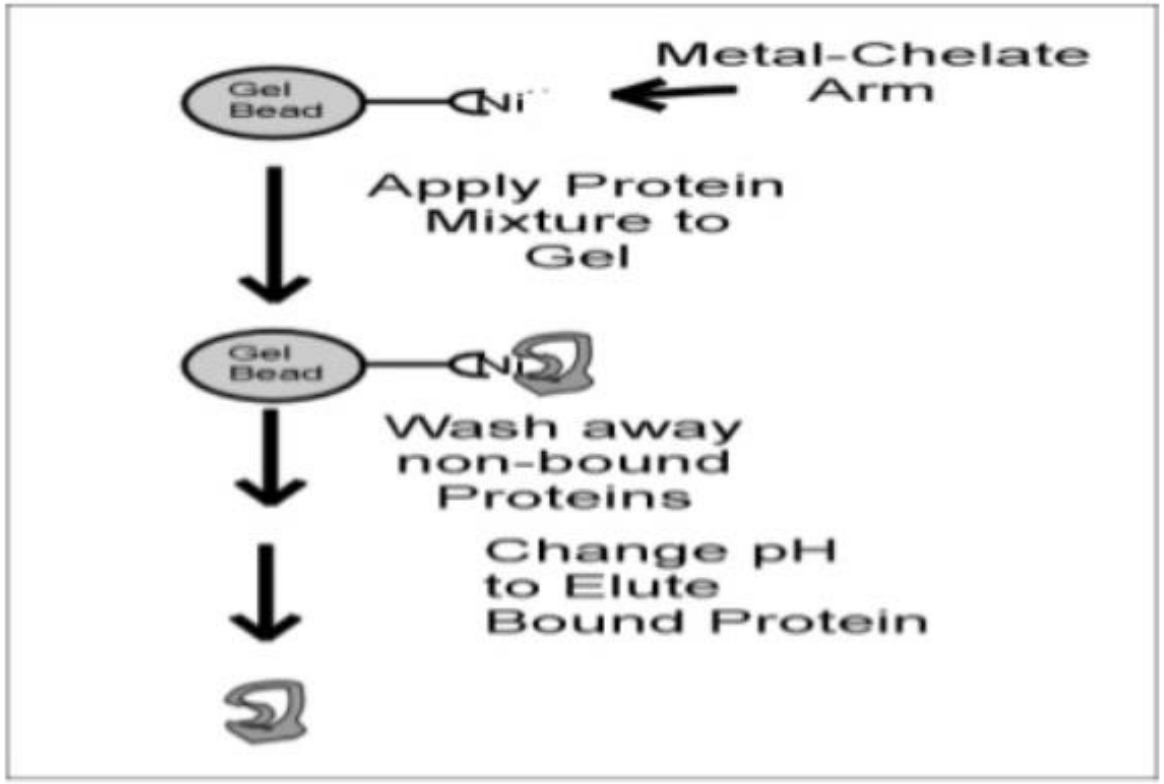
Types of Chromatography (IMAC, HIC, GC, LC)

Immobilized metal affinity chromatography

Metal-Chelate Affinity Chromatography (MCAC), also known as Immobilized Metal Affinity Chromatography (IMAC), was first successfully demonstrated in 1975 by Porath and collaborators for human serum proteins.



Principle

- Transition metal ions are used, electron pair acceptors.

e.g. Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{3+}

- Co-ordination between immobilized metal and electron donor from protein surface.
- Electron donors (N,S,O) present in chelating compound attached chromatographic support forms metal chelates, which can be monodentate or multidentate

Denticity : No. of donor groups in a single ligand that bind to central atom in coordination complex.

- Remaining metals sites are occupied by water molecules and are exchanged by electron donor from protein.

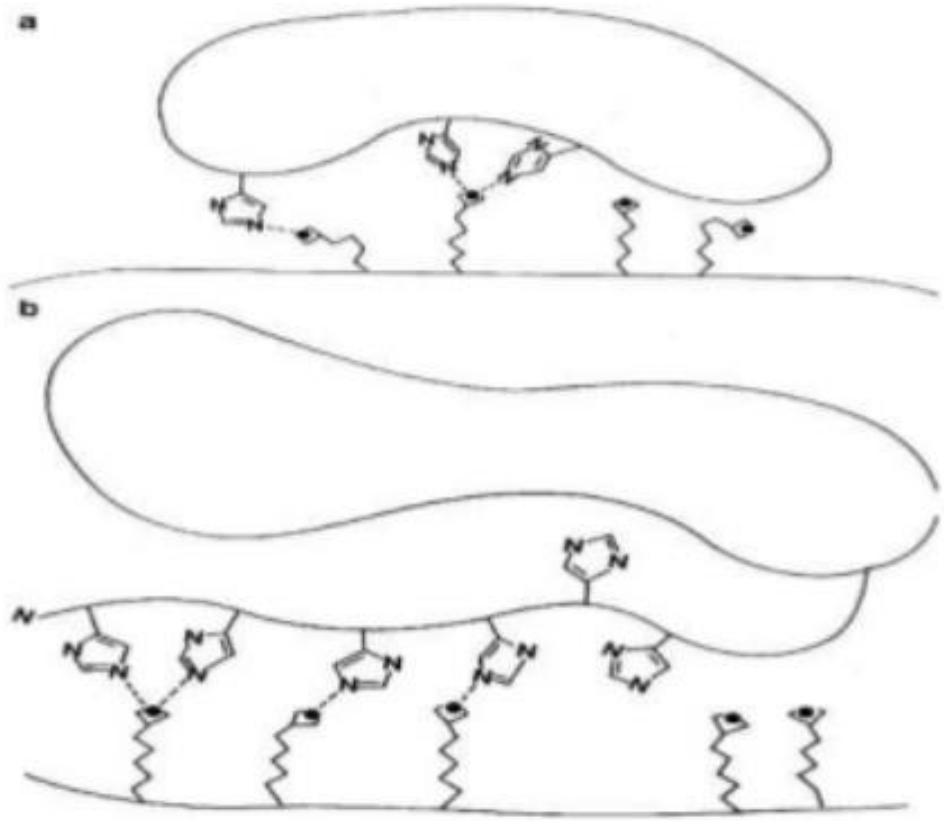


Fig. Schematic illustration of the protein binding to a metal-chelated affinity support. Strong binding of a protein onto the IMAC matrix is achieved predominately by multi-point attachment of native or engineered surface histidines ŽA, or by histidine tag ŽB added to the N- or C-terminus of the protein. There are many possibilities for the construction of efficient His tags considering the number of histidines, their location and microenvironment.

His-Tag for Purification of Recombinant Proteins

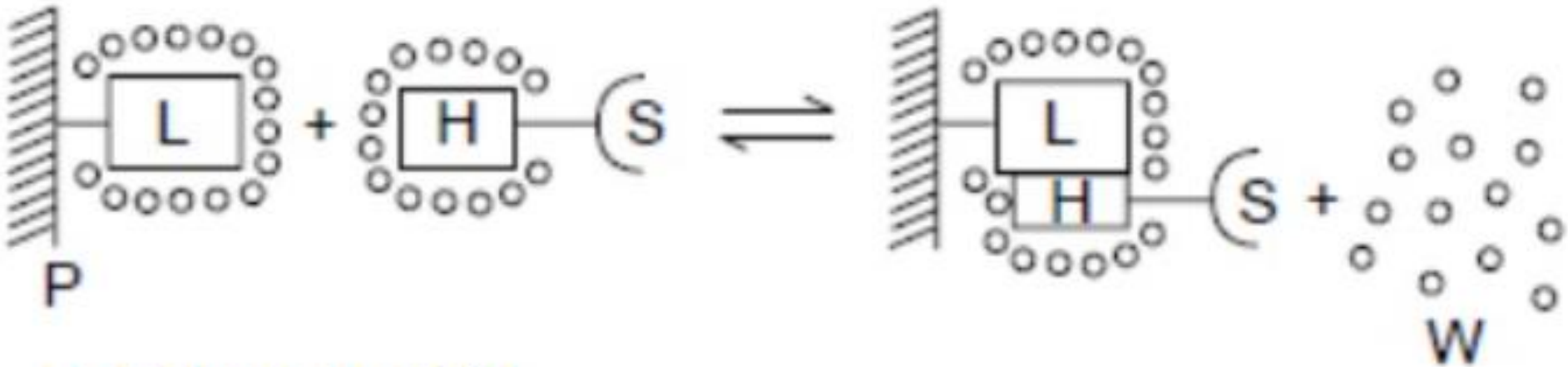
- It has been shown that an amino acid sequence consisting of 6 or more His residues in a row will also act as a metal binding site for a recombinant protein.
- A His-Tag sequence can be placed on the N-terminal of a target protein by using vectors

Met-Gly-Ser-Ser-**His-His-His-His-His-His**-Ser-Ser-Gly-**Leu-Val-Pro-Arg-Gly-Ser**....recombinant protein sequence

Hydrophobic interaction chromatography

Principle

- Separation of substances is based on their varying strength of interaction with hydrophobic groups attached to an uncharged gel matrix
- Hydrophobic groups on proteins are sufficiently exposed to bind to the hydrophobic groups on the matrix.
- How is this achieved?



P=Polymer matrix
S=Solute molecule
L=Ligand attached to polymer matrix
H=Hydrophobic patch on surface of solute molecule
W=Water molecules in the bulk solution

GAS CHROMATOGRAPHY

- Separation of gaseous & volatile substances
- Simple & efficient in regard to separation

GC consists of **GSC** (gas solid chromatography)
GLC (gas liquid chromatography

Gas → M.P

Solid / Liquid → S.P

GSC not used because of limited no. of S.P

GSC principle is **ADSORPTION**

GLC principle is **PARTITION**

Sample to be separated is converted into vapour

And mixed with gaseous M.P

Component more soluble in the S.P → travels slower

Component less soluble in the S.P → travels faster

Components are separated according to their **Partition Co-efficient**

Criteria for compounds to be analyzed by G.C

1.VOLATILITY:

2.THERMOSTABILITY:

Liquid Chromatography

Liquid Chromatography (LC) is a chromatographic technique in which the mobile phase is a liquid.

LC is a much older technique than GC, but was overshadowed by the rapid development of GC in the 1950's and 1960's.

LC is currently the dominate type of chromatography and is even replacing GC in its more traditional applications.

Advantages of LC compared to GC:

LC can be applied to the separation of any compound that is soluble in a liquid phase.

LC more useful in the separation of biological compounds, synthetic or natural polymers, and inorganic compounds

Advantages of LC compared to GC (continued):

Retention of solutes in LC depend on their interaction with both the mobile phase and stationary phase.

GC retention based on volatility and interaction with stationary phase
LC is more flexible in optimizing separations → change either stationary or mobile phase

Most LC detectors are non-destructive

most GC detectors are destructive
LC is better suited for preparative or process-scale separations

Disadvantage of LC compared to GC:

LC is subject to greater peak or band-broadening. RESOLUTION!!!!
much larger diffusion coefficients of solutes in gases vs. liquids

Low- and High-performance Liquid Chromatography: