# Lab 6 Liquid Column Chromatography

## Background

Column chromatography is a widely used laboratory technique used in biochemistry for separating and purifying mixtures of proteins based on their different properties. The goal is to separate a single protein from a mixture of proteins dissolved in a fluid. The typical chromatographic system includes two or three components:

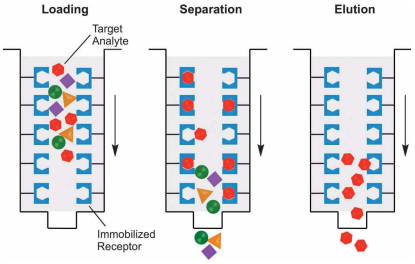
Stationary phase - This is the solid material that is usually packed in side a glass or metal column.

Mobile phase - is also called the solvent. This is what the protein mixture is dissolved in and is the delivery system.

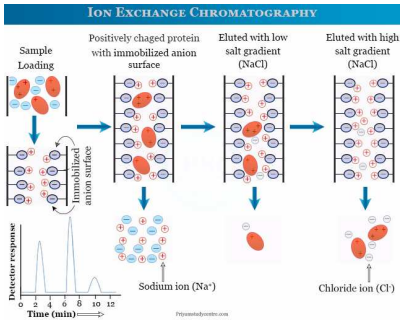
Eluting agent - may or may not be used to elute the desired protein off the column after adsorption.

Types of liquid chromatography:

**Affinity chromatography** - Here the stationary phase binds the protein of interest specifically. The other components of the mixture flow through the column freely. Thus the protein of interest is separated from the rest of the proteins. A common type of affinity chromatography involves the use of gel bound antibodies (Abs) specific for a certain protein. The mixture is passed through the column, where the Abs bind the specific protein and the rest of the proteins pass through. This usually results in lower yields since there are only so many Abs in the column. For elution a high salt solution is run down the column, which releases the proteins from the Abs. Thus the purified protein is isolated and captured in the fraction. Another type of affinity chromatography uses a nickel stationary phase and a repeated histidine (H) amino acid sequence on the protein of interest.




**Ion exchange chromatography** - Here the stationary phase is always an ion exchange resin with a charge. The protein mixture is loaded onto the column and allowed to pass through the column. Proteins with the correct charge will be bound by the opposite charged stationary phase. Some proteins will bind tightly and others will bind less tightly. For elution, a salt gradient is slowly run down the column. With the beginning low salt concentration weak binding proteins are released first. As the concentration of salt increased tighter binding proteins will be released. In this chromatography, what comes off the column (the eluent) is collected in fractions. The fractions are then analyzed for the protein of interest.




**Size exclusion chromatography** - is also known as gel filtration chromatography or gel permeation chromatography. Here the stationary phase contains porous beads. Large proteins are too large to fit into the pores of the beads and thus pass around the beads and elute from the column first. Smaller compounds (like salts) pass into the pores of the beads. The pores constitute a longer pathway so the smaller compounds take longer to pass through the column and thus elute from the column later. In this type of chromatography, the longer the column the better the separation. Here the same solution (buffer) is used to load the sample and elute the sample through the column.