



# Protein analysis

## *Part I: Protein purification*

Summer 2023-2024

# Bases of protein separation

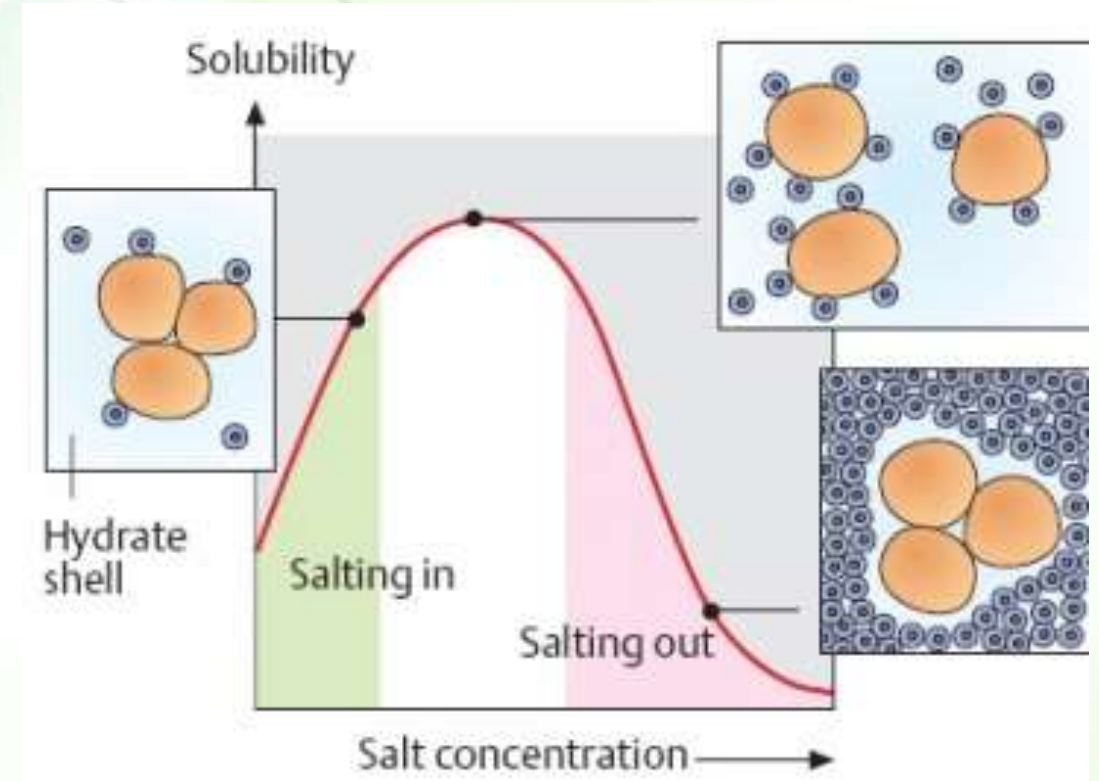


- Proteins can be purified on the basis
  - Solubility
  - Size
  - Charge
  - specific binding affinity

# Salting in...salting out



- Proteins are poorly soluble in pure water, but their solubility increases as the ionic strength increases.
  - salting in
- At very high concentrations of salt, most proteins become less soluble and precipitate.
  - salting out
- Why?



# Why?



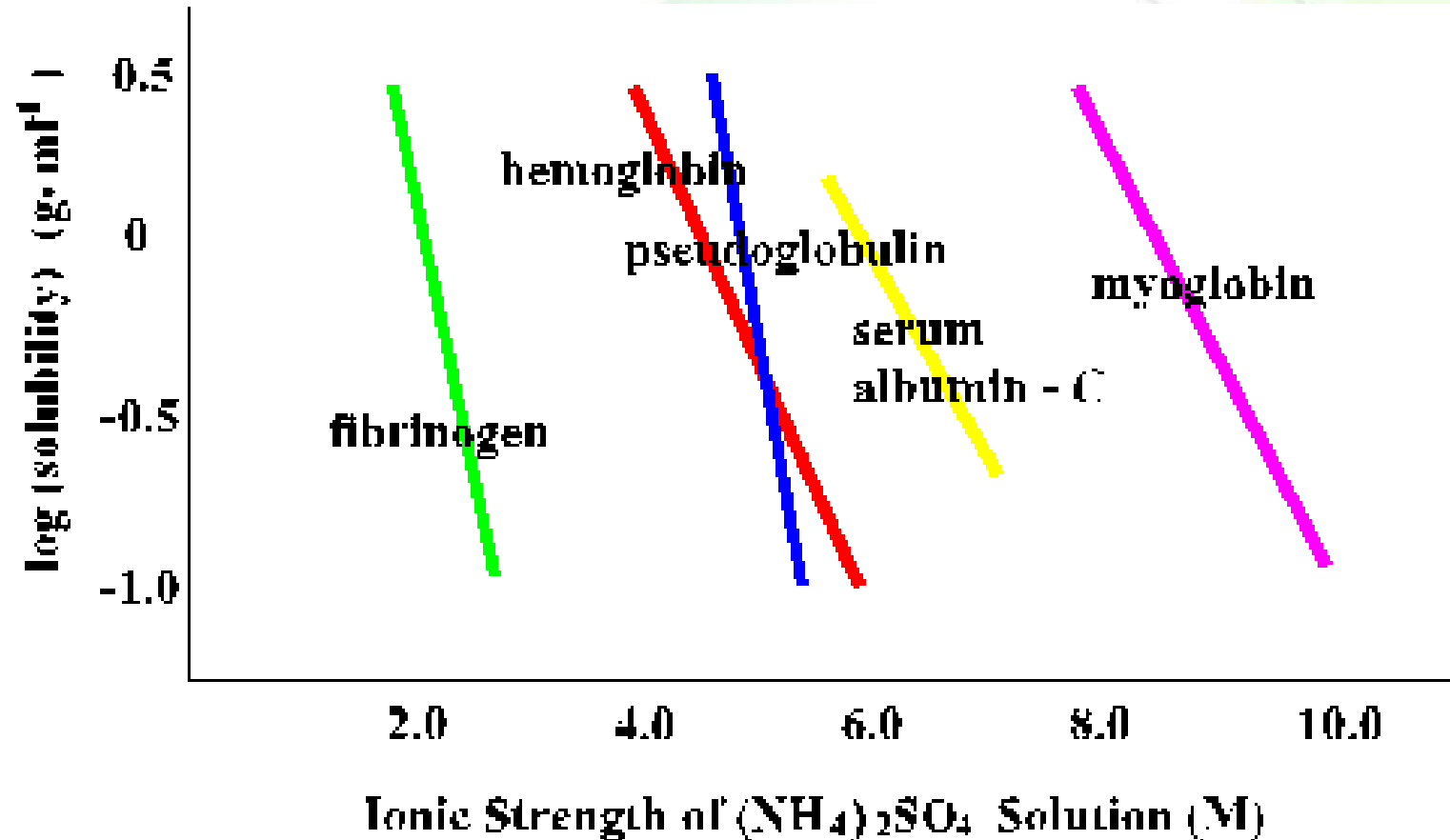
- When salt, like ammonium sulfate, is added to a protein solution, some of the water is taken away from the protein to make ion–dipole bonds with the ions.
- With less water available to hydrate the proteins, the protein molecules begin to interact with each other through hydrophobic bonds.
- Proteins precipitate at different and defined concentrations of the salt, resulting in precipitates that contain different proteins. These proteins can then be separated.



# Uses of salting out



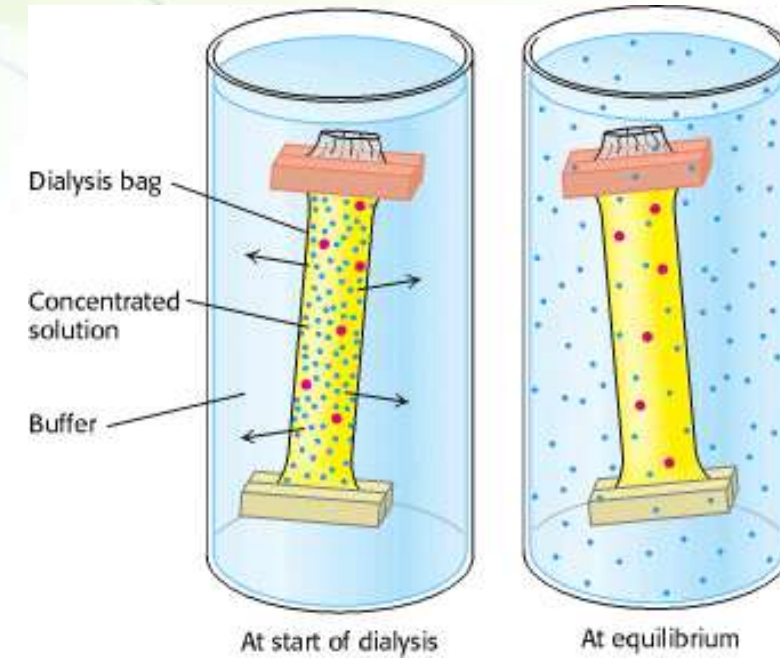
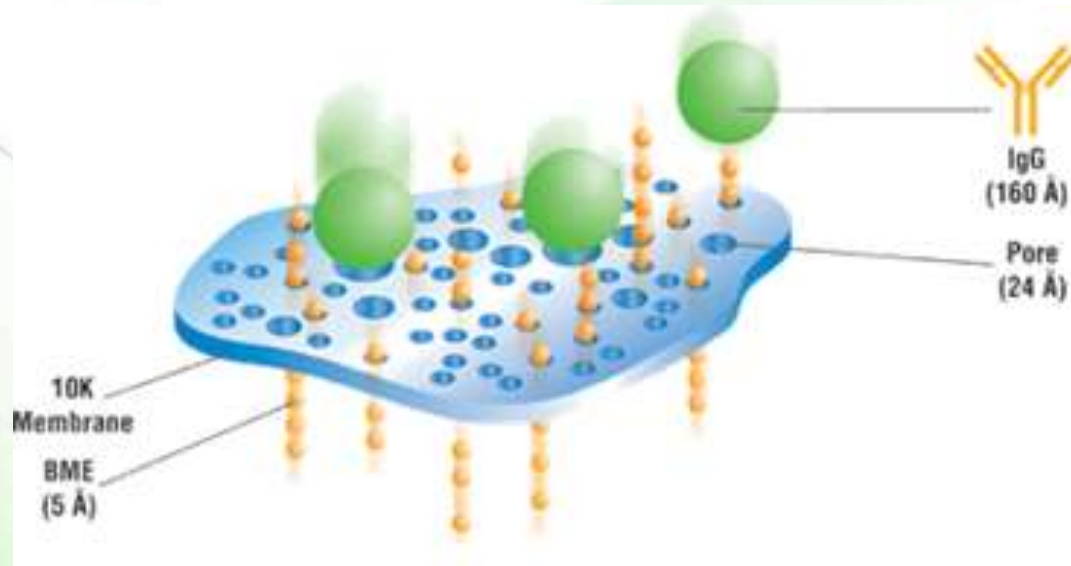
- Salting out can be used to fractionate proteins



# Dialysis



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

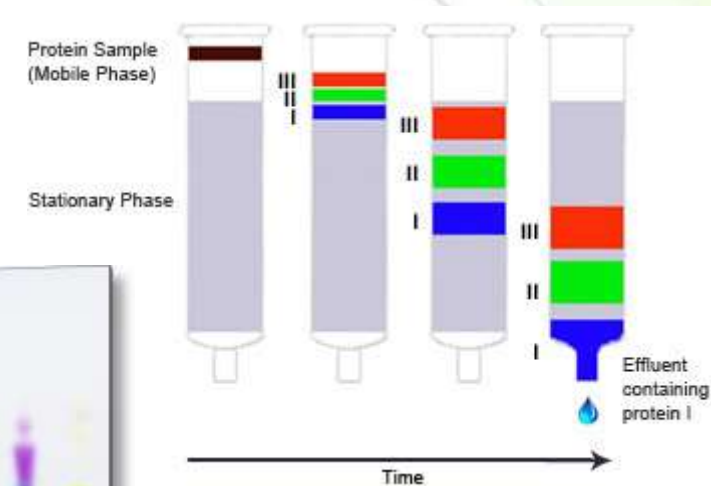
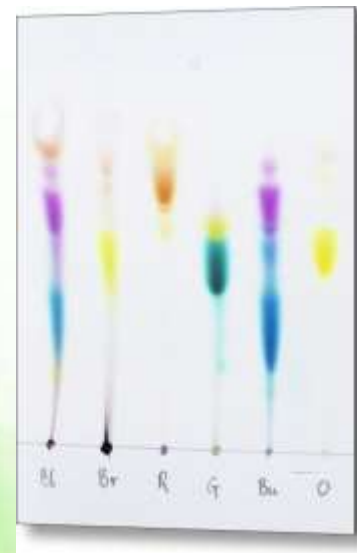


- Disadvantage of dialysis**
- Large number of larger proteins will still exist.**
  - Smaller proteins of significance are lost.**

# 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



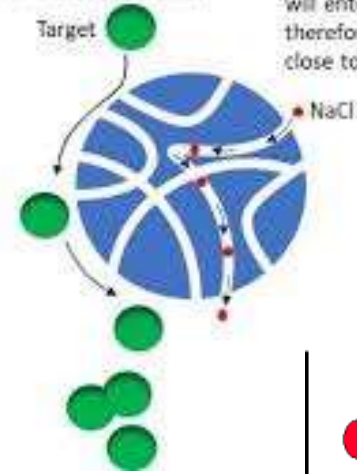
# Gel-filtration chromatography



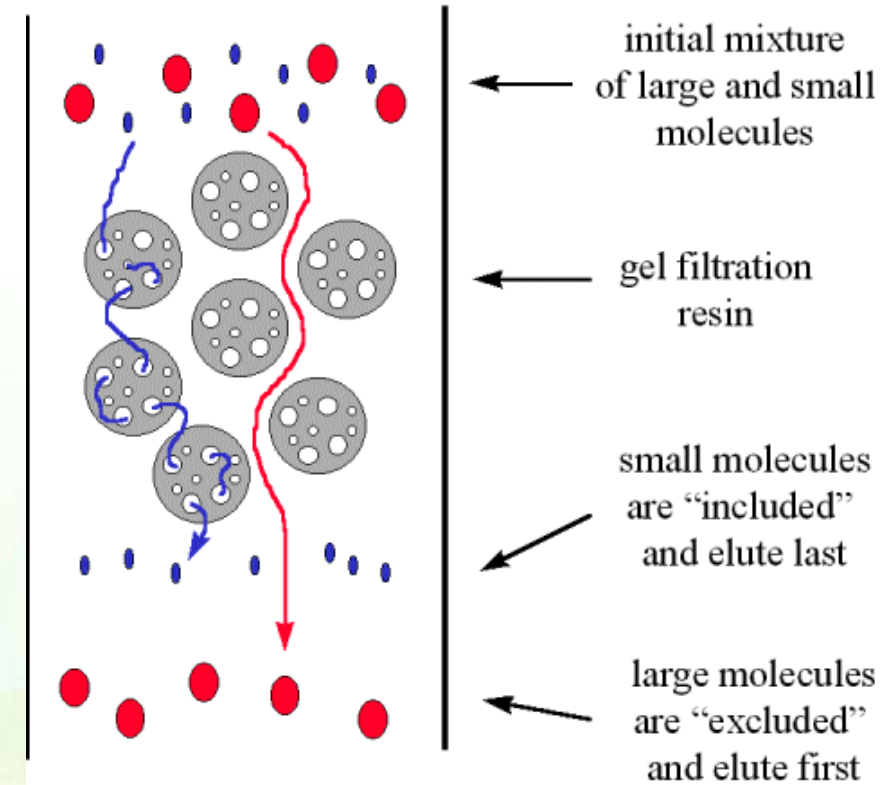
- Separations on the basis of size
  - Size-exclusion chromatography
  - Molecular sieve chromatography
- The stationary phase: porous beads
- Large molecules flow more rapidly and come out first
- Smaller molecules take a longer time in the column and exit late

Large molecules (> cut-off) will not enter into the pores, i.e. elute in the void volume.

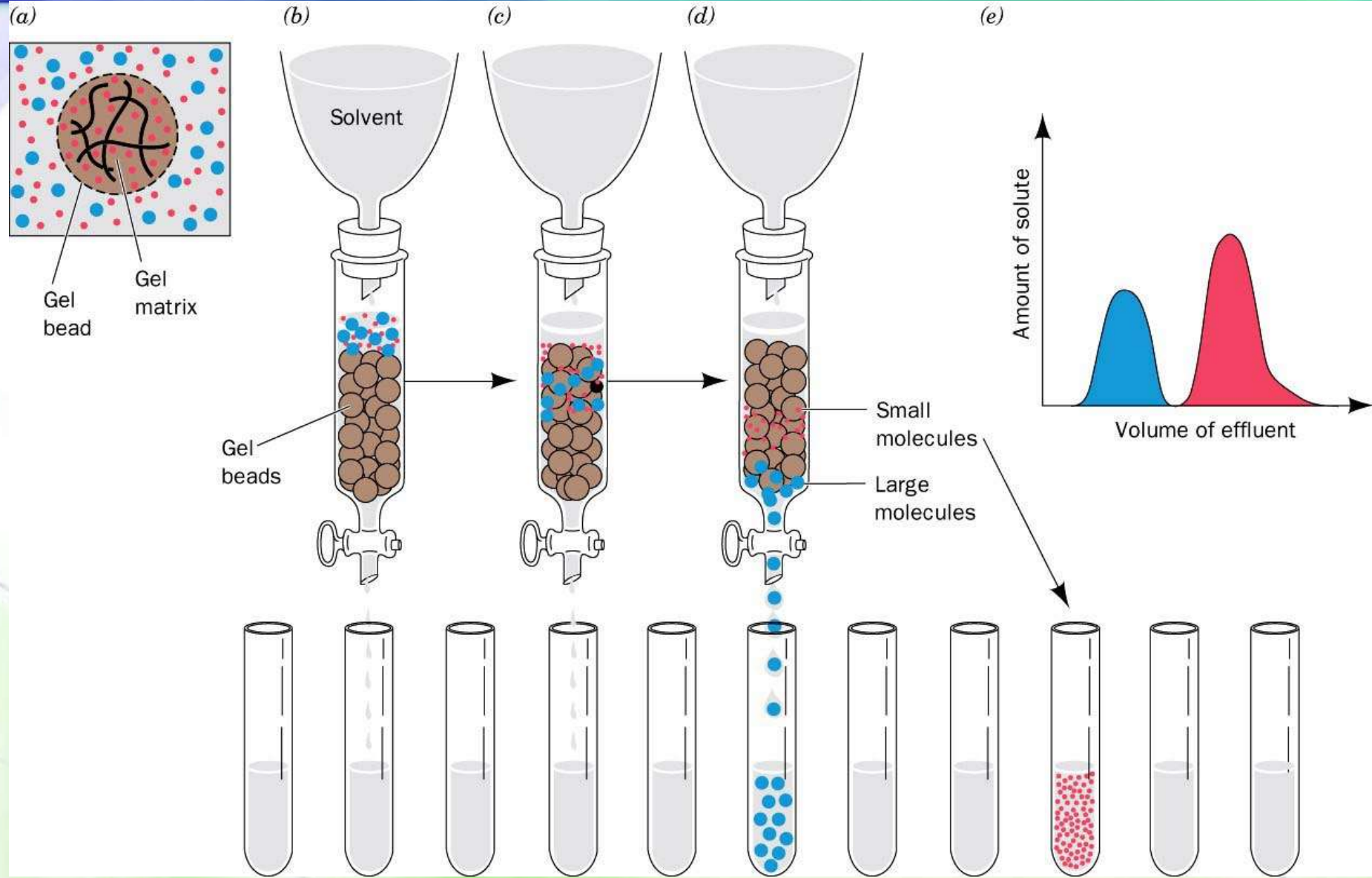
Small molecules, such as salt, will enter the pores and therefore be delayed and elute close to the column volume.



“Gel Filtration”









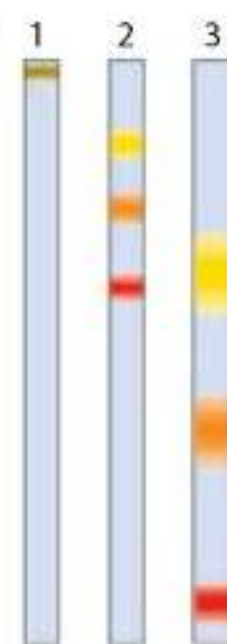
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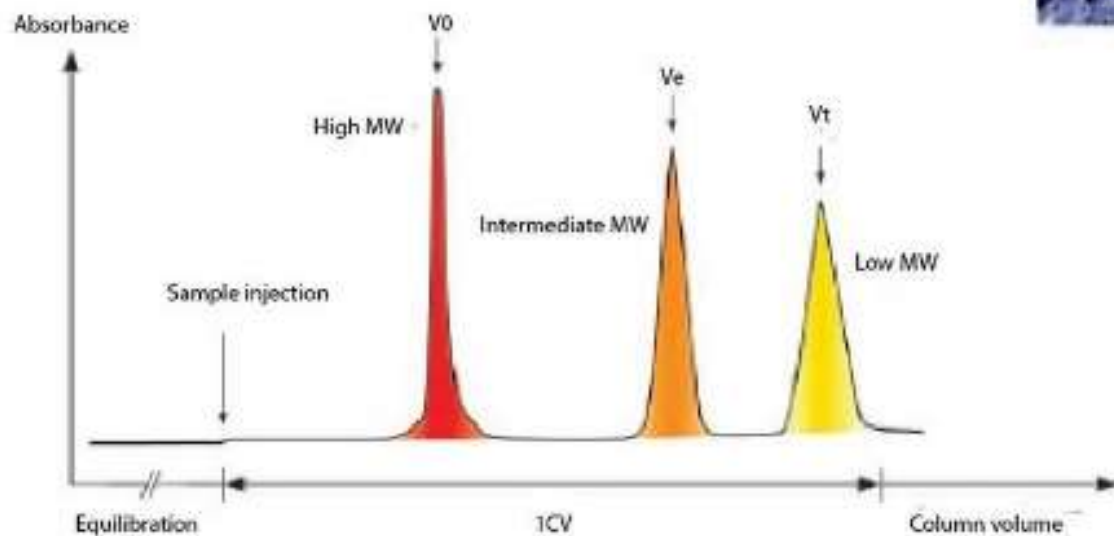
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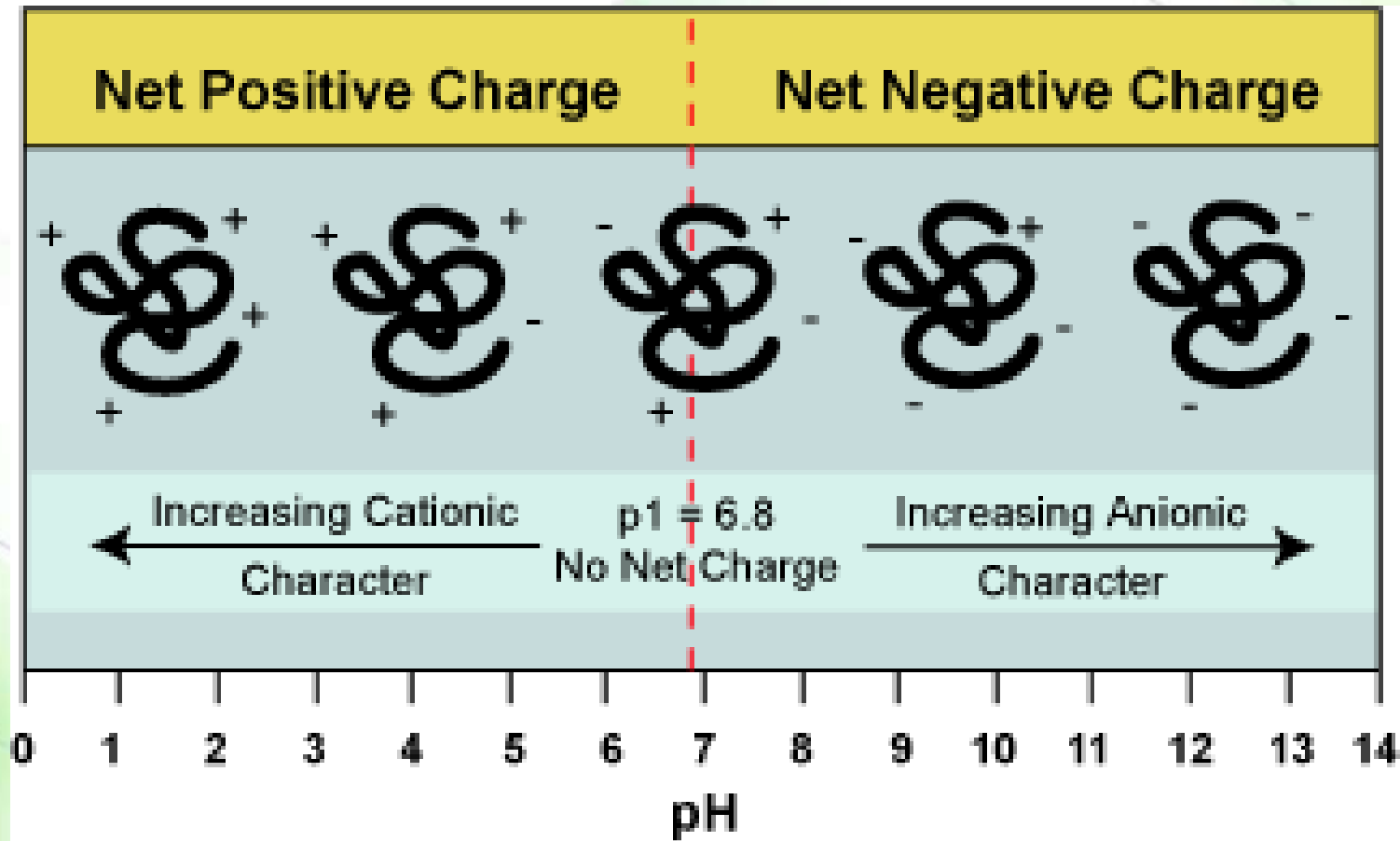
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# Ion-Exchange Chromatography



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

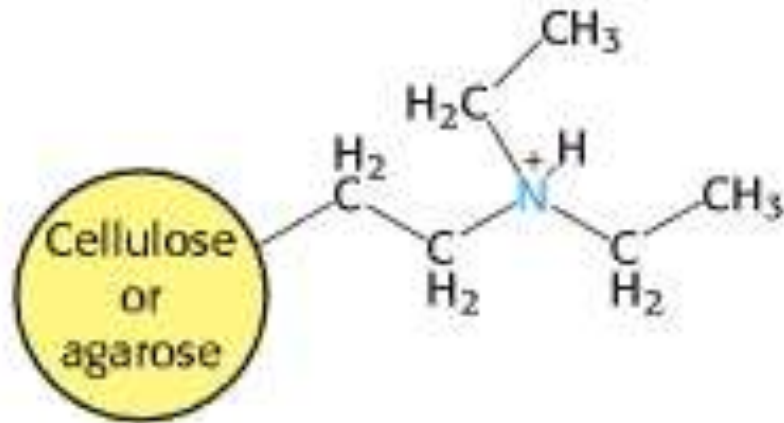




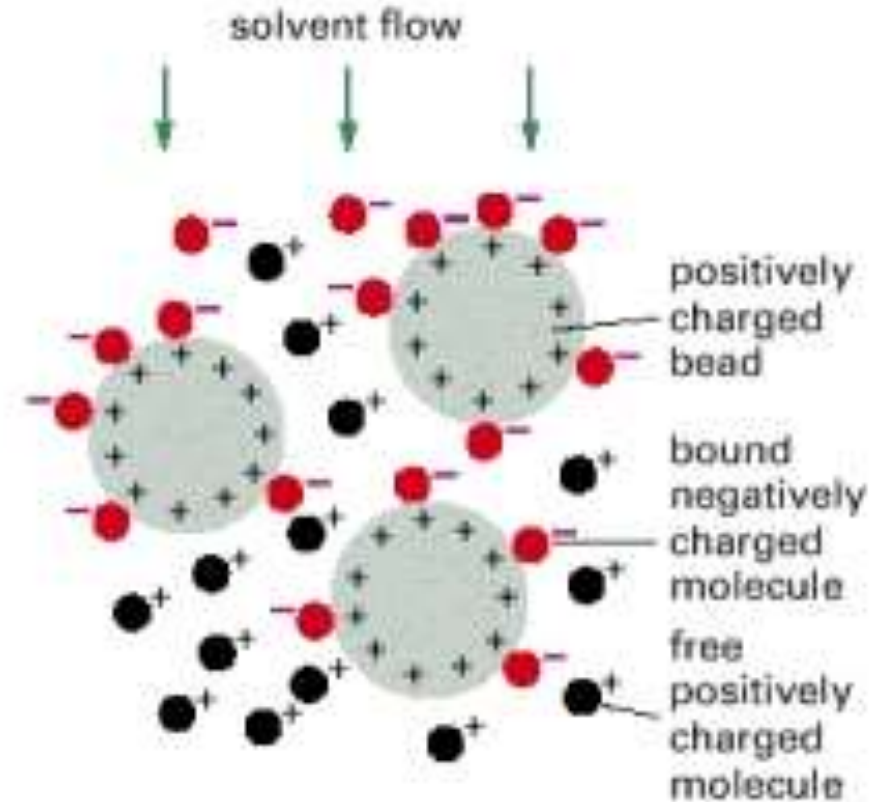
# Anionic-exchange chromatography



- The beads are positively-charged.
- Negatively charged proteins are separated.



**Diethylaminoethyl  
(DEAE) group  
(protonated form)**



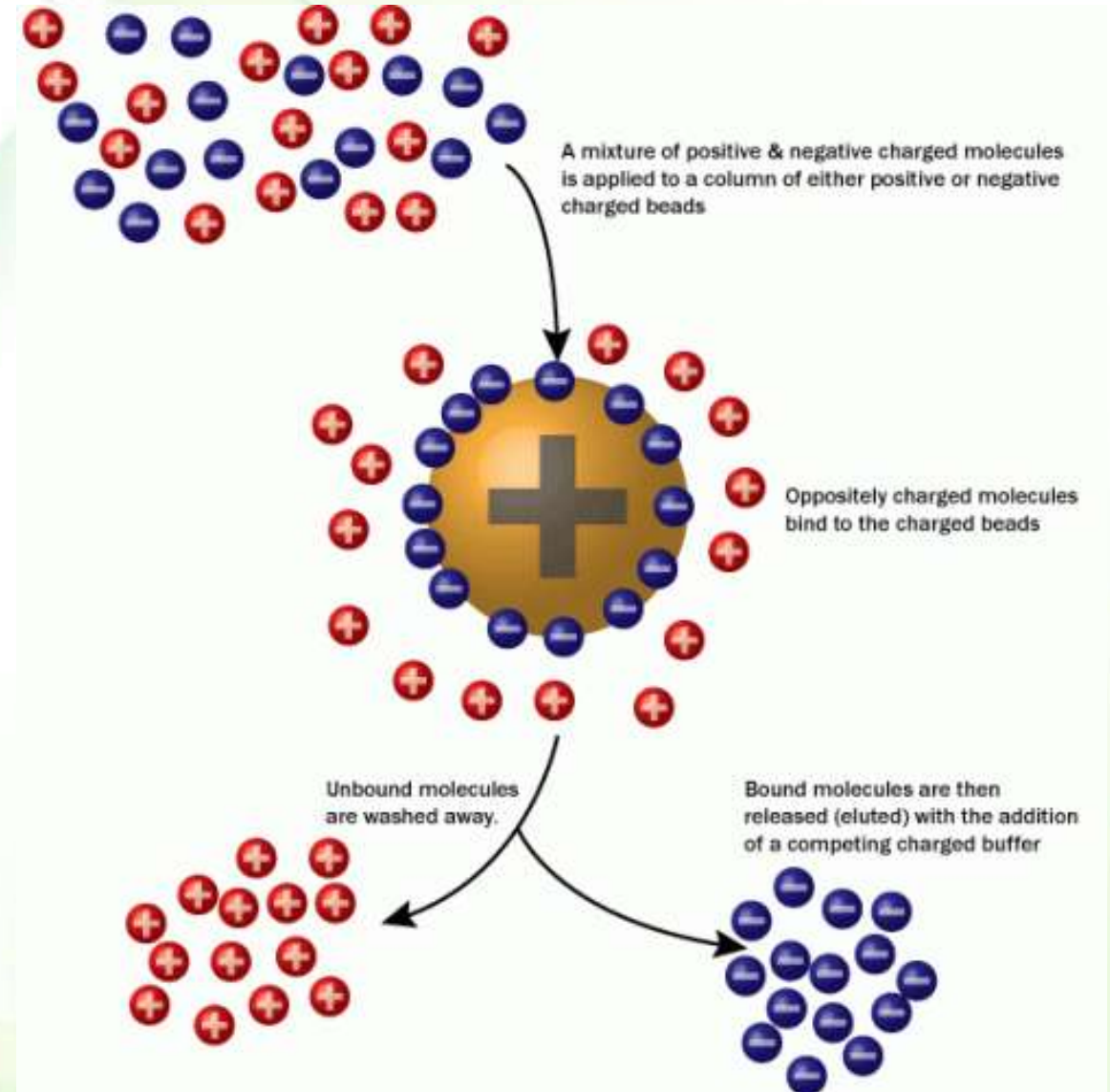
**(A) ION-EXCHANGE CHROMATOGRAPHY**



# Elution



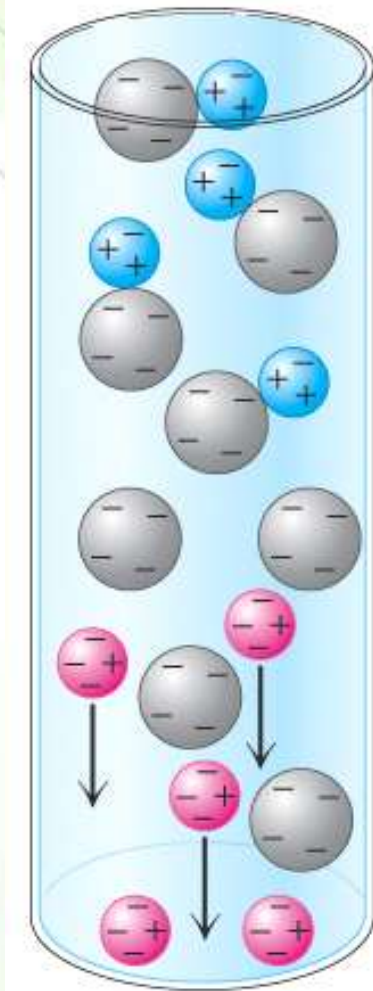
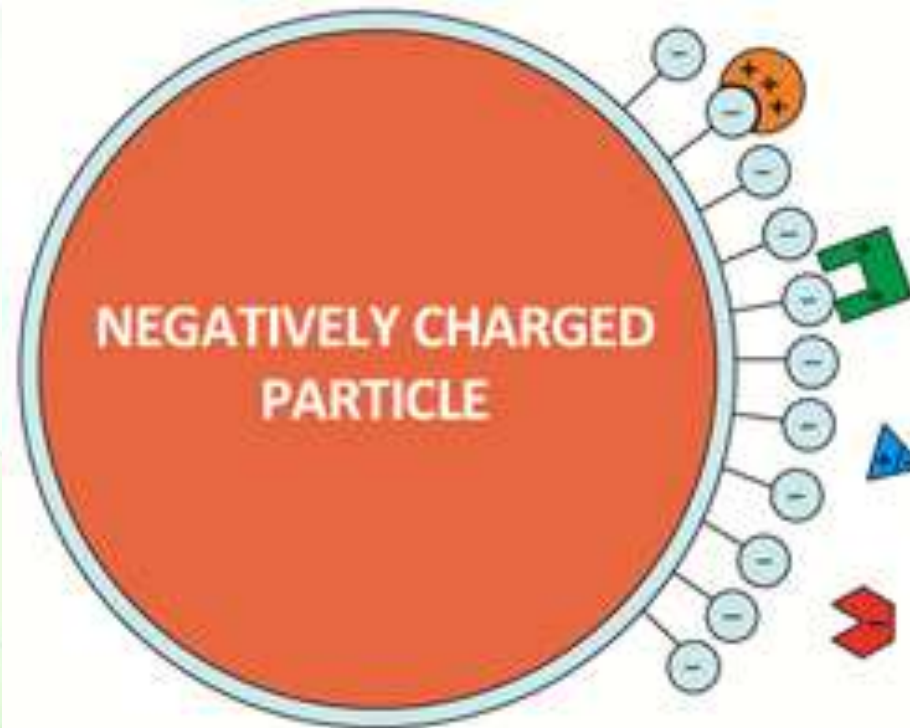
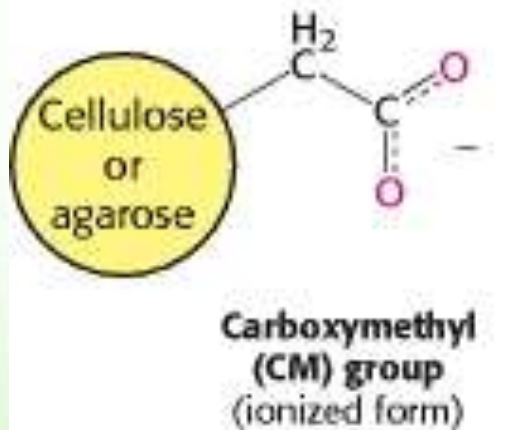
- A negatively-charged protein bound to such a column can then be eluted (released) by adding increasing concentrations of sodium chloride. Why?
  - Because chloride ions compete with negatively-charged groups on the protein for binding to the column.



# 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.



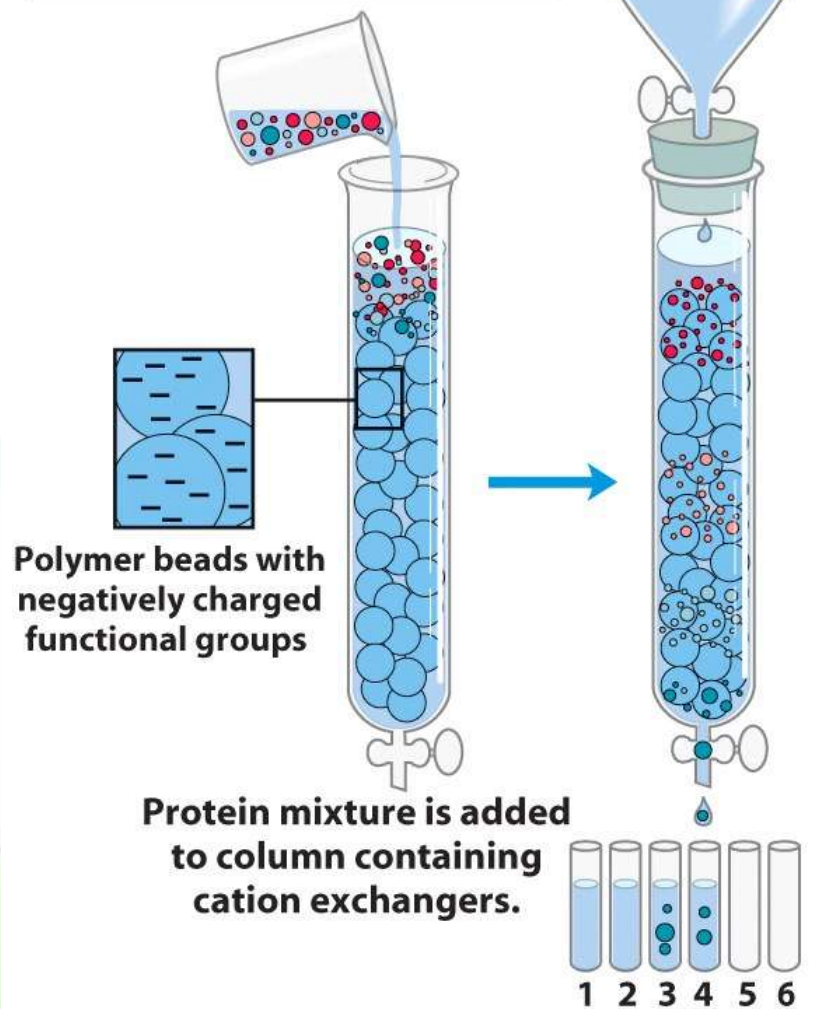
Positively charged protein binds to negatively charged bead

Negatively charged protein flows through





- Large net positive charge
- Net positive charge
- Net negative charge
- Large net negative charge



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



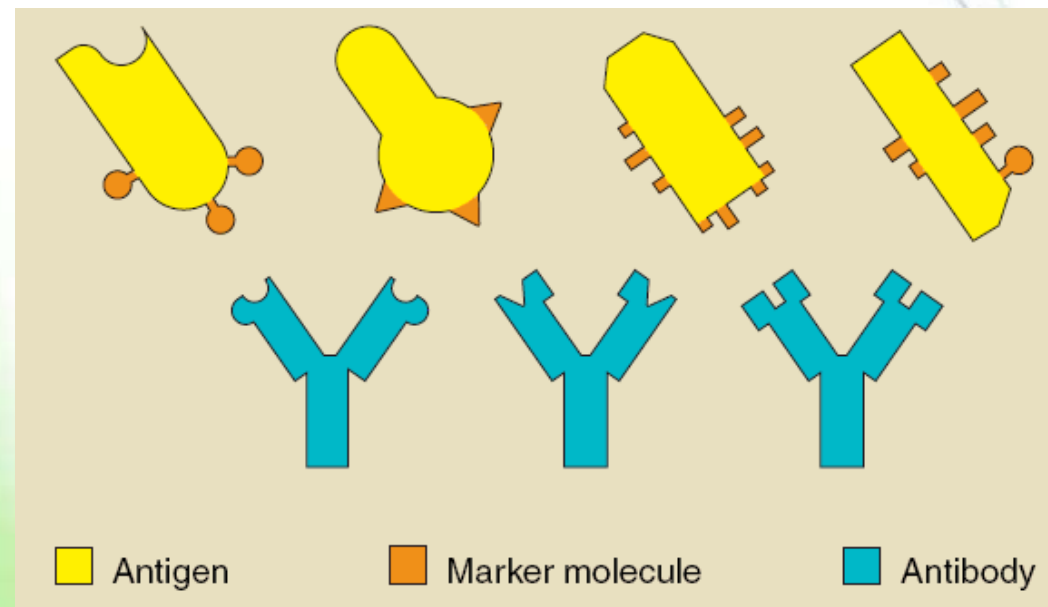
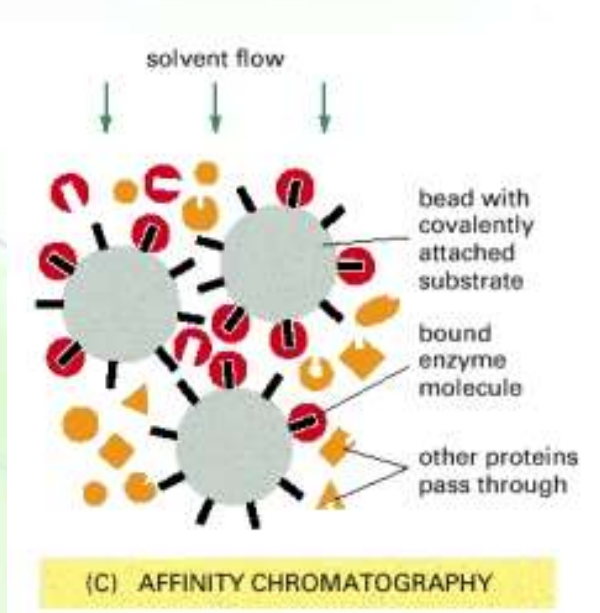
- You have 5 different proteins (#1, #2, #3, #4, and #5), with different isoelectric points (pI's).
  - pI#5 = 2.3
  - pI#4 = 4.7
  - pI#1 = 7.2
  - pI#2 = 9.1
  - pI#3 = 12.1
- Starting the column at pH 6.5, the sample is added and then washed to remove unbound molecules. What is the order of protein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

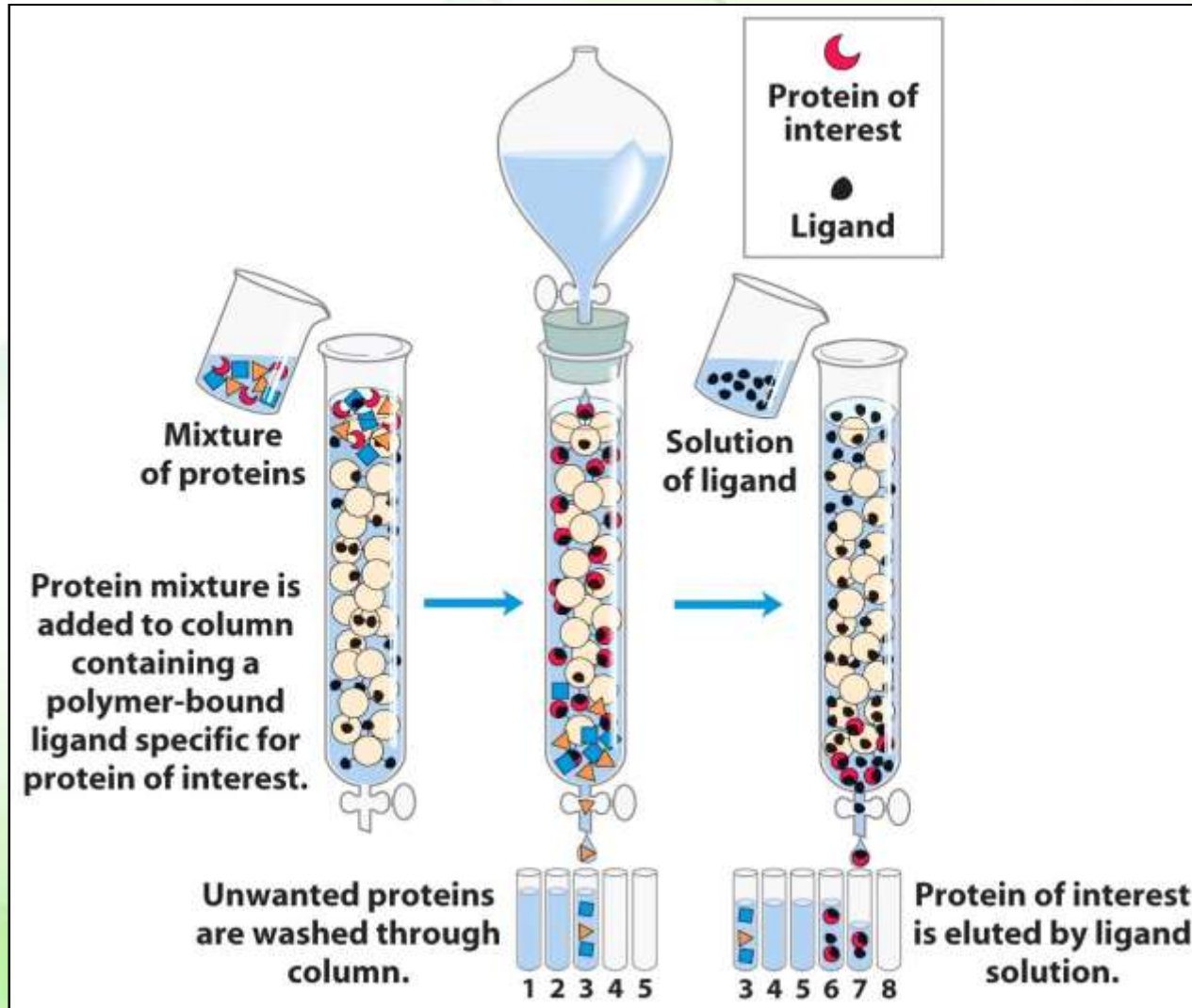
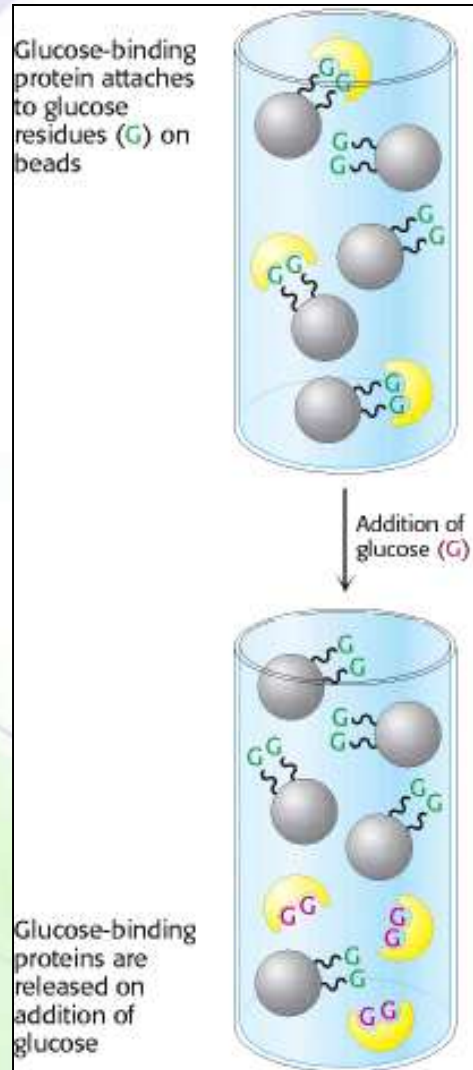


# Affinity Chromatography



- 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.

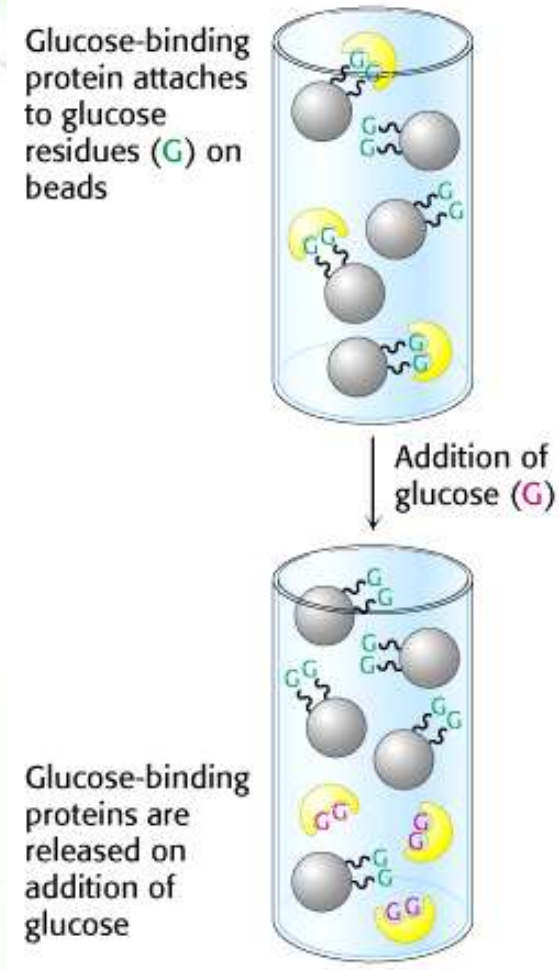




# Example



- 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?**