# CLAIMS

1. A method for purifying a multispecific antibody comprising a first heavy chain and a second heavy chain whose isoelectric points are different, wherein the method comprises the steps of:

providing a nucleic acid encoding at least a CH1, CH2, CH3 region of the first heavy chain and a nucleic acid encoding at least a CH1, CH2, CH3 region of the second heavy chain, such that the first encoded heavy chain and the second encoded heavy chain differ in isoelectric point, wherein the first or second heavy chain comprises a variation of an amino acid that is less than 50% surface exposed as determined using the program GETAREA 1.0 beta selected from an CH1 region amino acid variation at a position selected from N201, K147, D148, Y149, V154, A172, and K213 (EU-numbering), a CH2 region amino acid variation at position V303 (EU-numbering),a CH3 region amino acid variation at position E388 (EU-numbering) or a combination of said CH region amino acid variations and

culturing host cells to express the nucleic acid; and

purifying the multispecific antibody from the host cell culture by isoelectric focusing and separating the multispecific antibody from another antibody or an antibody fragment.

2. A method for producing a multispecific antibody comprising a first heavy chain and a second heavy chain whose isoelectric points are different, wherein the method comprises the steps of:

providing a nucleic acid encoding at least a CH1, CH2, CH3 region of the first heavy chain and a nucleic acid encoding at least a CH1, CH2, CH3 region of the second heavy chain, such that the isoelectric point of the first encoded heavy chain and that of the second encoded heavy chain differ, wherein the first or second heavy chain comprises a variation of an amino acid that is less than 50% surface exposed as determined using the program GETAREA 1.0 beta, selected from N201, K147, D148, Y149, V154, A172, and K213 (EU-numbering) a CH2 region amino acid variation at position V303 (EU-numbering), a CH3 region amino acid variation at position E388 (EU-numbering) or a combination of said CH region amino acid variations, and

culturing host cells to express the nucleic acid; and

collecting the multispecific antibody from the host cell culture, using the difference in isoelectric point further comprising the steps of

collecting the antibody from the host cell culture,

performing harvest clarification,

performing protein capture,

performing anion exchange chromatography, and

performing cation exchange chromatography to separate the antibody from another antibody or an antibody fragment.

3. The method of claim 1 or claim 2, wherein the nucleic acid encoding a homomultimer of the first heavy chain, a homomultimer of the second heavy chain, and a heteromultimer of the first and second heavy chain are expressed as proteins having different isoelectric points and produce different retention times in ion exchange chromatography.

4. The method of any one of claims 1-3, wherein the one or more amino acid variations are selected from
- a neutral amino acid to a negatively charged amino acid;
- a positively charged amino acid to a neutral amino acid;
- a positively charged amino acid to a negatively charged amino acid;
- a neutral amino acid to a positively charged amino acid;
- a negatively charged amino acid to a neutral amino acid; and
- a negatively charged amino acid to a positively charged amino acid.

5. The method of any one of claims 1-4, wherein said first heavy chain comprises one or more variations selected from
- a neutral amino acid to a negatively charged amino acid;
- a positively charged amino acid to a neutral amino acid;
- a positively charged amino acid to a negatively charged amino acid; and

said second heavy chain different from said first heavy chain that comprises one or more variations selected from
- a neutral amino acid to a positively charged amino acid;
- a negatively charged amino acid to a neutral amino acid; and
- a negatively charged amino acid to a positively charged amino acid

6. The method of any one of claims 1-5, wherein the first heavy chain and the second heavy chain comprise compatible CH3 heterodimerization regions.

7. The method of claim 6, wherein one of said compatible CH3 heterodimerization regions comprises an L351D and L368E variation and the other comprises a T366K and L351K variation.

8. The method of any one of claims 1-7, wherein the amino acid variations are selected from N201D, K147Q, K147T, K147S, K147D, D148R, D148E, Y149A, V154I, A172P, K213Q, K213T, K213S, K213D and K213E (EU-numbering).

9. A multispecific immunoglobulin protein comprising a first CH1 region containing immunoglobulin polypeptide and a second CH1 region containing immunoglobulin polypeptide, wherein the first or second CH1 region containing immunoglobulin polypeptides comprise one or more variations of one or more amino acids selected from selected from N201, K147, D148, Y149, V154, A172, and K213 (EU-numbering), that

are non-surface exposed, such that the isoelectric point of the immunoglobulin protein comprising the first CH1 region containing immunoglobulin polypeptide and the second CH1 region containing immunoglobulin polypeptide is different from the isoelectric points of immunoglobulin proteins containing only the first CH1 region immunoglobulin polypeptide and different from immunoglobulin proteins containing only the second CH1 region immunoglobulin polypeptide.

10. The multispecific immunoglobulin protein in accordance with claim 9, comprising a human CH1 region.

11. The multispecific immunoglobulin protein in accordance with claims 9 or 10, comprising a first CH1, CH2, and CH3 region containing immunoglobulin polypeptide and a second CH1, CH2, and CH3 region containing immunoglobulin polypeptide, wherein the first and/or second CH1, CH2, and CH3 region containing immunoglobulin polypeptides comprise one or more variations of one or more amino acids that are less than 50 % surface exposed, selected from T120, K147, D148, Y149, V154, N159, A172, Q175, S190, N201, K213, V303, K370, E382 and E388 (EU-numbering), such that the iso-electric point of the immunoglobulin protein comprising the first CH1, CH2, and CH3 region containing immunoglobulin polypeptide and the second CH1, CH2, and CH3 region containing immunoglobulin polypeptide is different from the iso-electric points of immunoglobulin proteins containing only the first CH1, CH2, and CH3 region containing immunoglobulin polypeptide and/or different from immunoglobulin proteins containing only the second CH1, CH2, and CH3 region containing immunoglobulin polypeptide.

12. The multispecific immunoglobulin protein in accordance with any one of claims 9-11, which is a multispecific antibody, preferably an IgG.

13. The antibody of claim 12, which is a bispecific antibody.

14. An immunoglobulin CH1, CH2, CH3 region comprising a variation of an amino acid that is non-surface exposed in an immunoglobulin or a combination of said regions, wherein the variation in CH1 is selected from N201D, K147Q, K147T, K147S, K147D, D148R, D148E, Y149A, V154I, A172P, K213Q, K213T, K213S, K213D and K213E (EU-numbering), the variation in CH2 comprises V303, preferably V303K, V303R, V303D, or V303E, and the variation in CH3 comprises E388, preferably E388Q, E388N, E388L, E388S, E388T, or E388M.

15. The immunoglobulin CH1 region in accordance with claim 14, wherein the amino acid variation comprises N201D, K147Q, K147T, K147S, K147D, D148R, D148E, Y149A, V154I, A172P, K213Q, K213T, K213S, K213D or K213E (EU-numbering).

16. The immunoglobulin CH1, CH2, CH3 region of claim 14 or claim 15, which is an IgG region.

17. The immunoglobulin CH1, CH2, CH3 region of any one of claims 14 to 16, comprising two or more variations of said amino acids.

18. The immunoglobulin CH1, CH2, CH3 region of claim 17, comprising variations of amino acids selected from the group A172/S190/N201, T197/K213, D148/Q175, N159/Q213, K147/Q175, Y149/V154/A172/S190, N201/K213, T120/N201, N201/N159, T120/N159, T120/N201/N159 and N201/K213/N159.

19. An immunoglobulin CH1/CL domain, comprising an immunoglobulin CH1 region of any one of claims 14-18.

20. A protein, antibody or multispecific antibody comprising an immunoglobulin CH1/CL domain of claim 19.

21. The multispecific antibody of claim 20, which comprises different heavy chains.

22. The multispecific antibody of claim 20 or 21, wherein the heavy chains comprise compatible heterodimerization regions.

23. The multispecific antibody of claim 22 comprising compatible CH3 heterodimerization regions.

24. The multispecific antibody of any one of claims 21-23, wherein one of the heavy chains comprises the CH3 variations L351D and L368E, and another of said heavy chains comprises the CH3 variations T366K and L351K.

25. The immunoglobulin region or domain of any one of claims 14-19, the protein of any one of claims 9-12, or the protein, antibody or multispecific antibody of any one of claims 20-24, comprising a CH1, a CH2 and/or a CH3 region sequence of any one of SEQ ID NO: 61-64, 67, 68, 71-77, 79-85, 90-92, and 95-97.

26. A composition comprising the immunoglobulin region, domain, protein or antibody of any one of claims 9-25.

27 A pharmaceutical composition comprising the immunoglobulin region, domain, protein or antibody of any one of claims 9-25, and preferably a pharmaceutically acceptable excipient.

28. Nucleic acid that encodes the immunoglobulin region, domain, protein or antibody of

any one of claims 9-25.

29. A recombinant host cell comprising the nucleic acid of claim 28.

30. A method of producing an antibody of any one of claims 20-25, wherein the method comprises the steps of

providing nucleic acid encoding a first heavy chain with a CH1, CH2, CH3 region of any one of claims 14-18, or a domain of claim 19;

providing a nucleic acid encoding a second heavy chain, wherein said first and second heavy chains are different;

providing a nucleic acid encoding a light chain;

introducing said nucleic acid into host cells and culturing said host cells to express the nucleic acid(s); and producing the antibody by performing at least one of the following steps:

collecting the antibody from the host cell culture,

performing harvest clarification,

performing protein capture,

performing anion exchange chromatography, and

performing cation exchange chromatography to separate the antibody from another antibody or an antibody fragment.

31. A method of producing an antibody of any one of claims 20-25, wherein the method comprises the steps of

providing a nucleic acid encoding a first heavy chain with a CH1, CH2, CH3 region of any one of claims 14-18, or a domain of claim 19;

providing a nucleic acid encoding a second heavy chain, wherein said first and second heavy chains are different;

providing a nucleic acid encoding a light chain;

introducing said nucleic acid into host cells and culturing said host cells to express the nucleic acid(s); and

collecting the antibody from the host cell culture, and

separating the antibody from other antibodies or antibody fragments in a separation step by isoelectric focusing on a gel.

32. A composition comprising the immunoglobulin region, immunoglobulin domain or antibody of any one of claims 9-25, further comprising a variation at an amino acid selected from T197 and at a hinge position E216.