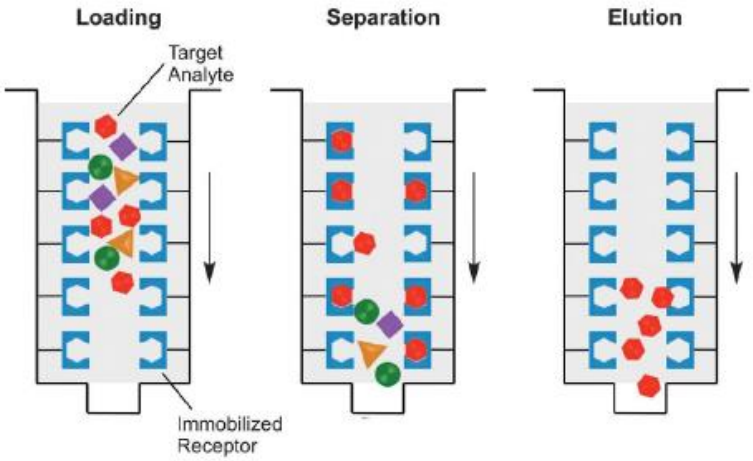
Affinity chromatography

- It is a method of separating biochemical mixtures based on a highly specific interaction that occurs between stationary phase and molecule of interest present in the mobile phase.
- Examples of such interactions include antigen- antibody/enzyme-substrate/receptor-ligand interaction etc.
- One of the members from the above example will be included in the stationary phase and the other will be in the mobile phase.
- Because these members are highly selective and have high affinity for each other, thus, method is called as affinity chromatography.
- The molecules bind with the stationary phase and those that do not bind are eluted first.
- The bound molecules are eluted with an eluting solvent of high salt concentration by *competitive interaction*.

Receptor Biomolecule	Ligand
Antigen	Antibody
Antibody	Antigen
Poly T or Poly U	Poly A tail of mRNA
Poly lysine (+ charge)	rRNA (- charge)
Ni^{2+}	6X His-tagged fusion protein
Glutathione	GST-tagged fusion protein
Maltose	MBP-tagged fusion protein
Concanavalin - A	Glycoproteins
Protein – A / G	Antibody



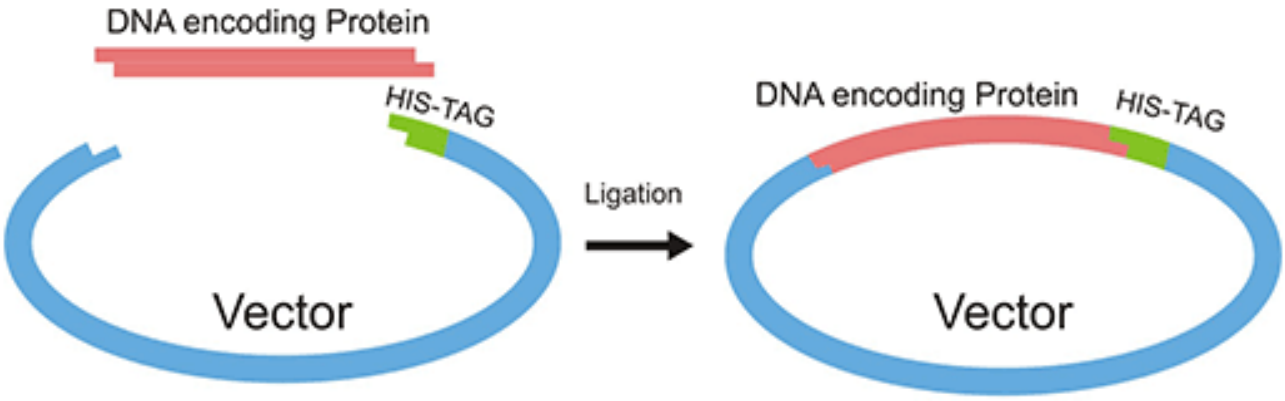
Significance:

- It is highly specific method for purification.
- It is efficient and quick as it is a single step purification method.
- It is used to separate glycoprotein from proteins.
- It is used to separate metalloenzyme from non-metal enzyme.

Significance in case of genetic engineering:

For easy purification of recombinant proteins from other host proteins this method is applied as is outlined below:

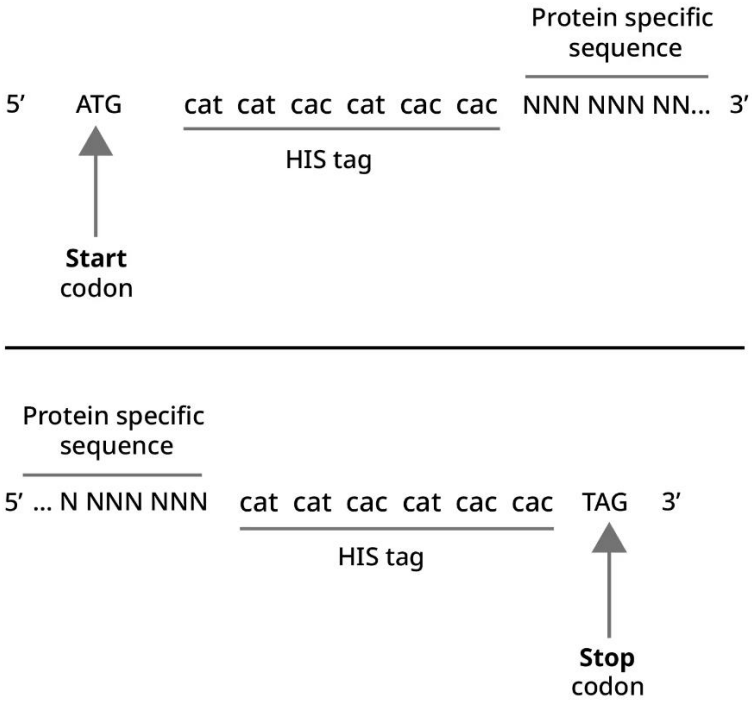
1. Vectors are designed in such a way that recombinant proteins will have affinity tags at one of their ends i.e. N-terminal or C-terminal. This is shown in the figure below for His-tag.



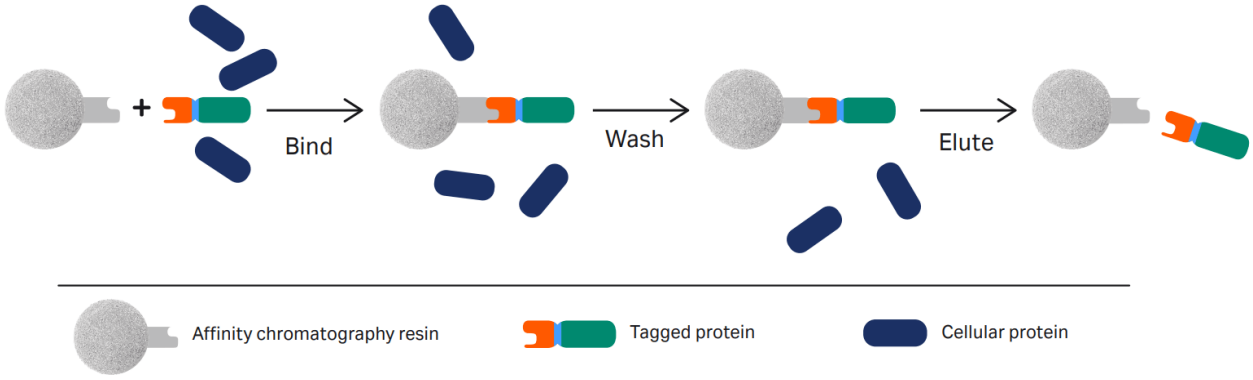
DNA sequence coding for histidine cluster is engineered in the vector in such a manner that after restriction digestion & ligation the gene of interest will have His-tag at N-terminal or C-terminal.

We call it histidine cluster because it has continuous presence of 6-9 or sometimes 10 Histidine amino acids. More commonly, it is referred to as 6X His-tag if sequence has 6 His amino acids or polyHis-tag for tags having more amino acids.

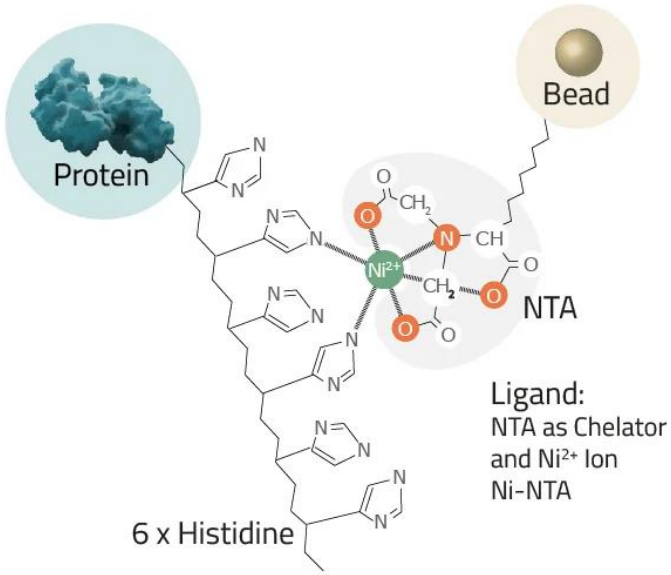
2. When recombinant protein is expressed, it will have the His-tag fused in its structure. This is the reason all such tags are also called as fusion tags. In the figure below you will find the tags at N-terminal on top and at C-terminal at the bottom.



3. These recombinant proteins having tags are present in the bacterial cell along with the usual bacterial proteins (usual bacterial proteins are called as endogenous protein).
4. When bacterial cells are lysed, the lysate contains endogenous proteins as well as recombinant proteins.
5. The presence of tags in the recombinant proteins helps in its separation from endogenous proteins. Principle for the same is shown in the figure below:

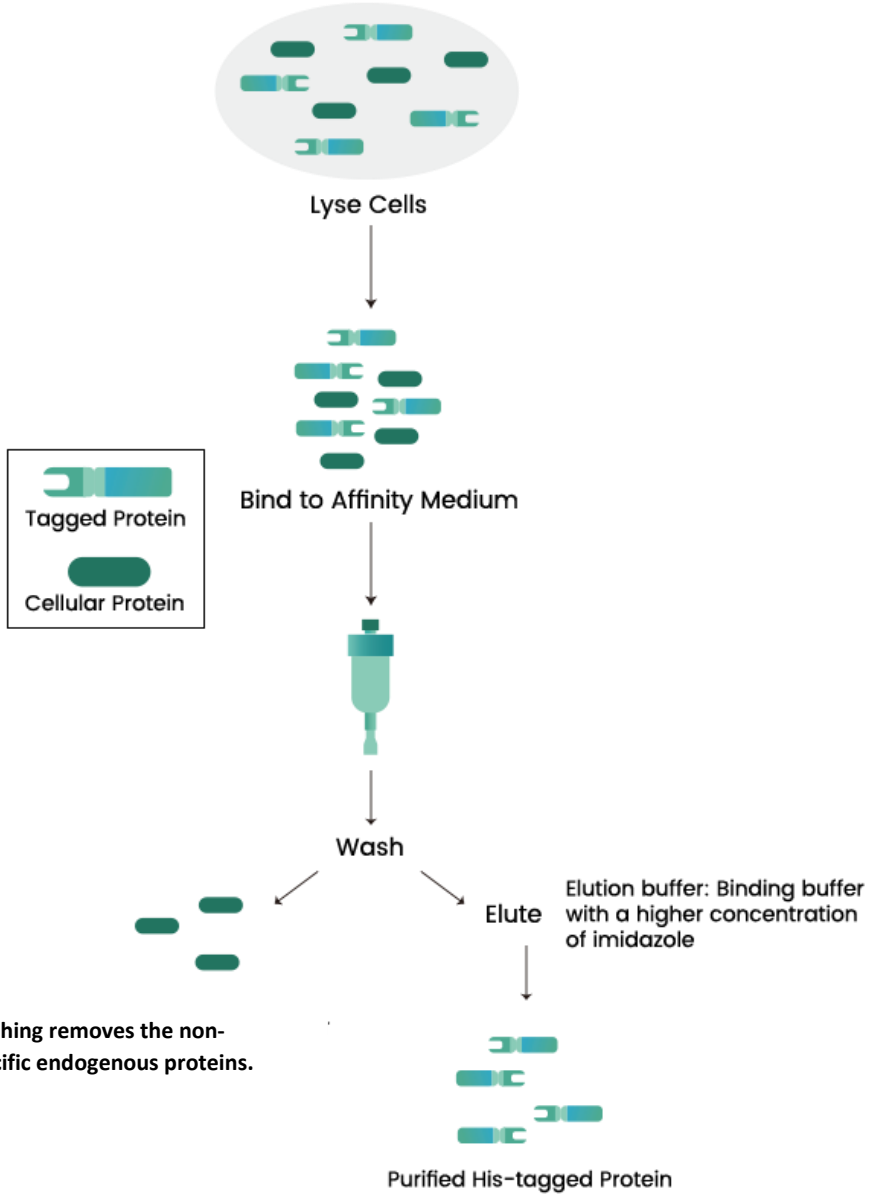


6. The method which is used for separation of His tagged proteins is affinity chromatography. The specific variant used here is called as Ni-NTA affinity chromatography. In this, NTA is the chelating agent and stands for Nitrilotriacetic acid. It is attached to the sepharose beads and chelates the Ni^{2+} ions. These Ni^{2+} ions bind to the imidazole ring present in the side chain of His-tag present in the recombinant fusion proteins. This is shown below.



7. After recombinant proteins are bound to the Ni-NTA beads present in the column, they are washed using a buffer that contains 10-25 mM of imidazole. This helps remove non-specific binding of endogenous proteins. After washing, elution is done using a higher concentration of imidazole usually in the range of 100-500 mM concentration.
8. The imidazole competes with the His side chain for binding to the Ni^{2+} and in this process the recombinant protein is released from the column and is collected. This process is called as elution.
9. We can also use cobalt (Co^{2+}) or copper (Cu^{2+}) ions in place of Ni^{2+} ions for isolation of His-tagged recombinant proteins using affinity chromatography.

The workflow outline for the whole process is shown in the figure below:



Washing removes the non-specific endogenous proteins.