Chromatography

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Chromatography

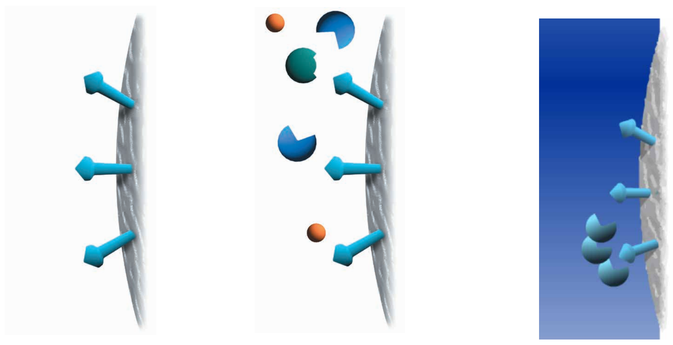
Pure proteins today. Powerful results tomorrow.

Affinity Chromatography

Affinity chromatography (AC) separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand attached to a chromatographic matrix. This technique can be used whenever a suitable ligand is available for the protein(s) of interest.

Affinity chromatography is frequently used as the only purification step or as the first step (capture step) of a two-step purification protocol, followed by gel filtration/size exclusion chromatography (polishing step) to remove remaining impurities.

Two examples when affinity chromatography is commonly used are purification of tagged proteins, e.g. polyhistidine (His), *Strep*-tag™ II, glutathione-S-transferase (GST) and Maltose Binding Protein (MBP) and purification of antibodies with protein A and protein G affinity media.



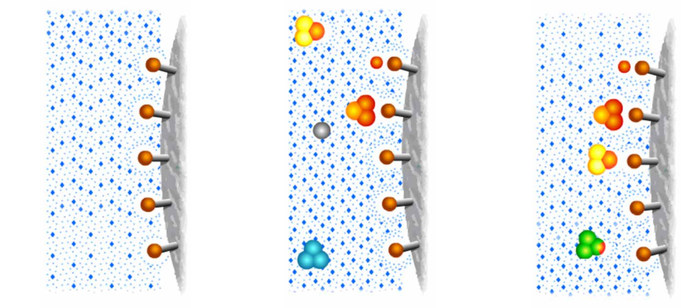
1 Equilibrate with binding buffer.
2 Load sample and wash out unbound materials.
3 Elute by changing buffer condition, e.g. pH or adding competing agent.

Hydrophobic Interaction Chromatography

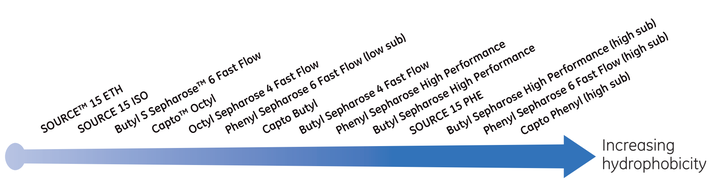
Hydrophobic interaction chromatography (HIC) is a versatile method for purification and separation of biomolecules based on differences in their surface hydrophobicity.

Hydrophobic interaction between a biomolecule and the matrix is enhanced by high ionic strength buffers. This makes HIC an ideal "next-step" for the purification of materials that have been precipitated with ammonium sulfate or eluted in high salt concentrations during ion exchange chromatography.

Compared to reversed phase chromatography, the density of the ligand on the matrix is much lower. This feature promotes the high selectivity of HIC, while allowing mild elution conditions to help preserve biological activity.



1 Equilibrate with starting buffer including a high salt concentration.
2 Load sample and wash out unbound materials.
3 Elute, most often by a linear or a step gradient of decreasing ionic strength.



Gel Filtration or Size Exclusion Chromatography

Gel filtration (GF) or as it is also commonly named, size exclusion chromatography (SEC) separates proteins on the basis of their size. The technique is well-suited for the final polishing step and can be used with most buffers suited for the target protein.

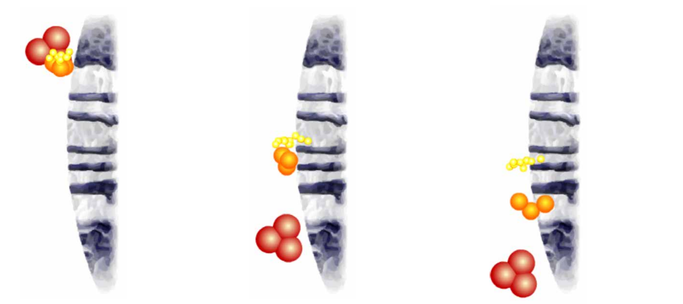
Gel filtration is a simple and reliable chromatographic technique and its versatility makes it generally applicable to the purification of all classes of biological substances.

Desalting or Buffer Exchange

Desalting (DS) or buffer exchange is a well-proven, simple, and fast method that rapidly removes low molecular weight contaminants, and at the same time, transfers the sample into the desired buffer in a single step.

The technique is often used between or after the last chromatography step to get the sample or target protein in the correct buffer condition.

Desalting provides several advantages over dialysis, as it is much faster which helps preserve biological activity of the target protein and it does not require large volumes of buffer.

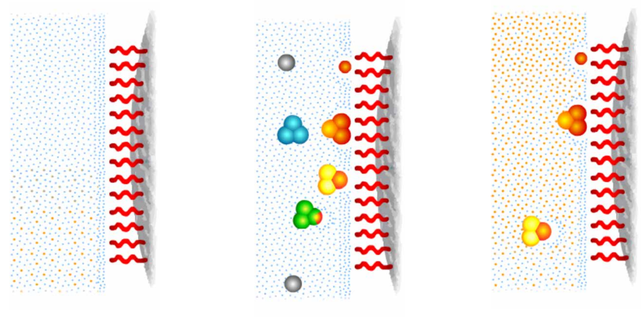


1 Equilibrate with buffer.
2 Load sample.
3 Sample is eluted isocratically (single buffer, no gradient). Largest molecules elute first.

Reversed Phase Chromatography

Reversed Phase Chromatography (RPC) separates molecules according to differences in their hydrophobicity. It is a reversible binding under conditions where the chromatography medium is more hydrophobic than the mobile phase.

This high-resolution technique is ideal for applications such as peptide mapping or purity checking and is often used for the final polishing of oligonucleotides and peptides.

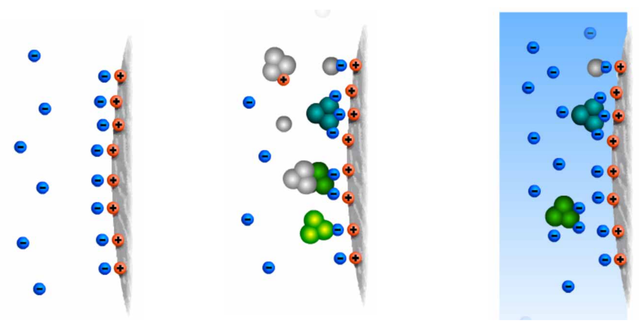


1 Equilibrate with starting buffer.
2 Load sample and wash out unbound materials.
3 Elute, most often by a linear or a step gradient increasing the hydrophobicity of the buffer.

Ion Exchange Chromatography

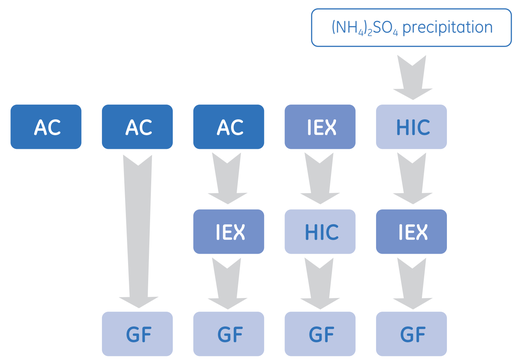
Ion exchange chromatography (IEX) separates proteins with only slight differences in charge. The separation is based on the reversible interaction between a charged protein and an oppositely charged chromatography medium.

Typically, conditions are selected to ensure that the proteins of interest bind to the medium as they are loaded onto the column. Conditions are then altered so that the bound proteins are eluted differentially. This is often done by applying a linear or a step gradient with increased ionic strength most commonly using NaCl.



1 Equilibrate with starting buffer.
2 Load sample and wash out unbound materials.
3 Elute, most often by a linear or a step gradient of increasing ionic strength.

Purification strategy combinations



Handbooks



Handbook	Code number
Affinity Chromatography	18-1022-29
Recombinant Protein Purification	18-1142-75
Antibody Purification	18-1037-46
Ion Exchange Chromatography and Chromatofocusing	11-0004-21
Hydrophobic Interaction and Reversed Phase Chromatography	11-0012-69
Gel Filtration	18-1022-18
Strategies for Protein Purification	28-9833-31

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