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 both or either one of a nucleic acid encoding a CH1, CH2, CH3 region or a combination thereof of the first heavy chain and a nucleic acid encoding a CH1, CH2, CH3 region or a combination thereof region of the second heavy chain, such that the first encoded heavy chain and the second encoded heavy chain differ in isoelectric point, wherein at least one of said regions comprises an CH1 region amino acid variation at a position selected from N159, N201, K147, D148, Y149, V154, A172, Q175 and K213 (EU-numbering), or a CH2 region amino acid variation at position V303 (EU-numbering), or a CH3 region amino acid variation at a position selected from K370, E382 and 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 antibodies 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 a CH1, CH2, CH3 region or combination thereof of the first heavy chain and a nucleic acid encoding a CH1, CH2, CH3 region or combination thereof 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 at least one of said CH1 regions comprises an amino acid variation at a position selected from N159, N201, K147, D148, Y149, V154, A172, Q175 and K213 (EU-numbering) or a CH2 region amino acid variation at position V303 (EU-numbering), or a CH3 region amino acid variation at a position selected from K370, E382 and 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 position(s) of said 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 the first heavy chain and the second heavy chain comprise compatible CH3 heterodimerization regions.

6. The method of any one of claims 1-5, 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

7. The method of claim 5 or 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 An immunoglobulin protein comprising a first CH1 region containing immunoglobulin polypeptide and a second CH1 region containing immunoglobulin polypeptide, wherein one CH1 region comprises one or more variations of an amino acid that are non-surface exposed selected from N159, N201, K147, D148, Y149, V154, A172, Q175, and K213 (EU-numbering),.

9. An immunoglobulin protein comprising a first CH1 region containing immunoglobulin polypeptide and a second CH1 region containing immunoglobulin polypeptide,

wherein one CH1 region comprises one or more variations of an amino acid that are non-surface exposed selected from N159, N201, K147, D148, Y149, V154, A172, Q175, and K213 (EU-numbering),
and the other CH1 region comprises one or more variations of an amino acid that are non-surface exposed 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.

10. An immunoglobulin protein comprising a first CH1 region containing immunoglobulin polypeptide and a second CH1 region containing immunoglobulin polypeptide, wherein the first and/or second CH1 region containing immunoglobulin polypeptides comprise one or more variations of one or more amino acids selected from selected from N159, N201, K147, D148, Y149, V154, A172, Q175, and K213 (EU-numbering), that are non-surface exposed, such that the iso-electric 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 iso-electric 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.

11. The immunoglobulin protein in accordance with any one of claims 8-10, comprising a human CH1 region.

12. The immunoglobulin protein in accordance with any one of claims 8-11, which is an antibody, preferably an IgG.

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

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

15. 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 N159R, N159E, N201D, K147Q, K147T, K147S, K147D, D148R, D148E, Y149A, V154I, A172P, Q175K, Q175R, Q175E, , 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 K370, preferably K370Q, a K370N, a K370H, a K370S, a K370T, or K370Y; E382, preferably E382Q, E382N, E382H, E382S, E382T, or E382Y; and E388, preferably E388Q, E388N, E388L, E388S, E388T, or E388M.

16. The immunoglobulin region of claim 15, which is an IgG region.

17. The immunoglobulin CH1 region of claim 15 or 16, comprising two or more variations of said amino acids.

18. The immunoglobulin CH1 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, a CH2 domain or CH3 domain comprising an immunoglobulin region of claims 15-18.

20. The immunoglobulin CH2 domain of claim 19 which further comprises an Fc-silent mutation.

21. The immunoglobulin CH3 domain of claim 19 which further comprises a CH3 heterodimerization domain.

22. A protein, antibody or multispecific antibody comprising an immunoglobulin domain of claims 19-21.

23. The multispecific antibody of claim 22, which comprises different heavy chains.

24. The multispecific antibody of claims 22-23, wherein the heavy chains comprise compatible heterodimerization regions.

25. The multispecific antibody of claim 24 comprising compatible CH3 heterodimerization regions.

26. The multispecific antibody of claims 23-25, 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.

27. The immunoglobulin region or domain of any one of claims 15-22, the protein of any one of claims 8-12 or 22, or the protein, antibody or multispecific antibody of any one of claims 22-26, comprising a CH1, a CH2 and/or a CH3 region sequence of Table 14 part B.

28. A composition comprising the immunoglobulin region, domain, protein or antibody of any one of claims 8-27.

29. A pharmaceutical composition comprising the immunoglobulin region, domain,

protein or antibody of any one of claims 8-27, and preferably a pharmaceutically acceptable excipient.

30. Nucleic acid that encodes the immunoglobulin region, domain, protein or antibody of any one of claims 8-29.

31. A recombinant host cell comprising the nucleic acid of claim 30.

32. A method of producing an antibody of any one of claims 22-27, wherein the method comprises the steps of

providing nucleic acid encoding a first heavy chain with a CH1, CH2, CH3 region or combination thereof of any one of claims 15-18 or a domain of any one of claims 19-21;

providing a nucleic acid encoding a second heavy chain, wherein said first and second heavy chain may be the same or 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.

33. A method of producing an antibody of any one of claims 22-27, wherein the method comprises the steps of

providing a nucleic acid encoding a first heavy chain with a CH1, CH2, CH3 region or combination thereof of any one of claims 15-18 or a domain of any one of claims 19-21;

providing a nucleic acid encoding a second heavy chain, wherein said first and second heavy chain may be the same or 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.

34. A CH1-containing immunoglobulin polypeptide comprising a first charged amino acid residue at position 159, position 201, position 120, position 147, position 148, position

149, position 154, position 172, position 175, position 190, or position 213, comprising in addition to the charged residue, a second charged amino acid residue at a different position selected from position 159, position 201, position 120, position 147, position 148, position 149, position 154, position 172, position 175, position 190, or position 213.

35. An immunoglobulin protein comprising a first CH1-containing immunoglobulin polypeptide and a second CH1-containing immunoglobulin polypeptide, wherein the first and/or second CH1-containing immunoglobulin polypeptides comprise one or more variations of one or more amino acids selected from N159, N201, K147, D148, Y149, V154, A172, Q175, and K213 (EU-numbering) that are non-surface exposed, such that the isoelectric point of the immunoglobulin protein comprising the first CH1-containing immunoglobulin polypeptide and the second CH1-containing immunoglobulin polypeptide is different from the isoelectric points of immunoglobulin proteins containing only the first CH1-immunoglobulin polypeptide or proteins containing only the second CH1-immunoglobulin polypeptide.

36. The immunoglobulin CH1 region in accordance with claim 15, wherein the amino acid variation comprises N159R, N159E, N201D, K147Q, K147T, K147S, K147D, D148R, D148E, Y149A, V154I, A172P, Q175K, Q175R, Q175E, K213Q, K213T, K213S, K213D and K213E (EU-numbering).

37. A composition comprising the immunoglobulin region, immunoglobulin domain or antibody of any one of claims 8-27-, further comprising a variation at an amino acid selected from T197 and at a hinge position E216.

38. An immunoglobulin protein comprising a first CH1 region containing immunoglobulin polypeptide and a second CH1 region containing immunoglobulin polypeptide, wherein the first and/or second CH1 region containing immunoglobulin polypeptides comprise one or more variations of amino acids T120 and/or S190 (EU-numbering) within the CH1 region, such that the iso-electric 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 iso-electric 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.