3.4.1. Affinity Chromatography

Affinity chromatography is another powerful technique of purifying proteins. This technique takes advantage of the high affinity of many proteins for specific chemical groups. Biological macromolecules, such as enzymes and other proteins, interact with other molecules with high specificity through several different types of bonds and interaction. Such interactions include hydrogen bonding, ionic interaction, disulfide bridges, hydrophobic interaction, and more. The high selectivity of affinity chromatography is caused by allowing the desired molecule to interact with the stationary phase and be bound within the column in order to be separated from the undesired material which will not interact and elute first. The molecules no longer needed are first washed away with a buffer while the desired proteins are let go in the presence of the eluting solvent (of higher salt concentration). This process creates a competitive interaction between the desired protein and the immobilized stationary molecules, which eventually lets the now highly purified proteins be released.

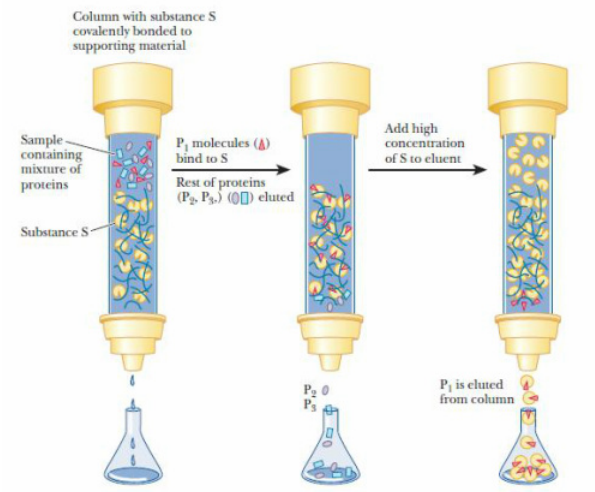
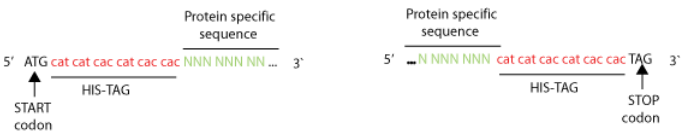


Figure 3.4.1.1: *Affinity chromatography is a powerful technique of purifying proteins.*

Purifying Proteins by Affinity Tag

Protein tags are peptide sequences genetically grafted onto a recombinant protein. Often these tags are removable by chemical agents or by enzymatic means, such as proteolysis or intein splicing. Tags are attached to proteins for various purposes.

Affinity tags are appended to proteins so that they can be purified from their crude biological source using an affinity technique. These include chitin binding protein (CBP), maltose binding protein (MBP), and glutathione-S-transferase (GST). The poly (His) tag is a widely-used protein tag; it binds to metal matrices.



Adding Polyhistidine Tags

This is an example of a primer designed to add a 6xHis-tag using PCR. Eighteen bases coding six histidines are inserted right after the START codon or right before the STOP codon. At least 16 bases specific to the gene of interest are needed next to the His-tag. With 6 His, the protein will have an added 1 kDa of molecular weight. Oftentimes, a linker (such as gly-gly-gly or gly-ser-gly) is placed between the protein of interest and the 6 His tag. This is to prevent the polyhistidine tag from affecting the activity of the protein being tagged.

Solubilization tags are used, especially for recombinant proteins expressed in chaperone-deficient species such as E. coli, so as to assist in the proper folding in proteins and keep them from precipitating. These include thioredoxin (TRX) and poly (NANP). Some affinity tags have a dual role as a solubilization agent, such as MBP and GST.

Chromatography tags are used to alter chromatographic properties of the protein to afford different resolution across a particular separation technique. These often consist of polyanionic amino acids, such as FLAG-tag.

Epitope tags are short peptide sequences which are chosen because high-affinity antibodies can be reliably produced in many different species. These are usually derived from viral genes, which explain their high immunoreactivity. Epitope tags include V5-tag, c-myc-tag, and HA-tag. These tags are particularly useful for western blotting, immunofluorescence and immunoprecipitation experiments, although they also find use in antibody purification.

Fluorescence tags are used to give visual readout on a protein. GFP and its variants are the most commonly used fluorescence tags. More advanced applications of GFP include using it as a folding reporter (fluorescent if folded, colorless if not).

Protein tags are also useful for specific enzymatic modification (such as biotin ligase tags) and chemical modification (FlAsH) tag. Often tags are combined to produce multifunctional modifications of the protein. However, with the addition of each tag comes the risk that the native function of the protein may be abolished or compromised by interactions with the tag.

Examples of peptide tags include

- AviTag, a peptide allowing biotinylation by the enzyme BirA and so the protein can be isolated by streptavidin (GLNDIFEAQKIEWHE)
- Calmodulin-tag, a peptide bound by the protein calmodulin (KRRWKKNFIAVSAANRFKKISSSGAL)
- FLAG-tag, a peptide recognized by an antibody (DYKDDDDK)
- HA-tag, a peptide recognized by an antibody (YPYDVPDYA)
- His-tag, 5-10 histidines bound by a nickel or cobalt chelate (HHHHHH)
- Myc-tag, a short peptide recognized by an antibody (EQKLISEEDL)
- S-tag (KETAAAKFERQHMDS)
- SBP-tag, a peptide which binds to streptavidin (MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP)
- Softag 1, for mammalian expression (SLAELLNAGLGGS)
- Softag 3, for prokaryotic expression (TQDPSRVG)
- V5 tag, a peptide recognized by an antibody (GKPIPNPLLGLDST)
- Xpress tag (DLYDDDDK)

Examples of protein tags include

- BCCP (Biotin Carboxyl Carrier Protein), a protein domain recognized by streptavidin
- Glutathione-S-transferase-tag, a protein which binds to immobilized glutathione
- Green fluorescent protein-tag, a protein which is spontaneously fluorescent and can be bound by nanobodies
- Maltose binding protein-tag, a protein which binds to amylose agarose
- Nus-tag
- Strep-tag, a peptide which binds to streptavidin or the modified streptavidin called streptactin (Strep-tag II: WSHPQFEK)
- Thioredoxin-tag