CLAIMS

1. A method for purifying a protein from solution comprising:

(a) a first chromatography step comprising:

- passing said solution over a first chromatography matrix;
- eluting a crude protein eluent from the first chromatography matrix using a first elution buffer;

(b) a second chromatography step comprising:

- passing the crude protein eluent obtained at the end of step (a) over a second chromatography matrix;
- eluting a protein eluate from the second chromatography matrix using a second elution buffer; and

(c) a third chromatography step comprising:

- passing the protein eluate obtained at the end of step (b) through a third chromatography matrix in the flow-through mode;
- recovering purified protein from the flow-through of the third chromatography matrix;

wherein each of the buffers comprises Bis Tris.

2. The method of claim 1, wherein the crude protein eluent obtained at the end of step (a) is directly passed over a second chromatography matrix, without undergoing any treatment such as pH adjustment, buffer exchange or dilution.

3. The method of claim 1 or 2, wherein the protein eluate obtained at the end of step (b) is directly passed over a third chromatography matrix, without undergoing any treatment such as pH adjustment, buffer exchange or dilution.

4. The method of any one of claims 1 to 3, wherein the method is run in a closed system from the first step to the last one.

5. The method of any one of claims 1 to 4, wherein each one of the two first chromatography steps comprises:

- passing equilibration buffer over the chromatography matrix;
- passing the solution or the crude protein eluent over the chromatography matrix;
- passing equilibration buffer over the chromatography matrix;

- optionally passing wash buffer over the chromatography matrix;
- optionally passing equilibration buffer over the chromatography matrix;
- eluting the crude protein eluent or the protein eluate from the chromatography matrix using an elution buffer,

wherein each of the buffers comprises Bis Tris.

6. The method of any one of claims 1 to 5, wherein each of the buffers consists of Bis Tris, acetic acid, NaCl, water, and optionally NH_4Cl.

7. The method of any one of claims 1 to 6, wherein said method comprises the steps of:

(a) a first chromatography step comprising:

(i) passing equilibration buffer over a first chromatography matrix;

(ii) passing the solution over the first chromatography matrix;

(iii) passing equilibration buffer over the first chromatography matrix;

(iv) passing wash buffer over the first chromatography matrix;

(v) passing equilibration buffer over the first chromatography matrix; and

(vi) eluting a crude protein eluent from the first chromatography matrix using a first elution buffer;

(b) a second chromatography step comprising:

(i) passing equilibration buffer over a second chromatography matrix;

(ii) passing the crude protein eluent from step (a) over the second chromatography matrix;

(iii) passing equilibration buffer over the second chromatography matrix; and

(iv) eluting a protein eluate from the second chromatography matrix using a second elution buffer;

and

(c) a third chromatography step comprising:

(i) passing equilibration buffer over a third chromatography matrix;

(ii) passing the protein eluate from step (b) over the third chromatography matrix in the flow-through mode;

(iii) optionally passing wash buffer over the third chromatography matrix; and

(iv) recovering purified protein from the flow-through of the third chromatography matrix.

8. The method of any one of claims 1 to 7, wherein the first, second and third chromatography matrices are chromatography columns.

9. The method of any one of claims 1 to 7, wherein the first, second and third chromatography matrices are chromatography membrane adsorbers.

10. The method of any one of claims 1 to 9, wherein the first chromatography matrix is an affinity chromatography matrix.

11. The method of claim 10, wherein said affinity chromatography matrix is a Protein A matrix.

12. The method of any one of claims 1 to 11, wherein the second chromatography matrix is a multi-modal resin chromatography matrix or a cation-exchange chromatography matrix.

13. The method of any one of claims 1 to 12, wherein the third chromatography matrix is an anion-exchange chromatography matrix.

14. The method of any one of claims 1 to 13, wherein the protein is a monoclonal antibody.

15. The method of claim 14, wherein said monoclonal antibody is selected from the group consisting of an antibody that specifically binds to the protofibrillar form of the human β-amyloid protein, an antibody that specifically binds to the bacterial surface polysaccharide poly-N-acetyl glucosamine (PNAG), an antibody that specifically binds to Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) and an antibody that specifically binds to the CD38 transmembrane glycoprotein.

16. The method of any one of claims 1 to 15, further comprising a nanofiltration step after step (c).

17. The method of claim 16, further comprising an ultrafiltration and diafiltration step after the nanofiltration step.

18. The method of any one of claims 1 to 17, wherein the first elution buffer comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 3 and 4 with acetic acid.

19. The method of any one of claims 1 to 8 or 10 to 18, wherein the second elution buffer comprises 15 to 25 mM Bis Tris, 40 to 50 mM NaCl, and 20 to 30 mM NH_4Cl, adjusted to a pH comprised between 7 and 8 with acetic acid.

20. The method of any one of claims 1 to 7 or 9 to 18, wherein the second elution buffer comprises 15 to 25 mM Bis Tris, 50 to 150 mM NaCl, and 20 to 30 mM NH_4Cl, adjusted to a pH comprised between 6 and 7 with acetic acid.

21. The method of any one of claims 5 to 20, wherein the equilibration buffer comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 7 and 8 with acetic acid.

22. The method of any one of claims 5 to 21, wherein the wash buffer comprises of 15 to 25 mM Bis Tris, and 0.9 to 1.1 M NaCl adjusted to a pH comprised between 7 and 8 with acetic acid.

23. The method of any one of claims 1 to 22, wherein the purified protein is recovered with a yield of at least 95%.

24. The method of any one of claims 1 to 23, wherein the recovered purified protein exhibits a purity of at least 99%.

25. The method of any one of claims 1 to 24, further comprising the step of formulating the recovered purified protein into a pharmaceutical composition.

26. A method for purifying a protein from solution in continuous mode comprising:

(a) a first chromatography step comprising:

(i) passing equilibration buffer over a Protein A column;

(ii) passing the solution over the Protein A column;

(iii) passing equilibration buffer over the Protein A column;

(iv) passing wash buffer over the Protein A column;

(v) passing equilibration buffer over the Protein A column; and

(vi) eluting a crude protein eluent from the Protein A column using a first elution buffer;

(b) a second chromatography step comprising:

(i) passing equilibration buffer over a multi-modal resin chromatography column;

(ii) passing the crude protein eluent from step (a) over the multi-modal resin chromatography column;

(iii) passing equilibration buffer over the multi-modal resin chromatography column; and

(iv) eluting a protein eluate from the multi-modal resin chromatography column using a second elution buffer;

(c) a third chromatography step comprising:

(i) passing equilibration buffer over an anion-exchange chromatography column;

(ii) passing the protein eluate from step (b) over the anion-exchange chromatography column in the flow-through mode; and

(iii) recovering purified protein from the flow-through of the anion-exchange chromatography column,

wherein each of the buffers consists of Bis Tris, acetic acid, NaCl, water, and optionally NH_4Cl.

27. A method for purifying a protein from solution in continuous mode comprising:

(a) a first chromatography step comprising:

(i) passing equilibration buffer over a Protein A column;

(ii) passing a part of the solution over the Protein A column;

(iii) passing equilibration buffer over the Protein A column;

(iv) passing wash buffer over the Protein A column;

(v) passing equilibration buffer over the Protein A column; and

(vi) eluting a crude protein eluent from the Protein A column using a first elution buffer;

(b) a second chromatography step comprising:

(i) passing equilibration buffer over a multi-modal resin chromatography column;

(ii) passing the crude protein eluent from step (a) over the multi-modal resin chromatography column;

(iii) passing equilibration buffer over the multi-modal resin chromatography column; and

(iv) eluting a protein eluate from the multi-modal resin chromatography column using a second elution buffer;

(c) a third chromatography step comprising:

(i) passing equilibration buffer over an anion-exchange chromatography column;

(ii) passing the protein eluate from step (b) over the anion-exchange chromatography column in the flow-through mode; and

(iii) recovering purified protein from the flow-through of the anion-exchange chromatography column,

(d) renewing successively steps a), b) and c) with another part of the solution until all the solution is used, and

(e) collecting the purified proteins recovered at the end of each third chromatography step;

wherein each of the buffers consists of Bis Tris, acetic acid, NaCl, water, and optionally NH_4Cl.

28. A method for purifying a protein from solution in a continuous mode comprising:

(a) a first chromatography step comprising:

(i) passing equilibration buffer over a Protein A membrane adsorber;

(ii) passing the solution over the Protein A membrane adsorber;

(iii) passing equilibration buffer over the Protein A membrane adsorber;

(iv) passing wash buffer over the Protein A membrane adsorber;

(v) passing equilibration buffer over the Protein A membrane adsorber; and

(vi) eluting a crude protein eluent from the Protein A membrane adsorber using a first elution buffer;

(b) a second chromatography step comprising:

(i) passing equilibration buffer over a cation-exchange membrane adsorber;

(ii) passing the crude protein eluent from step (a) over the cation-exchange membrane adsorber;

(iii) passing equilibration buffer over the cation-exchange membrane adsorber; and

(iv) eluting a protein eluate from the cation-exchange membrane adsorber using a second elution buffer;

(c) a third chromatography step comprising:

(i) passing equilibration buffer over an anion-exchange membrane adsorber;

(ii) passing the protein eluate from step (b) over the anion-exchange membrane adsorber in the flow-through mode; and

(iii) recovering purified protein from the flow-through of the anion-exchange membrane adsorber,

wherein each of the buffers consists of Bis Tris, acetic acid, NaCl, water, and optionally NH_4Cl.

29. A method for purifying a protein from solution in a continuous mode comprising:

(a) a first chromatography step comprising:

(i) passing equilibration buffer over a Protein A membrane adsorber;

(ii) passing a part of the solution over the Protein A membrane adsorber;

(iii) passing equilibration buffer over the Protein A membrane adsorber;

(iv) passing wash buffer over the Protein A membrane adsorber;

(v) passing equilibration buffer over the Protein A membrane adsorber; and

(vi) eluting a crude protein eluent from the Protein A membrane adsorber using a first elution buffer;

b) a second chromatography step comprising:

(i) passing equilibration buffer over a cation-exchange membrane adsorber;

(ii) passing the crude protein eluent from step (a) over the cation-exchange membrane adsorber;

(iii) passing equilibration buffer over the cation-exchange membrane adsorber; and

(iv) eluting a protein eluate from the cation-exchange membrane adsorber using a second elution buffer;

(c) a third chromatography step comprising:

(i) passing equilibration buffer over an anion-exchange membrane adsorber;

(ii) passing the protein eluate from step (b) over the anion-exchange membrane adsorber in the flow-through mode; and

(iii) recovering purified protein from the flow-through of the anion-exchange membrane adsorber,

(d) renewing successively steps a), b) and c) with another part of the solution until all the solution is used, and

(e) collecting the purified proteins recovered at the end of each third chromatography step;

wherein each of the buffers consists of Bis Tris, acetic acid, NaCl, water, and optionally NH_4Cl.

30. The method according to claim 26 or 27, wherein:

- said equilibration buffer comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 7 and 8 with acetic acid,
- said wash buffer comprises of 15 to 25 mM Bis Tris, and 0.9 to 1.1 M NaCl adjusted to a pH comprised between 7 and 8 with acetic acid,
- said first elution buffer comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 3 and 4 with acetic acid, and
- said second elution buffer comprises 15 to 25 mM Bis Tris, 40 to 50 mM NaCl, and 20 to 30 mM NH_4Cl, adjusted to a pH comprised between 7 and 8 with acetic acid.

31. The method according to claim 28 or 29, wherein:

- said equilibration buffer comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 7 and 8 with acetic acid,
- said wash buffer comprises of 15 to 25 mM Bis Tris, and 0.9 to 1.1 M NaCl adjusted to a pH comprised between 7 and 8 with acetic acid,
- said first elution buffer comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 3 and 4 with acetic acid, and
- said second elution buffer comprises 15 to 25 mM Bis Tris, 50 to 150 mM NaCl, and 20 to 30 mM NH_4Cl, adjusted to a pH comprised between 6 and 7 with acetic acid.

32. The method according to any one of claims 26 to 31, wherein the method is run in a closed system from the first step to the last one.

CLAIMS

1. A method for purifying a protein from solution comprising:

(a) a first chromatography step comprising:

- passing said solution over a first chromatography matrix;
- eluting a crude protein eluent from the first chromatography matrix using a first elution buffer;

(b) a second chromatography step comprising:

- passing the crude protein eluent obtained at the end of step (a) over a second chromatography matrix;
- eluting a protein eluate from the second chromatography matrix using a second elution buffer; and

(c) a third chromatography step comprising:

- passing the protein eluate obtained at the end of step (b) through a third chromatography matrix in the flow-through mode;
- recovering purified protein from the flow-through of the third chromatography matrix;

wherein each of the buffers comprises Bis Tris.

2. The method of claim 1, wherein the crude protein eluent obtained at the end of step (a) is directly passed over a second chromatography matrix, without undergoing any treatment such as pH adjustment, buffer exchange or dilution.

3. The method of claim 1 or 2, wherein the protein eluate obtained at the end of step (b) is directly passed over a third chromatography matrix, without undergoing any treatment such as pH adjustment, buffer exchange or dilution.

4. The method of any one of claims 1 to 3, wherein the method is run in a closed system from the first step to the last one.

5. The method of any one of claims 1 to 4, wherein each one of the two first chromatography steps comprises:

- passing equilibration buffer over the chromatography matrix;
- passing the solution or the crude protein eluent over the chromatography matrix;
- passing equilibration buffer over the chromatography matrix;
- optionally passing wash buffer over the chromatography matrix;
- optionally passing equilibration buffer over the chromatography matrix;
- eluting the crude protein eluent or the protein eluate from the chromatography matrix using an elution buffer,

wherein each of the buffers comprises Bis Tris.

6. The method of any one of claims 1 to 5, wherein each of the buffers consists of Bis Tris, acetic acid, NaCl, water, and optionally NH_4Cl.

7. The method of any one of claims 1 to 6, wherein said method comprises the steps of:

(a) a first chromatography step comprising:

(i) passing equilibration buffer over a first chromatography matrix;

(ii) passing the solution over the first chromatography matrix;

(iii) passing equilibration buffer over the first chromatography matrix;

(iv) passing wash buffer over the first chromatography matrix;

(v) passing equilibration buffer over the first chromatography matrix; and

(vi) eluting a crude protein eluent from the first chromatography matrix using a first elution buffer;

(b) a second chromatography step comprising:

(i) passing equilibration buffer over a second chromatography matrix;

(ii) passing the crude protein eluent from step (a) over the second chromatography matrix;

(iii) passing equilibration buffer over the second chromatography matrix; and

(iv) eluting a protein eluate from the second chromatography matrix using a second elution buffer;

and

(c) a third chromatography step comprising:

(i) passing equilibration buffer over a third chromatography matrix;

(ii) passing the protein eluate from step (b) over the third chromatography matrix in the flow-through mode;

(iii) optionally passing wash buffer over the third chromatography matrix; and

(iv) recovering purified protein from the flow-through of the third chromatography matrix.

8. The method of any one of claims 1 to 7, wherein the first, second and third chromatography matrices are chromatography columns.

9. The method of any one of claims 1 to 7, wherein the first, second and third chromatography matrices are chromatography membrane adsorbers.

10. The method of any one of claims 1 to 9, wherein the first chromatography matrix is an affinity chromatography matrix.

11. The method of claim 10, wherein said affinity chromatography matrix is a Protein A matrix.

12. The method of any one of claims 1 to 11, wherein the second chromatography matrix is a multi-modal resin chromatography matrix or a cation-exchange chromatography matrix.

13. The method of any one of claims 1 to 12, wherein the third chromatography matrix is an anion-exchange chromatography matrix.

14. The method of any one of claims 1 to 13, wherein the protein is a monoclonal antibody.

15. The method of claim 14, wherein said monoclonal antibody is selected from the group consisting of an antibody that specifically binds to the protofibrillar form of the human β-amyloid protein, an antibody that specifically binds to the bacterial surface polysaccharide poly-N-acetyl glucosamine (PNAG), an antibody that specifically binds to Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) and an antibody that specifically binds to the CD38 transmembrane glycoprotein.

16. The method of any one of claims 1 to 15, further comprising a nanofiltration step after step (c).

17. The method of claim 16, further comprising an ultrafiltration and diafiltration step after the nanofiltration step.

18. The method of any one of claims 1 to 17, wherein the first elution buffer comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 3 and 4 with acetic acid.

19. The method of any one of claims 1 to 8 or 10 to 18, wherein the second elution buffer comprises 15 to 25 mM Bis Tris, 40 to 50 mM NaCl, and 20 to 30 mM NH_4Cl, adjusted to a pH comprised between 7 and 8 with acetic acid.

20. The method of any one of claims 1 to 7 or 9 to 18, wherein the second elution buffer comprises 15 to 25 mM Bis Tris, 50 to 150 mM NaCl, and 20 to 30 mM NH_4Cl, adjusted to a pH comprised between 6 and 7 with acetic acid.

21. The method of any one of claims 5 to 20, wherein the equilibration buffer comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 7 and 8 with acetic acid.

22. The method of any one of claims 5 to 21, wherein the wash buffer comprises of 15 to 25 mM Bis Tris, and 0.9 to 1.1 M NaCl adjusted to a pH comprised between 7 and 8 with acetic acid.

23. The method of any one of claims 1 to 22, wherein the purified protein is recovered with a yield of at least 95%.

24. The method of any one of claims 1 to 23, wherein the recovered purified protein exhibits a purity of at least 99%.

25. The method of any one of claims 1 to 24, further comprising the step of formulating the recovered purified protein into a pharmaceutical composition.