Protein purification

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Protein purification

- Why do we purify proteins?
- To examine the function of the structure of the protein perfectly.
- If you have a mixture of proteins in a solution, then we need to purify it to have only one protein that function independently. Why?
- Other proteins and un-wanted molecules might have an impact on the protein function and structure understudy.

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How do we purify proteins?

- Once the Cells are lysed (from the previous lecture), the mixture is then loaded to a specific Column to analyze or purify a mixture of proteins.
- What is the technique that is used to purify proteins?
- Chromatography.
- FPLC system is now used to purify proteins.
- What is a FPLC ? Previously HPLC?
- Fast performance liquid chromatography. (High)

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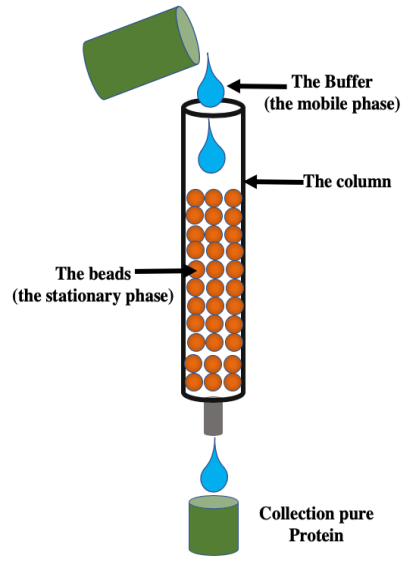
Tools and Techniques: Purification & analysis of Protein

- Since we got the (extracted from the Bactria)
- **Chromatography** is used to purify protein.
- It uses a **column** packed with a porous matrix (**the stationary phase**) and a buffered solution (**the mobile phase**)
- ***Proteins*** *or other solutes pass through the column at different rates, de- pending on how they interact with the stationary phase.*



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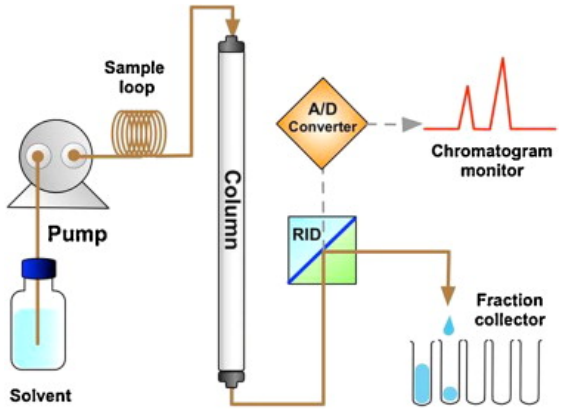
What is the Fast performance liquid chromatography.

- The method is based on loading or injecting the mixture of the protein into a specific column (where the protein can bind to its resin)
- Then it can be eluted by another solution to collect it and analyze it.
- Pump (with controlled pressure) is used to for loading.

Column



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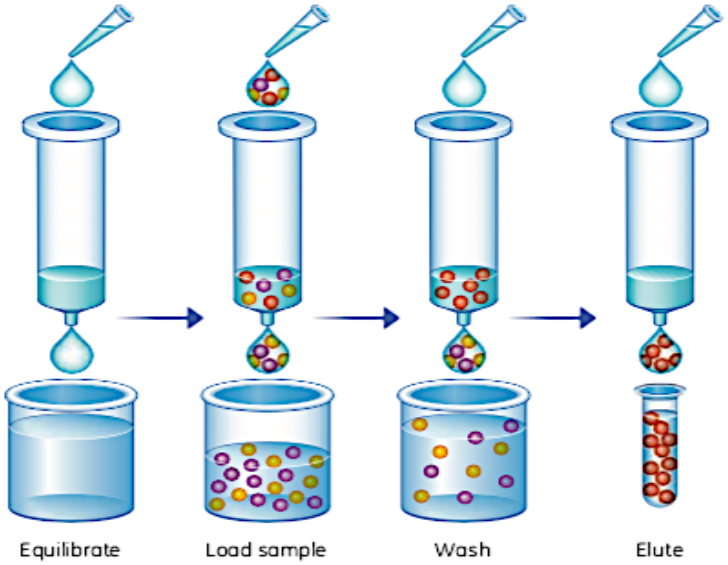
All purification methods are based on the same idea!



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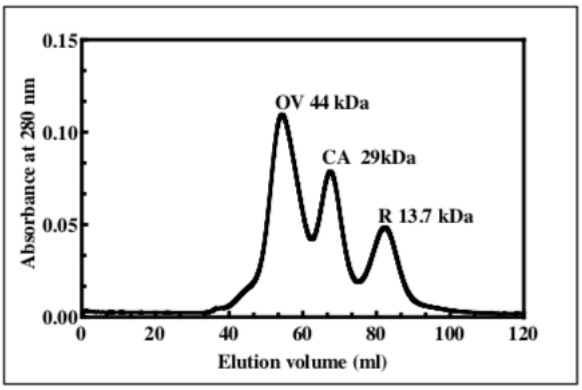
FPLC/HPLC

- Modern systems use an internal detector to show the chromatogram which shows the peaks of each protein.
- The purified protein can be fractionated and collected in small tubes for further analysis.

Give a chromatogram example of Separation three proteins by FPLC?



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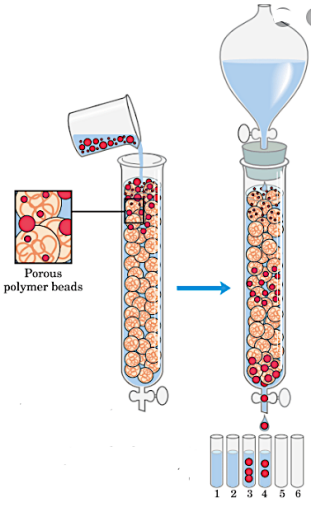
How can we choose the column to purify protein?

- We choose the columns depends on the ability of protein to bind the resins of the column.
- The resins of the columns have specific dimeter.
- Each column has different properties.
- So, lets know….



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The types of columns?

- What are the different common types of columns?

1. Ion exchange IXC chromatography .
2. Gel filtration GF chromatography.
3. Hydrophobic interaction chromatography HIC.
4. Affinity chromatography.

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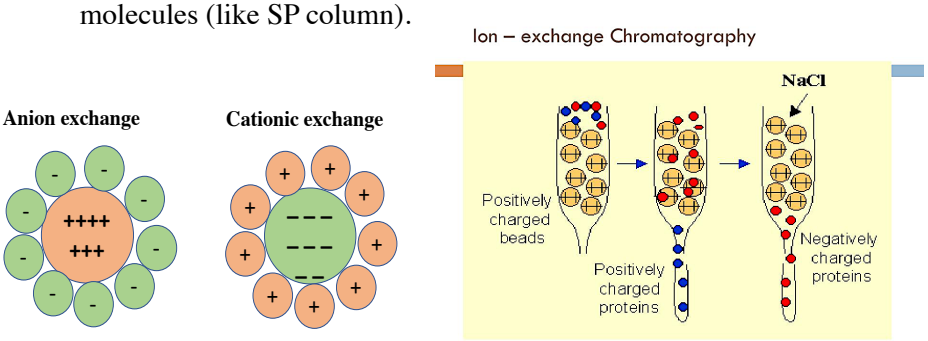
Ion exchange IXC chromatography .

- It separates the compounds according to the nature and degree of their ionic charge.
- Anion exchange resins have positive charge and used to separate negatively charged molecules.
- Cation exchange resins have a negative charge and are used to separate positively charged molecules (like SP column).



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Gel filtration GF chromatography

- In this type, the protein is separated in base of molecule size to which the molecules have different degrees of access.
- The smaller molecules have greater access, but the large molecules are excluded from the matrix (elute first).
- It is also called ‘’ size exclusion chromatography’’

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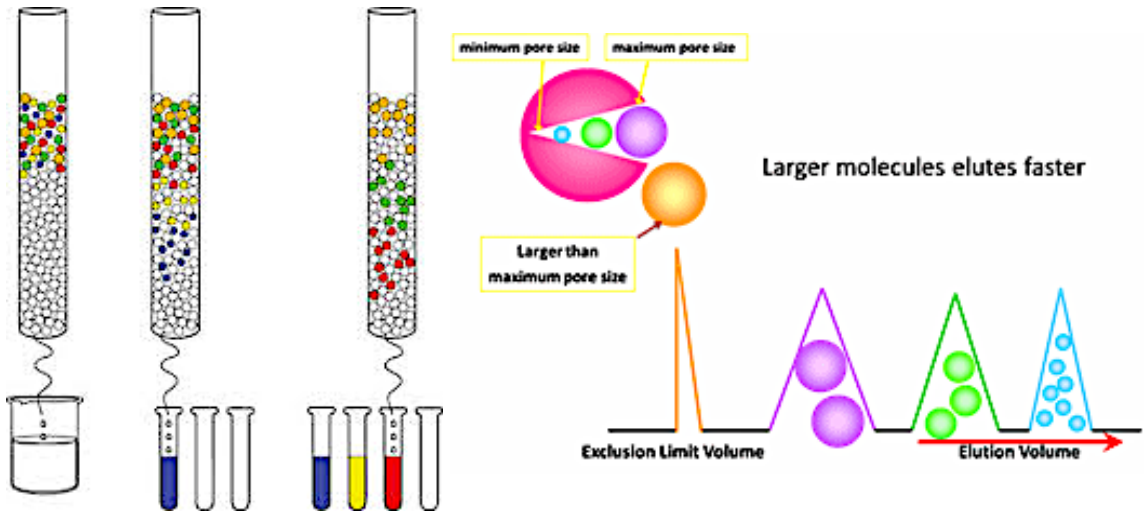
GF column



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Hydrophobic interaction chromatography HIC.

- HIC media is amphiphilic (means a protein has both hydrophilic and hydrophilic) to allow the separation on proteins based on their surface hydrophobicity.
- In the low salt buffer, interaction between the hydrophobic region and the resin is very weak, however, the interaction is enhanced in a high ionic strength buffer.

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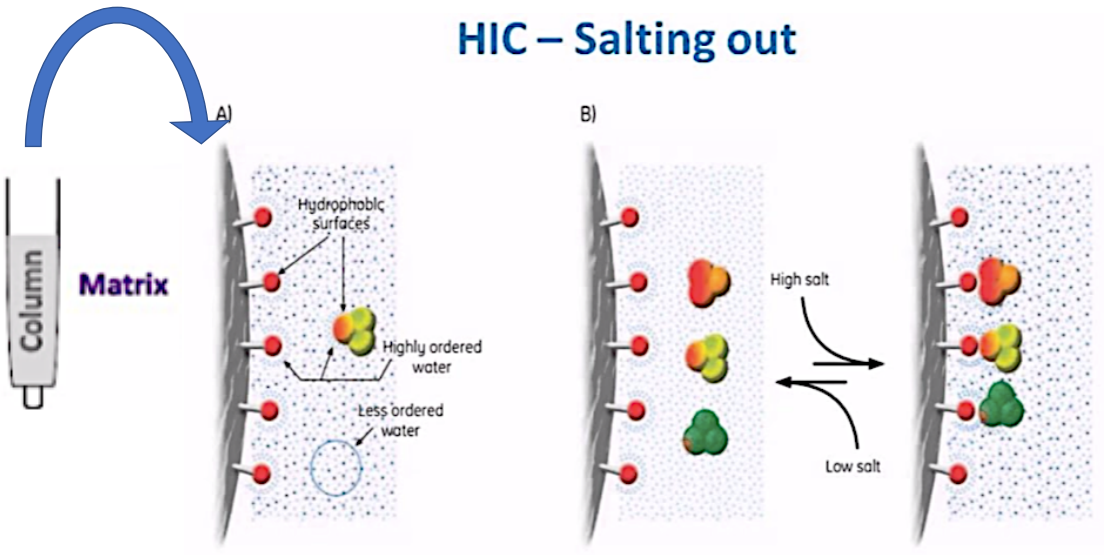
How does HIC work?



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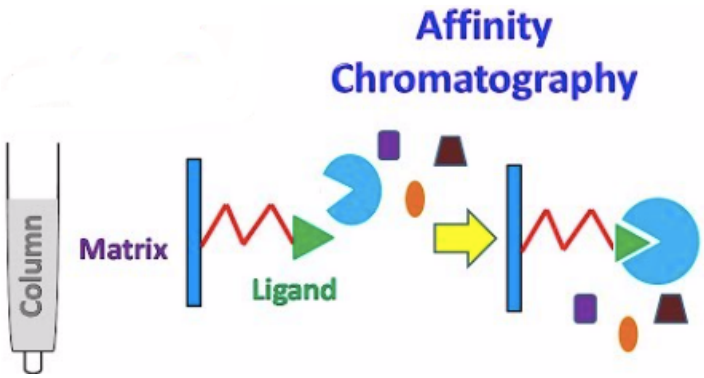
Affinity chromatography:

- The resins have ligands attached to their surfaces which are specific for a particular molecule.



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His 6-tag method

- What is his-tag method?
- It is a type of affinity chromatography.
- What is based on?
- Ni Nickle on the resin binds to specific molecules (six 6 Histidine residues that are attached to the protein).
- Histidine residues can coordinate to make a complex with Nickle (transition metal).

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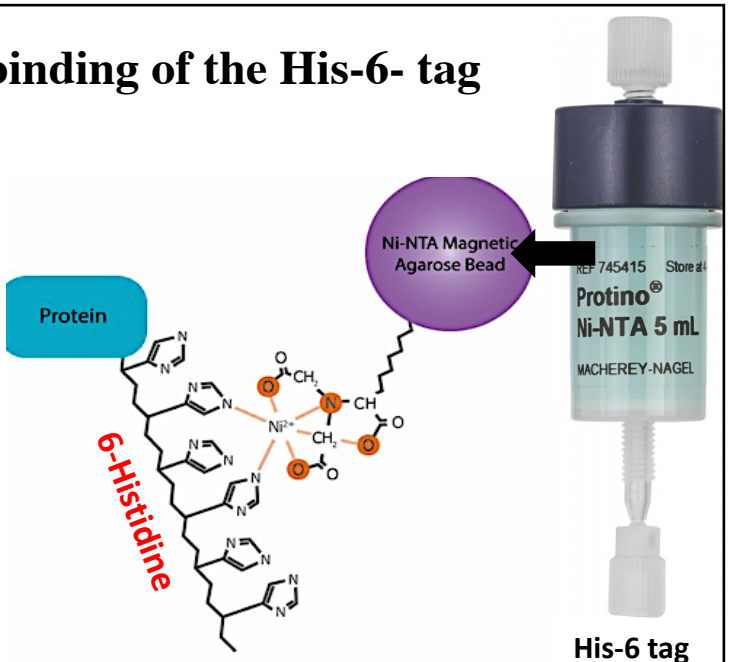
Describe the chemical binding of the His-6- tag

1. The Ni makes six bonds with the ligands like indole ring.
2. So, the Histidine contains an indole ring.
3. The 6 histidine binds the Ni on the column.
4. Un-wanted protein are washed by a buffer.
5. The pure protein is eluted and collected.



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Describe the tag process?

- Before the purification, the 6 H histidine (amino acid molecules) need to be genetically engineered and connect them to the protein.
- After the purification, they need to be cut out ‘’ cleaved out’’ by an enzyme called TEV.

•M HHHHHH- linker-  protein.

TEV enzyme

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Last but no the least

1. The purity of the protein must be checked. How?
2. The molecular weight MW of the protein must be analyzed and conformed at the expected size. How?
3. The buffer solution needs to be changed sometimes. How?
4. The protein concentration needs to be measured. How?
5. The low concentration protein needs to be concentrated. How?
6. Finally, the protein needs to be frozen and stored at -20 to -80 ˚C. How?

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