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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. |
| **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) |
| **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) |
| **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. |
| **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. |