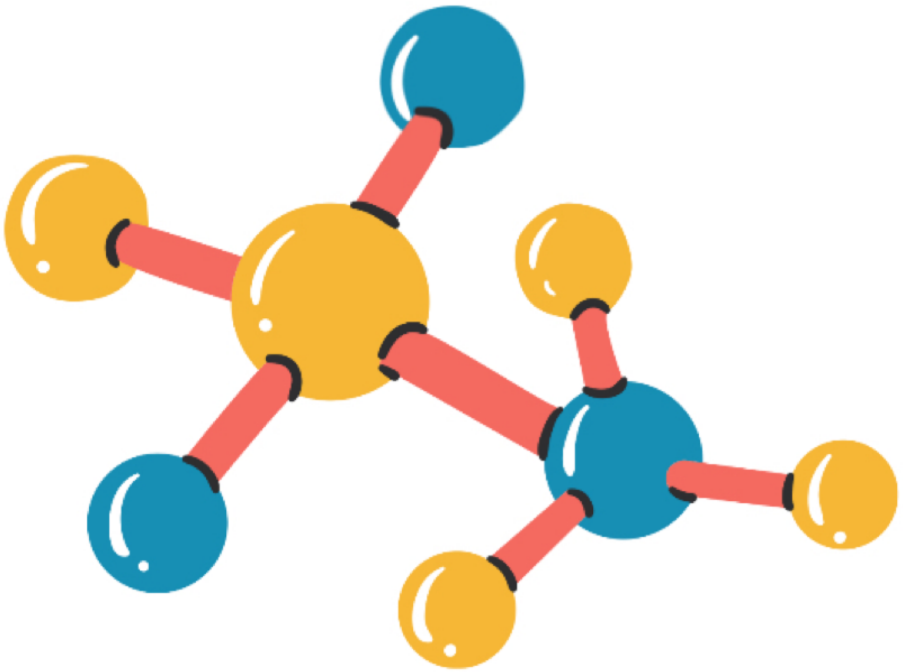
Biochemistry



Summer 2022

Writer: Doctor 2020
Corrector: Doctor 2020
Doctor : Dr.Nafeth AbuTarboush

Protein Purification and Characterization Techniques

Extracting Pure Proteins from Cells:
Many different proteins exist in a single cell. A detailed study of the properties of any one protein requires a homogeneous sample consisting of only one kind of molecule. The separation and isolation, or purification, of proteins constitutes an essential first step to further experimentation.

-Purification techniques focus on size and charge mainly.

Many techniques are performed to eliminate contaminants and to arrive at a pure sample of the protein of interest.

How do we get the proteins out of the cells?

Before the real purification steps can begin, the protein must be released from the cells and subcellular organelles.

- The first step, called ***homogenization***, involves breaking open the cells … BY :

1) The simplest approach is **grinding ,** it has been used in the past by **Potter–Elvehjem** homogenizer {in the figure }.

2) **Sonication (like ultrasounds)**, involves using sound waves to break open the cells (it is being used in the medical field not to break up the cells) but increasing the frequency of these waves to a higher level, it will break up the cells.

3) Cells can also be ruptured by cycles of **freezing and thawing.**

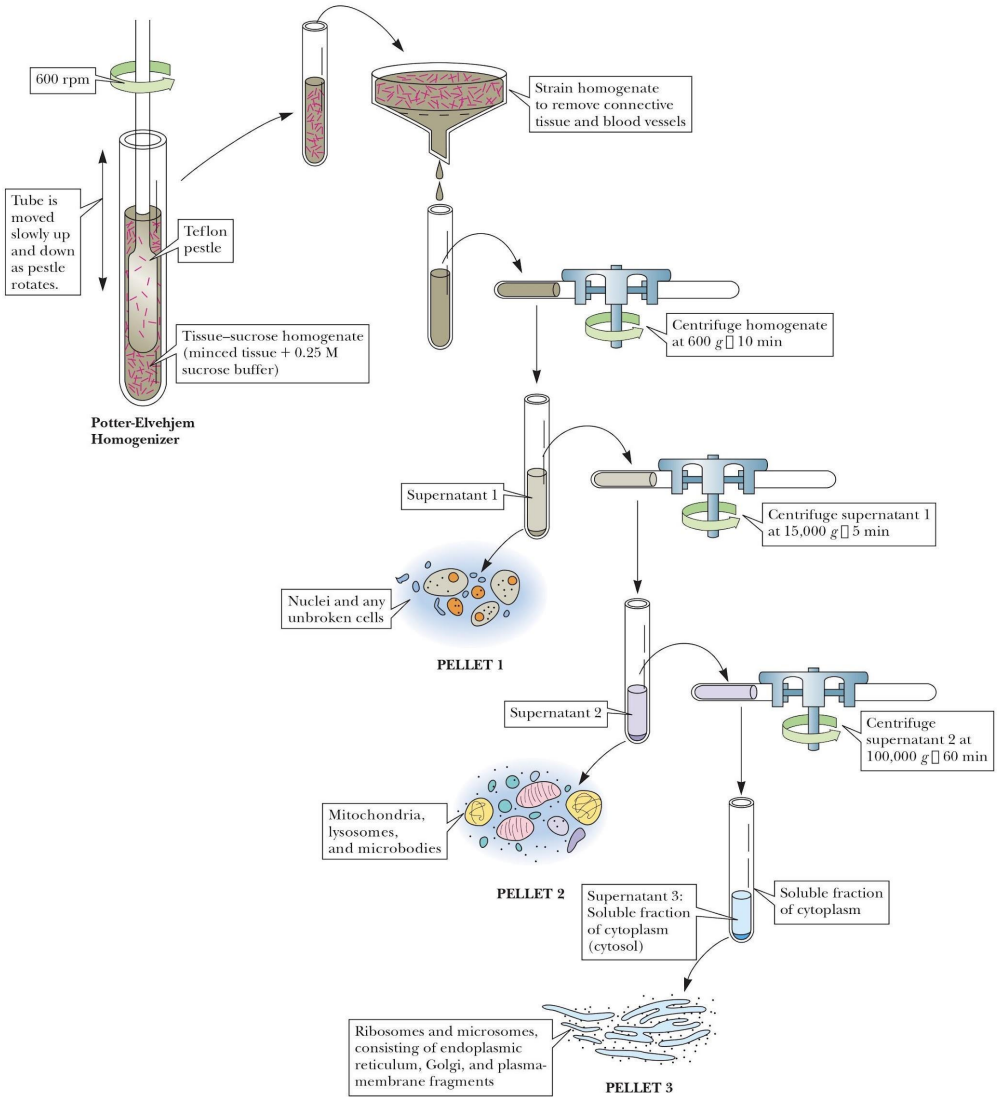
- The second step, after the cells are homogenized, they are subjected to **differential centrifugation.**

Explanation ~ [just to understand the process]

Spinning the sample at 600 times the force of gravity (600*g) results in a pellet of unbroken cells and nuclei.

If the protein of interest is not found in the nuclei, this precipitate is discarded. The supernatant can then be centrifuged at higher speed, such as 15,000 *g, to bring down the mitochondria. Further centrifugation at 100,000 * g brings down the microsomal fraction, consisting of ribosomes and membrane fragments. If the protein of interest is soluble, the supernatant from this spin will be collected and will already be partially purified because the nuclei and mitochondria will have been removed.

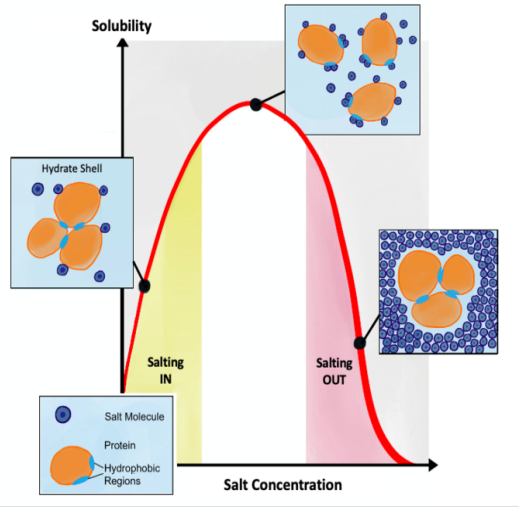
*this figure is from the book, for clarification



Salting in & out

- Are proteins soluble? If yes, to which limit?

Proteins have varying solubilities in polar and ionic compounds, but what will happen if we add salt to the protein solution?



Salting in

Proteins remain soluble because of their interactions with water. When salt is added to a protein solution ,Salt stabilizes the various charged groups on a protein molecule and enhances the polarity of water and increases the ionic strength, thus attracting protein into the solution and enhancing the solubility of protein.

Salting out

If we add more and more salt , some of the water is taken away from the protein . With less water available to hydrate the proteins, so decreasing of proteins solubility , they begin to interact with each other through hydrophobic bonds.

At a defined amount of salt, [FIRST] a precipitate that contains contaminating proteins forms. These proteins are the ones with a lower solubility . Then more salt is added, and a different set of proteins precipitates. This precipitate is collected and saved.

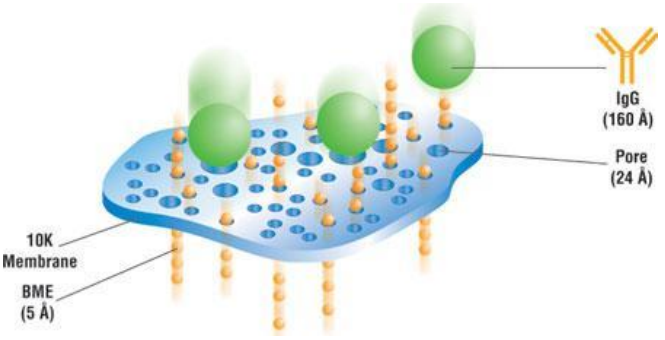
*Ammonium sulfate is the most common reagent to use at this step

*This technique is important but results are crude ….. but cheap , simple, and convenient .

Dialysis

This process is depending on Principle of diffusion , and Molecular weight. (MW cut-off)

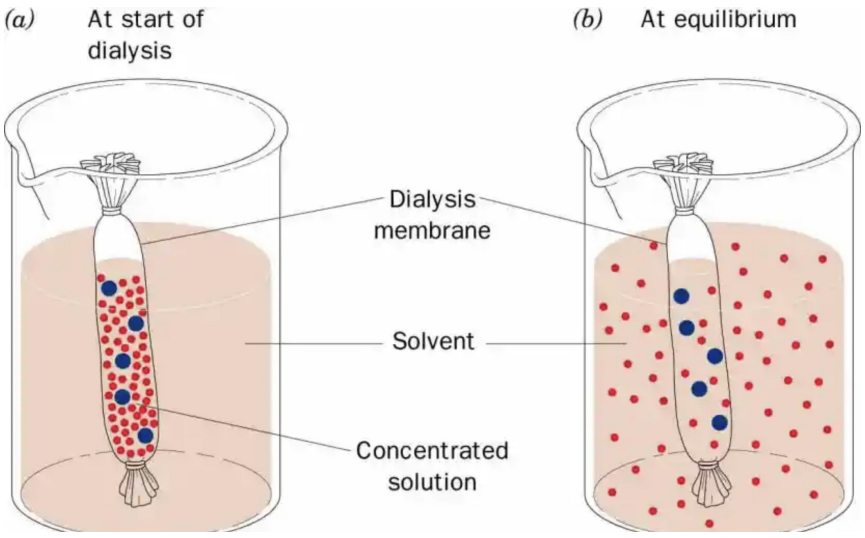
1. In dialysis a semipermeable membrane is used to separate small molecules and proteins based upon their size (depending on MW).



2. A dialysis bag made of a semipermeable membrane, and has small pores.
3. The bag is filled with a concentrated solution containing proteins. Molecules that are small enough to pass through the pores of the membrane diffuse out of the bag into the buffer/water solution.

-results of this technique is crude , because any protein which has a MW above that cut (MW cut-off) is contained inside the membrane .

*cheap , simple, and convenient .



Column Chromatography

The word chromatography comes from the Greek **chroma**, “color,” and **graphein**, “to write”.

We have a sample that contains a colored protein, the idea ;

1. We have a material which has the ability to bind your protein of interest and then to have that material inside a column and
2. you will pull your sample which has your protein of interest over the column, now the sample will start to move inside the column and when the sample gets out (colored).
3. You will see the color of interest(protein of interest) start to get out ,collect it inside beakers .

- This column is connected to a pencil and it will write on a drum the intensity of the peaks and the wavelengths ,so the higher the concentration(of proteins of the sample), the higher the peaks.

- This technique depends on two concepts (phases) :

The **stationary phase**, and the other is the **mobile phase**. The mobile phase flows over the stationary material and carries the sample to be separated along with it. The components of the sample interact with the stationary phase to different extents. " Some components interact relatively strongly with the stationary phase and are therefore carried along more slowly by the mobile phase than are those that interact less strongly or Some components bind to the stationary phase . The differing mobilities of the components are the basis of the separation ."

e.g (positively charged stationary phase and the protein of interest is negatively charged: protein of interest will bind to the positively charged stationary phase, and the positively charged proteins won't bind, they will get out)

- Washing: means getting materials (which aren't bound to the stationary phase) outside the column .
- Elution: means getting materials (which are bound to the stationary phase and contains the protein of interest) outside of the column.