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Dialysis: Diffusion Based Separation

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Abstract

Dialysis is a common technique used in biochemistry for separating molecules based on diffusion. In this procedure, a semipermeable membrane allows the movement of certain molecules based on size. This method can be applied to the removal of buffer, known as desalting, or exchanging buffer molecules or ions from a protein solution.

This video covers the principles of dialysis along with a general procedure. Several applications of dialysis are reviewed, including the removal of gradient reagents following ultracentrifugation, removing detergent after a membrane protein extraction, and the reconstitution of proteins by changing the solution environment.

Biochemical samples typically have high buffer concentrations that can disrupt downstream processing and analysis. Dialysis is a common, inexpensive technique used to separate molecules based on diffusion. The method utilizes a semi-permeable membrane that allows the movement of certain components, based on size. This video will show the concepts of dialysis, a general procedure, and some of its uses in biochemistry.

The most important aspect of dialysis is a semi-permeable membrane, which has pores that impose a molecular weight cut-off, allowing molecules below a certain size to pass through. For example, a 10k membrane will generally retain molecules larger than 10 kilodaltons. However, the molecular weight cutoff is not a discrete or precise boundary. The membrane typically contains a broad range of pore sizes, so a small fraction of molecules near the cutoff may be lost.

Since the molecular weight cutoff is defined using globular proteins, linear molecules of similar mass, like DNA or RNA, may slip through. Membranes are typically chosen one half to one third the molecular weight of the desired molecule.

To perform the procedure, a sample is placed into the membrane, which is in turn added to a large volume of solution, called the dialysate. Over time, smaller molecules will diffuse freely across the membrane between the sample and the dialysate, while the larger biomolecules are held within. Dialysis is a slow process. It is common to allow it to run overnight, or even across multiple days.

If the dialysate is pure water, the overall buffer concentration will decrease, a process known as desalting. If the solution contains other small particles, some will move into the sample, leading to buffer exchange. Because dialysis is an equilibrium process, the dialysate can be refreshed multiple times to further displace small molecules. Once the process is complete the sample is re-collected for further processing.

Now that you've seen the basics of dialysis, let's take a look at a general procedure.

Before beginning the procedure, the membrane is presoaked in dialysate. This makes it easier to use, and removes any preservatives. Once ready, the sample is collected, typically with a syringe and is then added to the dialysis container. This can be bare tubing, or contained within a cassette. Excess air is removed from the dialysis setup to maximize the sample's surface area with the membrane. The setup is then placed into the dialysate with stirring to maximize the diffusion. It should float to not inhibit stirring.

The dialysate is changed at relevant intervals as equilibrium between sample and dialysate is reached. After the last change, the reaction is typically left to run overnight. After a sufficient time period, the buffer-free or -exchanged sample is removed from the cassette. Once collected, the sample can be analyzed or further processed, depending on the nature of the experiment.

Now that we've looked at a general dialysis procedure, let's see some of the ways this technique is used in biochemistry.

Density gradients are a common way to separate complex biological samples. This concept relies on the distribution on small particles, typically sucrose or cesium chloride ions. Once complete, these reagents typically need to be removed before the collected sample can be processed. Dialysis makes it possible to utilize the purified sample for future analysis.

Certain proteins are found within a cell's lipid bilayer, and are usually studied by interspersing them into spherical lipid vesicles known as liposomes. The proteins and lipids are first extracted with a detergent. Dialysis can be used to slowly remove the detergent, forming proteoliposomes.

After purification, some proteins are misfolded, or denatured, leading to a loss in functionality. The compounds that cause these changes in structure can be removed with dialysis, leading to the reformation of functioning analytes.

You've just watched JoVE's video on dialysis. You should now understand this diffusion-based method, a simple experimental procedure, and the use of this technique.

Thanks for watching!