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| --- | --- | --- | --- |
| **Type** | **Type of Resin** | **Principal of Separation** | **How to Elute the Protein** |
| **Gel Filtration:***Separation by size* |  | * Proteins don't "stick".
* Small proteins enter the interior of the beads, and therefore take longer to wash off of the column.
 | * Simply washing the column with buffer will eventually wash the proteins out of the column.
* Smaller proteins elute last.
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| **Anion Exchange***Separation by charge.* | * Beads with a **positive** charge

 | Protein stick to resin because of:* Overall **negative** charge (*anions*)
* Proteins have patches of negative charge

 | * Wash, bound proteins **may** elute.
* Increase salt concentration to weaken electrostatic interaction.
* Change of pH to pH < pI (protein becomes positively charged)

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| **Cation Exchange***Separation by charge.* | * Beads with a **negative** charge

 | Protein stick to resin because of:* Overall **positive** charge (*cations*)
* Proteins have patches of positive charge

 | * Wash, bound proteins **may** elute.
* Increase salt concentration to weaken electrostatic interactions.
* Change of pH to pH > pI (protein becomes negatively charged)

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| **Affinity Chromato-graphy***Separation by affinity, either ligand affinity, or antibody..* | * Beads with a ligand:.

 | Protein stick to resin because of:* Binding site for ligand

 | * Excess ligand
* Change in pH, Salt, solvent to weaken protein-ligand interaction.

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| **Common:** Ni+2 ions attached to resin. | Protein has 6 His residues on N or C-term. “His-tag“ | Eluted with free histidine or imidazole (=His sidechain). |
| * or Antibody

 | Protein stick to resin because of:* Binding to antibody

 | * Changes in solution conditions (pH, Salt, solvent) to weaken protein-antibody interaction.

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