Affinity Chromatography

What is affinity chromatography?

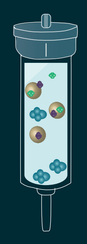
Purification via affinity chromatography is a powerful technique to separate your protein of interest from your sample. The proteins are marked with "tags" which give them specific binding properties. A suitable immobilized ligand binds to the tagged protein.

Why do we need purified proteins?

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

Chromatography methods in comparison

Size exclusion

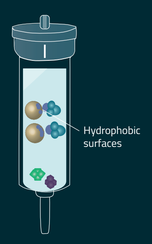


Molecular size

Ion exchange

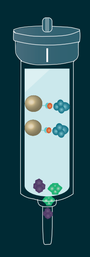
Net charge

Hydrophobic Interaction

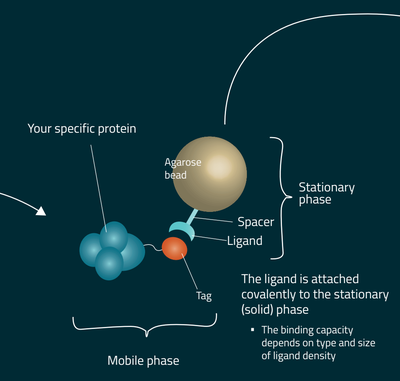


Hydrophobicity

Affinity



Specific binding with tagged proteins



The ligand is attached covalently to the stationary (solid) phase

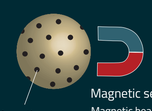
- The binding capacity depends on type and size of ligand density

Affinity bead (Core)

Agarose beads

- Cross-linked and porous
- Proteins can flow through pores
- Hydrophilic
- Specific bonds

Agarose bead with magnetic core



Magnetic seperator

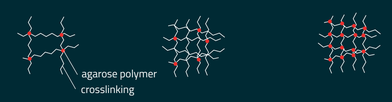
Magnetic beads are attracted by seperator

Agarose concentration of affinity bead

Low concentrated agarose

High concentrated agarose
Low degree of crosslinking

High concentrated agarose
High degree of crosslinking



Low initial agarose concentration:

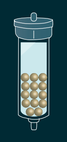
- Large proteins need large pores

High initial agarose concentration:

- Higher degree of crosslinking
- Smaller pore sizes
- Higher pressure resistance
- Mechanically stable

Affinity bead size in the colomn

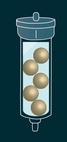
The bead sizes range from 30 µm to 800 µm



Small affinity beads

- Higher ratio of surface to volume
- Higher binding capacity of the beads
- Higher pressure resistence

The binding capacity decreases when pressure builds up inside the column at higher flow rates



Large affinity beads

- Allows faster flow rate in batch and FPLC experiments
- Useful for viscous media
- Useful for scaling-up a purification process

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 lable (MNR)

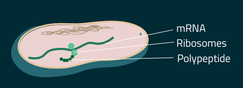
Properties:

- Easy to manipulate
- Costly if large amounts of protein are required

2. Choose the organism for protein expression

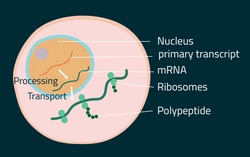
Prokaryotic cell

Processes of transcription and translation occur simultaneously



Eukaryotic cell

1. Transkription
2. mRNA is processed
3. modified mRNA is exported to the cytoplasm
4. Translation



Advantages

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

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

Bacteria

Application

- Structure determination
- Protein function assays
- Protein interactions assay

Properties

- Secretion to periplasm
- No post-translational modifications

Insect cells

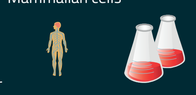
Application

- Structure determination
- Protein function assay
- Intracellular protein production
- Synthesis of protein complexes
- Expression of membrane proteins

Properties

- Secretion to medium
- Simple N-linked glycosylation
- O-linked glycosylation, Phosphorylation, Acetylation, Acylation

Mammalian cells



Application

- Structure determination
- Protein function assays
- Amplification of viral culture
- Protein interaction assay
- Synthesis of protein complexes
- Expression of membrane proteins

Properties

- Secretion to medium
- Complex N-linked glycosylation
- O-linked glycosylation, Phosphorylation, Acetylation, Acylation, gamma-Carboxylation

3. Vector construction

Gen of your target protein (wildtype or modified) with affinity tag

Vector insertion

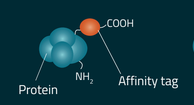
via Transformation

via Transduction

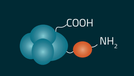
via Transfection

4. Choose the affinity tag and the position

C-terminal

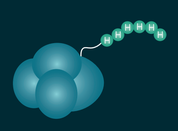


N-terminal



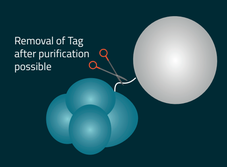
Affinity tags

Poly Histidin-tag



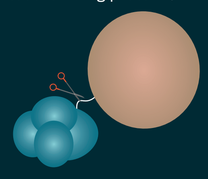
- Sequence: HHHHHH
- Specificity of interaction (KD):10 µM
- Protein Yield: Up to 80 [mg] / resin [ml]
- Size: 840.8-1,937.9 Dalton
- Eluent: Imidazol, histidine, or at low pH

Glutathione S-transferase tag (GST)



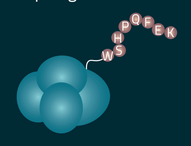
- Specificity of interaction (KD): 1 µM
- Protein Yield:10-12 [mg] / resin [ml]
- Size: 26 kDa
- Eluent: Reduced glutathione

Maltose-binding protein (MBP)



- Specificity of interaction (KD): 1,2 µM
- Protein Yield: 7-16 mg / resin ml
- Size: 45 kDa
- Eluent: Maltose

Strep-tag

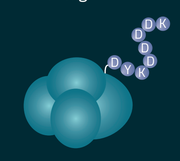


- Sequence: WSHPQFEK
- Specificity of interaction (KD): 300 nM
- Protein Yield: 9 mg / resin ml
- Size: 1,058.1 Dalton
- Eluent: Desthiobiotin

Rho1D4-tag

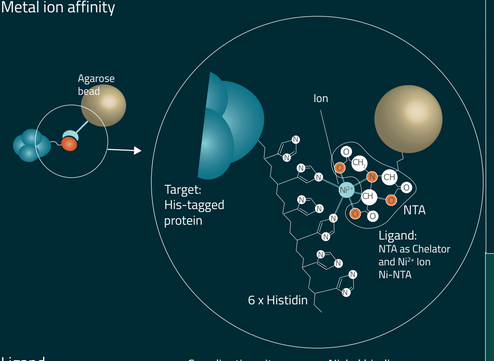
- Sequence: TETSQVAPA
- Specificity of interaction (KD): 20 nM
- Protein Yield: 3-4 mg / resin ml
- Size: 902.9 Dalton
- Eluent: Rho1D4 peptide, low pH, or protease digest

FLAG-tag



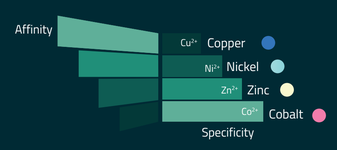
- Sequence: DYKDDDDK
- Specificity of interaction (KD): 100 nM
- Protein Yield: 0.6-1 mg / resin ml
- Size: 1,012.9 Dalton
- Eluent: DYKDDDDK (FLAG)peptide

Metal ion affinity

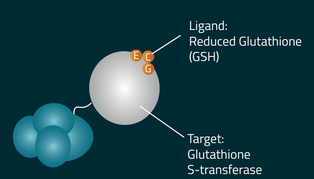


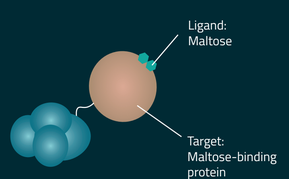
Ligand	Coordination sites	Nickel binding
Nitrilotriacetic acid (NTA)	4	Light
Iminodiacetic acid (IDA)	3	Weak
INDIGO	5	Strong

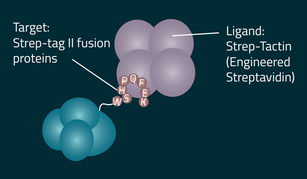
Coupled metal Ion



Enzym substrate specificity

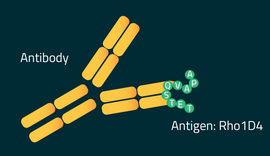


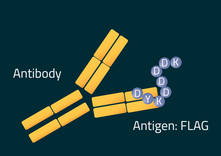




Antibody-Antigen-Interaction :

Each antibody is capable of binding only to a specific antigen





How to chose tag?

His-tag

GST Tag
MBP Tag

Strep-Tactin tag

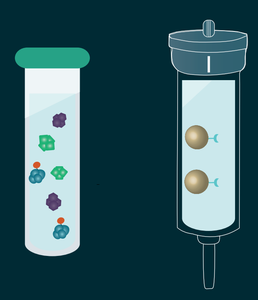
Rho1D4-tag
FLAG-tag

higher protein yield

more expensive
more specific and pure

Workflow of an Affinity Chromatography

1. Cell opening of expression organism by sonification, mechanical disruption etc. followed by centrifugation and cell separation



2. The mobile (liquid) phase: Cell lysate with the target protein

The stationary (solid) phase: Column/ Cartridge filled with affinity beads

3. Add lysate to the column

4. Protein of interest connects to the beads (matching affinity)

Unspecific proteins passes the beads

5. Add wash buffer to remove remaining unspecific proteins

6. Apply elution conditions: e.g. by competition with eluent (or pH change)

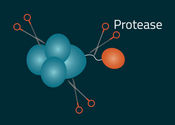
7. Concentrated purified target protein

Limitations of Affinity Chromatography

1. Inaccessible tag sequence

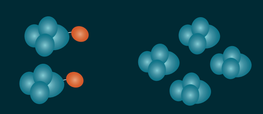
Solution approach: Move the tag to the other terminus of the protein or to an accessible loop within the protein

2. Tag degradation by cellulase proteases



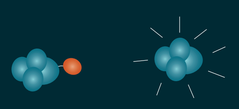
3. Affinity tag influences protein expression

- Tagged proteins may show a lower expression level



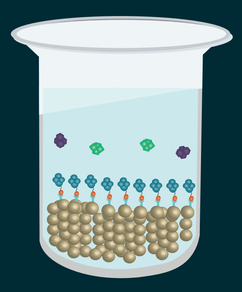
- Affinity tag may cause misfolded proteins

- Affinity tag may hamper protein activity



Separation methods in the lab

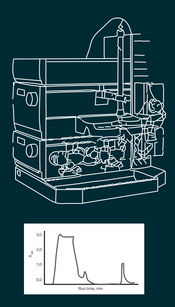
Batch Spin



- Simple and fast method
- No pressure or flow rate control

Gravity Flow Columns

Fast Protein Liquid Chromatography (FPLC)



- Automated system
- Reproducible
- Direct control on pressure, flow rate and the yield

Magnetic Beads

Magnetic bead separator

- Simple method
- Automatable and scalable system
- Applicable for small volumes

Subsequent studies

1. Combination with an additional chromatography steps
   - Different chromatography e.g. affinity chromatography and size exclusion chromatography

2. Control of the purification
   - SDS Page
     Purity and quantity of different proteins

   - Western Blot
     Analytical technique to detect specific proteins with antibody directed against affinity tag

   - Protein aktivity detection
     ELISA: Immunological assay used to detect and quantify soluble proteins in biological samples

     SPR (Surface plasmon resonance): Biosensor technique for studying interactions between all classes of biomolecules

Cube Biotech

https://cube-biotech.com/affinity-chromatography-which-tag-to-use