

CLAIMS

1. An industrial-scale process for the purification of a product biomolecule from a feedstock comprising the product biomolecule and at least one impurity, the process comprising the steps of:
 - a) loading feed from the feedstock to a chromatography matrix such that the product biomolecule binds to the chromatography matrix;
 - b) eluting the product biomolecule from the chromatography matrix in an eluate by applying an elution solution to the chromatography matrix;
wherein the eluate comprises:
 - a first fraction comprising purified product biomolecule; and
 - a second fraction comprising both the product biomolecule and at least one impurity, the second fraction comprising one or more leading and/or trailing fraction(s);and wherein the first fraction is collected separately from the second fraction;
 - c) holding the second fraction in one or more container(s);
 - d) loading the second fraction from the container(s) and additional feed from the feedstock to the chromatography matrix such that the product biomolecule in the second fraction binds to the chromatography matrix; wherein the additional feed is loaded simultaneously with or sequentially to the second fraction;
and
 - e) eluting the product biomolecule from the chromatography matrix in an eluate by applying an elution solution to the chromatography matrix, wherein the eluate comprises purified product biomolecule;wherein the chromatography matrix in step (a), step (b), step (d) and step (e) is the same chromatography matrix.
2. A process according to claim 1 wherein steps (b), (c) and (d) are repeated.
3. A process according to claim 1 wherein steps (b), (c) and (d) are performed at least twice.

4. A process according to any one of the preceding claims wherein the second fraction comprises (i) the leading fraction immediately preceding the first fraction; and/or (ii) the trailing fraction immediately following the first fraction.
5. A process according to any one of the preceding claims wherein:
 - steps (a) and (b) are repeated multiple times;
 - the second fractions collected in each step (b) are pooled together;
 - step (c) comprises holding the pooled second fractions in the container(s);and
 - step (d) comprises loading the pooled second fractions from the container(s) to the chromatography matrix such that the product biomolecule in the pooled second fractions binds to the chromatography matrix.
6. A process according to any one of the preceding claims, wherein step (a) further comprises collecting flow-through containing unbound product biomolecule that does not bind to the chromatography matrix; and step (d) further comprises loading the flow-through to the chromatography matrix simultaneously with or sequentially to the second fraction and/or additional feed.
7. A process according to any one of the preceding claims, further comprising processing the second fraction(s) to promote binding of the biomolecule to the chromatography matrix.
8. A process according to claim 7 wherein said processing takes place during step (c) and/or step (d).
9. A process according to claim 7 or claim 8 wherein said processing comprises altering the pH, ionic strength, concentration, temperature and/or solvent of the second fraction(s) and/or mixing and/or degassing the second fraction(s).
10. A process according to any one of the preceding claims, further comprising testing the second fraction(s).

11. A process according to claim 10 wherein testing the second fraction(s) comprises determining one or more of the concentration of the product biomolecule; the concentration of impurities; the identity of impurities; the pH; the ionic strength; the temperature; the solvent; and/or the gas concentration of the second fraction(s).
12. A process according to any one of the preceding claims wherein step (c) comprises holding the second fraction(s) for at least 5 minutes.
13. A process according to any one of the preceding claims wherein step (c) comprises holding the second fraction(s) overnight.
14. A process according to claim 12 or claim 13 wherein the chromatography matrix is exchanged, cleaned or renewed during the hold time.
15. A process according to any one of the preceding claims wherein the product biomolecule is a protein, an antibody, an antibody fragment, a polynucleotide or a polypeptide.
16. A process according to any one of the preceding claims wherein the feedstock has a volume of at least 20 litres, optionally at least 100 litres.
17. A process according to any one of the preceding claims wherein the chromatography matrix has a bed volume of at least 4 litres.
18. A process according to any one of the preceding claims wherein in step (b) the volume of the eluate is at least 2 times the bed volume of the chromatography matrix, optionally wherein the volume of the eluate is between 2 times and 20 times the bed volume of the chromatography matrix.
19. A process according to any one of the preceding claims wherein the chromatography matrix is selected from:
 - an ion exchange chromatography matrix;
 - a hydrophobic interaction chromatography matrix;
 - an affinity chromatography matrix;

- a mixed-mode chromatography matrix;
- a chiral chromatography matrix; and
- a dielectric chromatography matrix.

20. A process according to any one of the preceding claims wherein the chromatography matrix is in a chromatography column.

21. A process according to any one of claims 1 to 20 wherein the chromatography matrix is a chromatography membrane or a monolith adsorber.

22. A process according to any one of the preceding claims wherein eluting the product biomolecule from the chromatography matrix is conducted at a flow rate of at least about 0.2 chromatography matrix volumes per minute.

23. A process according to any one of the preceding claims further comprising combining each of the first fractions.

24. A process according to any one of the preceding claims further comprising diafiltering and/or concentrating the purified product biomolecule.

25. A process according to any one of the preceding claims further comprising nanofiltering the purified product biomolecule.

26. A process according to any one of the preceding claims further comprising subjecting the purified product biomolecule to further chromatographic purification.

27. A process according to any one of the preceding claims further comprising chemically modifying the purified product biomolecule.

28. A process according to any one of the preceding claims further comprising formulating the purified product biomolecule with a pharmaceutically acceptable excipient, diluent or adjuvant.