

# Affinity Chromatography

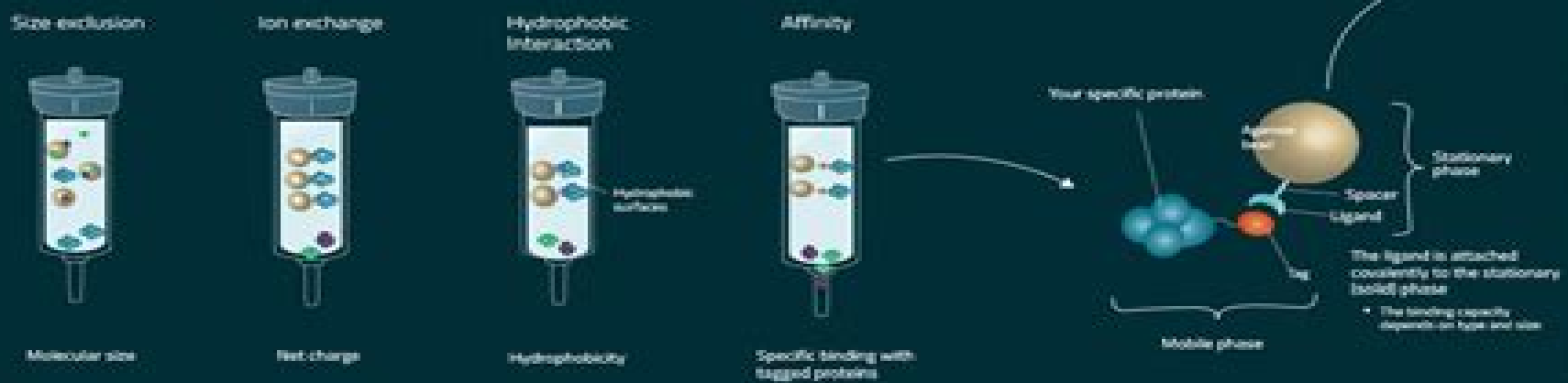
## What is affinity chromatography?

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

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# Affinity Chromatography A Practical Approach

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