

multispecific antibody is constructed as a single-chain Fab (scFab), a 5/4 antibody by-product (comprising an additional heavy or light chain variable domain) may be observed. Such corresponding product-specific impurities would not arise in standard antibody production.

[0006] Conventional purification techniques designed to remove process-related impurities such as HCPs, DNA, endotoxins, and other materials that have very different characteristics and properties from the antibodies can be inadequate when implemented to remove impurities that are more similar to the multispecific antibodies. As such, there is a need to develop manufacturing and purification schemes that effectively remove product-specific impurities and yield sufficient amount of the correct and complete multispecific antibody.

[0007] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0008] As described and exemplified herein, Applicants have discovered that the use of at least two mixed mode (also referred to herein as multi-modal or multimodal) chromatography steps after an initial capture chromatography step results in greater removal of product-specific impurities and an improved process for purifying multispecific antibodies. Thus, in one aspect, provided is a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and an impurity, wherein the multispecific antibody comprises multiple arms, each arm comprising a VH/VL unit, the method comprising the sequential steps of: a) subjecting the composition to a capture chromatography to produce a capture chromatography eluate; b) subjecting the capture chromatography eluate to a first mixed mode chromatography to generate a first mixed mode eluate; c) subjecting the first mixed mode eluate to a second mixed mode chromatography to generate a second mixed mode eluate; and d) collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of a product-specific impurity from the composition, wherein the product specific impurity is one or more of non-paired antibody arms, antibody homodimers, aggregates, high molecular weight species (HMWS), low molecular weight species (LMWS), acidic variants, or basic variants. In some embodiments according to (or as applied to) any of the embodiments above, the capture chromatography eluate is subjected to an ion exchange chromatography (*e.g.*, anion exchange) or to hydrophobic interaction chromatography prior to the first mixed mode chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the second mixed mode eluate is subjected to an ion exchange chromatography (*e.g.*, anion exchange) or to hydrophobic interaction chromatography.

[0009] In another aspect, provided is a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and an impurity, wherein the multispecific antibody comprises multiple arms, each arm comprising a VH/VL unit, wherein each arm of the multispecific antibody is produced separately, the method comprising the sequential steps of a) subjecting each arm of the multispecific antibody to capture chromatography to produce capture eluates for each arm of the multispecific antibody, b) forming a mixture comprising capture eluates of each arm of the multispecific antibody under conditions sufficient to produce a composition comprising the multispecific antibody, c) subjecting the composition comprising the multispecific antibody to a first mixed mode chromatography to generate a first mixed mode eluate, and d) subjecting the first mixed mode eluate to a second mixed

mode chromatography to generate a second mixed mode eluate; and e) collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of a product-specific impurity from the composition, wherein the product specific impurity is one or more of non-paired antibody arms, antibody homodimers, aggregates, high molecular weight species (HMWS), low molecular weight species (LMWS), acidic variants, or basic variants. In some embodiments according to (or as applied to) any of the embodiments above, the capture chromatography eluate is subjected to an ion exchange chromatography (*e.g.*, anion exchange) or to hydrophobic interaction chromatography prior to the first mixed mode chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the second mixed mode eluate is subjected to an ion exchange chromatography (*e.g.*, anion exchange) or to hydrophobic interaction chromatography.

[0010] In some embodiments according to (or as applied to) any of the embodiments above, the capture chromatography is affinity chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the affinity chromatography is protein L chromatography, protein A chromatography, protein G chromatography, protein A and protein G chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the affinity chromatography is protein A chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the capture chromatography is carried out in bind and elute mode.

[0011] In some embodiments according to (or as applied to) any of the embodiments above, the first mixed mode chromatography and the second mixed mode chromatography are contiguous. In some embodiments according to (or as applied to) any of the embodiments above, the first mixed mode chromatography is a mixed mode anion exchange chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the second mixed mode chromatography is a mixed mode cation exchange chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the first mixed mode chromatography is a mixed mode cation exchange chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the second mixed mode chromatography is a mixed mode anion exchange chromatography.

[0012] In some embodiments according to (or as applied to) any of the embodiments above, the first mixed mode chromatography is carried out in bind and elute mode or in flow-through mode. In some embodiments according to (or as applied to) any of the embodiments in which the first mixed mode chromatography is carried out in bind and elute mode, the elution is a gradient elution.

[0013] In some embodiments according to (or as applied to) any of the embodiments above, the second mixed mode chromatography is carried out in bind and elute mode or in flow-through mode. In some embodiments according to (or as applied to) any of the embodiments in which the second mixed mode chromatography is carried out in bind and elute mode, the elution is a gradient elution.

[0014] In some embodiments according to (or as applied to) any of the embodiments above, the method further comprises the step of subjecting the second mixed mode eluate to ultrafiltration. In some embodiments according to (or as applied to) any of the embodiments above, the ultrafiltration comprises sequentially a first ultrafiltration, a diafiltration and a second ultrafiltration.

[0015] In some embodiments according to (or as applied to) any of the embodiments above, the protein A chromatography comprises protein A linked to agarose. In some embodiments according to (or

bispecific antibody. In some embodiments according to (or as applied to) any of the embodiments above, the bispecific antibody is a CrossMab bispecific antibody.

[0026] In some embodiments according to (or as applied to) any of the methods above, a fraction is collected after the last chromatography step, comprising at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% multispecific antibody. In some embodiments according to (or as applied to) any of the embodiments above, a fraction is collected after the last chromatography step, comprising a reduced amount of a product-specific impurity, wherein the product-specific impurity is one or more of: non-paired antibody arms, antibody homodimers, high molecular weight species (HMWS), low molecular weight species (LMWS), or $\frac{3}{4}$ antibodies. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% non-paired antibody arms. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% antibody homodimers. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains no more than about 1% or no more than about 2% HMWS. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains no more than about 2% or no more than about 1% LMWS. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains no more than about 5%, no more than about 4%, no more than about 3%, no more than about 2%, or no more than about 1% of $\frac{3}{4}$ antibodies.

[0027] In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains

- a) at least about 95% - 100% multispecific antibody;
- b) less than about 1%- 5% non-paired antibody arms;
- c) less than about 1%- 5% antibody homodimers;
- d) no more than about 1% or 2% HMWS;
- e) no more than about 1% or 2% LMWS; and/or
- f) no more than about 5% of $\frac{3}{4}$ antibodies.

[0028] Disclosed is a composition comprising a multispecific antibody purified by the method of any one of the methods described above.

[0029] In some embodiments according to (or as applied to) any of the methods above, provided is a composition comprising a multispecific antibody, wherein the composition comprises at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% multispecific antibody. In some embodiments according to (or as applied to) any of the embodiments above, provided is a composition comprising a multispecific antibody, wherein the composition comprises a reduced amount of a product-specific impurity, wherein the product-specific impurity is one or more of: non-paired antibody arms, antibody homodimers, high molecular weight species (HMWS), low molecular weight species (LMWS), or $\frac{3}{4}$ antibodies. In some embodiments according to (or as

that binds ANG2 and VEGF) purified by any one of the methods described above for the treatment of cancer or eye disease. Disclosed is a composition comprising a multispecific antibody purified by the method as described above for the treatment of cancer or eye disease.

[0032] In some embodiments according to (or as applied to) any of the embodiments above, provided is the use of a composition comprising a multispecific or bispecific antibody (such as a bispecific antibody that binds ANG2 and VEGF) purified by any one of the methods described above for the manufacture of a medicament for the treatment of cancer or eye disease.

[0033] In some embodiments according to (or as applied to) any of the embodiments above, the methods provided herein are used for the purification of an Fc-containing heterodimeric polypeptide.

[0034] In some embodiments according to (or as applied to) any of the embodiments above, provided is the use of any of the methods provided herein for the reduction of Fc-containing heterodimeric polypeptide-related impurities in a composition.

[0035] In one aspect, provided is a method for purifying an Fc-region containing heterodimeric polypeptide with a multi-step chromatography method wherein the method comprises an affinity chromatography step followed by two different multimodal ion exchange chromatography steps, and thereby purifying the Fc-region containing heterodimeric polypeptide. In some embodiments according to (or as applied to) any of the embodiments above, the multi-step chromatography method comprises (i) an affinity chromatography step, followed by a multimodal anion exchange chromatography step, followed by a multimodal cation exchange chromatography step; or (ii) an affinity chromatography step, followed by a multimodal cation exchange chromatography step, followed by a multimodal anion exchange chromatography step.

[0036] In some embodiments according to (or as applied to) any of the embodiments above, the multi-step chromatography method comprises an affinity chromatography step, followed by a multimodal anion exchange chromatography step, followed by a multimodal cation exchange chromatography step. In some embodiments according to (or as applied to) any of the embodiments above, the multi-step chromatography method comprises exactly three chromatography steps. In some embodiments according to (or as applied to) any of the embodiments above, the multimodal anion exchange chromatography step is performed in flow-through mode. In some embodiments according to (or as applied to) any of the embodiments above, the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value of less than 7 mS/cm. In some embodiments according to (or as applied to) any of the embodiments above, in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value in the range of about 6 mS/cm to about 2 mS/cm. In some embodiments according to (or as applied to) any of the embodiments above, in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value of about 4.5 mS/cm. In some embodiments according to (or as applied to) any of the embodiments above, the multimodal anion exchange chromatography step is performed at a pH of about 7. In some embodiments according to (or as applied to) any of the embodiments above, in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide

- [0042a] Disclosed is a composition comprising a bispecific antibody, wherein the composition contains at least about 95%, bispecific antibody.
- [0042b] Disclosed is a composition comprising a CrossMab antibody, wherein the composition contains at least 95% CrossMab antibody.
- [0042c] Disclosed is a composition comprising a bispecific antibody that binds to ANG-2 and VEGF, wherein the composition contains no more than about 5% , about 4%, about 3%, about 2%, or about 1% of $\frac{3}{4}$ antibodies.
- [0043] In one aspect, provided is the use of any method according to (or as applied to) any of the aspects or embodiments above for the reduction of Fc-containing heterodimeric polypeptide related impurities. In another aspect, provided is the use of any method according to (or as applied to) any of the aspects or embodiments above for the purification of an Fc-containing heterodimeric polypeptide.
- [0044] Disclosed is an Fc-containing heterodimeric polypeptide obtained with the method according to (or as applied to) any of the aspects or embodiments above, or a bispecific antibody that binds to ANG-2 and VEGF obtained using the method according to (or as applied to) any of the aspects or embodiments above for the manufacture of a medicament for the treatment of cancer or eye disease.
- [0045] Disclosed is an Fc-containing heterodimeric polypeptide obtained from the method according to (or as applied to) any of the aspects or embodiments above or a bispecific antibody that binds to ANG-2 and VEGF obtained using the method according to (or as applied to) any of the aspects or embodiments above for use in the treatment of cancer or eye disease.
- [0046] In one aspect, provided is a method for producing an Fc-containing heterodimeric polypeptide comprising the steps of (i) cultivating a cell comprising a nucleic acid encoding an Fc-containing heterodimeric polypeptide; (ii) recovering the Fc-containing heterodimeric protein from the cell or the cultivation medium; (iii) purifying the Fc-containing heterodimeric polypeptide using a method according to (or as applied to) any of the aspects or embodiments above, and thereby producing the Fc-containing heterodimeric polypeptide.
- [0047] In another aspect, provided is a method of producing a bispecific antibody that binds to ANG-2 and VEGF obtained using the method according to any of the aspects or embodiments above comprising the steps of: (i) cultivating a cell comprising a nucleic acid encoding the bispecific antibody; (ii) recovering the bispecific antibody from the cell or the cultivation medium; (iii) purifying the bispecific antibody using a method according to (or as applied to) any of the aspects or embodiments above, and thereby producing the bispecific antibody that binds to ANG-2 and VEGF.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0048] **FIG. 1A** depicts a first purification scheme used in Example 1.
- [0049] **FIG. 1B** depicts a second purification scheme used in Example 1.
- [0050] **FIG. 1C** depicts a third purification scheme used in Example 1.
- [0051] **FIG. 2** depicts a purification scheme used in Example 2.
- [0052] **FIG. 3A** depicts a first purification scheme used in Example 3.
- [0053] **FIG. 3B** depicts a second purification scheme used in Example 3.
- [0054] **FIG. 4** depicts a purification scheme used in Example 4.
- [0055] **FIG. 5A** depicts a first purification scheme used in Example 6.
- [0056] **FIG. 5B** depicts a second purification scheme used in Example 6.
- [0057] **FIG. 6A** depicts a first purification scheme used in Example 7.
- [0058] **FIG. 6B** depicts a second purification scheme used in Example 7.
- [0059] **FIG. 7A** depicts a first purification scheme described in Example 8.
- [0060] **FIG. 7B** depicts a second purification scheme described in Example 8.
- [0061] **FIG. 7C** depicts a third purification scheme used described in Example 8.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and an impurity, wherein the multispecific antibody comprises multiple arms, each arm comprising a VH/VL unit, the method comprising the sequential steps of:
 - a) subjecting the composition to a capture chromatography to produce a capture chromatography eluate;
 - b) subjecting the capture chromatography eluate to a first mixed mode chromatography to generate a first mixed mode eluate; and
 - c) subjecting the first mixed mode eluate to a second mixed mode chromatography to generate a second mixed mode eluate; and
 - d) collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of a product-specific impurity from the composition, wherein the product specific impurity is one or more of non-paired antibody arms, antibody homodimers, aggregates, high molecular weight species (HMWS), low molecular weight species (LMWS), acidic variants, or basic variants.
2. The method of claim 1, wherein the capture chromatography eluate is subjected to ion exchange or HIC chromatography prior to the first mixed mode chromatography.
3. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and an impurity, wherein the multispecific antibody comprises multiple arms, each arm comprising a VH/VL unit, wherein each arm of the multispecific antibody is produced separately, the method comprising the sequential steps of
 - a) subjecting each arm of the multispecific antibody to capture chromatography to produce capture eluates for each arm of the multispecific antibody,
 - b) forming a mixture comprising capture eluates of each arm of the multispecific antibody under conditions sufficient to produce a composition comprising the multispecific antibody,
 - c) subjecting the composition comprising the multispecific antibody to a first mixed mode chromatography to generate a first mixed mode eluate, and
 - d) subjecting the first mixed mode eluate to a second mixed mode chromatography to generate a second mixed mode eluate; and
 - e) collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of a product-specific impurity from the composition,

wherein the product specific impurity is one or more of non-paired antibody arms, antibody homodimers, aggregates, high molecular weight species (HMWS), low molecular weight species (LMWS), acidic variants, or basic variants.

4. The method of claim 3, wherein the composition comprising the multispecific antibody is subjected to ion exchange or HIC chromatography prior to the first mixed mode chromatography.
5. The method of any one of claims 1-4, wherein the capture chromatography is affinity chromatography.
6. The method of claim 5, wherein the affinity chromatography is protein L chromatography, protein A chromatography, protein G chromatography, protein A and protein G chromatography.
7. The method of claim 5 or claim 6, wherein the affinity chromatography is protein A chromatography.
8. The method of any one of claims 1-7, wherein the capture chromatography is carried out in bind and elute mode.
9. The method of any one of claims 1-8, wherein the first mixed mode chromatography and the second mixed mode chromatography are contiguous.
10. The method any one of claims 1-9, wherein the first mixed mode chromatography is a mixed mode anion exchange chromatography.
11. The method of any one of claims 1-10, wherein the second mixed mode chromatography is a mixed mode cation exchange chromatography.
12. The method any one of claims 1-9, wherein the first mixed mode chromatography is a mixed mode cation exchange chromatography.
13. The method of any one of claims 1-10 and 12, wherein the second mixed mode chromatography is a mixed mode anion exchange chromatography.
14. The method of any one of claims 1-13, wherein the first mixed mode chromatography is carried out in bind and elute mode.
15. The method of claim 14, wherein the elution is a gradient elution.

16. The method of any one of claims 1-13, wherein the first mixed mode chromatography is carried out in flow through mode.
17. The method of any one of claims 1-16, wherein the second mixed mode chromatography is carried out in bind and elute mode.
18. The method of claim 17, wherein the elution is a gradient elution.
19. The method of any one of claims 1-16, wherein the second mixed mode chromatography is carried out in flow through mode.
20. The method of any one of claims 1-19 further comprising the step of subjecting the second mixed mode eluate to ultrafiltration.
21. The method of claim 20 wherein the ultrafiltration comprises sequentially a first ultrafiltration, a diafiltration and a second ultrafiltration.
22. The method of any one of claims 7-21, wherein the protein A chromatography comprises protein A linked to agarose.
23. The method of any one of claims 7-22, wherein the protein A chromatography uses one or more of a protein A equilibration buffer, a protein A loading buffer or a protein A wash buffer wherein the equilibration buffer, a loading buffer, and/or wash buffer is between pH 7 and pH 8.
24. The method of claim 23, wherein the protein A equilibration buffer is about pH 7.7.
25. The method of claim 23 or claim 24, wherein the protein A equilibration buffer comprises about 25 mM Tris and about 25 mM NaCl.
26. The method of any one of claims 23-25, wherein the protein A chromatography is washed with equilibration buffer following load.
27. The method of any one of claims 7-26, wherein the multispecific antibody is eluted from the protein A chromatography by a pH step elution.
28. The method of any one of claims 7-27, wherein the multispecific antibody is eluted from the protein A by applying a protein A elution buffer with low pH to the protein A chromatography.

29. The method of claim 28, wherein the protein A elution buffer comprises about 150 mM acetic acid, about pH 2.9.
30. The method of any one of claims 7-29 wherein the protein A eluate is pooled where the OD₂₈₀ of the eluate is greater than 0.5.
31. The method of any one of claims 10-30, wherein the anion exchange mixed mode chromatography comprises a quaternary amine and a hydrophobic moiety.
32. The method of claim 31, wherein the anion exchange mixed mode chromatography comprises a quaternary amine and a hydrophobic moiety linked to highly crosslinked agarose.
33. The method of any one of claims 11-32, wherein the cation exchange mixed mode chromatography comprises a N-benzyl-n-methyl ethanolamine.
34. The method of claim 33, wherein the mixed mode chromatography is a Capto™ MMC chromatography or a Capto™ MMC ImpRes chromatography.
35. The method of any one of claims 1-34 wherein the first mixed mode chromatography uses one or more of a mixed mode pre-equilibration buffer, a mixed mode equilibration buffer, a mixed mode loading buffer, or a mixed mode wash buffer, and wherein the mixed mode pre-equilibration buffer, the mixed mode equilibration buffer, the mixed mode loading buffer and/or the mixed mode wash buffer is between pH 6 and pH 7.
36. The method of any one of claims 1-35 wherein the second mixed mode chromatography uses one or more of a mixed mode pre-equilibration buffer, a mixed mode equilibration buffer, a mixed mode loading buffer or a mixed mode wash buffer wherein the mixed mode pre-equilibration buffer, the mixed mode equilibration buffer, and/or mixed mode wash buffer is between pH 5 and pH 8.
37. The method of claim 35 or claim 36, wherein the mixed mode pre-equilibration buffer, the mixed mode equilibration buffer, and/or mixed mode wash buffer is about pH 6.5.
38. The method of any one of claims 35-37, wherein the mixed mode pre-equilibration buffer comprises about 500 mM acetate.
39. The method of any one of claims 35-38, wherein the mixed mode equilibration buffer comprises about 50 mM acetate.

40. The method of any one of claims 35-39, wherein the first mixed mode chromatography is washed with wash buffer following load.
41. The method of any one of claims 35-40, wherein the second mixed mode chromatography is washed with wash buffer following load.
42. The method of any one of claims 15 and 18-41 wherein the multispecific antibody is eluted from the first mixed mode chromatography by pH gradient.
43. The method of any one of claims 15 and 18-42, wherein the multispecific antibody is eluted from the first mixed mode chromatography by applying a mixed mode elution buffer with low pH to the mixed mode ion exchange chromatography.
44. The method of any one of claims 15 and 18-43 wherein the multispecific antibody is eluted from the second mixed mode chromatography by pH gradient.
45. The method of any one of claims 15 and 18-44, wherein the multispecific antibody is eluted from the second mixed mode chromatography by applying a mixed mode elution buffer with low pH to the mixed mode exchange chromatography.
46. The method of any one of claims 2 and 4-45, wherein the ion exchange chromatography comprises a quaternary amine.
47. The method of claim 46, wherein the ion exchange chromatography is an anion exchange chromatography, and wherein the anion exchange chromatography comprises a quaternary amine linked to crosslinked agarose.
48. The method of claim 47, wherein the mixed mode anion exchange chromatography is a CaptoAdhere chromatography.
49. The method of any one of claims 46-48, wherein the anion exchange chromatography uses one or more of an anion exchange pre-equilibration buffer, an anion exchange equilibration buffer or an anion exchange loading buffer wherein the anion exchange pre-equilibration buffer, the anion exchange equilibration buffer and/or anion exchange the load buffer is between pH 6 and pH 8.

50. The method of claim 49, wherein the anion exchange pre-equilibration buffer, the anion exchange equilibration buffer and/or the anion exchange load is about pH 6.5.
51. The method of claim 49 or claim 50, wherein the anion exchange pre-equilibration buffer comprises about 50 mM Tris, 500 mM sodium acetate.
52. The method of any one of claims 49-51, wherein the anion exchange equilibration buffer comprises about 50 mM Tris.
53. The method of any one of claims 49-52, wherein the anion exchange chromatography is washed with anion exchange equilibration buffer following load.
54. The method of any one of claims 49-53, wherein the multispecific antibody is eluted from the anion exchange chromatography by salt gradient.
55. The method of any one of claims 49-54, wherein the multispecific antibody is eluted from the anion exchange chromatography by applying an anion exchange elution buffer with increased salt concentration to the anion exchange chromatography.
56. The method of claim 55, wherein the anion exchange elution buffer comprises about 50 mM Tris, 100 mM sodium acetate at about pH 8.5.
57. The method of any one of claims 49-56 wherein the anion exchange eluate is pooled where the OD₂₈₀ of the eluate is greater than 0.5 to 2.0.
58. The method of any one of claims 1-57, wherein the arms of the multispecific antibody are produced in a cell.
59. The method of claim 58, wherein the cell is a prokaryotic cell.
60. The method of claim 59, wherein the prokaryotic cell is an *E. coli* cell.
61. The method of claim 59 or claim 60, wherein the cell is engineered to express one or more chaperones.
62. The method of claim 61, wherein the chaperone is one or more of FkpA, DsbA or DsbC.

63. The method of claim 61 or claim 62, wherein the chaperone is an *E. coli* chaperone.
64. The method of any one of claims 1-58, wherein the cell is a eukaryotic cell.
65. The method of claim 64, wherein the eukaryotic cell is a yeast cell, an insect cell, or a mammalian cell.
66. The method of claim 64 or claim 65, wherein the eukaryotic cell is a CHO cell.
67. The method of any one of claims 58-66, wherein the cells are lysed to generate a cell lysate comprising the multispecific antibody or an arm of the multispecific antibody prior to capture chromatography.
68. The method of claim 67, wherein the cells are lysed using a microfluidizer.
69. The method of claim 67 or claim 68, wherein polyethylenimine (PEI) is added to the cell lysate prior to chromatography.
70. The method of any one of claims 1-69 wherein the method reduces the amount of any one of host cell protein (HCP), leached protein A, nucleic acid, cell culture media components, or viral impurities in the composition.
71. The method of any one of claims 1-70, wherein the multispecific antibody is a bispecific antibody.
72. The method of claim 71, wherein the bispecific antibody is a knob-in-hole (KiH) bispecific antibody.
73. The method of claim 71 or claim 72, wherein the bispecific antibody is a CrossMab bispecific antibody.
74. The method of any one of claims 1-73, wherein the fraction contains at least 95% multispecific antibody.
75. The method of any one of claims 1-74, wherein the fraction contains no more than 5% non-paired antibody arms.

76. The method of any one of claims 1-75, wherein the fraction contains no more than 5% antibody homodimers.
77. The method of any one of claims 1-76, wherein the fraction contains no more than 2% aggregates or high molecular weight species (HMWS).
78. The method of any one of claims 1-77, wherein the fraction contains no more than 2% LMWS.
79. The method of any one of claims 1-78 wherein the fraction contains no more than 50% acidic variants.
80. The method of any one of claims 1-79, wherein the fraction contains no more than 35% basic variants.
81. The method of any one of claims 1-80, wherein the fraction contains no more than 5% of $\frac{3}{4}$ antibodies.
82. The method of claim any one of claims 1-81, wherein the fraction contains
- a) at least 95% -100% multispecific antibody;
 - b) no more than 1%-5% non-paired antibody arms;
 - c) no more than 1%-5% antibody homodimers;
 - d) no more than 1% or 2% HMWS;
 - e) no more than 1% or 2% LMWS; and
 - f) no more than 5% of $\frac{3}{4}$ antibodies.
83. A method for purifying an Fc-region containing heterodimeric polypeptide with a multi-step chromatography method wherein the method comprises an affinity chromatography step followed by two different multimodal ion exchange chromatography steps,
and thereby purifying the Fc-region containing heterodimeric polypeptide.
84. The method according to claim 83, wherein the multi-step chromatography method comprises
- i. an affinity chromatography step, followed by a multimodal anion exchange chromatography step, followed by a multimodal cation exchange chromatography step
 - or
 - ii. an affinity chromatography step, followed by a multimodal cation exchange chromatography step, followed by a multimodal anion exchange chromatography step.

85. The method according to any one of claims 83-84, wherein the multi-step chromatography method comprises an affinity chromatography step, followed by a multimodal anion exchange chromatography step, followed by a multimodal cation exchange chromatography step.
86. The method according any one of claims 83-85, wherein the multi-step chromatography method comprises exactly three chromatography steps.
87. The method according to any one of claims 84-86, wherein the multimodal anion exchange chromatography step is performed in flow-through mode.
88. The method according to any one of claims 84-87, wherein in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value of less than 7 mS/cm.
89. The method according to any one of claims 84-88, wherein in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value in the range of 6 mS/cm to 2 mS/cm.
90. The method according to any one of claims 84-89, wherein in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value of about 4.5 mS/cm.
91. The method according to any one of claims 84-90, wherein the multimodal anion exchange chromatography step is performed at a pH of about 7.
92. The method according to any one of claims 84-91, wherein in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity of about 4.5 mS/cm and a pH of about 7.
93. The method according to any one of claims 84-92, wherein in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in the range of from 100 g to 300 g per liter of chromatography material.
94. The method according to any one of claims 84-93, wherein the multimodal anion exchange chromatography material is a multimodal strong anion exchange chromatography material.

95. The method according to any one of claims 84-94, wherein the multimodal anion exchange chromatography material has a matrix of high-flow agarose, a multimodal strong anion exchanger as ligand, an average particle size of 36-44 μm and an ionic capacity of 0.08 to 0.11 mmol Cl-/mL medium.
96. The method according to any one of claims 84-95, wherein the multimodal cation exchange chromatography medium is a multimodal weak cation exchange chromatography medium.
97. The method according to any one of claims 84-96, wherein the multimodal cation exchange chromatography medium has a matrix of high-flow agarose, a multimodal weak cation exchanger as ligand, an average particle size of 36-44 μm and ionic capacity of 25 to 39 $\mu\text{mol/mL}$.
98. The method according to any one of claims 84-97, wherein the multimodal anion exchange chromatography step is performed in bind and elute mode.
99. The method according to any one of claims 84-98, wherein the affinity chromatography is a protein A affinity chromatography or a Protein G affinity chromatography or a single chain Fv ligand affinity chromatography or a chromatography step with CaptureSelect chromatography material or a chromatography step with CaptureSelect FcXL chromatography material.
100. The method according to any one of claims 83-99, wherein the affinity chromatography step is a protein A chromatography step.
101. The method according to any one of claims 83-100, wherein the affinity chromatography step is a chromatography step with CaptureSelect™ chromatography material.
102. The method according to any one of claims 83-101, wherein the Fc-region containing heterodimeric polypeptide is an antibody, a bispecific antibody or Fc-fusion proteins.
103. The method according to any one of claims 83-102, wherein the Fc-region containing heterodimeric polypeptide is a bispecific antibody.
104. The method according to any one of claims 85-103, wherein the Fc-region containing heterodimeric polypeptide is a CrossMab.
105. The method according to any one of claims 85-104, wherein the Fc-region containing heterodimeric polypeptide is a bispecific antibody comprising

- a) a heavy chain and a light chain of a first full length antibody that specifically binds to a first antigen; and
- b) a modified heavy chain and a modified light chain of a full length antibody that specifically binds to a second antigen, wherein the constant domains CL and CH1 are replaced by each other.

106. The method according to any one of claims 103-105, wherein the bispecific antibody binds to ANG2 and VEGF.

107. The method according to any one of claims 103-105, wherein the CrossMab binds to ANG2 and VEGF.

108. The method according to any one of claims 103-107, wherein the bispecific antibody is vanucizumab.

109. The method according to any one of claims 103-107 wherein the bispecific antibody comprises a first antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 1, and as light chain variable domain (VL) the SEQ ID NO: 2; and a second antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 3, and as light chain variable domain (VL) the SEQ ID NO: 4.

110. The method according to any one of claims 103-107, wherein the bispecific antibody comprises a first heavy chain with the amino acid sequence of SEQ ID NO: 9 and a second heavy chain with the amino acid sequence of SEQ ID NO: 10 and a first light chain with the amino acid sequence of SEQ ID NO: 11 and a second light chain with the amino acid sequence of SEQ ID NO: 12.

111. The method according to any one of claims 103-107, wherein the bispecific antibody comprises a first antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 5, and as light chain variable domain (VL) the SEQ ID NO: 6; and a second antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 7, and as light chain variable domain (VL) the SEQ ID NO: 8.

112. The method according to any one of claims 103-107, wherein the bispecific antibody comprises a first heavy chain with the amino acid sequence of SEQ ID NO: 13 and a second heavy chain with the amino acid sequence of SEQ ID NO: 14 and a first light chain with the amino acid sequence of SEQ ID NO: 15 and a second light chain with the amino acid sequence of SEQ ID NO: 16.

113. The method of any one of claims 103-112, wherein the purified Fc-region containing heterodimeric polypeptide contains no more than 5% of $\frac{3}{4}$ antibodies.
114. A method for purifying a bispecific antibody that binds to ANG-2 and VEGF with a multi-step chromatography method wherein the method comprises an affinity chromatography step, followed by a multimodal anion exchange chromatography step, followed by a multimodal cation exchange chromatography step,
and thereby purifying the bispecific antibody that binds to ANG-2 and VEGF,
wherein bispecific antibody comprises a first antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 1, and as light chain variable domain (VL) the SEQ ID NO: 2; and a second antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 3, and as light chain variable domain (VL) the SEQ ID NO: 4
or that comprises a first antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 5, and as light chain variable domain (VL) the SEQ ID NO: 6; and a second antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 7, and as light chain variable domain (VL) the SEQ ID NO: 8.
115. The method according to claim 114, wherein the bispecific antibody binds to ANG-2 and VEGF comprises
a) the heavy chain and the light chain of a first full length antibody that comprises the first antigen-binding site; and
b) the modified heavy chain and modified light chain of a full length antibody that comprises the second antigen-binding site, wherein the constant domains CL and CH1 are replaced by each other.
116. Use of the method according to any one of claims 83-113 for the purification of an Fc-containing heterodimeric polypeptide.
117. Use of the method according to any one of claims 83-113 for the reduction of Fc-containing heterodimeric polypeptide related impurities.
118. A method for producing an Fc-containing heterodimeric polypeptide comprising the steps of
i. cultivating a cell comprising a nucleic acid encoding an Fc-containing heterodimeric polypeptide,
ii. recovering the Fc-containing heterodimeric protein from the cell or the cultivation medium,
iii. purifying the Fc-containing heterodimeric polypeptide with a method according to any one of claims 83 to 113,

and thereby producing the Fc-containing heterodimeric polypeptide.

119. A method for producing a bispecific antibody that binds to ANG-2 and VEGF obtained using the method of any one of claims 114-115 comprising the steps of

- i. cultivating a cell comprising a nucleic acid encoding the bispecific antibody,
 - ii. recovering the bispecific antibody from the cell or the cultivation medium,
 - iii. purifying the bispecific antibody with a method according to any one of claims 114-115,
- and thereby producing the bispecific antibody that binds to ANG-2 and VEGF.