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.

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 affinity chromatography column~~.~~, more particularly

~~**7. The method of claim 6,**~~ wherein one of ~~said affinity~~the chromatography columns is a Protein A affinity chromatography column.

~~8.~~7. The method of any one of claims 1 to ~~7~~6, wherein one of the chromatography columns is a multi-modal resin chromatography column.

~~9.~~8. The method of any one of claims 1 to ~~8~~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 ~~**10. The method of claim 9, wherein the pH is adjusted**~~ to a value comprised between 6 and 7 using a Bis Tris solution.

~~11.~~9. The method of any one of claims 1 to ~~8~~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.

~~12.~~10. The method of claim any one of claims 1 to ~~11~~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.

~~13.~~11. The method of any one of claims 1 to ~~12~~10, wherein the first chromatography column is an affinity chromatography column~~.~~, more particularly wherein the first

~~14. The method of claim 13, wherein said affinity~~ chromatography column is a Protein A affinity chromatography column.

~~15.~~12. The method of any one of claims 1 to ~~14~~11, wherein the second chromatography column is a multi-modal resin chromatography column.

~~16.~~13. The method of any one of claims 1 to ~~11~~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.

~~17.~~**14.** The method of any one of claims 1 to ~~11~~9 and ~~16~~13, wherein the first chromatography column is a multi-modal resin chromatography column.

~~18.~~**15.** The method of any one of claims 1 to ~~11~~9, ~~16~~13 and ~~17~~14, wherein the second chromatography column is an affinity chromatography column~~.~~, more particularly wherein the second

~~19. The method of claim 18, wherein said affinity~~ chromatography column is a Protein A affinity chromatography column.

~~20.~~**16.** The method of any one of claims 1 to ~~19~~15, wherein the protein is an antibody~~.~~, more particularly

~~21. The method of claim 20,~~ wherein the ~~antibody~~ protein is a monoclonal antibody~~.~~ even more particularly

~~22. The method of claim 20 or 21,~~ wherein the protein is a ~~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), and an antibody that specifically binds to the CD38 transmembrane glycoprotein.

~~23.~~**17.** The method of any one of claims 1 to ~~22~~16, further comprising, after step (b), a step (c) of passing the crude protein eluent over a membrane adsorber.

~~24.~~18. The method of claim ~~23~~17, wherein said membrane adsorber is a salt tolerant interaction chromatography membrane adsorber.

~~25.~~19. The method of any one of claims 1 to ~~24~~18, further comprising a nanofiltration step after step (b) or (c).

~~26.~~20. The method of claim ~~25~~19, further comprising an ultrafiltration and diafiltration step after the nanofiltration step.

~~27.~~21. The method of any one of claims 1 to ~~15~~12 and ~~20~~16 to ~~26~~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 3 and 4 with acetic acid.

~~28.~~22. The method of any one of claim 1 to ~~15~~12 and ~~20~~16 to ~~27~~21, 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
- ~~29. The method of any one of claim 1 to 15 and 20 to 27, wherein the second elution buffer~~ 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.

~~30.~~23. The method of any one of claims 1 to ~~11~~9 and ~~16~~13 to ~~26~~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
- ~~31. The method of any one of claims 1 to 11 and 16 to 26, wherein the first elution buffer~~ 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.

~~32.~~24. The method of any one of claims 1 to ~~11~~9, ~~16~~13 to ~~26~~20, ~~30~~ and ~~31~~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.

~~33.~~25. The method of any one of claims 2 to ~~32~~24, 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.

~~34.~~26. The method of any one of claims 2 to ~~33~~25, 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.

~~35.~~27. The method of any one of claims 1 to ~~34~~26, wherein the purified protein is recovered with a yield of at least 85%.

~~36.~~28. The method of any one of claims 1 to ~~35~~27, wherein the recovered purified protein exhibits a purity of at least 95%.

~~37.~~29. The method of any one of claims 1 to ~~36~~28, further comprising the step of formulating the recovered purified protein into a pharmaceutical composition.

~~38.~~30. A method for preparing buffers suitable for use in the method of any one of claims 1 to ~~37~~29, 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.

~~39.~~31. A method for preparing buffers suitable for use in the method of any one of claims 1 to ~~37~~29, 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. 1M), thereby obtaining a wash and sanitation buffer.

~~40.~~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.

~~41.~~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.

~~42.~~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.

~~43.~~**35.** The use of claim ~~42~~34, wherein the chromatography step is a multi-modal resin chromatography step and/or an affinity chromatography column such as a Protein A column.