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 example, 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 examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples above, the second mixed mode eluate is subjected to an ion exchange chromatography (*e.g.*, anion exchange) or to hydrophobic interaction chromatography.

[0008a] In one aspect, the present disclosure refers to 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 and antibody homodimers, wherein a) the first mixed mode chromatography is a mixed mode cation exchange chromatography and the second mixed mode chromatography is a mixed mode anion exchange chromatography; or wherein b) the first mixed mode chromatography is a mixed mode anion exchange chromatography and the second mixed mode chromatography is a mixed mode cation exchange chromatography; wherein the anion exchange mixed mode chromatography comprises a quaternary amine and a hydrophobic moiety and the cation exchange mixed mode chromatography comprises a N-benzyl-n-methyl ethanolamine, wherein the multispecific antibody is a bispecific antibody; and wherein the bispecific antibody is a knob-in-hole (KiH) bispecific antibody.

[0009] In another example, 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 examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples above, the capture chromatography is affinity chromatography. In some examples according to (or as applied to) any of the examples above, the affinity chromatography is protein L chromatography, protein A chromatography, protein G chromatography, protein A and protein G chromatography. In some examples according to (or as applied to) any of the examples above, the affinity chromatography is protein A chromatography. In some examples according to (or as applied to) any of the examples above, the capture chromatography is carried out in bind and elute mode.

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

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

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

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

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

as applied to) any of the examples above, the protein A chromatography is a MabSelect™, MabSelect™ SuRe and MabSelect™ SuRe LX, Prosep-VA, Prosep-VA Ultra Plus, Protein A sepharose fast flow, or Toyopearl Protein A chromatography. In some examples according to (or as applied to) any of the examples above, 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 about pH 7 and about pH 8. In some examples according to (or as applied to) any of the examples above, the protein A equilibration buffer is about pH 7.7. In some examples according to (or as applied to) any of the examples above, the protein A equilibration buffer comprises about 25 mM Tris and about 25 mM NaCl. In some examples according to (or as applied to) any of the examples above, the protein A chromatography is washed with equilibration buffer following load. In some examples according to (or as applied to) any of the examples above, the multispecific antibody is eluted from the protein A by applying a protein A elution buffer with low pH to the protein A chromatography. In some examples according to (or as applied to) any of the examples above, the protein A elution buffer comprises about 150 mM acetic acid, about pH 2.9. In some examples according to (or as applied to) any of the examples above, the multispecific antibody is eluted from the protein A chromatography by pH gradient.

[0016] In some examples according to (or as applied to) any of the examples above, the anion exchange mixed mode chromatography comprises a quaternary amine and a hydrophobic moiety. In some examples according to (or as applied to) any of the examples above, the anion exchange mixed mode chromatography comprises a quaternary amine and a hydrophobic moiety linked to highly crosslinked agarose. In some examples according to (or as applied to) any of the examples above, the mixed mode chromatography is a Capto™ Adhere chromatography or a Capto™ Adhere ImpRes chromatography. In some examples according to (or as applied to) any of the examples above, the cation exchange mixed mode chromatography comprises N-benzyl-n-methyl ethanolamine. In some examples according to (or as applied to) any of the examples above, the mixed mode chromatography is a Capto™ MMC chromatography or a Capto™ MMC ImpRes chromatography.

[0017] In some examples according to (or as applied to) any of the examples above, 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, 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 about pH 6 and about pH 7. In some examples, an anion mixed mode equilibration buffer is between about pH 6.5 and about pH 8.

[0018] In some examples according to (or as applied to) any of the examples above, 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 about pH 5 and about pH 8, optionally between about pH 5 and about pH 7, or between about pH 5 and about pH 6, or between about pH 6 and about pH 7.

[0019] In some examples according to (or as applied to) any of the examples above, the mixed mode pre-equilibration buffer, the mixed mode equilibration buffer, and/or mixed mode wash buffer is about pH 5.5. In some examples according to (or as applied to) any of the examples above, the mixed mode pre-equilibration buffer comprises about 500 mM acetate. In some examples according to (or as applied to) any of the examples above, the mixed mode equilibration buffer comprises about 50 mM acetate.

[0020] In some examples according to (or as applied to) any of the examples above, the first mixed mode chromatography is washed with wash buffer following load. In some examples according to (or as applied to) any of the examples above, the second mixed mode chromatography is washed with wash buffer following load. In some examples according to (or as applied to) any of the examples above, the multispecific antibody is eluted from the first mixed mode chromatography by salt gradient and/or pH gradient or pH step elution. In some examples according to (or as applied to) any of the examples above, 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 exchange chromatography. In some examples according to (or as applied to) any of the examples above, the multispecific antibody is eluted from the second mixed mode chromatography by salt gradient and/or pH gradient or pH step elution. In some examples according to (or as applied to) any of the examples above, 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. In some examples according to (or as applied to) any of the examples above, the mixed mode elution buffer comprises about 25 mM acetate, about pH 5.5.

[0021] In some examples according to (or as applied to) any of the examples above, the anion exchange chromatography comprises a quaternary amine. In some examples according to (or as applied to) any of the examples above, the anion exchange chromatography comprises a quaternary amine linked to crosslinked agarose. In some examples according to (or as applied to) any of the examples above, the anion exchange chromatography is a QSFF chromatography. In some examples according to (or as applied to) any of the examples above, 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 about pH 8 and about pH 9. In some examples according to (or as applied to) any of the examples above, the anion exchange pre-equilibration buffer, the anion exchange equilibration buffer and/or the anion exchange load buffer is about pH 8.5. In some examples according to (or as applied to) any of the examples above, the anion exchange pre-equilibration buffer comprises about 50 mM Tris, 500 mM sodium acetate. In some examples according to (or as applied to) any of the examples above, the anion exchange equilibration buffer comprises about 50 mM Tris. In some examples

according to (or as applied to) any of the examples above, the anion exchange chromatography is washed with anion exchange equilibration buffer following load. In some examples according to (or as applied to) any of the examples above, the multispecific antibody is eluted from the anion exchange chromatography by salt gradient. In some examples according to (or as applied to) any of the examples above, 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. In some examples according to (or as applied to) any of the examples above, the anion exchange elution buffer comprises about 50 mM Tris, 100 mM sodium acetate at about pH 8.5.

[0022] In some examples according to (or as applied to) any of the examples above, the arms of the multispecific antibody are produced in a cell. In some examples according to (or as applied to) any of the examples above, the cell is a prokaryotic cell. In some examples according to (or as applied to) any of the examples above, the prokaryotic cell is an *E. coli* cell. In some examples according to (or as applied to) any of the examples above, the cell is engineered to express one or more chaperones. In some examples according to (or as applied to) any of the examples above, the chaperone is one or more of FkpA, DsbA or DsbC. In some examples according to (or as applied to) any of the examples above, the chaperone is an *E. coli* chaperone. In some examples according to (or as applied to) the examples above, the cell is a eukaryotic cell. In some examples according to (or as applied to) the examples above, the eukaryotic cell is a yeast cell, an insect cell, or a mammalian cell. In some examples according to (or as applied to) the examples above, the eukaryotic cell is a CHO cell.

[0023] In some examples according to (or as applied to) any of the examples above, the cells are lysed to generate a cell lysate comprising the multispecific antibody or an arm of the multispecific antibody prior to capture chromatography. In some examples according to (or as applied to) any of the examples above, the cells are lysed using a microfluidizer. In some examples according to (or as applied to) any of the examples above, polyethlyeneimine (PEI) is added to the cell lysate prior to chromatography. In some examples according to (or as applied to) any of the examples above, the PEI is added to the lysate to a final concentration of about 0.4%. In some examples according to (or as applied to) any of the examples above, the cell lysate is clarified by centrifugation. In some examples according to (or as applied to) any of the examples above, the cell lysate from a mammalian cell, *e.g.*, a CHO cell, is subject to one or more the following treatments: heat inactivation, low pH inactivation, viral inactivation by addition of detergent.

[0024] In some examples according to (or as applied to) any of the examples above, the method reduces the amount of a process-specific impurity such as any one of host cell protein (HCP), leached protein A, nucleic acid, cell culture media components, or viral impurities in the composition.

[0025] In some examples according to (or as applied to) any of the examples above, the multispecific antibody is a bispecific antibody. In some examples according to (or as applied to) any of the examples above, the bispecific antibody is a knob-in-hole (KiH) antibody, *e.g.*, a KiH

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

[0026] In some examples 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 examples according to (or as applied to) any of the examples 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 ¾ antibodies. In some examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples above, the fraction contains no more than about 1% or no more than about 2% HMWS. In some examples according to (or as applied to) any of the examples above, the fraction contains no more than about 2% or no more than about 1% LMWS. In some examples according to (or as applied to) any of the examples 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 ¾ antibodies.

[0027] In some examples according to (or as applied to) any of the examples 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 ¾ 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 examples 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 examples according to (or as applied to) any of the examples 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 ¾ antibodies. In some examples according to (or as

applied to) any of the examples above, the composition 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 examples according to (or as applied to) any of the examples above, the composition 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 examples according to (or as applied to) any of the examples above, the composition contains no more than about 1% or no more than about 2% HMWS. In some examples according to (or as applied to) any of the examples above, the composition contains no more than about 2% or no more than about 1% LMWS. In some examples according to (or as applied to) any of the examples above, the composition 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 ¾ antibodies. In some examples according to (or as applied to) any of the examples above, the multispecific antibody in the composition is a bispecific antibody. In some examples according to (or as applied to) any of the examples above, the bispecific antibody is a knob-in-hole (KiH) antibody, *e.g.*, a KiH bispecific antibody. In some examples according to (or as applied to) any of the examples above, the bispecific antibody is a CrossMab bispecific antibody. In some examples according to (or as applied to) any of the examples above, the bispecific antibody binds to ANG-2 and VEGF. In some examples according to (or as applied to) any of the examples above, 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.

[0030] In some examples according to (or as applied to) any of the examples above, the composition 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 ¾ antibodies. In some examples according to (or as applied to) any of the examples above, the multispecific antibody in the composition is a bispecific antibody. In some examples according to (or as applied to) any of the examples above, the bispecific antibody is a knob-in-hole (KiH) antibody, *e.g.*, a KiH bispecific antibody. In some examples according to (or as applied to) any of the examples above, the bispecific antibody is a CrossMab bispecific antibody. In some examples according to (or as applied to) any of the examples above, the bispecific antibody binds to ANG-2 and VEGF. In some examples according to (or as applied to) any of the examples above, 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.

[0031] In some examples according to (or as applied to) any of the examples above, provided is 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 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 examples according to (or as applied to) any of the examples above, provided is the use of a 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 examples according to (or as applied to) any of the examples above, the methods provided herein are used for the purification of an Fc-containing heterodimeric polypeptide.

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

[0035] In one example, 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 examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples above, the multi-step chromatography method comprises exactly three chromatography steps. In some examples according to (or as applied to) any of the examples above, the multimodal anion exchange chromatography step is performed in flow-through mode. In some examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples 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 examples according to (or as applied to) any of the examples above, the multimodal anion exchange chromatography step is performed at a pH of about 7. In some examples according to (or as applied to) any of the examples above, 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. In some examples according to (or as applied to) any of the examples above, in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in the range of from about 100 g to about 300 g per liter of chromatography material.

[0037] In some examples according to (or as applied to) any of the examples above, the multimodal anion exchange chromatography material is a multimodal strong anion exchange chromatography material. In some examples according to (or as applied to) any of the examples above, 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. In some examples according to (or as applied to) any of the examples above, the multimodal cation exchange chromatography medium is a multimodal weak cation exchange chromatography medium. In some examples according to (or as applied to) any of the examples above, 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 µmol/mL. In some examples according to (or as applied to) any of the examples above, the multimodal anion exchange chromatography step is performed in bind and elute mode. In some examples according to (or as applied to) any of the examples above, the capture chromatography is carried out by affinity chromatography. In some examples, 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. In some examples according to (or as applied to) any of the examples above, the affinity chromatography step is a protein A chromatography step. In some examples according to (or as applied to) any of the examples above, the affinity chromatography step is a chromatography step with CaptureSelect™ chromatography material.

[0038] In some examples according to (or as applied to) any of the examples above, the Fc-region containing heterodimeric polypeptide is an antibody, a bispecific antibody or Fc-fusion proteins. In some examples according to (or as applied to) any of the examples above, the Fc-region containing heterodimeric polypeptide is a bispecific antibody. In some examples according to (or as applied to) any of the examples above, the Fc-region containing heterodimeric polypeptide is a CrossMab. In some examples according to (or as applied to) any of the examples above, 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.

[0039] In some examples according to (or as applied to) any of the examples above, the bispecific antibody binds to ANG2 and VEGF. In some examples according to (or as applied to) any of the examples above, the CrossMab binds to ANG2 and VEGF. In some examples according to

(or as applied to) any of the examples above, the bispecific antibody is vanucizumab. In some examples according to (or as applied to) any of the examples above, 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. In some examples according to (or as applied to) any of the examples above, 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.

[0040] In some examples according to (or as applied to) any of the examples above, 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. In some examples according to (or as applied to) any of the examples above, 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.

[0041] In some examples according to (or as applied to) any of the examples above, the purified Fc-region containing heterodimeric polypeptide contains no more than about 5% of ¾ antibodies.

[0042] In one example, provided is a method for purifying a bispecific antibody that binds to ANG2 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 ANG2 and VEGF, wherein bispecific antibody that binds to ANG2 and VEGF 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. In some examples according to (or as applied to) any of the examples above, the bispecific antibody that binds to ANG2 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.

[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 ¾ antibodies.

[0043] In one example, provided is the use of any method according to (or as applied to) any of the aspects or examples above for the reduction of Fc-containing heterodimeric polypeptide related impurities. In another example, provided is the use of any method according to (or as applied to) any of the aspects or examples 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 examples 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 examples 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 examples 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 examples above for use in the treatment of cancer or eye disease.

[0046] In one example, 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 examples above, and thereby producing the Fc-containing heterodimeric polypeptide.

[0047] In another example, 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 examples 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 examples 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, 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 and antibody homodimers,

wherein a) the first mixed mode chromatography is a mixed mode cation exchange chromatography and the second mixed mode chromatography is a mixed mode anion exchange chromatography; or

wherein b) the first mixed mode chromatography is a mixed mode anion exchange chromatography and the second mixed mode chromatography is a mixed mode cation exchange chromatography;

wherein the anion exchange mixed mode chromatography comprises a quaternary amine and a hydrophobic moiety and the cation exchange mixed mode chromatography comprises a N-benzyl-n-methyl ethanolamine,

wherein the multispecific antibody is a bispecific antibody; and

wherein the bispecific antibody is a knob-in-hole (KiH) bispecific antibody.

2. The method of claim 1, wherein the first mixed mode chromatography is carried out in bind and elute mode or in flow through mode.

3. The method of any one of claims 1-2, wherein the second mixed mode chromatography is carried out in bind and elute mode or in flow through mode.

4. The method of any one of claims 1-3, wherein the anion exchange mixed mode chromatography comprises a quaternary amine and a hydrophobic moiety linked to highly crosslinked agarose.

5. The method of any one of claims 1-4, wherein the fraction contains at least 95% multispecific antibody.

6. The method of claim 5, wherein the fraction contains no more than 5% non-paired antibody arms or no more than 5% antibody homodimers.