



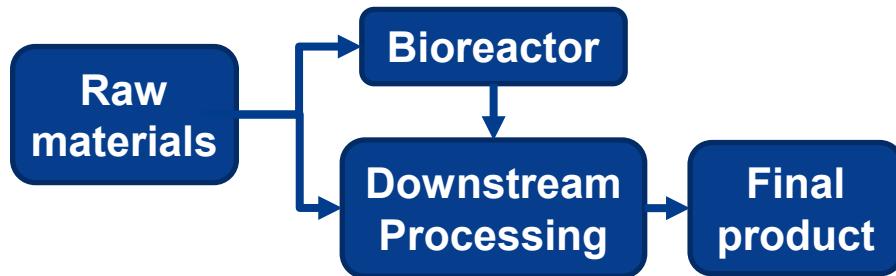
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What is bioprocessing?



Bioprocessing





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Cell removal / enrichment



Cell removal/enrichment

A common first step if the cells themselves are the product or the product is outside the cell.

The step involves either filtration or centrifugation.

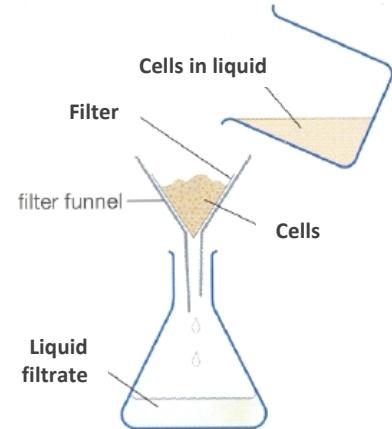
- Filtration relies on size – cells versus proteins in the medium
- Centrifugation relies on density – cells are more dense than proteins.

Filtration

Conventional filtration – solid particles are separated from fluid-solid mixture by forcing the fluid through a filter medium or filter cloth that retains the particles.

Cell suspensions are passed through a filter cloth that retains the cells while the liquid passes through the filter cloth.

Difficult to achieve on a large scale under aseptic techniques.



Microfiltration uses membranes with a pore size of 0.1 – 10 µm.
Remove all bacteria, but only some viral contamination.

Ultra filtration completely removes viruses with membrane pores of 0.001 – 0.1 µm.

Centrifugation

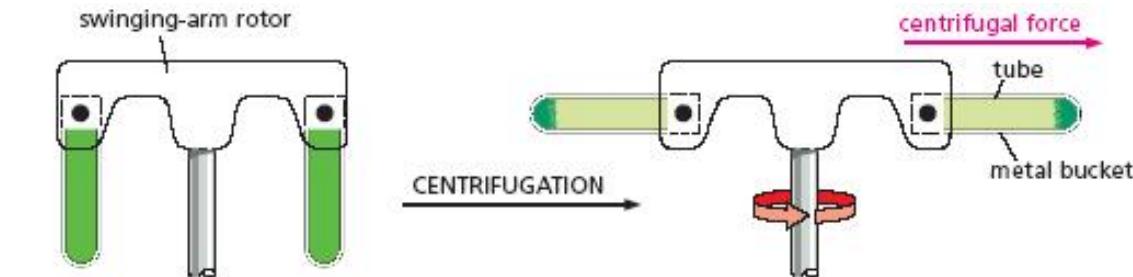
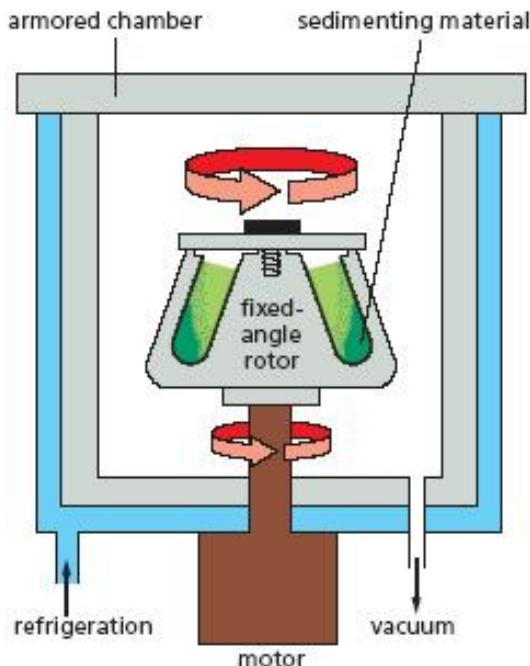
Separate materials of different density by applying a force greater than gravity.

Used to:

- Remove cells from suspension
- Remove cell debris from suspension
- Collect precipitates

Centrifugation example

THE CENTRIFUGE



Many cell fractionations are done in a second type of rotor, a swinging-arm rotor.





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Cell disruption



Cell disruption

Some products remain inside the cell requiring the cells to be broken to release the product.

Mechanical



Grinding

rotating blades grind and burst cells

Sonication (Ultrasound)

pulsed, high frequency sound waves to agitate and lyse cells. Generates heat.

Homogenisation

Cells are lysed by forcing the cell suspension through a narrow space, thereby shearing the cell membranes.

Cell disruption

Physical

Freeze thaw

freezing a cell suspension in a dry ice/ethanol bath or freezer and then thawing the material at room temperature or 37°C. This method of lysis causes cells to swell and ultimately break as ice crystals form during the freezing process and then contract during thawing. Multiple cycles are necessary for efficient lysis, and the process can be quite lengthy.

Osmotic shock

Step change in solution that causes excess water to enter the cell

Chemical

Enzymes

Enzymes that break down cell walls –eg lysozyme. Costly, not reproducible for commercial scale.

Detergents

Bursts cell wall, denatures proteins. Can be hard to remove.



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Primary isolation

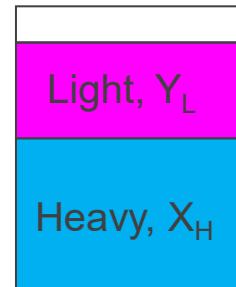


Aqueous 2 phase separation

Liquids that form 2 distinct phases are shaken together and the two phases are allowed to separate in a separating funnel.

The heavy phase can then be withdrawn from the bottom of the funnel.

The phase containing the solute can then be further processed.



Aqueous 2 phase separation

Organic solvents are frequently used to generate 2 phase systems, but are not suitable for proteins.

Two-phase aqueous solutions can be produced with polymers and salt. eg polyethylene glycol and ammonium sulphate.

Biomolecules and cell fragments partition between the phases and partitioning is influenced by size, electric charge, hydrophobicity and affinity for the polymers.

Requirements of liquid extractants:

- Nontoxic
- Selective
- Inexpensive
- immiscible with cell culture medium



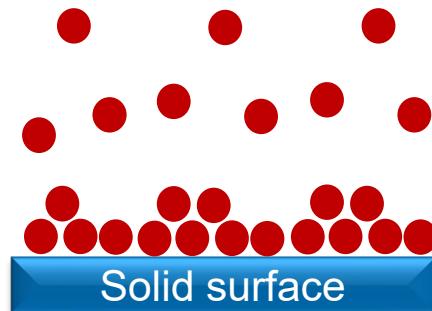
Adsorption

Adsorb soluble product from cell culture medium onto solid using physical adsorption and relies upon differences in the affinity of a product in the solid and liquid phase.

This is affected by properties of :

Adsorbents: functional groups, their nature and density, and surface properties

Solution: solutes, pH, ionic strength, temperature



Precipitation

Can be used to remove unwanted components from the process stream.

Precipitants include salts, solvents and polymers that change the pH, ionic strength or temperature of the solution.

These reduce the solubility of the product or contaminant, causing it to precipitate and form an insoluble solid that can be recovered by centrifugation or filtration.

Often used early in the process to reduce the volume to be processed.



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Enrichment



Chromatography

Separate the components of a mixture by passing them through a matrix.

A solvent is used for carrying sample mixture through the matrix

Separation occurs because each compound in a mixture interacts differently with the matrix.

Usually protein separation

Main types:

Gel filtration - based on size

Affinity – binding affinity

Ion exchange - ionic interaction

Gel filtration

Also called molecular exclusion or gel permeation chromatography.

Separation based on size.

Volume between the beads is called the void volume – accessible to all molecules.

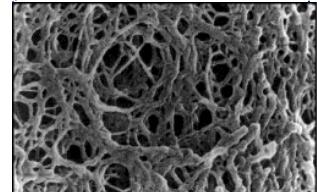
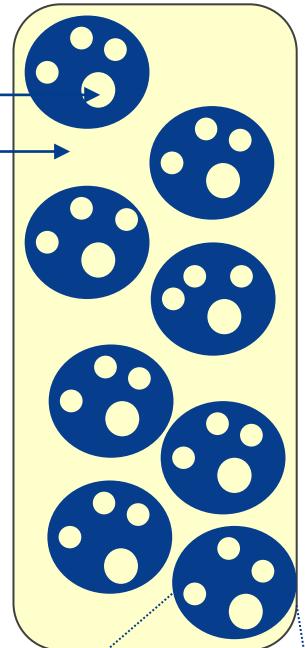
Volume in the pore is the pore volume

Non-porous part of the beads is the backbone volume not accessible to samples.

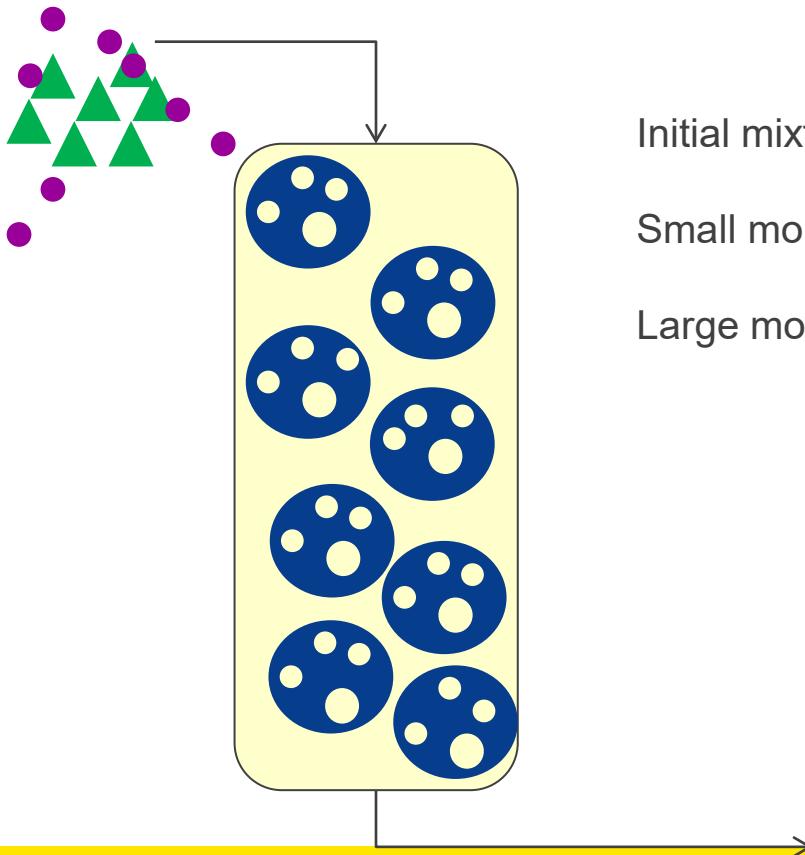
Partitioning occurs between the molecules that have access to the void volume and molecules that have access to both the void and pore volumes.

Pore volume

Void volume



Gel filtration

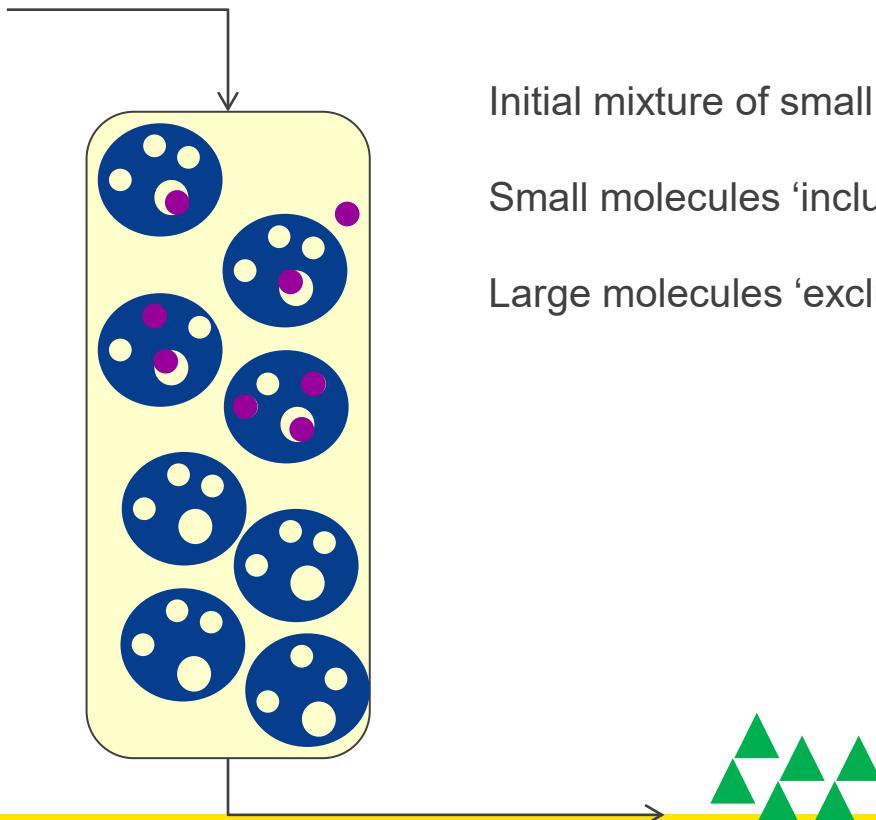


Initial mixture of small and large particles

Small molecules 'included' and elute last

Large molecules 'excluded' and eluted first

Gel filtration chromatography

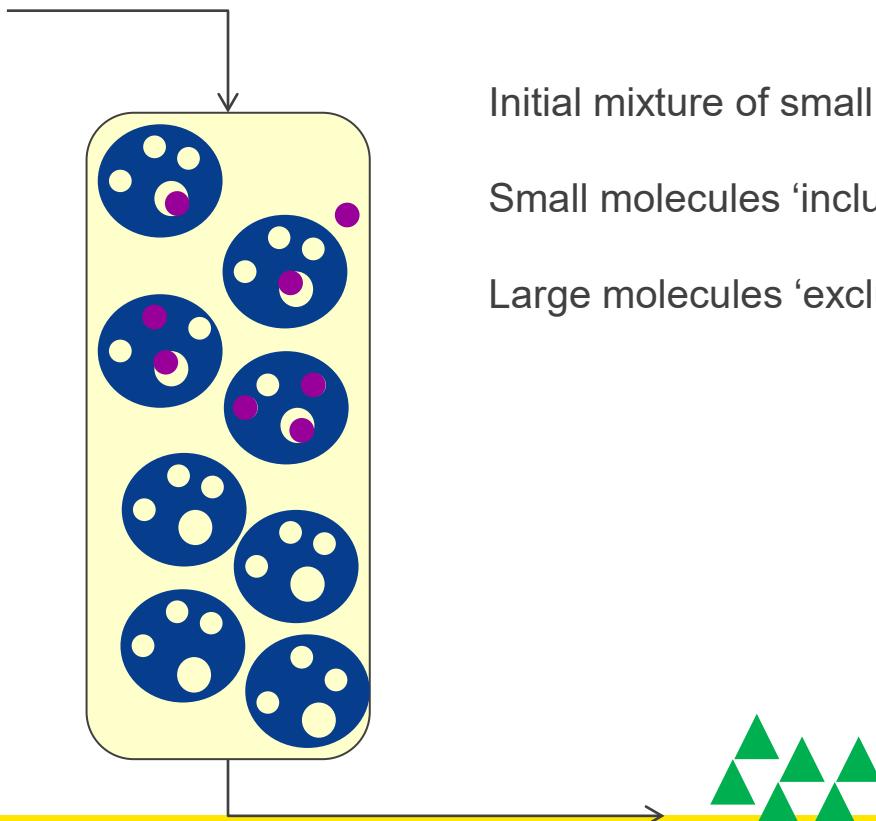


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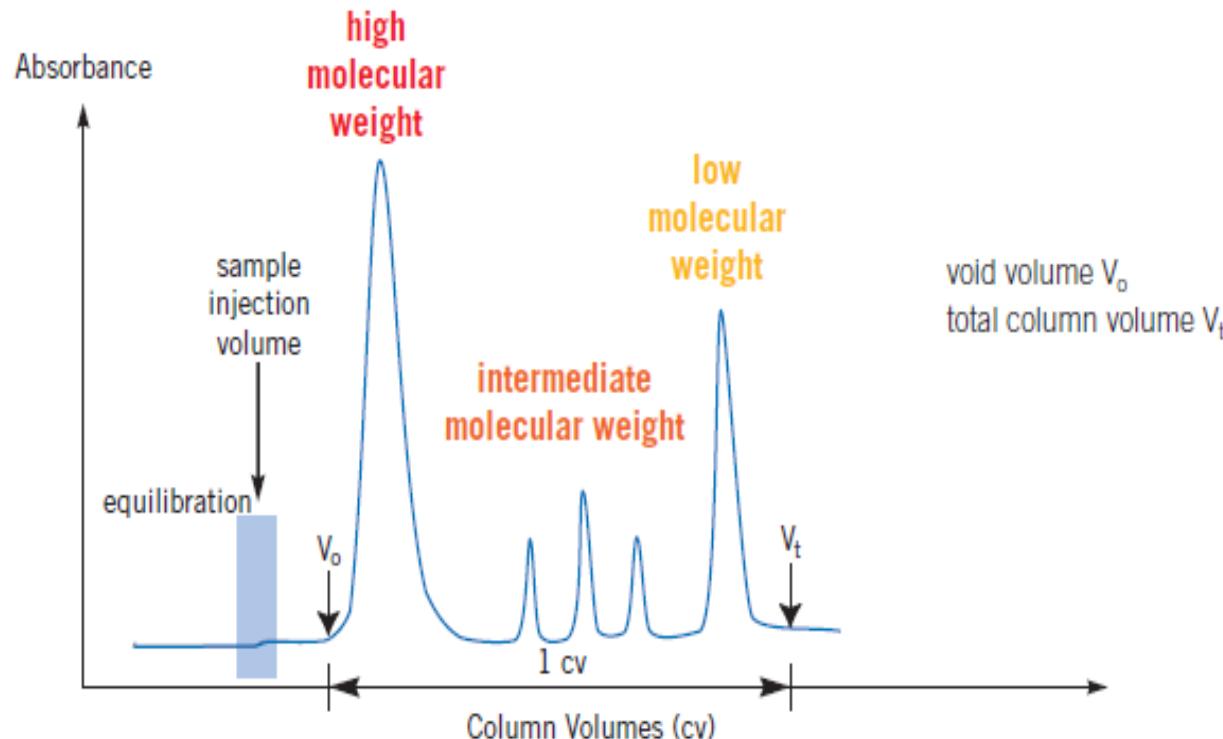


Initial mixture of small and large particles

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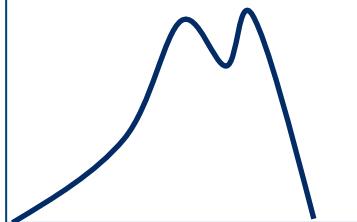
Large molecules 'excluded' and eluted first

Gel filtration chromatography

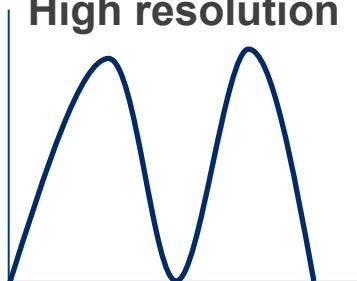


Gel filtration chromatography

Low resolution

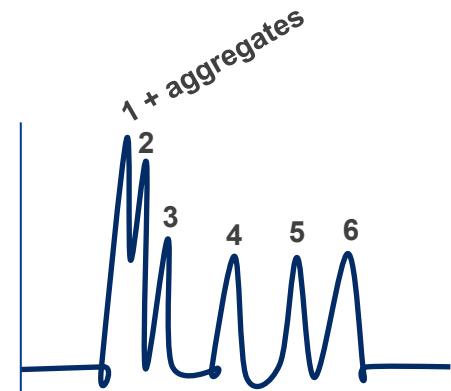


High resolution

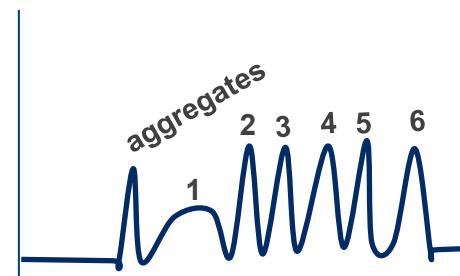


1. 970,000 Da
2. 669,000 Da
3. 440,000 Da
4. 66,000 Da
5. 17,000 Da
6. 1,355 Da

Superdex 200 (10,000 – 600,000)



Superose 6 (5,000 – 5,000,000)



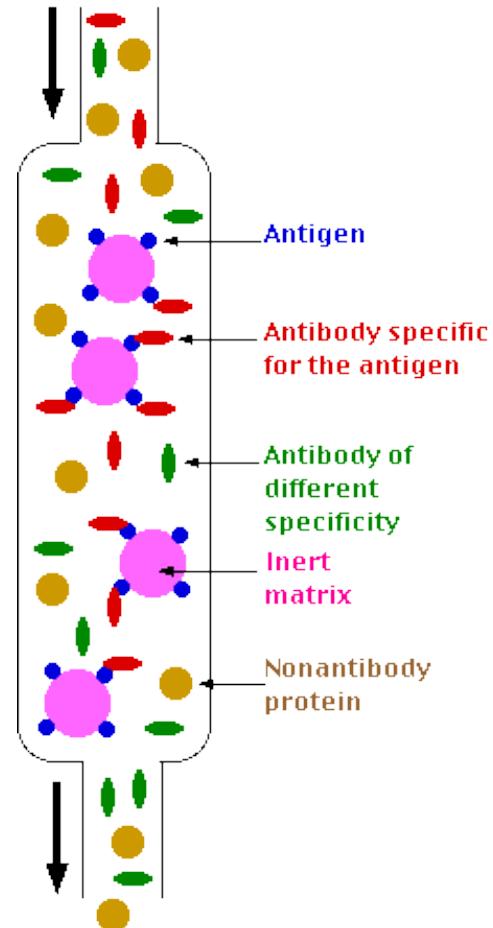
Affinity chromatography

Substance to be purified is specifically and reversibly adsorbed to a ligand immobilised by a covalent bond to the column matrix.

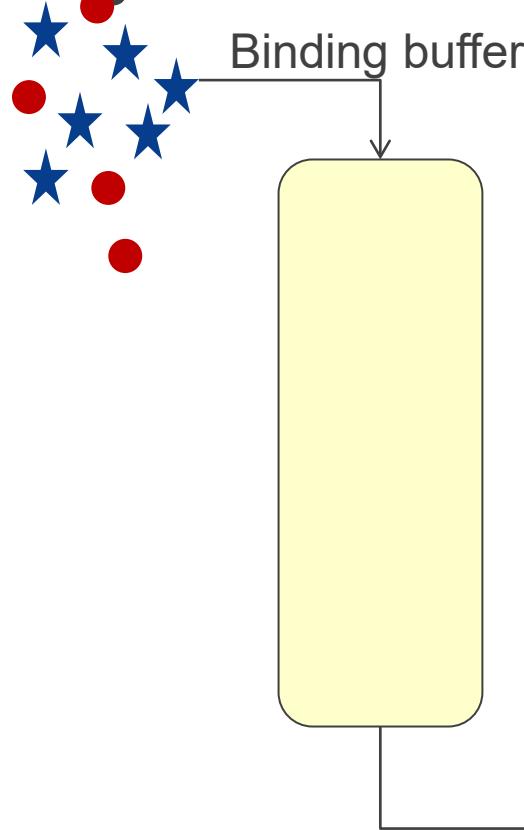
Samples are applied under conditions that favour specific binding to the ligand.

Unbound material is washed away.

Recovery of molecules of interest can be achieved by changing experimental conditions to favour desorption.



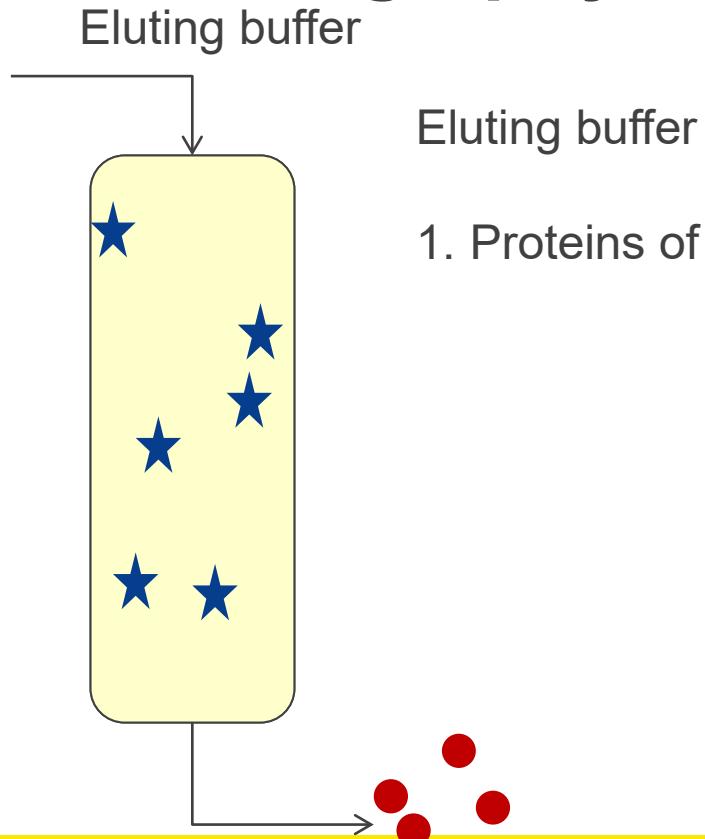
Affinity Chromatography



Binding buffer

1. Load sample
2. Proteins of interest bind to the column.
3. Unbound fraction passes through the column.

Affinity Chromatography

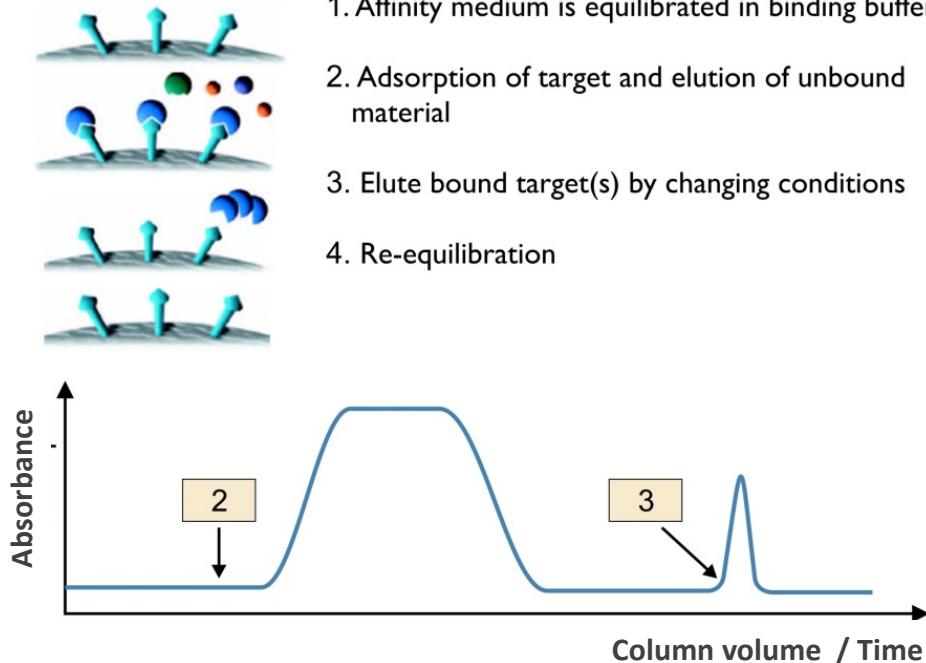


1. Proteins of interest elute from the column.

Affinity Chromatography

Key stages in an affinity purification:

1. Affinity medium is equilibrated in binding buffer
2. Adsorption of target and elution of unbound material
3. Elute bound target(s) by changing conditions
4. Re-equilibration



5.1B Affinity chromatography

Commonly used for purification of:

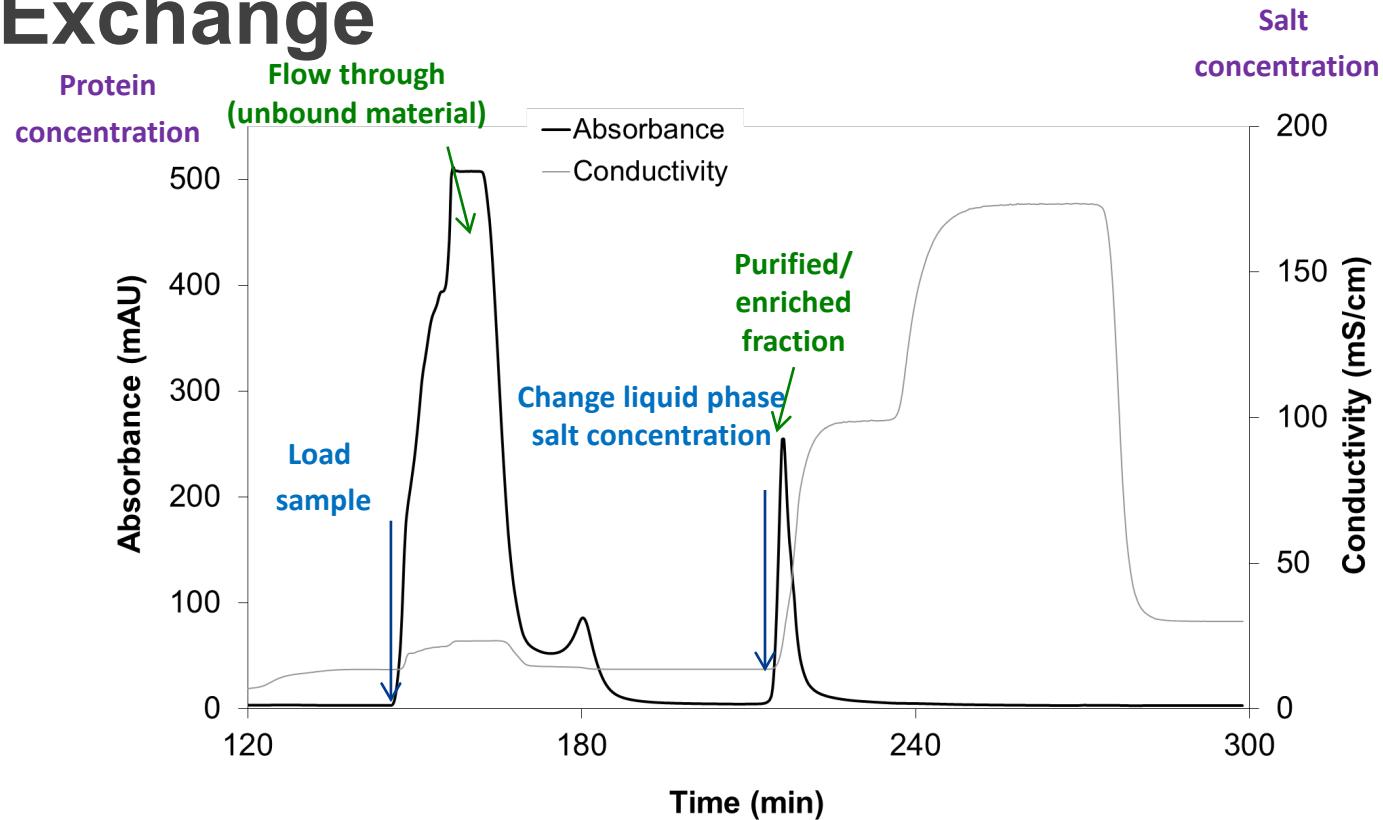
Application	Column functional group	Specificity	pH
Recombinant proteins - Expressed with His tag - Expressed with GST tag	Ni ²⁺ glutathione	Histidine GST	1 – 14
Antibodies	Protein A or G Protein for specific antibody	IgG antibodies	2 – 10
Proteins	Antibody to specific protein	Protein of interest	

Ion exchange

Separates molecules based on electrostatic attraction between the solute and charged groups on the column resin.

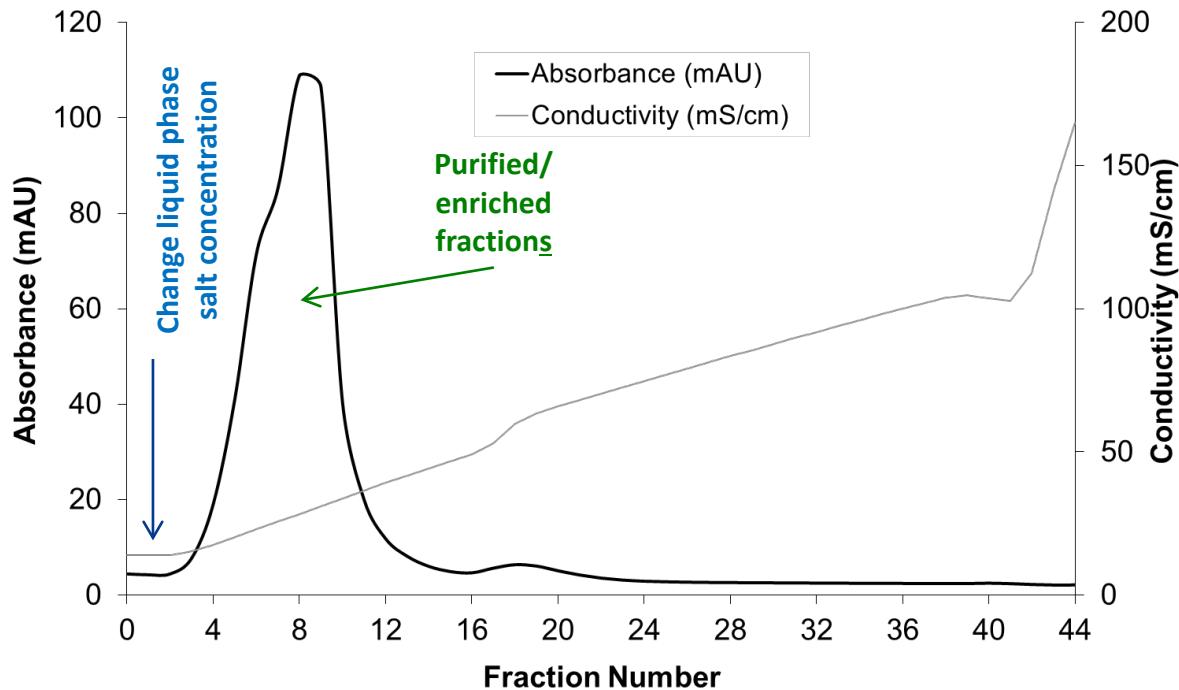
Molecules that bind to the column can be released by changing the pH or salt concentration of the liquid phase. Changes in salt are more common.

Ion Exchange



Ion Exchange

Flow through not shown



Lambert – Beer Law

$$A = \epsilon c l$$

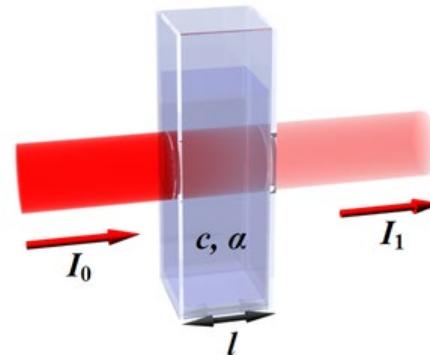
where

A = absorbance (AU)

ϵ = molar extinction coefficient ($M^{-1}cm^{-1}$ or $L mol^{-1}cm^{-1}$)

c = concentration (M)

l = path length (cm)



Absorbance measurements rely on measuring the ratio of transmitted light : $T = I_t / I_o$ where

- I_o = intensity of incident light
- I_t = intensity of light after passing
- through the material

Crystallisation

Last step in producing highly purified products such as antibiotics.

Supersaturated solution, low temperature, crystals are separated by filters.

Precipitation

Inorganic salts such as ammonium sulfate, or sodium sulfate added to increase high ionic strength (factors: pH, temperature)

The solubility of globular proteins increases upon the addition of salt (<0.15 M), an effect termed salting-in. At higher salt concentrations, protein solubility usually decreases, leading to precipitation; this effect is termed salting-out.

Added salts interact more strongly with water so that the proteins precipitate.

inexpensive

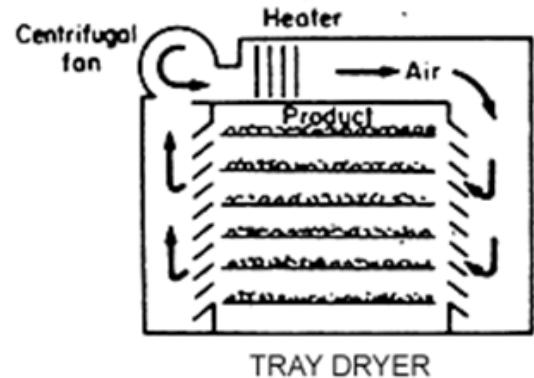
Drying

Remove solvent from purified wet product

Vacuum-tray dryers eg pharmaceutical products

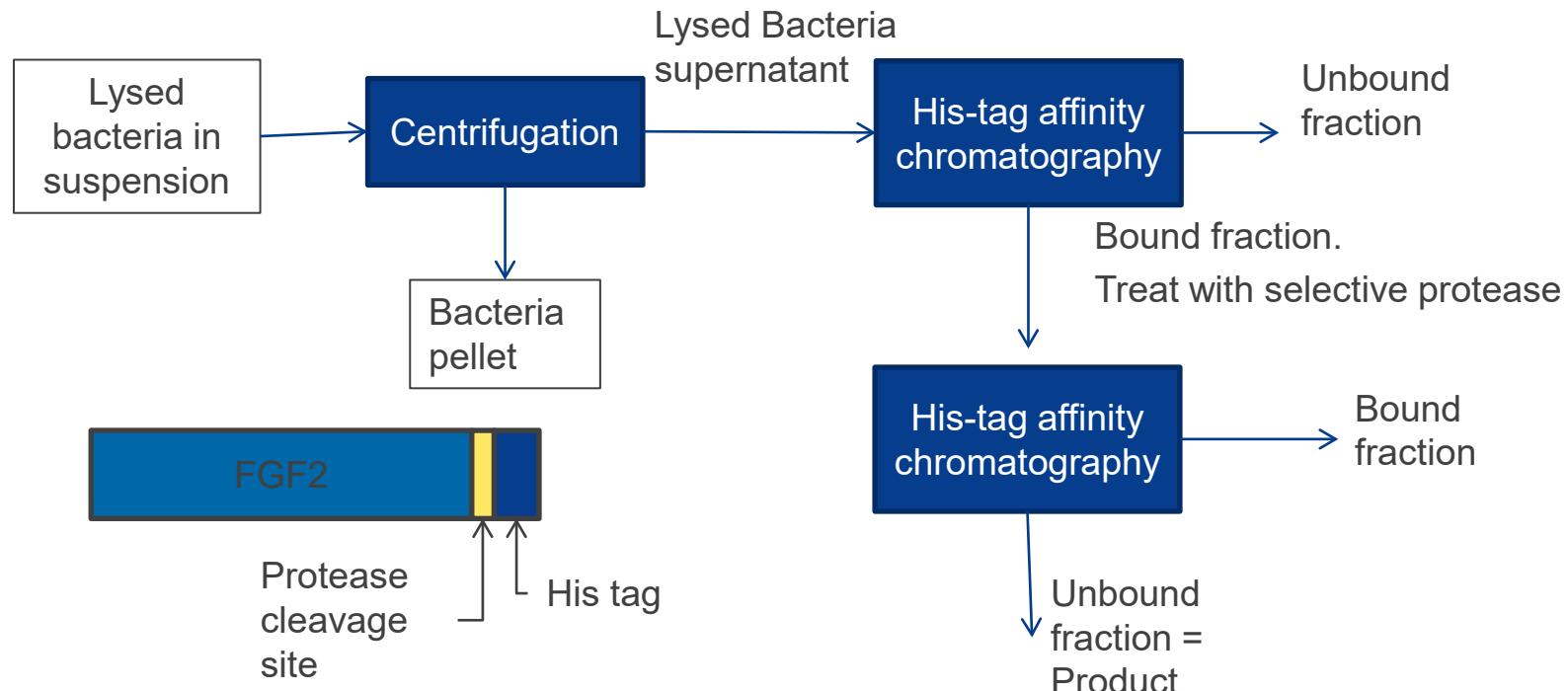
Freeze drying: by sublimation (from solid ice to vapour): eg antibiotics, enzyme, bacteria

Spray dryer: heat-sensitive materials



Industrial examples

Isolation of recombinant protein from bacterial cultures – eg fibroblast growth factor 2.

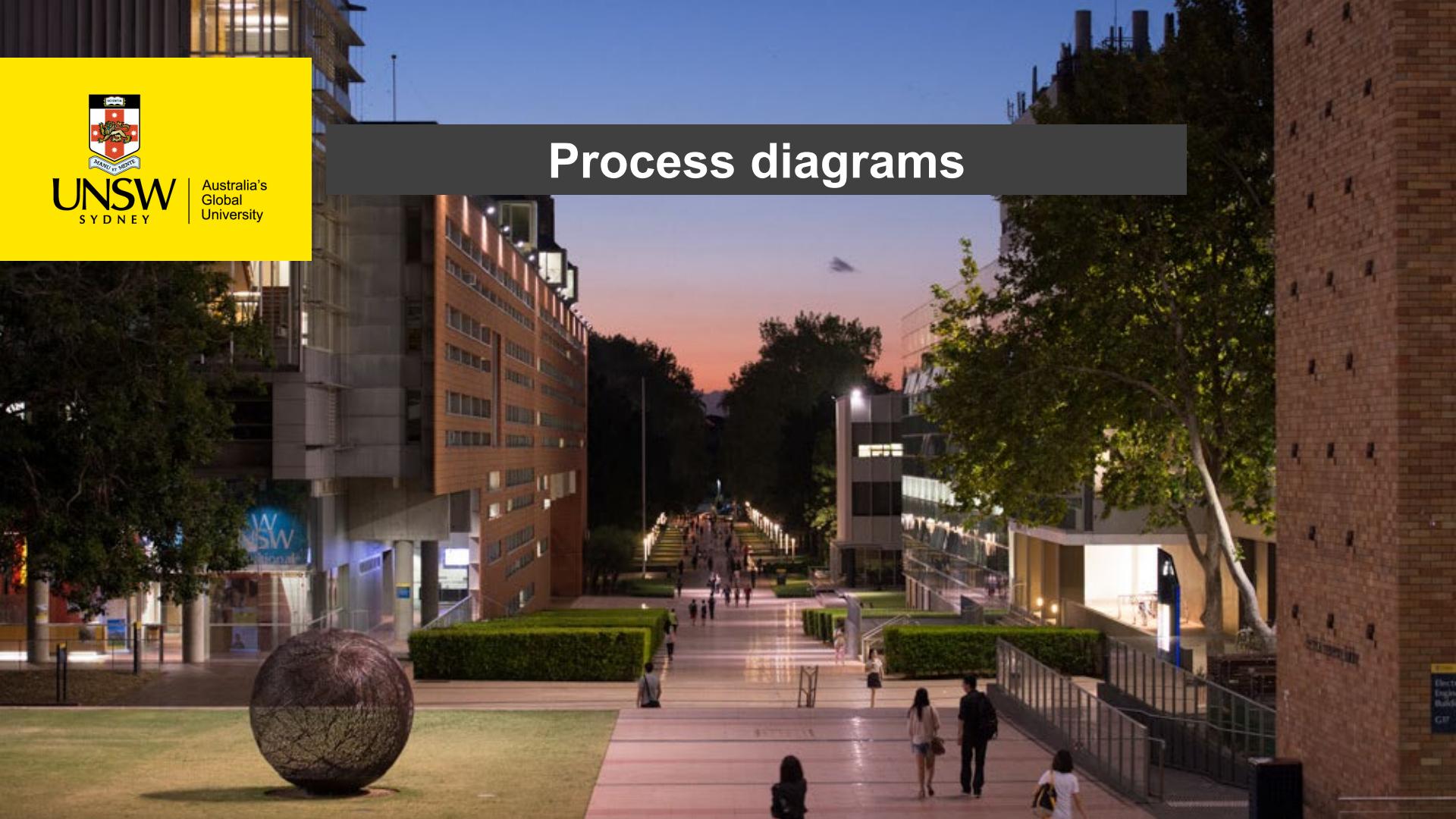




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Process diagrams



Process diagrams

Overview of a process to produce a product from raw materials.

Primarily includes equipment and flows.

Can be simple boxes and lines or images to represent each of the pieces of equipment.

