

Affinity Chromatography

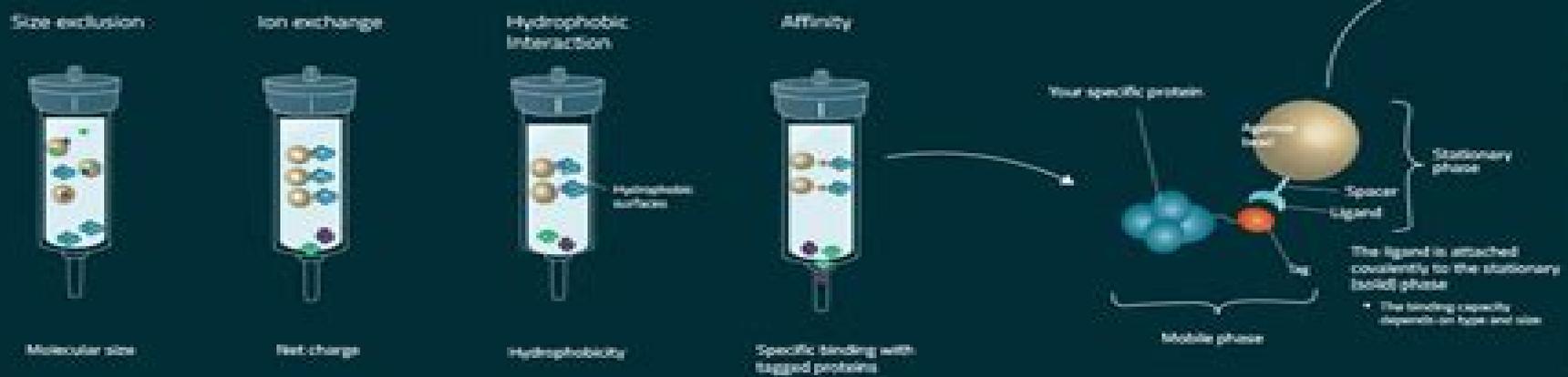
What is affinity chromatography?

Purification via affinity chromatography is a powerful technique to separate your protein of interest from your sample. The proteins are marked with "tags" which give them specific binding properties. A suitable immobilized ligand binds to the tagged protein.

Why do we need purified proteins?

Characterisation of proteins e.g. functional assays, structure determination and protein interactions.

Chromatography methods in comparison



Design the target protein with an Affinity tag

1. Choose expression system

In vivo
In vitro

Cell-free expression



- Application**
- Toxic proteins
 - DNA-binding proteins
 - Unnatural label or aminoacids e.g. isotope label (NMR)

- Properties**
- Easy to scale up

2. Choose the organism for protein expression

Prokaryotic cell
Process of transcription and translation occur simultaneously



- Eukaryotic cell**
- Transcription
 - mRNA is processed
 - modified mRNA is exported to the cytoplasm
 - Translation



Advantages

- Scalable
 - Simple culture conditions
 - High expression level
 - Less expensive
-
- High-level protein processing
 - Low - moderate expression level

Affinity Chromatography A Practical Approach

IM Harris



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