

# Affinity Chromatography

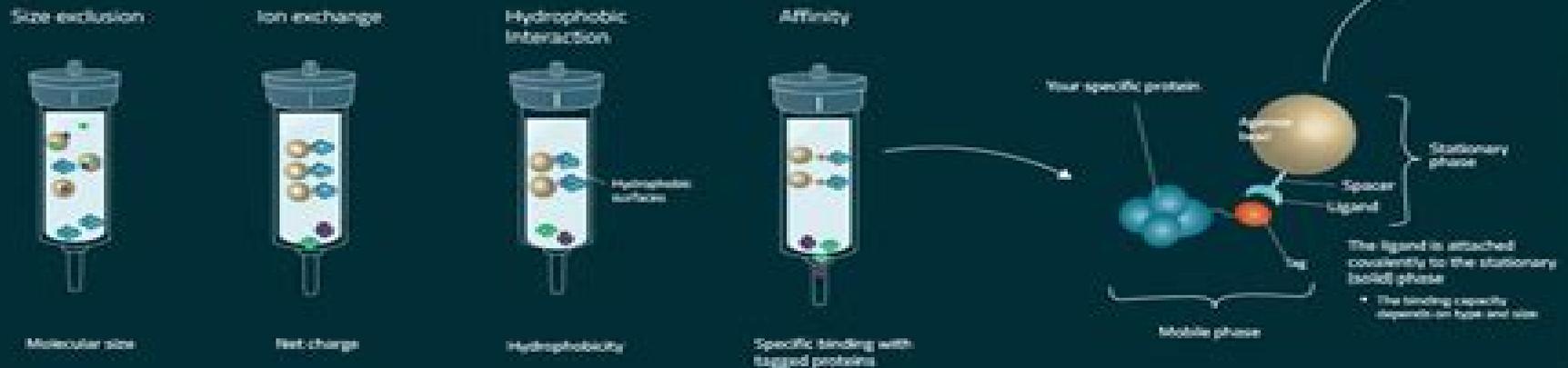
## What is affinity chromatography?

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

Amino acids  
DNA  
Energy components  
Plasmid  
rNTPs  
Water

#### 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**  
1. Transcription  
2. mRNA is processed  
3. modified mRNA is exported to the cytoplasm  
4. Translation



#### Advantages

- Scalable
- Simple culture conditions
- High expression level
- Less expensive

- High-level protein processing
- Low - moderate expression level

# Affinity Chromatography A Practical Approach

**D Keegan**



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