

Introduction to Downstream Process – Protein Purification

How do you separate the protein you want
from all the other cellular proteins?

Module 3, Lesson 1

Downstream Process

When the culture in the bioreactor has produced the desired protein product, it must be harvested and separated from the other materials in the solution.

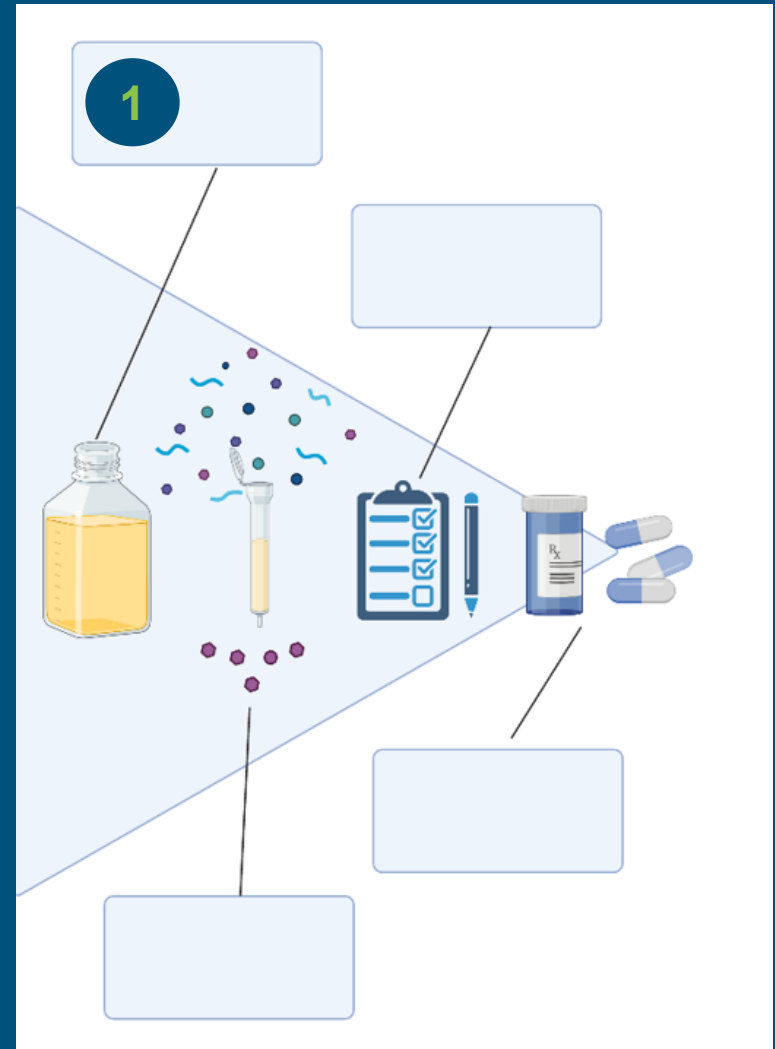
The processes of harvesting and purifying the product of interest is referred to as **downstream**.



Downstream Process

1 - The protein product often is secreted into the liquid media. In this case, the media (contain the protein of interest) needs to be separated from the cells.

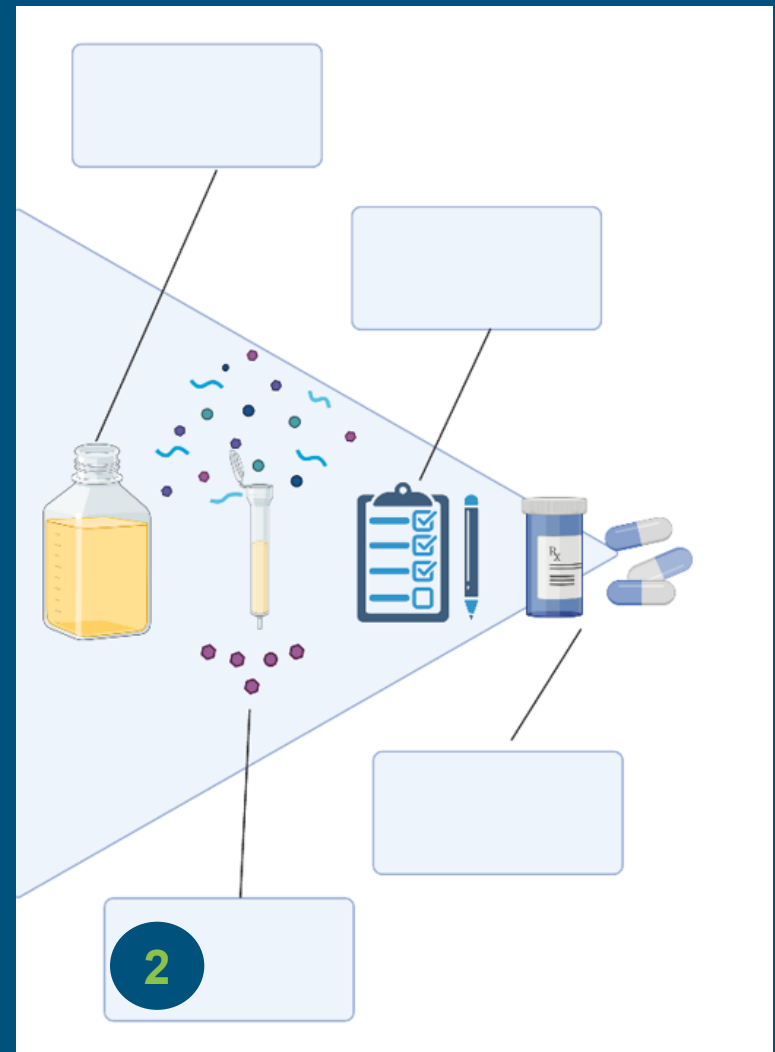
Other times, the proteins are inside the cells. In this case, the cells must be opened up, then separated.



Downstream Process

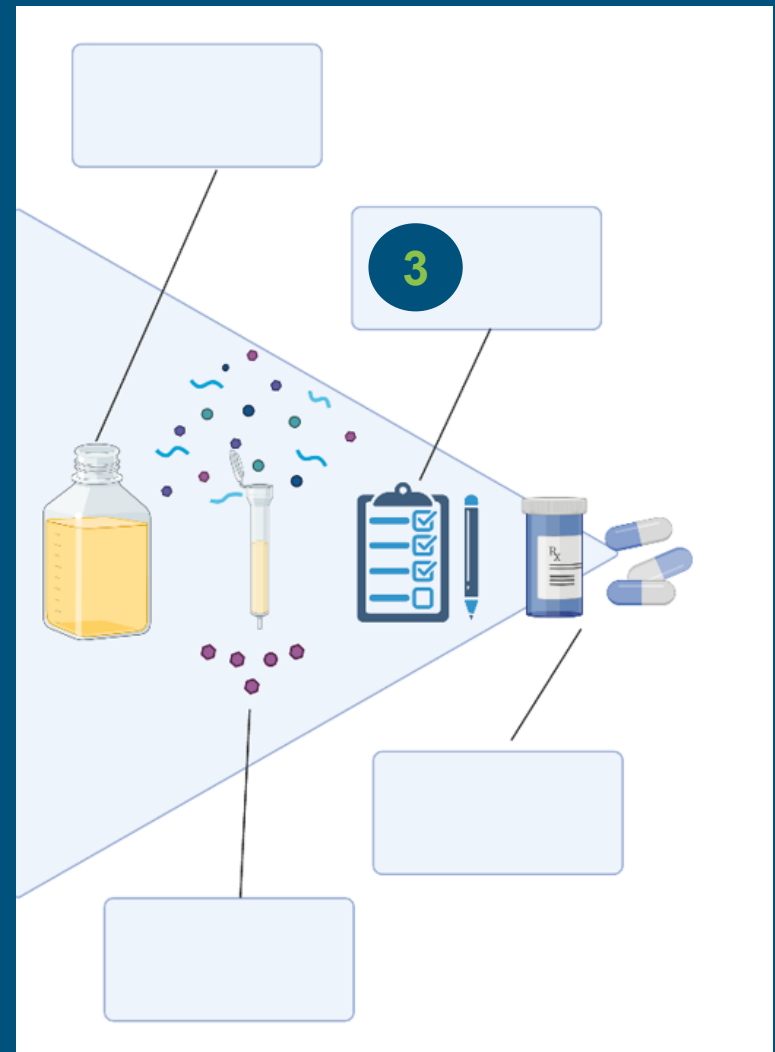
2 - The protein of interest is purified - separated from all the other proteins and molecules found in the solution.

A common method of purification is called **chromatography**.



Downstream Process

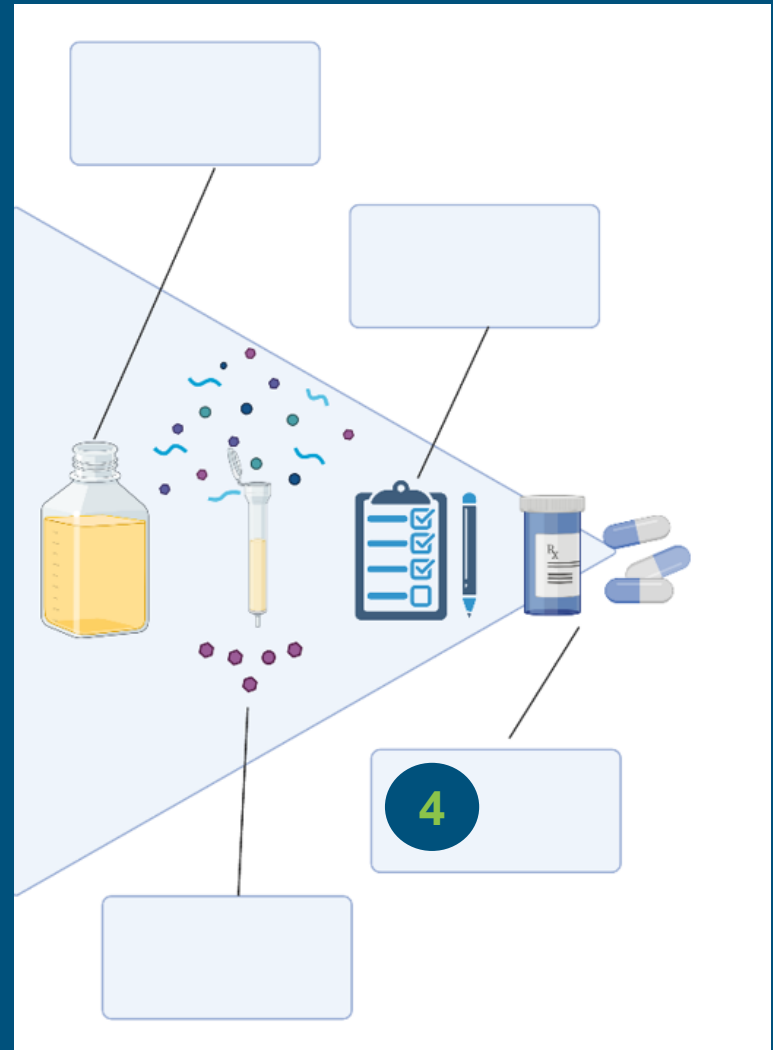
3 - Numerous checks and tests are performed to ensure the purity and quality of the protein.



Downstream Process

4 - The final therapeutic product includes the protein, along with different chemical substances. Combining these in the correct concentrations is termed **formulation**.

Bulk fill is the process of dispensing the therapeutic into bottles or vials.



Chromatography is a commonly used method of protein purification

- Separation techniques based on the differential interaction of molecules between a stationary and a **mobile** phase
- In Greek "Chroma" means "color" and "Graphein" means "to write"

Chromatography is a commonly used method of protein purification

- In this case the stationary phase is the packed resin in the column.
- The mobile phase is the liquid that we pass over the column. Initially it is the bacterial lysate, which contains the protein we want to purify (RFP or GFP).

Chromatography is a commonly used method of protein purification

- Molecules in the mobile phase move through or bind to the stationary phase depending on their size, charge etc.
- Molecules that bind to the stationary phase (the column resin) are removed or eluted by the gradual or step-wise change of the composition of the mobile phase (liquid buffer that moves past the stationary phase).

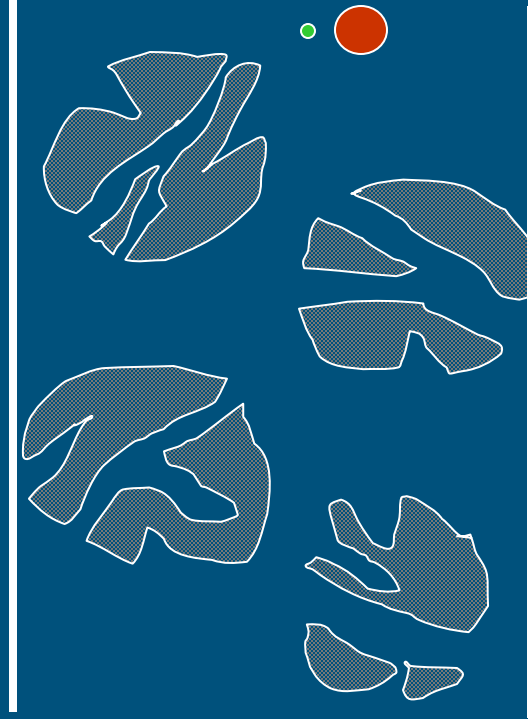
Methods of Chromatography

- Size-exclusion methods - also known as molecular sieve chromatography, are a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight.
- Flow-Through methods – product passes through while unwanted elements are bound to resin
- Bind-elute methods – product binds to resin and bound components removed (eluted) by the gradual or step-wise change of the composition of the mobile phase (buffers)

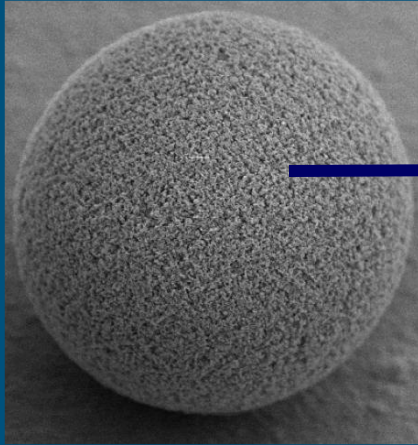
Types of column separation: Size

Everything flows through the column.

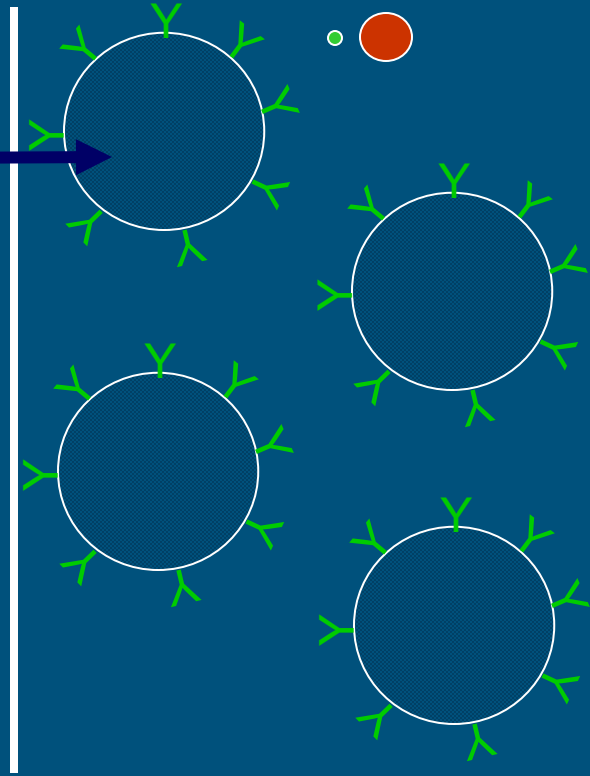
But different proteins flow through at different speeds.



Types of column separation: Flow through

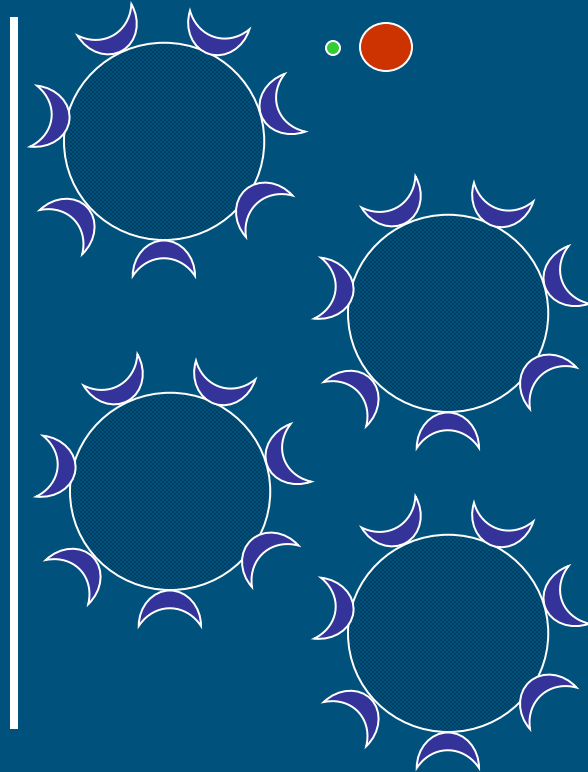


Resin bead



Types of column separation: Bind and elute

1. Therapeutic binds to resin
2. Therapeutic is eluted from resin with another solution

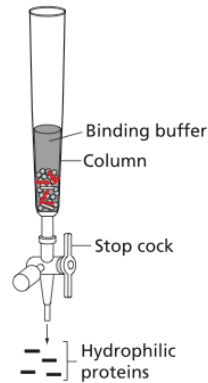


Hydrophobic Interaction Chromatography (HIC) – a method of Bind and Elute Chromatography

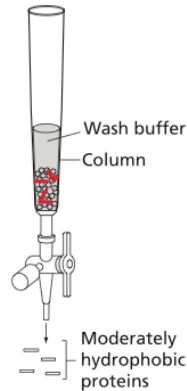
- The bacterial lysate, which contains hydrophobic and hydrophilic regions, is applied to an HIC column in a high-salt buffer.
- The salt contained in the binding buffer exposes the hydrophobic regions of RFP. These exposed hydrophobic regions bind to the column resin.
- A decreasing salt gradient is used to elute RFP from the column resin.

Column Purification of RFP

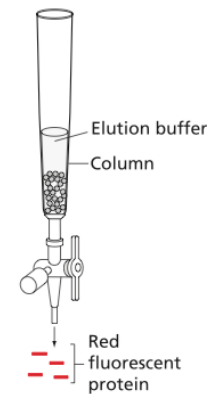
Binding buffer is
passed through
the column



Wash buffer is
passed through
the column



Elution buffer is
passed through
the column



This image outlines how a series of buffers of different concentrations of salt can be used to separate many proteins from each other by column chromatography. Three buffers are used to separate the highly hydrophobic protein RFP from the column.