CLAIMS

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

(a) a first chromatography step comprising:

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

(b) a second chromatography step comprising:

- passing the crude protein eluent obtained at the end of step (a) over a second chromatography column;
- recovering purified protein from the second chromatography column using a second elution buffer

wherein each of the buffers comprises Bis Tris,
and wherein the first chromatography column is an affinity chromatography column.

2. The method of claim 1, wherein each one of the two chromatography steps comprises:

- passing equilibration buffer over the chromatography column;
- passing the solution or the crude protein eluent over the chromatography column;
- passing equilibration buffer over the chromatography column;
- optionally passing wash and sanitation buffer over the chromatography column;
- optionally passing equilibration buffer over the chromatography column;
- eluting the crude protein eluent or recovering purified protein from the chromatography column using an elution buffer,

wherein each of the buffers comprises Bis Tris.

3. The method of claim 1 or 2, wherein each of the buffers consists of varying concentrations of the same chemicals.

4. The method of any one of claims 1 to 3, wherein each of the buffers consists of Bis Tris, acetic acid, NaCl, and water.

5. The method of any one of claims 1 to 4, wherein the method for purifying a protein from solution only comprises two chromatographic steps.

6. The method of any one of claims 1 to 5, wherein ~~one of the chromatography columns is an~~the affinity chromatography column~~, more particularly wherein one of the chromatography columns~~ is a Protein A affinity chromatography column.

7. The method of any one of claims 1 to 6, wherein ~~one of~~ the second chromatography column~~s~~ is a multi-modal resin chromatography column.

8. The method of any one of claims 1 to 7, wherein step (a) further comprises adjusting the pH of the crude protein eluent using a Bis Tris solution, particularly wherein step (a) further comprises adjusting the pH of the crude protein eluent to a value comprised between 6 and 7 using a Bis Tris solution.

9. The method of any one of claims 1 to 7, wherein the crude protein eluent obtained at the end of step (a) is directly passed over a second chromatography column, without undergoing any treatment such as pH adjustment, buffer exchange or dilution.

10. The method of any one of claims 1 to 9, wherein said method comprises the steps of:

(a) a first chromatography step comprising:

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

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

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

(iv) passing wash and sanitation buffer over the first chromatography column;

(v) passing equilibration buffer over the first chromatography column;

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

(vii) optionally adjusting the pH of the crude protein eluent using a Bis Tris solution;

and

(b) a second chromatography step comprising:

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

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

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

(iv) recovering purified protein from the second chromatography column using a second elution buffer.

~~11. The method of any one of claims 1 to 10, wherein the first chromatography column is an affinity chromatography column, more particularly wherein the first chromatography column is a Protein A affinity chromatography column.~~

~~12. The method of any one of claims 1 to 11, wherein the second chromatography column is a multi-modal resin chromatography column.~~

~~13. The method of any one of claims 1 to 9, wherein said method comprises the steps of:~~

~~(a) a first chromatography step comprising:~~

~~(i) passing equilibration buffer over a first chromatography column;~~

~~(ii) passing the solution over the first chromatography column;~~

~~(iii) passing equilibration buffer over the first chromatography column;~~

~~(iv) eluting a crude protein eluent from the first chromatography column using a first elution buffer; and~~

~~(v) optionally adjusting the pH of the crude protein eluent using a Bis Tris solution;~~

~~and~~

~~(b) a second chromatography step comprising:~~

~~(i) passing equilibration buffer over a second chromatography column;~~

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

(iii) passing equilibration buffer over the second chromatography column;

(iv) passing wash and sanitation buffer over the second chromatography column;

(v) passing equilibration buffer over the second chromatography column; and

(vi) recovering purified protein from the second chromatography column using a second elution buffer.

14. The method of any one of claims 1 to 9 and 13, wherein the first chromatography column is a multi-modal resin chromatography column.

15. The method of any one of claims 1 to 9, 13 and 14, wherein the second chromatography column is an affinity chromatography column, more particularly wherein the second chromatography column is a Protein A affinity chromatography column.

~~16~~11. The method of any one of claims 1 to ~~15~~10, wherein the protein is an antibody, more particularly wherein the protein is a monoclonal antibody, even more particularly wherein the protein is a monoclonal antibody 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), and an antibody that specifically binds to the CD38 transmembrane glycoprotein.

~~17~~12. The method of any one of claims 1 to ~~16~~11, further comprising, after step (b), a step (c) of passing the crude protein eluent over a membrane adsorber.

1813. The method of claim 1712, wherein said membrane adsorber is a salt tolerant interaction chromatography membrane adsorber.

1914. The method of any one of claims 1 to 1813, further comprising a nanofiltration step after step (b) or (c).

2015. The method of claim 1914, further comprising an ultrafiltration and diafiltration step after the nanofiltration step.

2116. The method of any one of claims 1 to 12 and 16 to 2015, 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.

2217. The method of any one of claim 1 to 12 and 16 to 2116, wherein the second elution buffer :

- comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl, adjusted to a pH comprised between 4 and 5 with acetic acid, or
- comprises 15 to 25 mM Bis Tris, and 150 to 250 mM NaCl, adjusted to a pH comprised between 8 and 9 with acetic acid.

~~23. The method of any one of claims 1 to 9 and 13 to 20, wherein the first elution buffer :~~

- ~~comprises 15 to 25 mM Bis Tris, and 15 to 25 mM NaCl adjusted to a pH comprised between 4 and 5 with acetic acid, or~~
- ~~comprises 15 to 25 mM Bis Tris, and 150 to 250 mM NaCl adjusted to a pH comprised between 8 and 9 with acetic acid.~~

~~24. The method of any one of claims 1 to 9, 13 to 20, and 23, wherein the second 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.~~

2518. The method of any one of claims 2 to 2417, wherein 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.

2619. The method of any one of claims 2 to 2518, wherein the wash and sanitation buffer comprises of 15 to 25 mM Bis Tris, and 0.9 to 1.1 mM NaCl adjusted to a pH comprised between 7 and 8 with acetic acid.

2720. The method of any one of claims 1 to 2619, wherein the purified protein is recovered with a yield of at least 85%.

2821. The method of any one of claims 1 to 2720, wherein the recovered purified protein exhibits a purity of at least 95%.

2922. The method of any one of claims 1 to 2821, further comprising the step of formulating the recovered purified protein into a pharmaceutical composition.

3023. A method for preparing buffers suitable for use in the method of any one of claims 1 to 2922, comprising the steps of:

i) creating a solution with a final concentration of 15 to 25 mM Bis Tris and of 15 to 25 mM NaCl;

ii) adjusting the pH of the solution to a value comprised between 7 and 8 with acetic acid;

iii) collecting half of the solution, thereby obtaining an equilibration buffer;

iv) adjusting the pH of the remaining half of solution from step (iii) to a value comprised between 4 and 5 with acetic acid;

v) collecting half of the solution obtained at step (iv), thereby obtaining an elution buffer.

vi) adjusting the pH of the remaining half of solution from step (v) to a value comprised between 3 and 4 with acetic acid, thereby obtaining a further elution buffer.

vii) collecting half of the equilibration buffer obtained at step (iii) and adding NaCl to obtain a final NaCl concentration comprised between 0.9 to 1.1 mM, thereby obtaining a wash and sanitation buffer.

~~31~~24. A method for preparing buffers suitable for use in the method of any one of claims 1 to ~~29~~22, comprising the steps of:

i) creating a solution with a final concentration of 15 to 25 mM Bis Tris and of 15 to 25 mM NaCl;

ii) adjusting the pH of the solution to a value comprised between 8 and 9 with acetic acid;

iii) collecting one fourth of the solution, thereby obtaining an elution buffer;

iv) adjusting the pH of the remaining solution from step (iii) to a value comprised between 7 and 8 with acetic acid;

v) collecting two third of the solution obtained at step (iv), thereby obtaining an equilibration buffer.

vi) adjusting the pH of the remaining solution from step (v) to a value comprised between 3 and 4 with acetic acid, thereby obtaining a further elution buffer.

vii) collecting half of the equilibration buffer obtained at step (v) and adding NaCl to obtain a final NaCl concentration comprised between 0.9 to 1.1 mM (e.g. 1mM), thereby obtaining a wash and sanitation buffer.

~~32. A kit comprising:~~

~~(a) a multi-modal resin chromatography column and/or an affinity chromatography column such as a Protein A column;~~

~~and~~

~~(b) at least one buffer comprising or consisting of Bis Tris, acetic acid, NaCl, and water.~~

~~33. A kit comprising:~~

~~(a) a multi-modal resin chromatography column and/or an affinity chromatography column such as a Protein A column;~~

~~and~~

~~(b) instructions for preparing at least one buffer comprising or consisting of Bis Tris, acetic acid, NaCl, and water.~~

~~34. Use of a buffer comprising or consisting of Bis Tris, acetic acid, NaCl, and water for purifying a protein from solution by at least one chromatography step.~~

35. The use of claim 34, wherein the chromatography step is a multi-modal resin chromatography step and/or an affinity chromatography column such as a Protein A column.