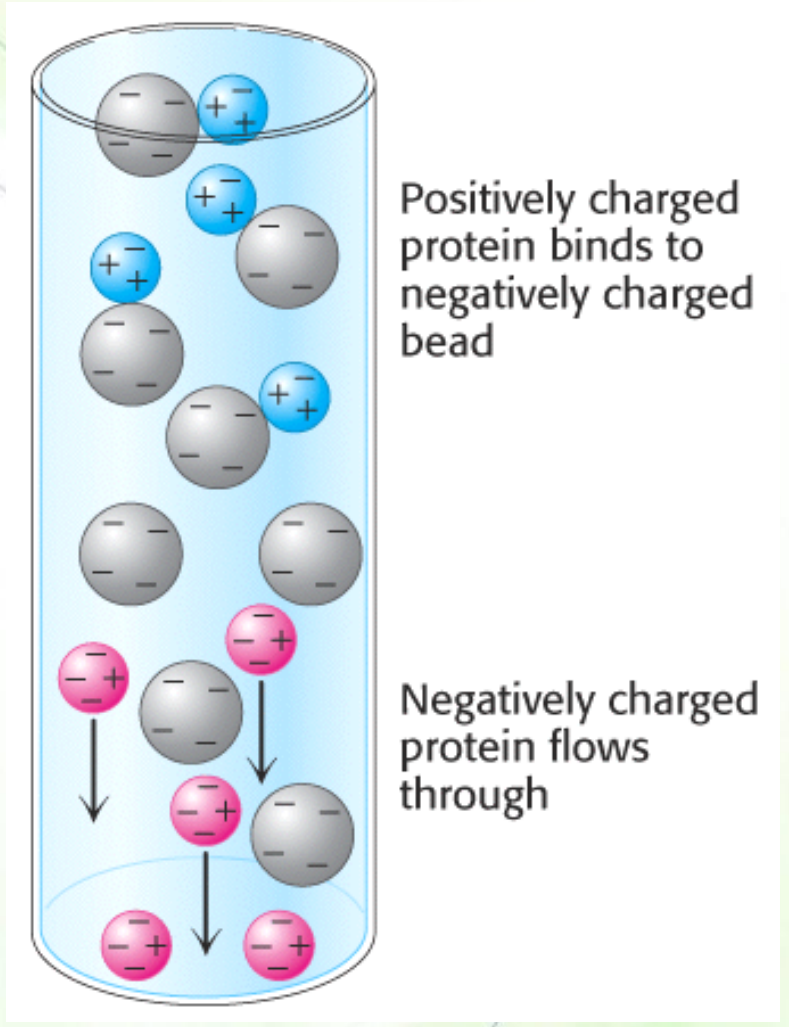


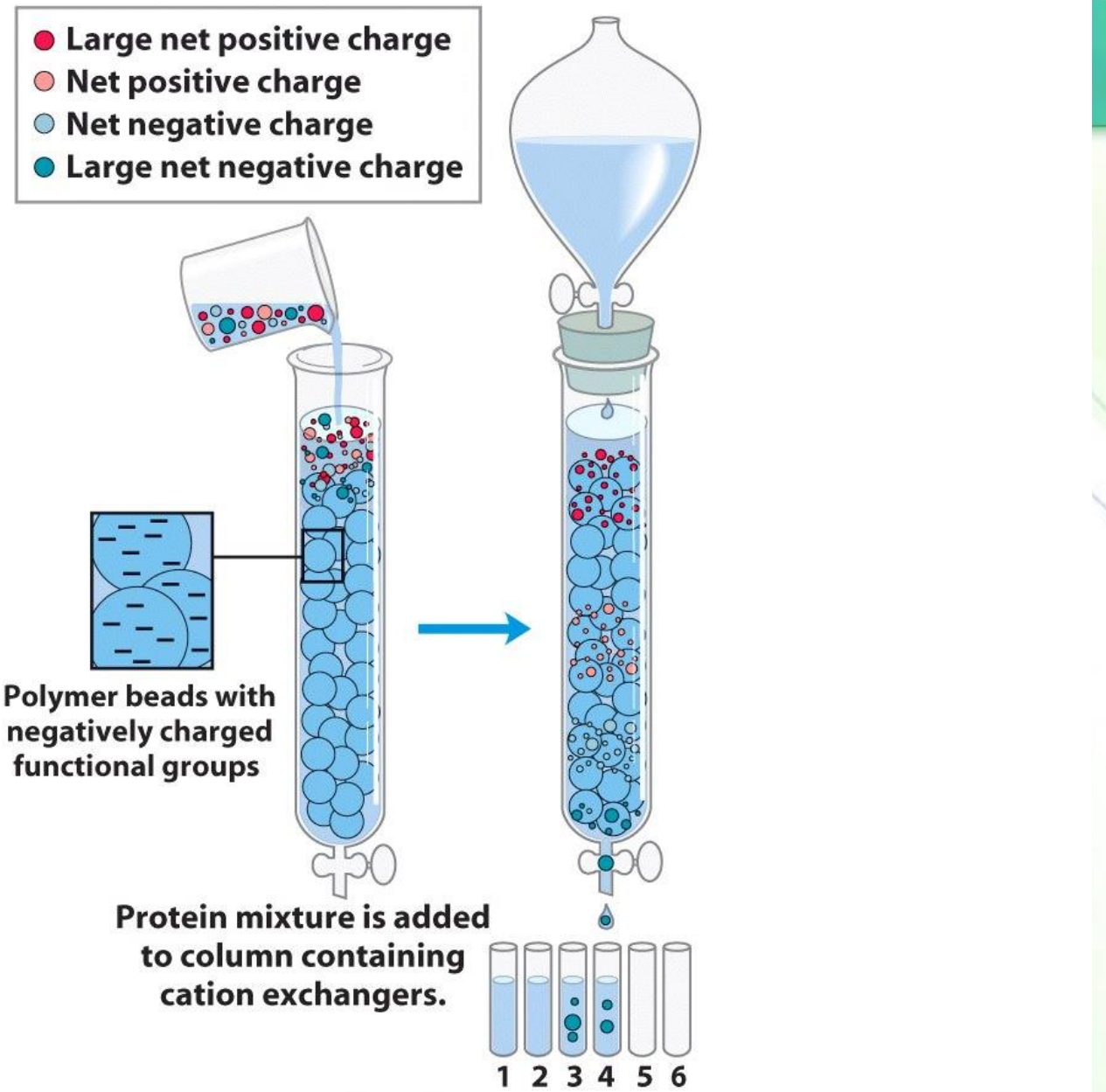
Cationic-exchange chromatography

- The beads are negatively-charged.
- Proteins that have a net positive charge will tend to emerge first, followed by those having a higher positive charge density.









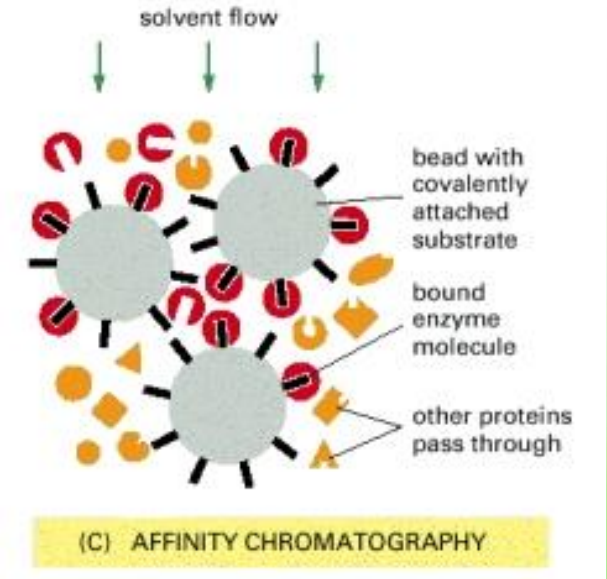
Proteins move through the column at rates determined by their net charge at the pH being used. With cation exchangers, proteins with a more negative net charge move faster and elute earlier.

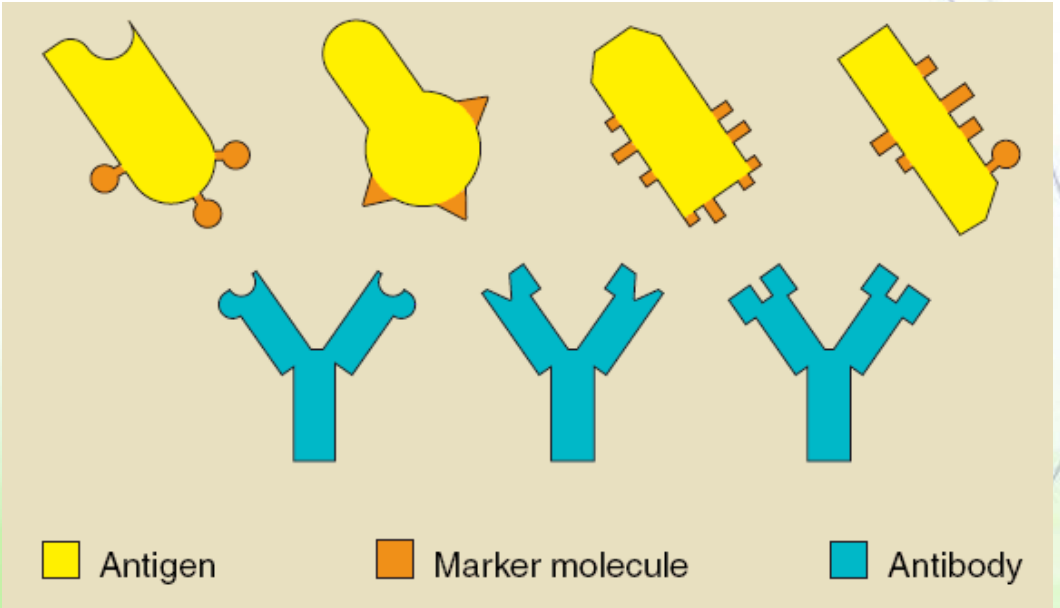
Problem

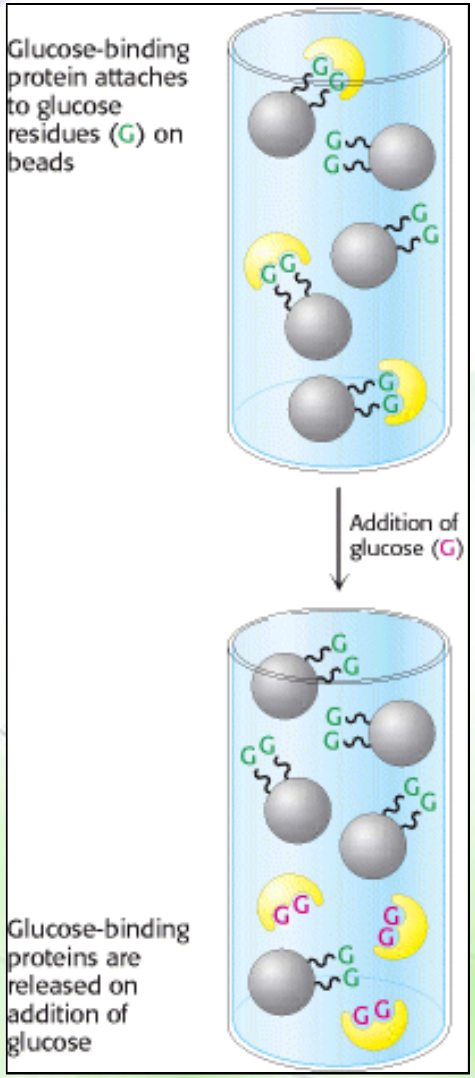
- You have 5 different proteins (#1, #2, #3, #4, and #5), with different isoelectric points (pI's).
  - pI#5 = 2.3
  - pI#4 = 4.7
  - pI#1 = 7.2
  - pI#2 = 9.1
  - pI#3 = 12.1
- Starting the column at pH 6.5, the sample is added and, then, washed to remove unbound molecules. What is the order of protein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

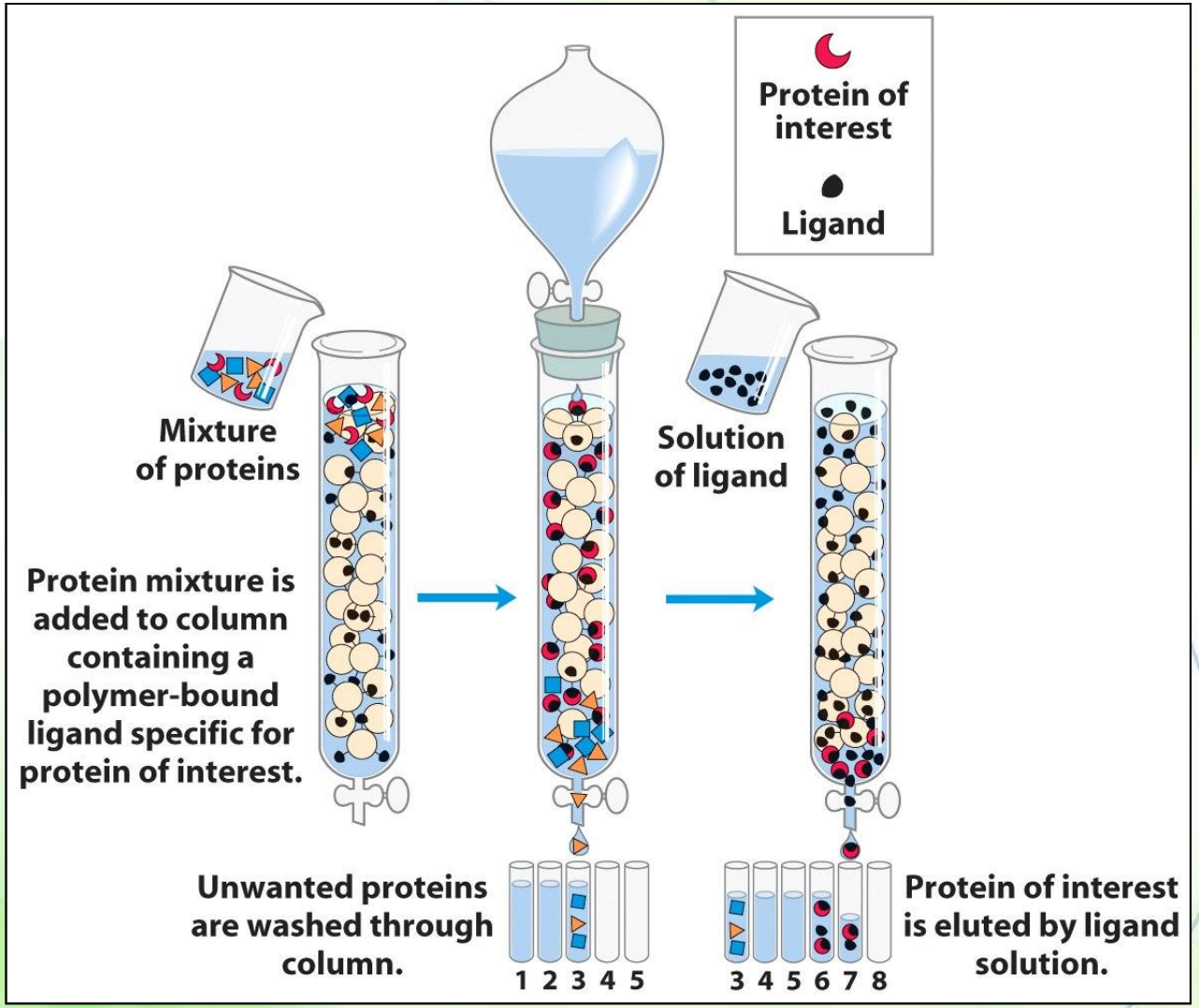
Affinity Chromatography

- Affinity: strength of binding between two molecules
- Affinity chromatography takes advantage of the high affinity of many proteins for specific chemical groups or other proteins (antibodies).
- Affinity chromatography is most effective when the interaction of the protein and the molecule that is used as the bait (trap) is highly specific.



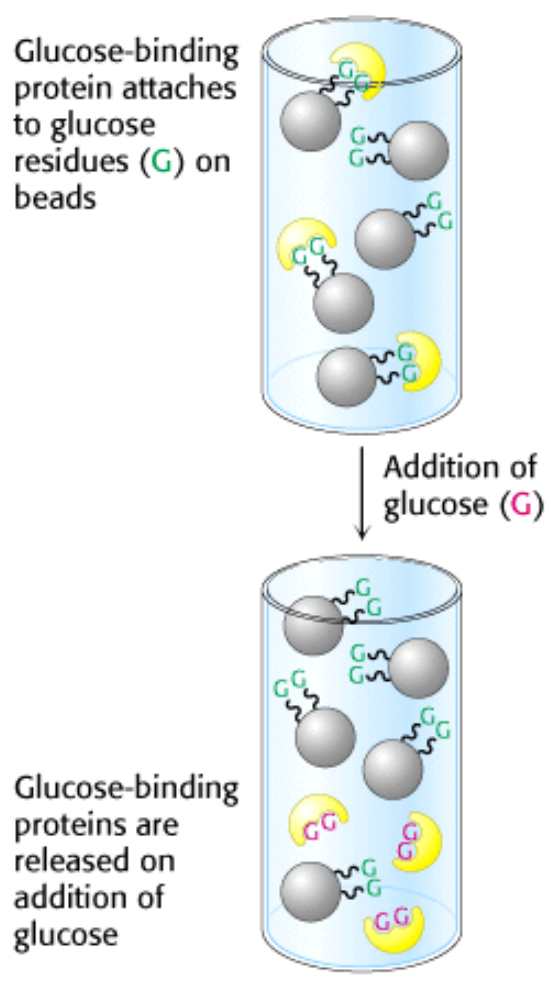



Glucose-binding protein attaches to glucose residues (G) on beads
Addition of glucose (G)
Glucose-binding proteins are released on addition of glucose


Protein of interest
Ligand
Mixture of proteins
Solution of ligand
Protein mixture is added to column containing a polymer-bound ligand specific for protein of interest.
Unwanted proteins are washed through column.
1 2 3 4 5
3 4 5 6 7 8
Protein of interest is eluted by ligand solution.

Example

- The plant protein concanavalin A, which binds to glucose with high affinity, can be purified by passing a protein mixture through a column of beads attached to glucose residues.
- Concanavalin A, but not other proteins, binds to the beads.
- The bound concanavalin A can then be released by adding a concentrated solution of glucose.



What kind of proteins would a concanavalin A-linked bead separate?