

CLAIMS

1. A process for the purification of a protein of interest from a mixture comprising the steps of
 - a) in an operational chromatography cycle loading a first volume of a mixture containing the protein of interest from a first container to a chromatography matrix operated such that the protein binds to the chromatography matrix until 40% to 100% of the static binding capacity of the chromatography matrix is reached;
 - b) collecting flow-through containing unbound protein of interest in a second container, and
 - c) in a further operational chromatography cycle re-loading the flow-through from the second container and loading a second volume of the protein of interest from the first container to the same chromatography matrix, operated such that the protein of interest binds to the chromatography matrix until 40% to 100% of the maximum static binding capacity of the chromatography matrix is reached,

wherein the re-loading of the flow-through from the second container to the chromatography matrix is performed prior to the loading of the second volume of the mixture containing the protein of interest from the first container to the chromatography matrix.

2. A process for the purification of a protein of interest from a mixture comprising the steps of:
 - a) in an operational chromatography cycle loading a first volume of a mixture containing the protein of interest from a first container to a chromatography matrix operated such that the dynamic binding capacity of the chromatography matrix is exceeded;
 - b) collecting flow-through containing unbound protein of interest in a second container, and
 - c) in a further operational chromatography cycle re-loading the flow-through from the second container and loading a second volume of the protein of interest from the first container to the same chromatography matrix, operated such that the dynamic binding capacity of the chromatography matrix is exceeded;

wherein the re-loading of the flow-through from the second container to the same chromatography matrix is performed prior to the loading of the second volume of the mixture containing the protein of interest from the first container to the same

chromatography matrix.

3. The process according to claim 2, wherein in step (a) the loading of the protein of interest is stopped when at least 40% of the maximum static binding capacity is reached.
4. The process according to claim 1, 2 or 3, wherein the further operational chromatography cycle is the chromatography cycle immediately following.
5. The process according to claim 1, 2, 3 or 4, wherein the collection of the flow-through is started at a predetermined first concentration of protein of interest in the flow-through and stopped at a predetermined second concentration of protein of interest in the flow-through.
6. The process according to any one of claims 1 to 5, wherein a predetermined fraction of the flow-through is collected in the second container.
7. The process according to any one of claims 1 to 6, wherein the flow-through is processed prior to being re-loaded onto the chromatography matrix, the processing being selected from stirring or agitation, dilution, concentration adjustment, pH adjustment, conductivity adjustment, buffer or solvent exchange, cooling or heating or any combination thereof.
8. The process according to any one of claims 1 to 7, wherein the chromatography is selected from affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, chiral chromatography or dielectric chromatography.
9. The process according to any one of claims 1 to 8, wherein the process comprises three chromatography steps.
10. The process according to claim 9, wherein the process comprises Protein A chromatography followed by cation exchange chromatography followed by anion exchange chromatography.
11. The process according to any one of claims 1 to 10, wherein one, two, three or all of the chromatography matrix/matrices is/are a chromatography column.
12. The process according to any one of claims 1 to 11, wherein the protein of interest is an antibody or an antibody fragment.
13. A method of manufacture of a protein of interest comprising the process for purification of the protein according to any one of claims 1 to 12.