

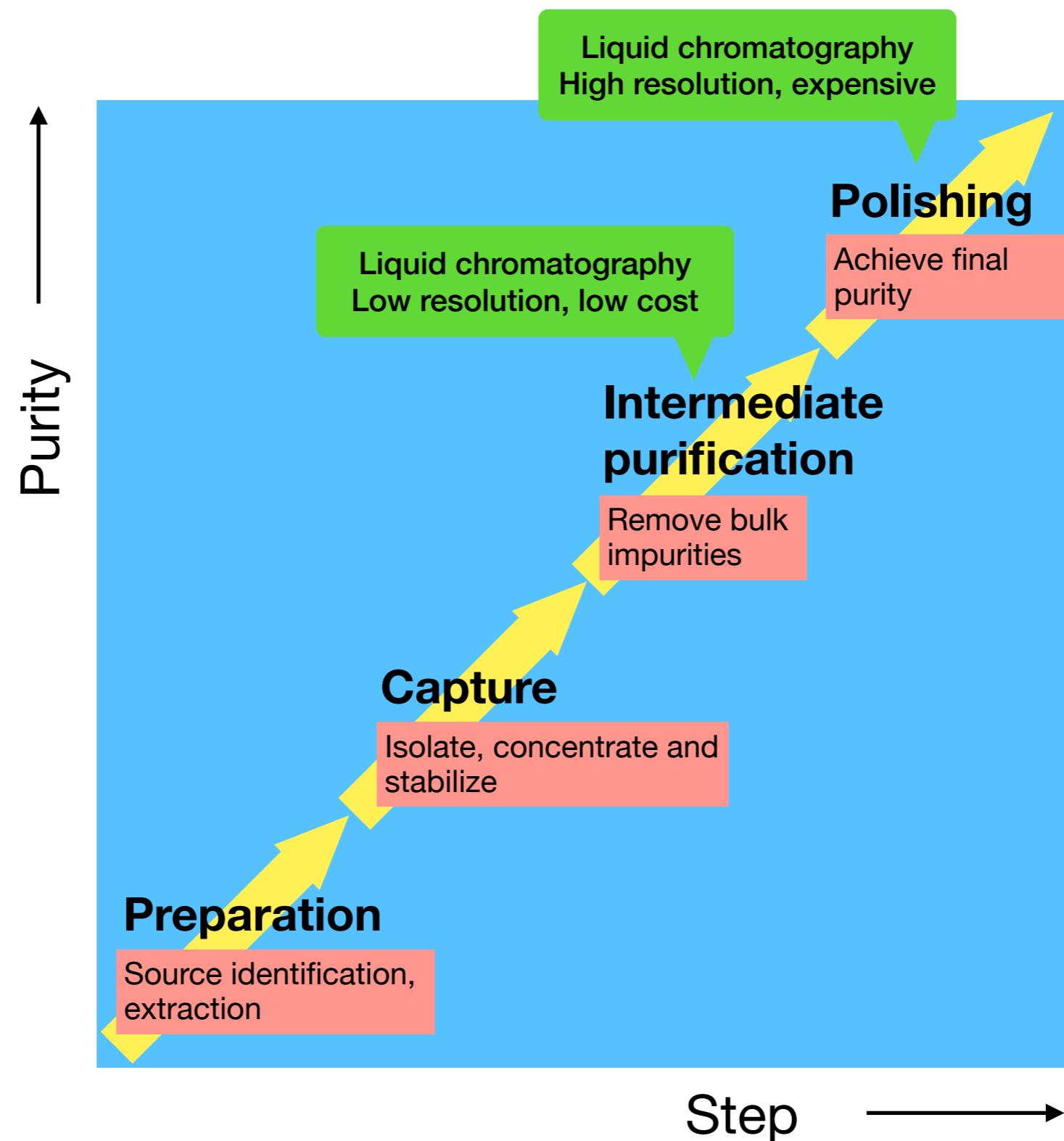
Today's class:

Purification of Biomolecules - part 2

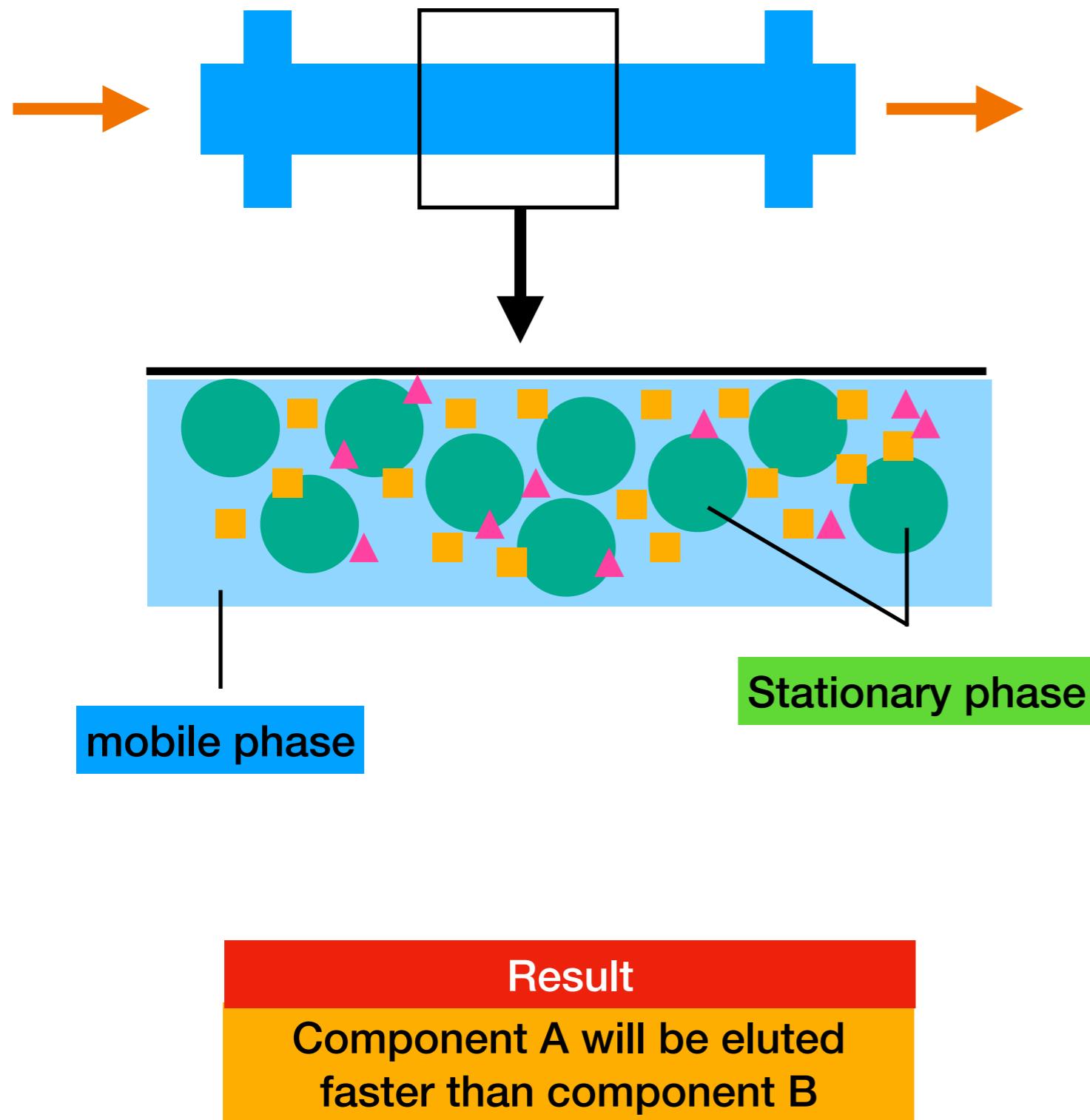
This lecture follows the materials from different sources

- *Fundamentals of Biochemistry by Voet, Voet and Pratt, 5th Ed, Wiley*
- *Proteins: Structure and Function by David Whitford, Wiley*

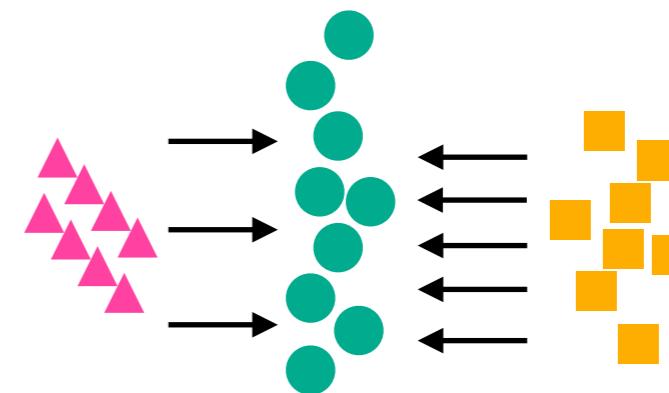
Steps of protein purification



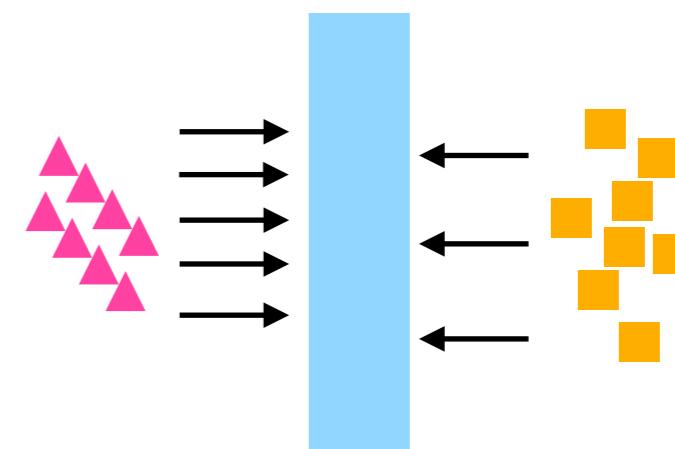
Liquid chromatography: basics



Affinity for the stationary phase



Affinity for the mobile phase



Types of liquid chromatography

Adsorption chromatography

- Proteins bind to the stationary phase
- Proteins eluted by altering mobile phases
- E.g. affinity, hydrophobic interaction, ion-exchange

Solution-phase chromatography

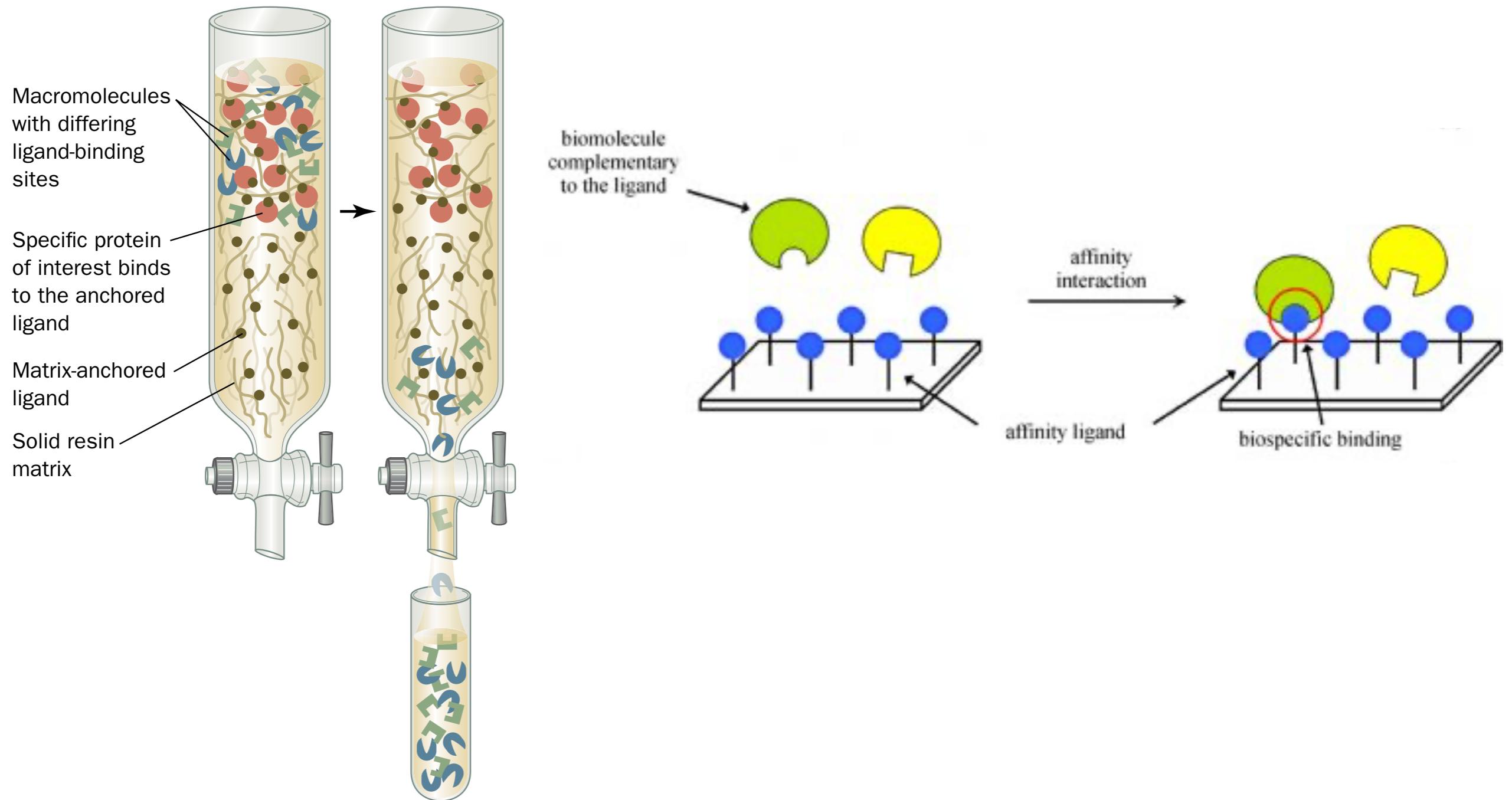
- Proteins do not bind to the stationary phase
- Migration of proteins impeded by matrix of the stationary phase
- E.g. size-exclusion or gel filtration

Advantages and disadvantages of liquid chromatography

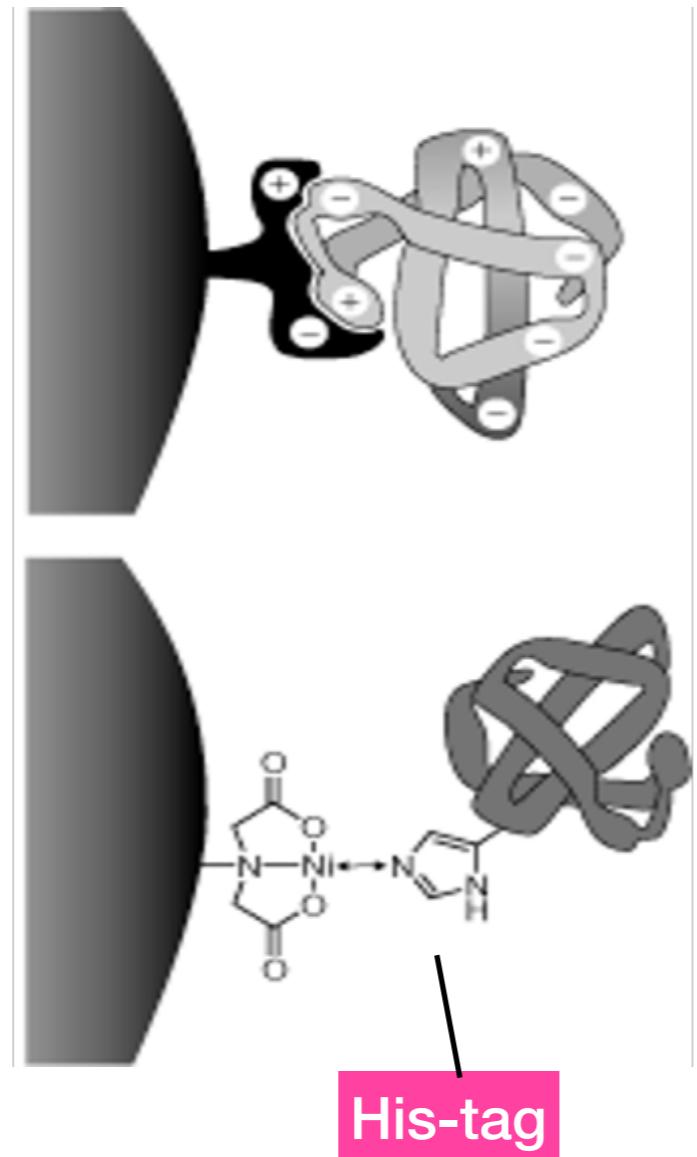
Type of Chromatography	Advantages	Disadvantages	Resolution
Affinity	Quick and specific	Resins and ligands can be expensive	Low to Medium
Hydrophobic Interaction	Can be used directly from ammonium sulfate precipitation	Relatively low resolution and binding capacity	Low to Medium
Ion Exchange	Versatile resin choices	Protein solution must start at low [salt]	Medium to High
Size Exclusion	Distinct from other techniques, Can be used analytically or for buffer exchange	Long run time	Low to High

Affinity chromatography

This method is based on the ability of proteins to interact with specific molecules.



Two types of substrates are used in affinity chromatography



Surface bound with
Epoxy, aldehyde or aryl ester groups

Surface bound with
Iminodiacetic acid + $\text{Ni}^{2+}/\text{Zn}^{2+}/\text{Co}^{2+}$

Recombinant DNA techniques can be used to append a segment of six consecutive His residues, known as a His tag, to the N- or C-terminus of the polypeptide to be isolated. This creates a metal ion–binding site.

Hydrophobic interaction chromatography

This method exploits hydrophobic interactions between proteins and the chromatographic matrix

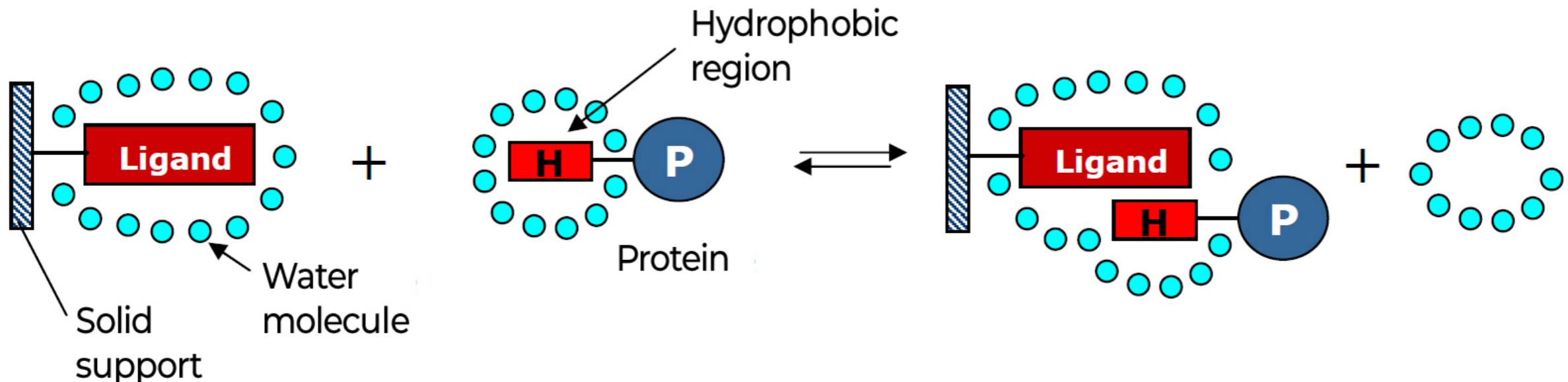
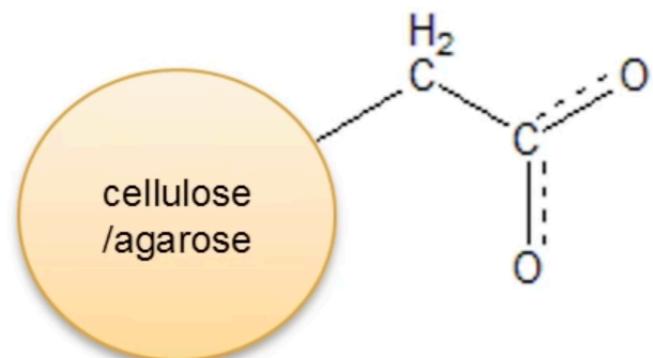


Image courtesy: intercom.com

- The matrix material is lightly substituted with octyl or phenyl groups
- At high salt concentrations, nonpolar groups on the surface of proteins “interact” with the hydrophobic groups on the matrix and get excluded by the polar solvent — entropy effect
- Hydrophobic effects are augmented by increased ionic strength
- The eluant is typically an aqueous buffer with decreasing salt concentrations, increasing concentrations of detergent (which disrupts hydrophobic interactions), or changes in pH.

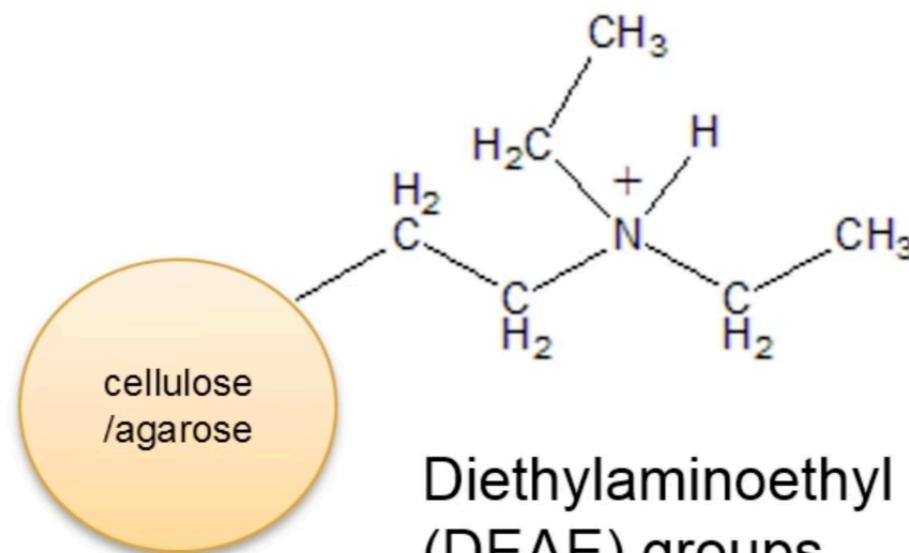
Ion-exchange chromatography

Cation exchanger matrix

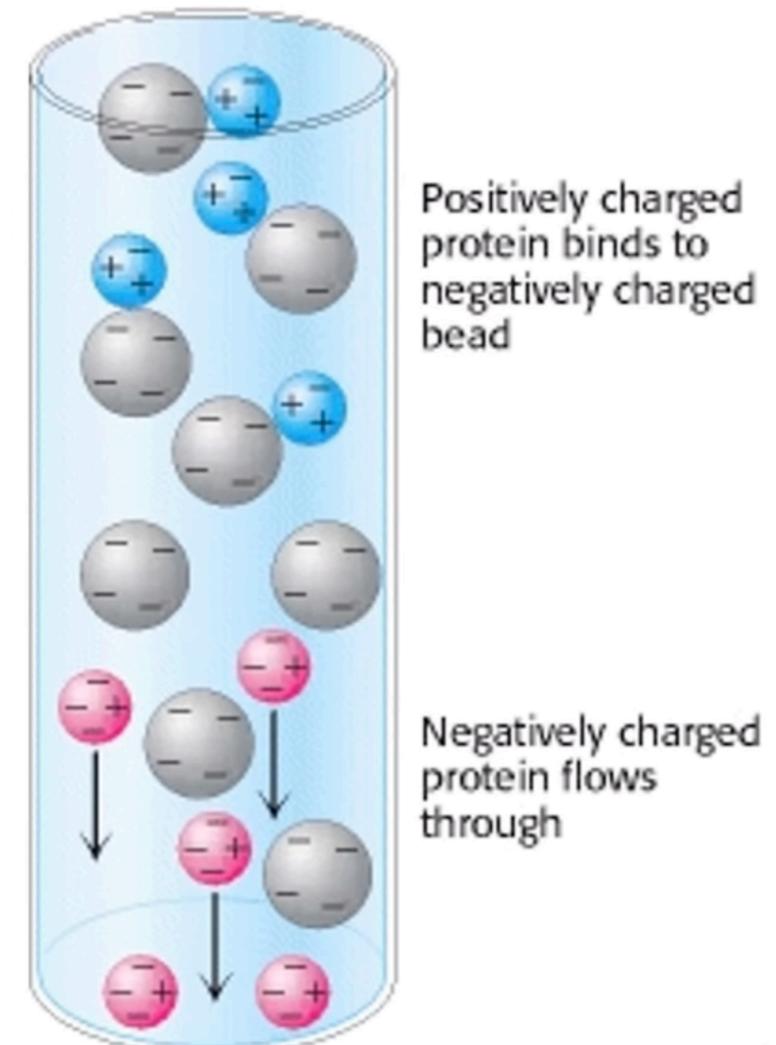


Carboxylate(CM)
groups

Anion exchanger matrix



Diethylaminoethyl
(DEAE) groups



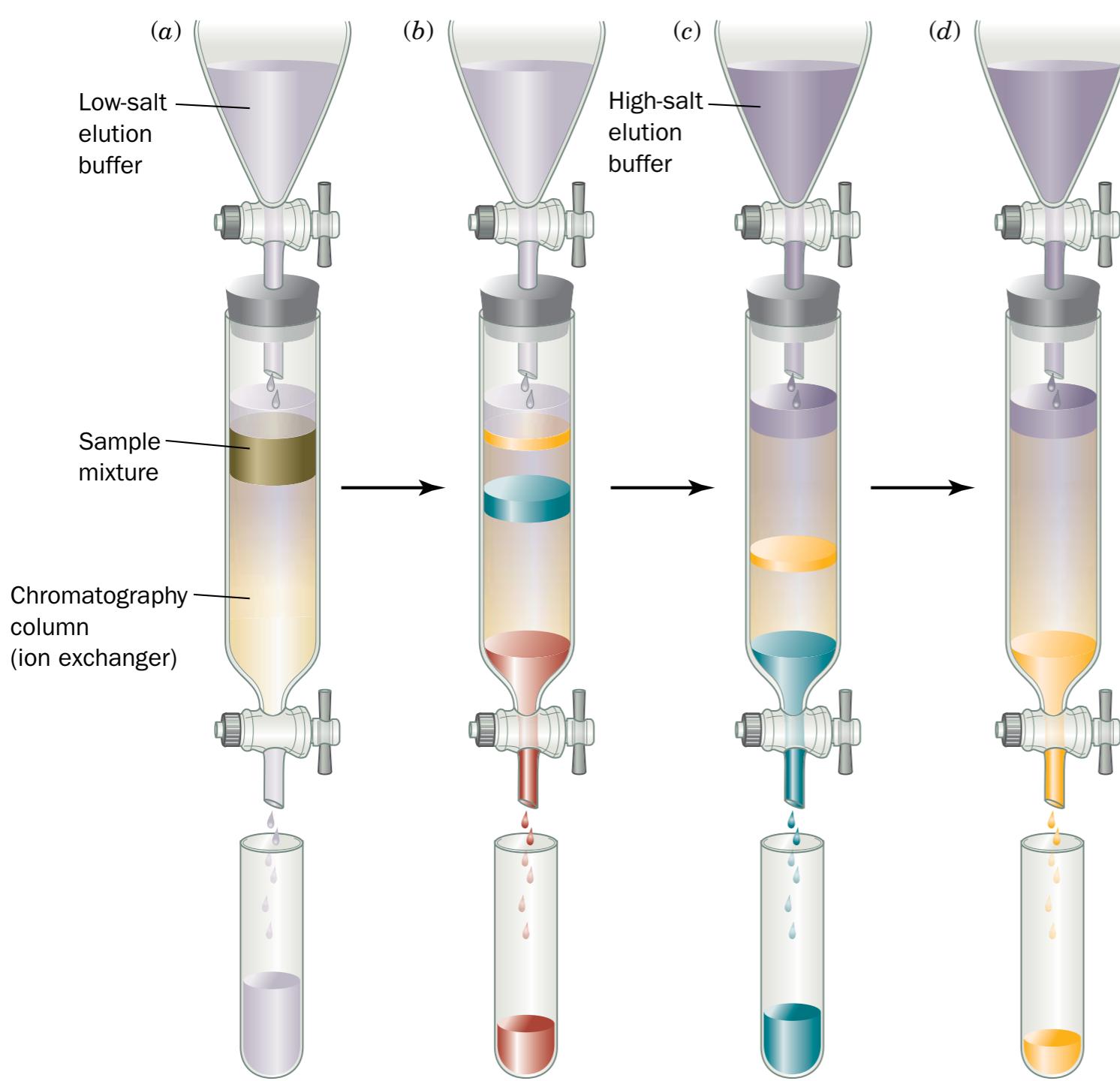
Positively charged protein binds to negatively charged bead

Negatively charged protein flows through

Image courtesy: creative BioMart

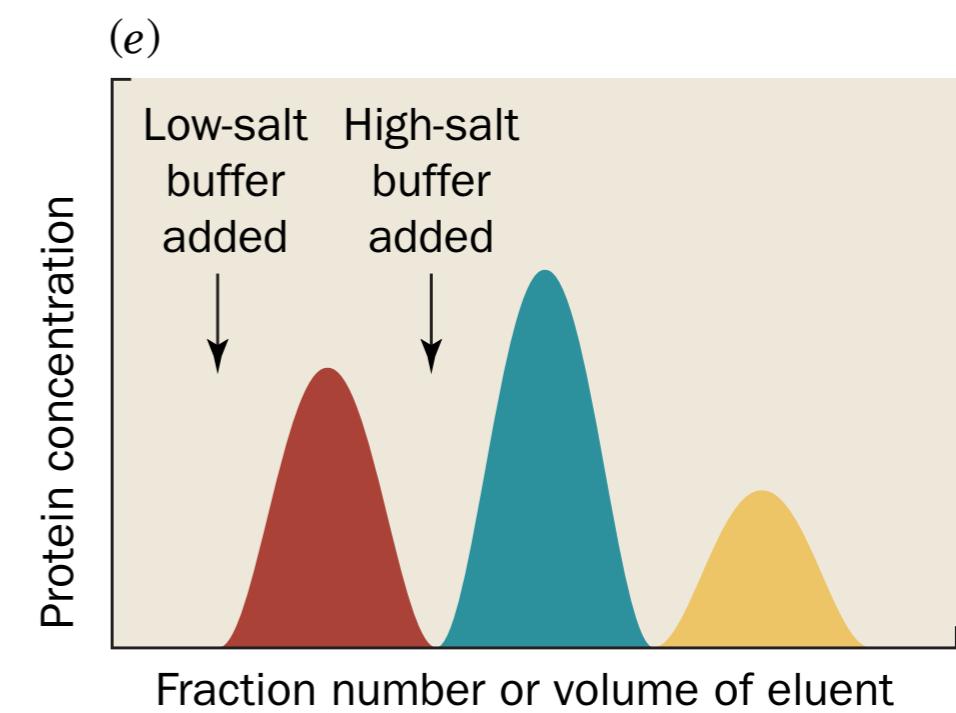
- Proteins and other polyelectrolytes (polyionic polymers) that bear both positive and negative charges can bind to both cation and anion exchangers.
- The binding affinity of a particular protein depends on the presence of other ions that compete with the protein for binding to the ion exchanger and on the pH of the solution, which influences the net charge of the protein.

Ion-exchange chromatography



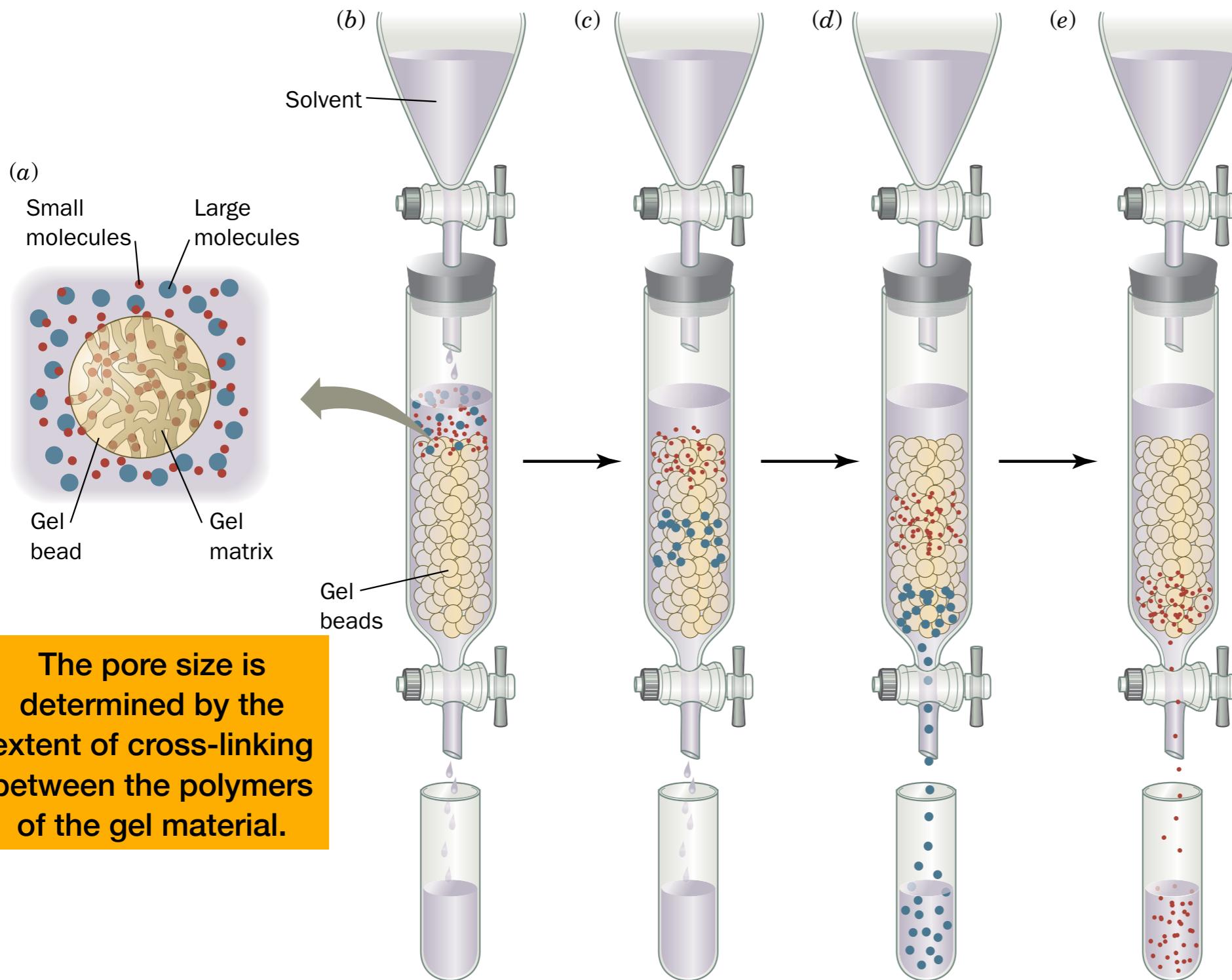
Here salt conc in the eluant (buffer used to elute the proteins tightly bound to the matrix) is slowly increased to elute different proteins

Salt changes the ionic strength of the eluant that alters the affinity of the protein to the matrix



Gel-filtration chromatography

aka size-exclusion chromatography



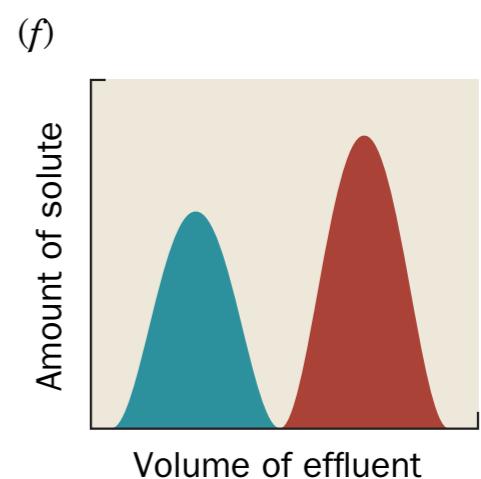
The pore size is determined by the extent of cross-linking between the polymers of the gel material.

Proteins larger than pore size moves rapidly and eluted early

Different pore sizes can be used in same gel to separate different proteins

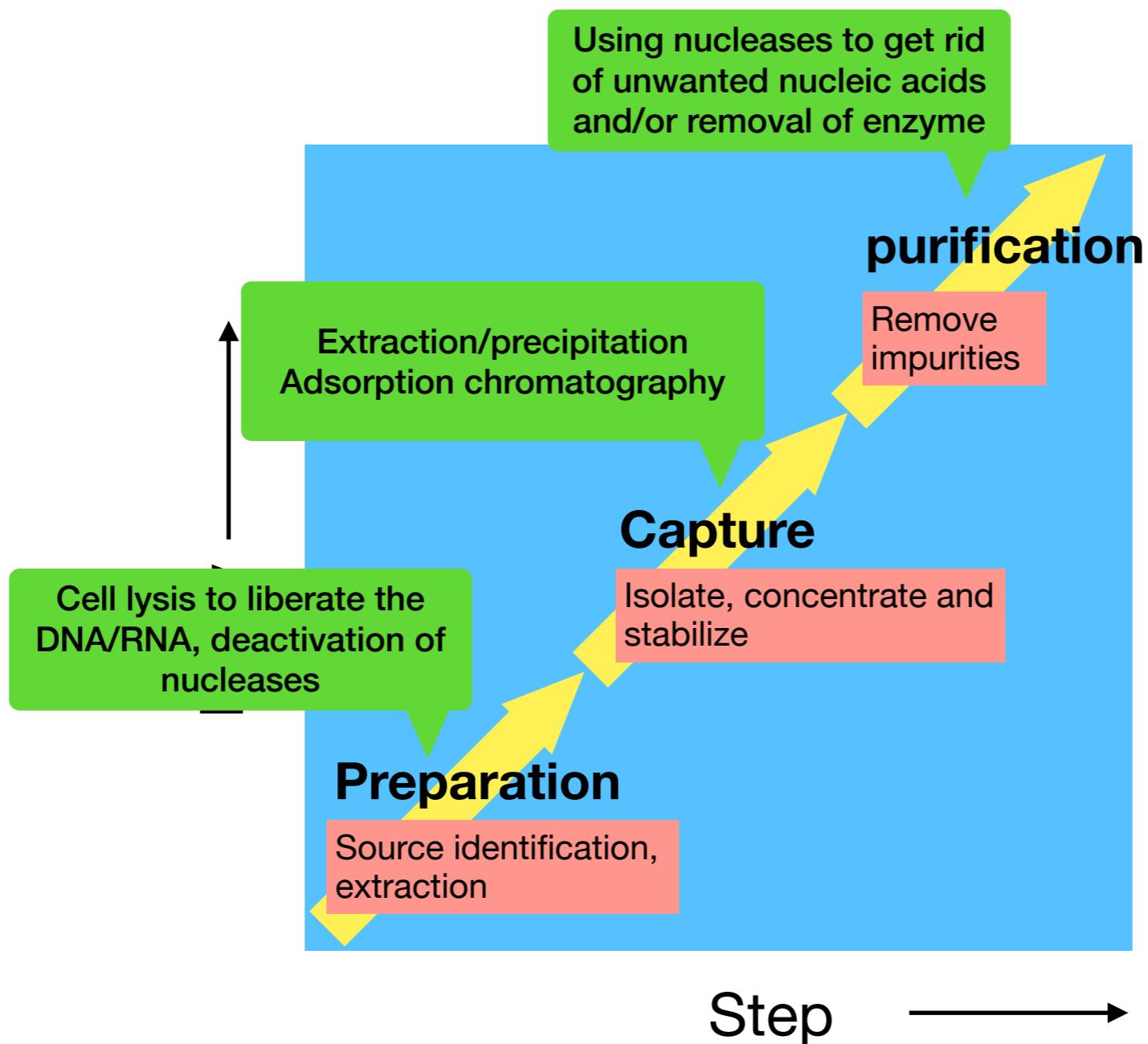
Relative elution volume of a protein
 $V_{elute} \propto \log(\text{MW})$

So a gel column can be calibrated to find MW of an unknown protein from its elution position



Nucleic acid purification

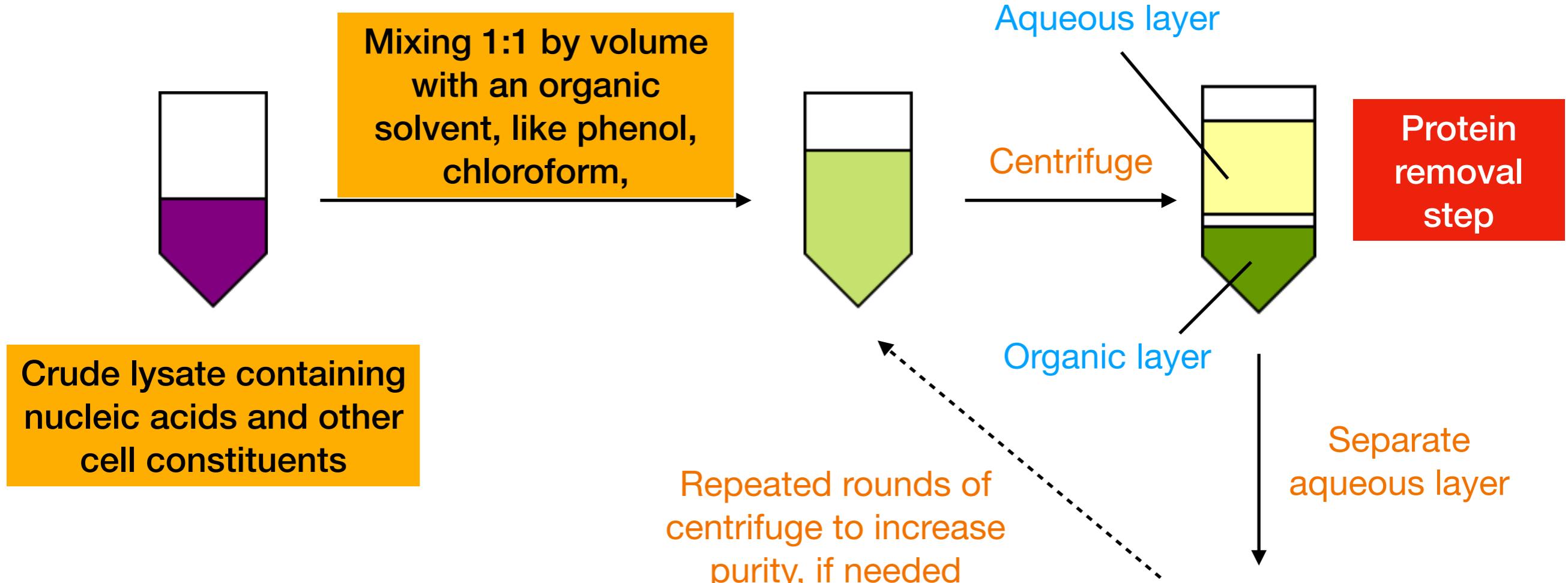
Steps of nucleic acid purification



Deactivation of nucleases

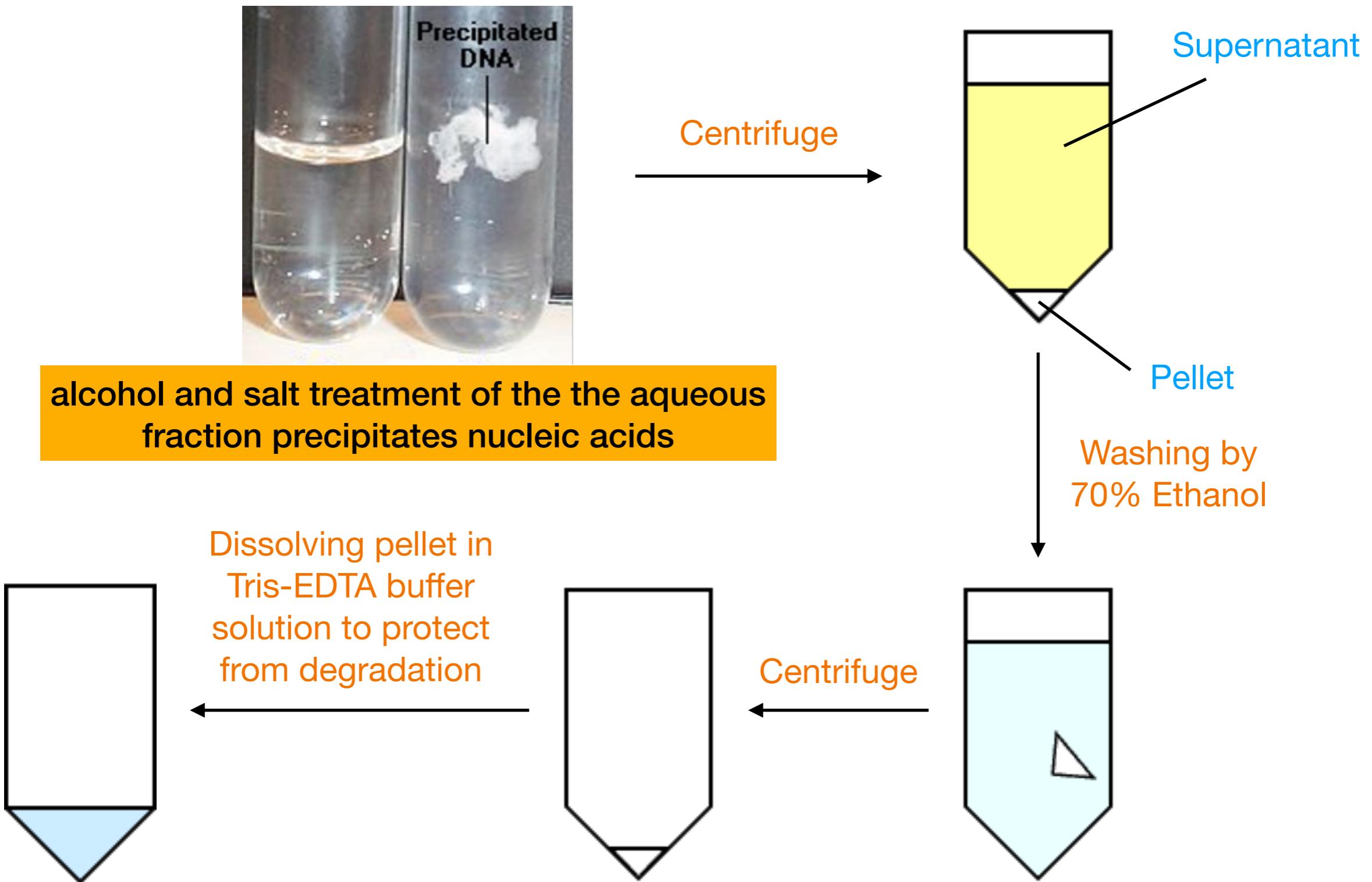
- Autoclaving solutions — This is usually sufficient for removal of DNases, and most RNases
- Using Diethyl Pyrocarbonate (DEPC)-treated water — 0.1% DEPC deactivates RNases by covalent modifications of His residues. In Tris or HEPES buffers, commonly used for nucleic acid extraction higher conc of DEPC is required.
- Baking glass, metal, or ceramic equipment at high temp and maintaining the same set of equipments to avoid contamination.
- Wearing gloves — this avoids RNase7 naturally produced by skin cells,

Organic extraction/precipitation of nucleic acids



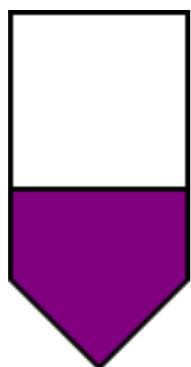
- The aqueous phase contains all the water-soluble ingredients, including nucleic acids.
- Proteins and lipids become trapped in the organic phase, and are thus separated away.
- Insoluble plant debris become trapped in the interphase between the two layers

Organic extraction/precipitation of nucleic acids...*contd*



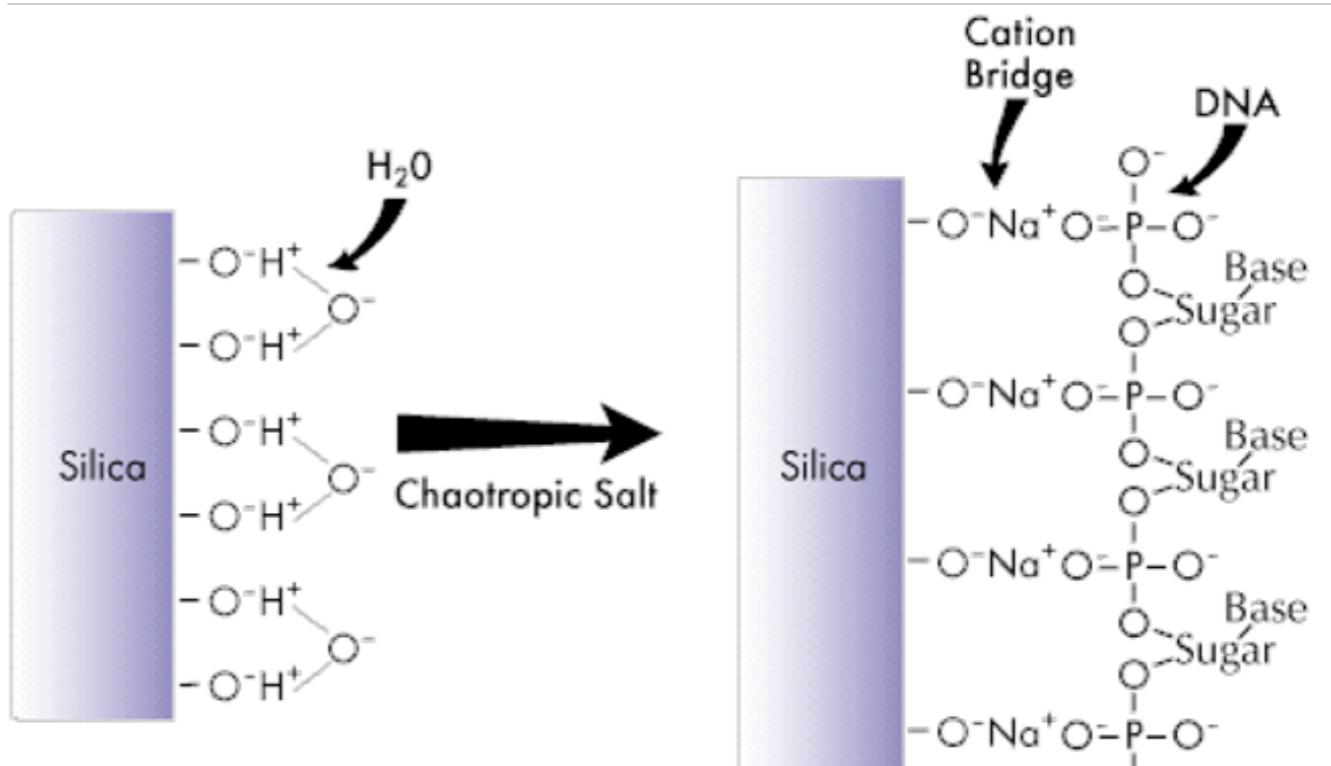
Adsorption chromatography of nucleic acids

Crude lysate containing nucleic acids and other cell constituents



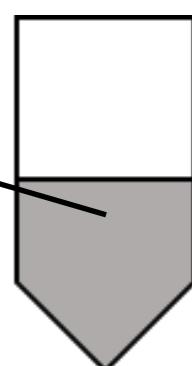
Extraction Buffer composition favors DNA and RNA adsorption to silica:

- Low pH
- High ionic strength
- Chaotropic salt (Guanidinium chloride)

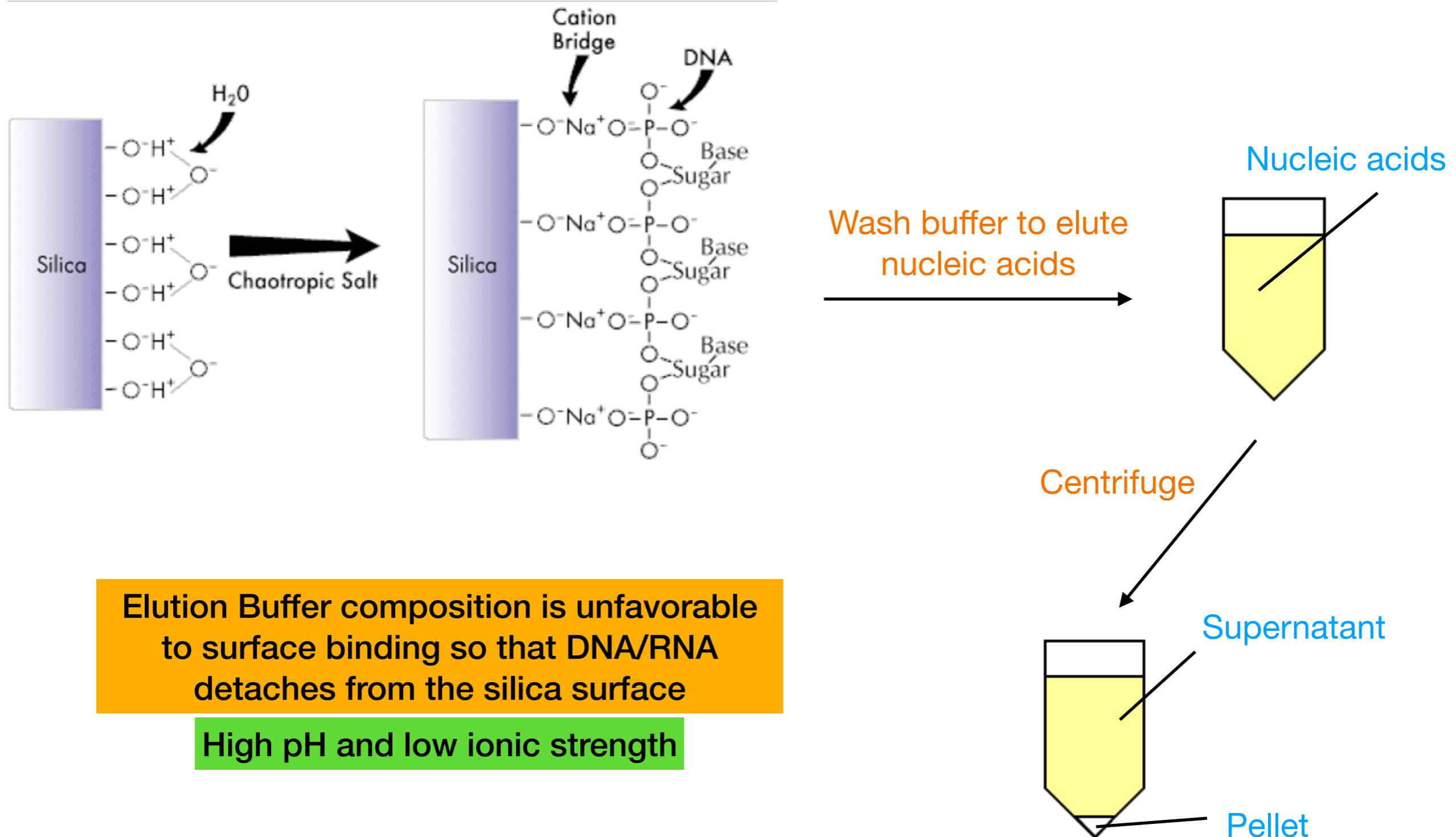


Nucleic acids bind to the membrane, while contaminants pass through the column.

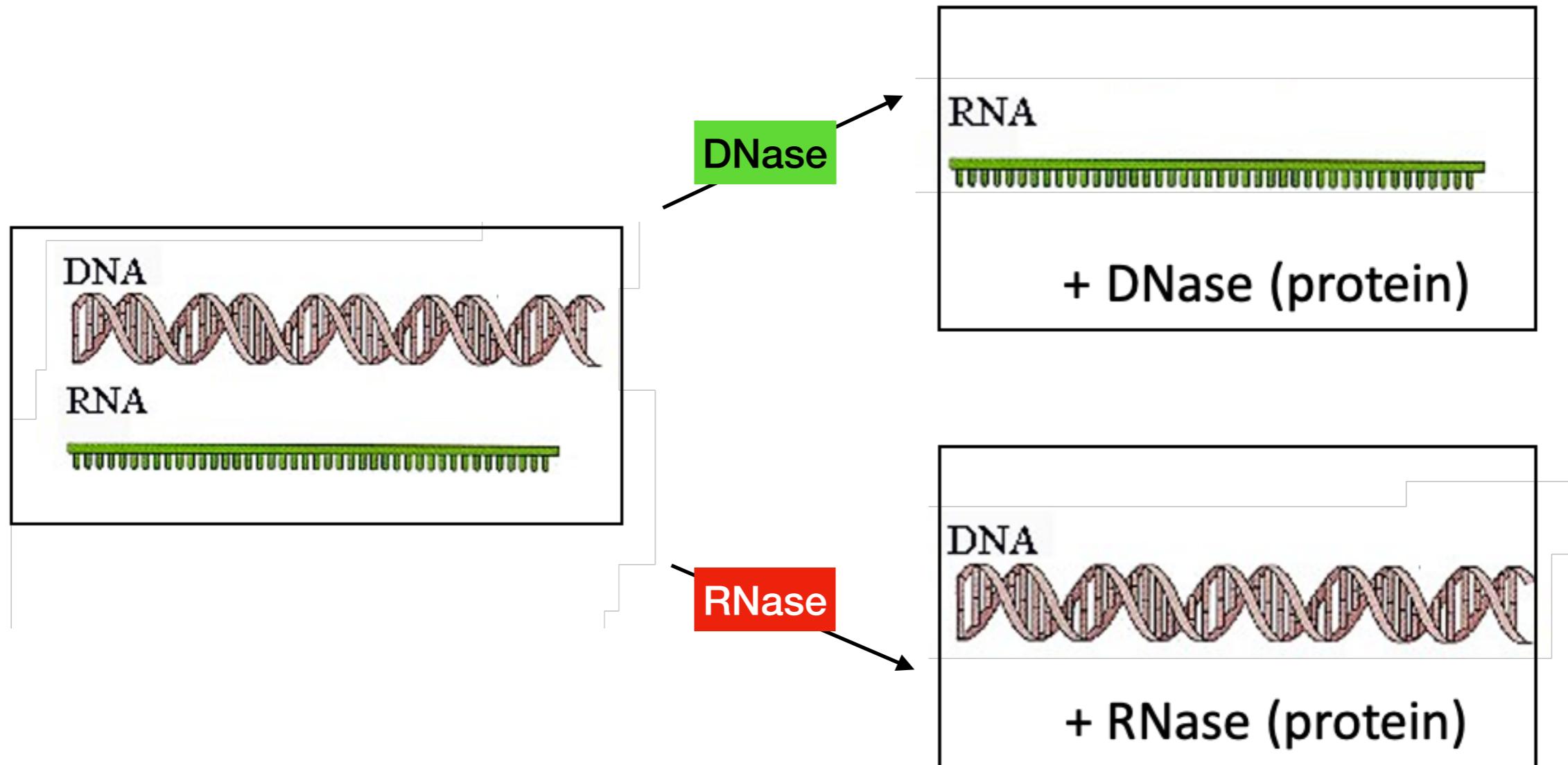
Flow through (discarded)



Adsorption chromatography of nucleic acids...*contd*



Final step if needed to remove unwanted DNA or RNA



It may be necessary to repeat purification steps for protein removal
(e.g. phenol/chloroform extraction).