CLAIMS

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

(a) a filtration step comprising:
- passing said solution over at least one chelating agent matrix in the flow-through mode,
- recovering a filtered protein solution from the flow-through of said at least one chelating agent matrix;

(b) an exchanging step comprising:
- passing the filtered protein solution obtained at the end of step (a) over at least one diafiltration membrane using only one buffer for the exchange,
- recovering the partly purified protein-containing retentate of said at least one diafiltration membrane;

(c) a polishing step comprising:
- passing the retentate obtained at the end of step (b) over a combination of membrane adsorbers in the flow-through mode, wherein two membrane adsorbers of said combination of membrane adsorbers are orthogonal in terms of mechanisms of action, and said combination of membrane adsorbers has been equilibrated beforehand with an equilibration buffer which is identical to the one buffer used for exchange at step (b),
- recovering purified protein from the flow-through of said combination of membrane adsorbers;

wherein said purifying method does not include a Protein A chromatography step.

2. The method of claim 1, wherein the filtered protein solution obtained at the end of step (a) is directly passed over the at least one diafiltration membrane, without undergoing any treatment such as pH adjustment, buffer exchange or dilution.

3. The method of claim 1 or 2, wherein the retentate obtained at the end of step (b) is directly passed over said combination of membrane adsorbers, 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 does not comprise any intermediate storage between the three steps (a), (b) and (c).

5. The method of any one of claims 1 to 4, wherein the method has a flowpath functionally closed.

6. The method of any one of claims 1 to 5, wherein only one buffer is used over the whole purification method.

7. The method of any one of claims 1 to 6, wherein the one buffer comprises Tris, Tris-HCl, Bis Tris, phosphate and/or citric acid, in particular comprises or consists of (i) Bis Tris, Tris or Tris-HCl, (ii) acetic acid, (iii) water and (iv) optionally salt.

8. The method of any one of claims 1 to 7, wherein the at least one chelating agent matrix of the filtration step is selected from the group consisting of activated carbons, diatomite earth, free cationic exchange resin, free anionic exchange resin, and free mixed mode resin.

9. The method of claim 8, wherein the at least one chelating agent matrix is a combination of two different chelating agent matrices.

10. The method of claim 9, wherein the combination of two different chelating agent matrices is a combination of activated carbons and free anionic exchange resin.

11. The method of any one of claims 1 to 10, wherein the at least one diafiltration membrane of the exchanging step is a single-path tangential flow filtration (SPTFF) module or a tangential flow filtration (TFF) module.

12. The method of any one of claims 1 to 11, wherein the at least one diafiltration membrane of the exchanging step is in the form of a cassette, a hollow fiber or a spiral wound.

13. The method of any one of claims 1 to 12, wherein the filtered protein solution is concentrated during the exchanging step.

14. The method of any one of claims 1 to 13, wherein the combination of membrane adsorbers of the polishing step is a combination of at least two membrane adsorbers selected from the group consisting of cationic-exchange membrane adsorbers, anionic-

exchange membrane adsorbers, multi-modal membrane adsorbers and hydrophobic interaction membrane adsorbers.

15. The method of any one of claims 1 to 14, wherein the combination of membrane adsorbers of the polishing step is a combination of a cationic-exchange membrane adsorber and an anionic-exchange membrane adsorber.

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 a final ultrafiltration and/or diafiltration step after the nanofiltration step.

18. The method of any one of claims 1 to 17, wherein the protein is a monoclonal antibody.

19. The method of any one of claims 1 to 18, wherein the one buffer comprises:

- 5 to 40 mM Bis Tris, 15 to 150 mM NaCl, adjusted to a pH comprised between 6 and 9 with acetic acid,
- 5 to 40 mM Tris or Tris-HCl, 15 to 150 mM NaCl, adjusted to a pH comprised between 6 and 9 with acetic acid,
- 5 to 40 mM Tris or Tris-HCl, 15 to 150 mM NaCl, adjusted to a pH comprised between 6 and 9 with citric acid, or
- 15 to 150 mM NaCl, adjusted to a pH comprised between 6 and 9 with Na_2HPO_4/NaH_2PO_4.

20. The method of any one of claims 1 to 19, further comprising the step of formulating the recovered purified protein into a pharmaceutical composition.

21. Use of a kit in purifying a protein from solution according to the method of any one of claims 1 to 20, wherein said kit comprises or consists of:

(a) at least one chelating agent matrix, at least one diafiltration membrane and a combination of membrane adsorbers, wherein two membrane adsorbers of said combination of membrane adsorbers are orthogonal in terms of mechanisms of action; and

(b) one buffer comprising Tris, Tris-HCl, Bis Tris, phosphate and/or citric acid,

wherein the kit does not include Protein A matrix.