

which are process-related impurities in the biologic product derived from recombinant DNA technology, for example, chaperones such as FkpA, DsbA and DsbC that are overexpressed to facilitate protein folding. While HCPs are typically present in the final drug substance in small quantities (in parts-per-million or nanograms per milligram of the intended recombinant polypeptide), it is recognized that HCPs are undesirable and their quantities should be minimized. For example, the U.S. Food and Drug Administration (FDA) requires that biopharmaceuticals intended for in vivo human use should be as free as possible of extraneous impurities, and requires tests for detection and quantitation of potential impurities, such as HCPs. In addition, the International Conference on Harmonization (ICH) provides guidelines on test procedures and acceptance criteria for biotechnological/biological products. The guidelines suggest that for HCPs, a sensitive immunoassay capable of detecting a wide range of protein impurities be utilized. Assays and reagents to detect immunoglobulins, DNA, endotoxins, viruses, and total HCPs, *e.g.*, total *E. coli* proteins (ECP) or total CHO proteins (CHOP) have been developed but such assays and reagents may not accurately detect accessory protein impurities such as FkpA. There are currently no commercial reagents or analytical methods of sufficient specificity and sensitivity for the detection and quantification of proteins such as FkpA that are typically not expressed at high levels in bacteria but may be overexpressed in recombinant bacterial host cells to facilitate folding and secretion of biologic products.

[0006] Reagents, methods and kits for the detection of FkpA are particularly needed where there are no existing assays and reagents of sufficient consistency, sensitivity, specificity or efficiency. The invention described herein meets certain of the above described needs and provides other benefits. In addition, the preparation of multispecific antibodies have unique product-specific impurities such as non-paired antibody arms and misassembled antibodies. As such, there is a need to develop purification schemes for multispecific antibodies that remove these product-specific impurities.

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

BRIEF SUMMARY

[0008] The disclosure provides methods for purifying a FkpA polypeptide from a cell lysate comprising the FkpA polypeptide, the method comprising a) clarifying the cell lysate by centrifugation, b) applying the clarified cell lysate comprising the FkpA polypeptide to a cation exchange chromatography material, c) eluting the FkpA polypeptide from the cation exchange chromatography material to generate a cation exchange eluate comprising the FkpA polypeptide, d) applying the cation exchange eluate comprising the FkpA polypeptide to a hydrophobic interaction chromatography (HIC) material, e) eluting the FkpA polypeptide from the HIC material to generate a HIC eluate, f) applying the HIC eluate comprising the FkpA polypeptide to a size exclusion chromatography material, g) collecting fraction from the size exclusion chromatography comprising the purified FkpA polypeptide. In some embodiments, the lysate comprising the FkpA polypeptide is at about pH 6.0.

[0009] In some embodiments, the cation exchange chromatography material is a strong cation exchanger. In some embodiments, the strong cation exchanger comprises a sulfopropyl group. In some embodiments, the sulphopropyl group is linked to crosslinked agarose. In some embodiments, the cation exchange material is washed in 25 mM 2-(N-morpholino)ethanesulfonic acid (MES) prior to elution. In some embodiments, the FkpA is eluted from the cation chromatography material using a salt gradient. In some embodiments, the salt gradient is a linear gradient. In some embodiments, the salt gradient is a 0-30% gradient from about 25 mM MES, 300 mM NaCl to 25 mM MES 300 mM NaCl over 9 column volumes. In some embodiments, the cation exchange eluate is collected in fractions. In some embodiments, the fractions are analyzed by size exclusion chromatography prior to hydrophobic interaction chromatography. In some embodiments, fractions comprising at least about 25% FkpA are selected for further purification.

[0010] In some embodiments of the disclosure, the HIC material comprises a butyl moiety. In some embodiments, the butyl moiety is linked to a crosslinked agarose. In some embodiments, the cation exchange eluate is conditioned to comprise about 0.6 M sodium sulfate and about 50 mM PO₄, about pH 7 before HIC. In some embodiments, the FkpA-bound HIC material is washed with 40 mM phosphate, 300 mM sodium sulfate, pH 7.0 prior to elution. In some embodiments, the FkpA is eluted from the HIC material using water. In some embodiments, the HIC eluate is collected in fractions. In some embodiments, fractions comprising FkpA are pooled. In some embodiments, fractions comprising at least about 25% FkpA are pooled.

[0011] In some embodiments of the disclosure, the size exclusion chromatography material comprises a spherical composite of cross-linked agarose and dextran. In some embodiments, the size exclusion flow through is collected in fractions. In some embodiments, the HIC eluate is concentrated prior to size exclusion chromatography. In some embodiments, the HIC eluate is concentrated about 6-fold to about 10-fold prior to size exclusion chromatography. In some embodiments, the size exclusion fractions comprising FkpA are pooled.

[0012] In some embodiments of the above embodiments, the FkpA polypeptide is an *Escherichia coli* FkpA polypeptide. In some embodiments, the FkpA polypeptide comprises the amino acid sequence of SEQ ID NO:1. In some embodiments, the amino acid sequence of the FkpA polypeptide comprises at least about 80% identical to the amino acid sequence of SEQ ID NO:1. In some embodiments, the FkpA is expressed in the cell. In some embodiments, the cell is a prokaryotic cell. In some embodiments, the cell is an *E. coli* cell. In some embodiments, the cell is engineered to express FkpA at levels greater than endogenous expression of FkpA. In some embodiments, the cell is lysed using a microfluidizer.

[0013] In some embodiments, the disclosure provides a composition comprising a FkpA polypeptide purified by the methods described herein. In some embodiments, the composition comprises at least about 98% FkpA polypeptide (*e.g.*, 98% monomeric FkpA polypeptide; for example, as measured by size exclusion chromatography). In some embodiments, the composition comprises less than about 2% low molecular weight species. In some embodiments, the composition comprises no more than about 5% high molecular

weight species. In some embodiments, the composition comprises less than about 2% high molecular weight species. In some embodiments, the percentage of FkpA polypeptide (*e.g.*, monomeric FkpA) is detected by size exclusion chromatography. In some embodiments, the composition comprises less than about 5%, about 4%, about 3%, about 2%, about 1% impurities or is substantially free of impurities. In some embodiments, the impurities are high molecular weight and/or low molecular weight polypeptide species relative to FkpA. In some embodiments, the impurities are one or more of an *E. coli* protein (ECP), aggregates of FkpA, fragments of FkpA, a nucleic acid or a cell culture media component.

[0014] In some embodiments of the above compositions, the purified FkpA is stable to one or more freeze-thaw cycles. In some embodiments, the FkpA is stable to three freeze-thaw cycles. In some embodiments, the composition comprises at least about 70% FkpA after freeze-thaw. In some embodiments, the composition comprises at least about 80% FkpA after freeze-thaw.

[0015] In some embodiments of the above compositions, the purity of the FkpA polypeptide in the composition is measured by one or more of chromatography, SDS polyacrylamide gel electrophoresis or western blot analysis. In some embodiments, the purity of the FkpA polypeptide in the composition is measured by high performance liquid chromatography (HPLC). In some embodiments, the purity of the FkpA polypeptide in the composition is measured by size exclusion chromatography (SEC). In some embodiments, the purity of the FkpA polypeptide in the composition is measured by SDS gel electrophoresis using a fluorescent imaging or a silver stain. In some embodiments, the presence of non-FkpA polypeptides in the composition are identified by the presence of species identified by gel electrophoresis that are not immunoreactive with anti-FkpA antibodies as shown by western blot analysis. In some embodiments, the presence of aggregates of the FkpA polypeptide in the composition are identified by the presence of species with a molecular weight greater than the native FkpA by western blot analysis.

[0016] In some embodiments, the disclosure provides methods for generating antibodies that specifically bind FkpA, comprising exposing or immunizing an animal to a composition comprising FkpA purified by the methods described herein. In some embodiments, the methods for generating antibodies that specifically bind FkpA comprise exposing or immunizing an animal to a composition of FkpA as described herein. In some embodiments, the methods further comprise collecting sera from the animal wherein the sera comprises antibodies that specifically bind FkpA. In some embodiments, the sera comprises polyclonal antibodies that specifically bind FkpA. In other embodiments, one or more monoclonal antibodies are isolated from the sera. In some embodiments, the animal is a goat, a rabbit, a mouse, a guinea pig, a hamster, a rat, a donkey or a chicken.

[0017] In some embodiments the disclosure provides compositions comprising polyclonal antibodies that specifically bind FkpA, wherein the polyclonal antibodies are generated by immunizing an animal to a composition comprising FkpA purified by any of the methods described herein. In some embodiments, the polyclonal antibodies are generated by immunizing an animal to a purified FkpA composition as described herein. In some embodiments, the polyclonal antibodies are collected from sera of the animal.

In other embodiments, the disclosure provides monoclonal antibodies that specifically bind FkpA, wherein the monoclonal antibodies are generated by immunizing an animal to a composition comprising FkpA purified by any of the methods described herein. In some embodiments, the monoclonal antibodies are generated by immunizing an animal to a purified FkpA composition as described herein. In some embodiments, the animal is a goat, a rabbit, a mouse, a guinea pig, a hamster, a rat, a donkey or a chicken.

[0018] In some embodiments, the disclosure provides methods for purifying antibodies that specifically bind FkpA, comprising contacting a composition that comprises anti-FkpA antibodies with ammonium sulfate to a final concentration of ammonium sulfate of about 60%, centrifuging the solutions, removing the supernatants and dissolving the pellets in phosphate buffer. In some embodiments, the phosphate buffer comprises about 50 mM sodium phosphate at about pH 7.0. In some embodiments, the method for purifying antibodies that specifically bind FkpA comprise a) contacting a composition that comprises anti-FkpA antibodies with ammonium sulfate to a final concentration of ammonium sulfate of about 60%, centrifuging the solutions, removing the supernatants and dissolving the pellets in phosphate buffer to generate an anti-FkpA antibody solution; b) applying the anti-FkpA antibody solution to an affinity chromatography material, c) eluting the antibodies from the affinity material to generate an affinity eluate comprising the anti-FkpA antibodies; and d) subjecting the affinity eluate to size exclusion chromatography, wherein fractions comprising the purified antibodies are collected from the size exclusion chromatography. In some embodiments, the phosphate buffer comprises about 50 mM sodium phosphate at about pH 7.0. In some embodiments, the affinity chromatography material comprises purified FkpA linked to a chromatography media. In some embodiments, the chromatography media is a cross-linked dextran. In some embodiments, the FkpA is purified by any of the method described herein. In some embodiments, the FkpA is derived from any of the compositions described herein. In some embodiments, the FkpA is covalently linked to the chromatography media. In some embodiments, the FkpA is linked to the chromatography media using Glyceryl Controlled Pore Glass (Glyceryl-CPG). In some embodiments, the anti-FkpA antibodies are eluted from the affinity column using phosphate buffered saline at about pH 2.0. In some embodiments, the elution pool is collected into about 1 M Tris, about pH 8.0. In some embodiments, the size exclusion chromatography material comprises a spherical composite of cross-linked agarose and dextran. In some embodiments, the spherical composite of cross-linked agarose and dextran has a separation range for molecules with molecular weights between 10,000 daltons and 600,000 daltons. In some embodiments, the size exclusion flow through is collected in fractions. In some embodiments, the size exclusion fractions comprising anti-FkpA antibodies are pooled. In some embodiments, the antibodies are polyclonal antibodies. In some embodiments, the antibodies are prepared according to any of the methods described herein. In some embodiments, less than about 1% of the antibodies specifically bind non-FkpA compounds. In some embodiments, the disclosure provides isolated anti-FkpA antibodies purified by any of the methods described herein.

[0019] In some embodiments, the disclosure provides methods for quantifying FkpA in a sample, comprising detecting FkpA in the sample using a detection system and comparing the amount of FkpA detected in the sample with the detection of one or more concentrations of an ultrapure FkpA reference standard. In some embodiments, the ultrapure FkpA reference standard comprises at least about 95%, about 96%, about 97%, or about 98% monomeric FkpA polypeptide. In some embodiments, the ultrapure FkpA reference standard is prepared by any of the methods described herein or comprises any of the FkpA compositions described herein. In some embodiments, the detection system is an immunoassay. In some embodiments, the immunoassay comprises antibodies that specifically binds the ultrapure FkpA reference standard.

[0020] In some embodiments, the disclosure provides methods for analyzing a recombinant polypeptide sample for the presence of and/or quantity of FkpA, comprising detecting FkpA in the sample using an immunoassay and comparing the amount of FkpA detected in the sample with the detection of one or more concentrations of an ultrapure FkpA reference standard. In some embodiments, the ultrapure FkpA reference standard comprises at least about 98% monomeric FkpA polypeptide. In some embodiments, the ultrapure FkpA reference standard is prepared by any of the methods described herein. In some embodiments, the immunoassay comprises antibodies that specifically bind the ultrapure FkpA reference standard. In some embodiments, the antibodies that specifically bind the ultrapure FkpA are polyclonal antibodies. In some embodiments, the antibodies that specifically bind the ultrapure FkpA are used as capture antibodies in the immunoassay. In some embodiments, the antibodies that specifically bind ultrapure FkpA are used as detection antibodies. In some embodiments, the detection antibodies are conjugated to a detection agent. In some embodiments, the detection agent is a horseradish peroxidase. In some embodiments, the antibodies that specifically bind FkpA are prepared according to any of the methods described herein. In some embodiments, the FkpA is an *E. coli* FkpA. In some embodiments, the sample comprises a recombinant polypeptide prepared in a host cell. In some embodiments, the host cell is an *E. coli* cell. In some embodiments, the host cell overexpresses FkpA. In some embodiments, the sample is a cell lysate. In some embodiments, the sample is obtained from a recombinant polypeptide preparation and wherein the recombinant polypeptide preparation has been subjected to one or more chromatographic purification steps. In some embodiments, the recombinant polypeptide preparation is a final purified product. In some embodiments, the recombinant polypeptide contained in the recombinant polypeptide sample is an antibody or an immunoadhesin. In some embodiments, the antibody is a multispecific antibody, a bispecific antibody, a half antibody or an antibody fragment. In some embodiments, the recombinant polypeptide is an IgG1, an IgG2, an IgG3, or an IgG4.

[0021] In some embodiments, the disclosure provides immunoassay methods for detecting FkpA in a sample, wherein the sample is obtained from a recombinant polypeptide preparation from a host cell line, the method comprising: (a) contacting a capture antibody that binds FkpA with the sample thereby generating a sample-capture antibody combination material; (b) contacting a detection antibody that binds FkpA with the sample-capture antibody combination material; and (c) detecting the antibody bound

domain of the bispecific antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, the IL17 is human IL17. In some embodiments, the second binding domain binds human IL17AA, IL17FF and IL17AF. In some embodiments, the IL17A comprises the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7, and the IL17F comprises the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:9. In some embodiments, the second binding domain of the bispecific antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO:23, HVR-H2 comprising the amino acid sequence of SEQ ID NO:24, HVR-H3 comprising the amino acid sequence of SEQ ID NO:25, HVR-L1 comprising the amino acid sequence of SEQ ID NO:26, HVR-L2 comprising the amino acid sequence of SEQ ID NO:27, and HVR-L3 comprising the amino acid sequence of SEQ ID NO:28. In some embodiments, the second binding domain of the bispecific antibody comprises a VH sequence comprising the amino acid sequence of SEQ ID NO:21 and a VL sequence comprising the amino acid sequence of SEQ ID NO:22. In some embodiments, the second binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:29. In some embodiments, the second binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:30. In some embodiments, the second binding domain of the bispecific antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:31.

[0023] In some embodiments, the disclosure provides a quality assay for a pharmaceutical composition comprising a recombinant polypeptide prepared from a bacterial cell, the quality assay comprising subjecting a sample of the pharmaceutical composition to the immunoassay of as described herein for detecting presence or amount of FkpA in the pharmaceutical composition. In some embodiments, the amount of FkpA detected in the pharmaceutical composition is no more than about 30 ppm, about 25 ppm, about 20 ppm, about 15 ppm, about 14 ppm, about 13 ppm, about 12 ppm, about 11 ppm, about 10 ppm, about 9 ppm, about 8 ppm, about 7 ppm, about 6 ppm, about 5 ppm, about 4 ppm, about 3 ppm, about 2 ppm, about 1 ppm, about 0.9 ppm, about 0.8 ppm, about 0.7 ppm, about 0.6 ppm, about 0.5 ppm, about 0.4 ppm, about 0.3 ppm, about 0.2 ppm, or about 0.1 ppm as determined by immunoassay. In some embodiments, the bacterial cell is an *E. coli* cell. In some embodiments, the bacterial cell expresses exogenous FkpA. In some embodiments, the sample is a cell lysate. In some embodiments, the sample is obtained from the recombinant polypeptide preparation and wherein the recombinant polypeptide preparation has been subjected to one or more chromatographic purification steps. In some embodiments, the recombinant polypeptide preparation is a final purified product. In some embodiments, the recombinant polypeptide is an antibody or an immunoadhesin. In some embodiments, the antibody is a multispecific antibody, a bispecific antibody, a half antibody or an antibody fragment. In some embodiments, the recombinant polypeptide is an IgG1, an IgG2, an IgG3, or an IgG4.

[0024] In some embodiments, the disclosure provides a quality assay for a bispecific antibody comprising a first binding domain that binds IL13 and a second binding domain that binds IL17. In some embodiments, the IL13 comprises the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:5. In some

embodiments, the first binding domain of the bispecific antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO:12, HVR-H2 comprising the amino acid sequence of SEQ ID NO:13, HVR-H3 comprising the amino acid sequence of SEQ ID NO:14, HVR-L1 comprising the amino acid sequence of SEQ ID NO:15, HVR-L2 comprising the amino acid sequence of SEQ ID NO:16, and HVR-L3 comprising the amino acid sequence of SEQ ID NO:17. In some embodiments, the first binding domain of the bispecific antibody comprises a VH sequence comprising the amino acid sequence of SEQ ID NO:10 and a VL sequence comprising the amino acid sequence of SEQ ID NO:11. In some embodiments, the first binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the first binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:19. In some embodiments, the first binding domain of the bispecific antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, the IL17 is human IL17. In some embodiments, the second binding domain binds human IL17AA, IL17FF and IL17AF. In some embodiments, the IL17A comprises the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7 and IL17F comprises the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:9. In some embodiments, the second binding domain of the bispecific antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO:23, HVR-H2 comprising the amino acid sequence of SEQ ID NO:24, HVR-H3 comprising the amino acid sequence of SEQ ID NO:25, HVR-L1 comprising the amino acid sequence of SEQ ID NO:26, HVR-L2 comprising the amino acid sequence of SEQ ID NO:27, and HVR-L3 comprising the amino acid sequence of SEQ ID NO:28. In some embodiments, the second binding domain of the bispecific antibody comprises a VH sequence comprising the amino acid sequence of SEQ ID NO:21 and a VL sequence comprising the amino acid sequence of SEQ ID NO:22. In some embodiments, the second binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:29. In some embodiments, the second binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:30. In some embodiments, the second binding domain of the bispecific antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:31. In some embodiments, the assay further comprising the step of detecting an amount of DsbA and/or DsbC in the pharmaceutical composition. In some embodiments, the amount of DsbA is no more than about 5 ppm, about 4 ppm, about 3 ppm, about 2 ppm, about 1 ppm, about 0.9 ppm, about 0.8 ppm, about 0.7 ppm, about 0.6 ppm, about 0.5 ppm, about 0.4 ppm, about 0.3 ppm, about 0.2 ppm, or about 0.1 ppm. In some embodiments, the amount of DsbC is no more than about 5 ppm, about 4 ppm, about 3 ppm, about 2 ppm, about 1 ppm, about 0.9 ppm, about 0.8 ppm, about 0.7 ppm, about 0.6 ppm, about 0.5 ppm, about 0.4 ppm, about 0.3 ppm, about 0.2 ppm, or about 0.1 ppm as determined by immunoassay.

[0025] In some embodiments, the disclosure provides kits for the detection of FkpA in a pharmaceutical composition comprising a recombinant polypeptide prepared from a bacterial cell, said kit comprising anti-FkpA antibodies prepared by any of the methods described herein or any of the

compositions of anti-FkpA antibodies described herein. In some embodiments, the kit further comprises ultrapure FkpA for use as a reference standard in generating standard curves for quantitating FkpA in a sample and/or for use as a positive control. In some embodiments, the ultrapure FkpA is prepared according to any of the methods described herein.

[0026] In some embodiments, the disclosure provides compositions comprising a recombinant polypeptide, wherein the recombinant polypeptide was produced in a host cell that expresses endogenous FkpA, wherein the composition comprises FkpA at a concentration of no more than about 6 ppm, about 5 ppm, about 4 ppm, about 3 ppm, about 2 ppm, about 1 ppm, about 0.9 ppm, about 0.8 ppm, about 0.7 ppm, about 0.6 ppm, about 0.5 ppm, about 0.4 ppm, about 0.3 ppm, about 0.2 ppm, or about 0.1 ppm. In some embodiments, the concentration of FkpA is determined by an immunoassay. In some embodiments, the concentration of FkpA is determined by any of the immunoassays described herein. In some embodiments, the recombinant polypeptide was produced in a host cell that is engineered to express exogenous FkpA, wherein the composition comprises no more than about 15 ppm FkpA, about 6 ppm, about 5 ppm, about 4 ppm, about 3 ppm, about 2 ppm, about 1 ppm, about 0.9 ppm, about 0.8 ppm, about 0.7 ppm, about 0.6 ppm, about 0.5 ppm, about 0.4 ppm, about 0.3 ppm, about 0.2 ppm, or about 0.1 ppm. In some embodiments, the recombinant polypeptide is an antibody. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the composition comprises at least about 0.1 ppm FkpA. In some embodiments, the host cell expresses exogenous FkpA, wherein the composition comprises no more than about 13 ppm. In some embodiments, the host cell expresses exogenous FkpA, wherein the composition comprises no more than about 0.7 ppm. In some embodiments, the host cell expresses exogenous FkpA, wherein the composition comprises no more than about 6 ppm.

[0027] In some embodiments, the disclosure provides compositions comprising a bispecific antibody, wherein the bispecific antibody comprises a first binding domain that binds IL13, and a second binding domain that binds IL17. In some embodiments, the IL13 comprises the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:5. In some embodiments, the first binding domain of the bispecific antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO:12, HVR-H2 comprising the amino acid sequence of SEQ ID NO:13, HVR-H3 comprising the amino acid sequence of SEQ ID NO:14, HVR-L1 comprising the amino acid sequence of SEQ ID NO:15, HVR-L2 comprising the amino acid sequence of SEQ ID NO:16, and HVR-L3 comprising the amino acid sequence of SEQ ID NO:17. In some embodiments, the first binding domain of the bispecific antibody comprises a VH sequence comprising the amino acid sequence of SEQ ID NO:10 and a VL sequence comprising the amino acid sequence of SEQ ID NO:11. In some embodiments, the first binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the first binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:19. In some embodiments, the first binding domain of the bispecific antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, the IL17 is human IL17. In some embodiments, the second binding domain binds human IL17AA,

IL17FF and IL17AF. In some embodiments, the IL17A comprises the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7 and IL17F comprises the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:9. In some embodiments, the second binding domain of the bispecific antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO:23, HVR-H2 comprising the amino acid sequence of SEQ ID NO:24, HVR-H3 comprising the amino acid sequence of SEQ ID NO:25, HVR-L1 comprising the amino acid sequence of SEQ ID NO:26, HVR-L2 comprising the amino acid sequence of SEQ ID NO:27, and HVR-L3 comprising the amino acid sequence of SEQ ID NO:28. In some embodiments, the second binding domain of the bispecific antibody comprises a VH sequence comprising the amino acid sequence of SEQ ID NO:21 and a VL sequence comprising the amino acid sequence of SEQ ID NO:22. In some embodiments, the second binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:29. In some embodiments, the second binding domain of the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:30. In some embodiments, the second binding domain of the bispecific antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:31. In some embodiments, the amount of DsbA is no more than about 5 ppm, about 4 ppm, about 3 ppm, about 2 ppm, about 1 ppm, about 0.9 ppm, about 0.8 ppm, about 0.7 ppm, about 0.6 ppm, about 0.5 ppm, about 0.4 ppm, about 0.3 ppm, about 0.2 ppm, or about 0.1 ppm. In some embodiments, the amount of DsbA is determined by an immunoassay. In some embodiments, the amount of DsbC is no more than about 5 ppm, about 4 ppm, about 3 ppm, about 2 ppm, about 1 ppm, about 0.9 ppm, about 0.8 ppm, about 0.7 ppm, about 0.6 ppm, about 0.5 ppm, about 0.4 ppm, about 0.3 ppm, about 0.2 ppm, or about 0.1 ppm. In some embodiments, the amount of DsbC is determined by an immunoassay. In some embodiments, the composition is substantially free of FkpA. In some embodiments, the composition is substantially free of DsbA. In some embodiments, the composition is substantially free of DsbC.

[0028] In some embodiments, the disclosure provides compositions comprising a bispecific antibody, wherein the recombinant polypeptide was produced by a method comprising a) introducing to a host cell i) nucleic acid encoding a first heavy chain and a first light chain, wherein the first heavy chain and the first light chain form a first antigen binding domain; ii) nucleic acid encoding a second heavy chain and a second light chain, wherein the second heavy chain and the second light chain form a second antigen binding domain; iii) nucleic acid encoding a FkpA polypeptide; wherein the first heavy chain and the second heavy chain form an Fc domain; and b) purifying the antibody, wherein the composition comprises less than about 5 ppm FkpA. In some embodiments, the disclosure provides a composition comprising a bispecific antibody, wherein the bispecific antibody was produced by a method comprising a) introducing to a first host cell i) nucleic acid encoding a first heavy chain and a first light chain, wherein the first heavy chain and the first light chain form a first antigen binding domain; ii) nucleic acid encoding a FkpA polypeptide; b) introducing to a second host cell i) nucleic acid encoding a second heavy chain and a second light chain, wherein the second heavy chain and the second light chain form a second antigen binding domain; ii) nucleic acid encoding a FkpA polypeptide; b) isolating the first

comprises no more than about 15 ppm DsbA. In some embodiments, the composition comprises no more than about 5 ppm DsbA. In some embodiments, the composition comprises no more than about 15 ppm DsbC. In some embodiments, the composition comprises no more than about 5 ppm DsbC. In some embodiments, the DsbA is determined by immunoassay. In some embodiments, the DsbC is measured by immunoassay.

[0029] The disclosure provides methods for purifying a polypeptide from a composition comprising the multispecific antibody and one or more impurities, the method comprising the sequential steps of a) subjecting the composition to affinity chromatography to produce an affinity eluate, b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to hydrophobic interaction chromatography and collecting a fraction comprising the polypeptide, wherein the method reduces the amount of product-specific impurities from the composition. In one aspect, there is provided a method for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities, a) subjecting the composition to affinity chromatography to produce an affinity eluate, b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to hydrophobic interaction chromatography and collecting a fraction comprising the polypeptide, wherein the method reduces the amount of product-specific impurities from the composition. In some embodiments, the mixed-mode chromatography is a mixed-mode anion exchange chromatography. In some embodiments, the affinity chromatography is carried out in bind and elute mode. In some embodiments, the mixed-mode chromatography is carried out in bind and elute mode. In some embodiments, the HIC is carried out in flow-through mode.

[0030] In one aspect, there is provided a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce a protein A eluate, b) subjecting the protein A eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to hydrophobic interaction chromatography and collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of product-specific impurities from the composition. In some embodiments, the mixed-mode chromatography is a mixed-mode anion exchange chromatography. In some embodiments, the affinity chromatography is carried out in bind and elute mode. In some embodiments, the mixed-mode chromatography is carried out in bind and elute mode. In some embodiments, the HIC is carried out in flow-through mode.

[0031] In some aspects, there is provided methods for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce protein A eluates for each arm of the multispecific antibody, b) forming a mixture comprising protein A 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 mixed-mode chromatography to generate a mixed-mode eluate, and d) subjecting the mixed-mode eluate to hydrophobic interaction chromatography (HIC) and collecting a fraction comprising the multispecific antibody wherein the method reduces the amount of product-specific impurities from the composition. In

some embodiments, the mixed-mode chromatography is a mixed-mode anion exchange chromatography. In some embodiments, the affinity chromatography is carried out in bind and elute mode. In some embodiments, the mixed-mode chromatography is carried out in bind and elute mode. In some embodiments, the hydrophobic interaction chromatography is carried out in flow-through mode. In some embodiments, the mixed-mode eluate is subjected to anion exchange chromatography prior to hydrophobic interaction chromatography. In some embodiments, the anion exchange chromatography is carried out in bind and elute mode.

[0032] In some embodiments of the above aspects, the methods further comprising the step of subjecting the hydrophobic interaction chromatography fraction comprising the multispecific antibody to cation exchange chromatography and collecting a fraction comprising the multispecific antibody. In some embodiments, the cation exchange chromatography is carried out in bind and elute mode.

[0033] In some embodiments, the disclosure provides methods for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities, the method comprising the sequential steps of a) subjecting the composition to affinity chromatography to produce an affinity eluate, b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate, d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the polypeptide, wherein the method reduces the amount of product-specific impurities from the composition. In one aspect, there is provided a method for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities, a) subjecting the composition to affinity chromatography to produce a affinity eluate, b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate, d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the polypeptide, wherein the method reduces the amount of product-specific impurities from the composition.

[0034] In some aspects, there is provided methods for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce a protein A eluate, b) subjecting the protein A eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate, d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of product-specific impurities from the composition.

[0035] In some aspects, there is provided methods for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce protein A eluates for each arm of the multispecific antibody, b) forming a mixture comprising protein A 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 mixed-mode chromatography to generate a mixed-mode eluate, and d) subjecting the mixed-mode eluate to anion

exchange chromatography to generate an anion exchange eluate, e) subjecting the anion exchange eluate to hydrophobic interaction chromatography in and collecting a fraction comprising the multispecific antibody and collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of product-specific impurities from the composition.

[0036] In some embodiments of the above aspects, the mixed-mode chromatography is a mixed-mode anion exchange chromatography. In some embodiments, the affinity chromatography is carried out in bind and elute mode. In some embodiments, the mixed-mode chromatography is carried out in bind and elute mode. In some embodiments, the anion exchange chromatography is carried out in bind and elute mode. In some embodiments, the hydrophobic interaction chromatography is carried out in flow-through mode. In some embodiments, the methods further comprise the step of subjecting the hydrophobic interaction chromatography fraction comprising the multispecific antibody to cation exchange chromatography. In some embodiments, the cation exchange chromatography is carried out in bind and elute mode. In some embodiments, the methods further comprise the step of subjecting the hydrophobic interaction chromatography fraction comprising the multispecific antibody to ultrafiltration. In some embodiments, the methods further comprise the step of subjecting the cation exchange fraction comprising the multispecific antibody to ultrafiltration. In some embodiments, the ultrafiltration comprises sequentially a first ultrafiltration, a diafiltration and a second ultrafiltration.

[0036a] In one aspect, there is provided a method for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities, a) subjecting the composition to affinity chromatography to produce a affinity eluate, b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate, d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the polypeptide, e) subjecting the hydrophobic interaction eluate to cation exchange chromatography and collecting a fraction comprising the polypeptide, wherein the method reduces the amount of product-specific impurities from the composition.

[0036b] In one aspect, there is provided a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce a protein A eluate, b) subjecting the protein A eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate, d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the multispecific antibody, e) subjecting the hydrophobic interaction eluate to cation exchange chromatography and collecting a fraction comprising the polypeptide, wherein the method reduces the amount of product-specific impurities from the composition.

[0036c] In one aspect, there is provided a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce protein A eluates for each arm of the multispecific antibody, b) forming a mixture comprising protein A 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 mixed-mode chromatography to generate a mixed-mode eluate, and d) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate, e) subjecting the anion exchange eluate to hydrophobic interaction chromatography in and collecting a fraction comprising the multispecific antibody and collecting a fraction comprising the multispecific antibody, f) subjecting the hydrophobic interaction eluate to cation exchange chromatography and collecting a fraction comprising the polypeptide, wherein the method reduces the amount of product-specific impurities from the composition.

[0037] In some aspects, there is provided a method for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities, the method comprising the sequential steps of: a) subjecting the composition to affinity chromatography to produce a affinity eluate, b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate, d) subjecting the anion exchange eluate to hydroxyapatite chromatography and collecting a fraction comprising the polypeptide, wherein the method reduces the amount of product-specific impurities from the composition. In some aspects, the invention provides a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce a protein A eluate, b) subjecting the protein A eluate to mixed-mode chromatography to generate a mixed-mode eluate, and c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate, d) subjecting the anion exchange eluate to hydroxyapatite chromatography and collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of product-specific impurities from the composition. In some aspects, the invention provides a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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

species (HMW), low molecular weight species (LMW), acidic variants, or basic variants. In some embodiments, the methods reduce the amount of any one of *E. coli* protein (ECP), FkpA, DsbA, DsbC, leached protein A, nucleic acid, cell culture media components, or viral impurities in the composition.

[0048] In some embodiments, the multispecific antibody is a bispecific antibody. In some embodiments, a first arm of the multispecific antibody binds IL13. In some embodiments, the IL13 is human IL13. In some embodiments, the IL13 comprises the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2. In some embodiments, the first arm of the multispecific antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO:7, HVR-H2 comprising the amino acid sequence of SEQ ID NO:8, HVR-H3 comprising the amino acid sequence of SEQ ID NO:9, HVR-L1 comprising the amino acid sequence of SEQ ID NO:10, HVR-L2 comprising the amino acid sequence of SEQ ID NO:11, and HVR-L3 comprising the amino acid sequence of SEQ ID NO:12. In some embodiments, the first arm of the multispecific antibody comprises a VH sequence comprising the amino acid sequence of SEQ ID NO:13 and a VL sequence comprising the amino acid sequence of SEQ ID NO:14. In some embodiments, the first arm of the multispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:15. In some embodiments, the first arm of the multispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:16. In some embodiments, the first arm of the multispecific antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:17. In some embodiments, a second arm of the antibody binds IL17. In some embodiments, the IL17 is human IL17. In some embodiments, the IL17 comprises the amino acid sequence of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6. In some embodiments, a second arm of the multispecific antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO:18, HVR-H2 comprising the amino acid sequence of SEQ ID NO:19, HVR-H3 comprising the amino acid sequence of SEQ ID NO:20, HVR-L1 comprising the amino acid sequence of SEQ ID NO:21, HVR-L2 comprising the amino acid sequence of SEQ ID NO:22, and HVR-L3 comprising the amino acid sequence of SEQ ID NO:23. In some embodiments, the second arm of the multispecific antibody comprises a VH sequence comprising the amino acid sequence of SEQ ID NO:25 and a VL sequence comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the second arm of the multispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:27. In some embodiments, the second arm of the multispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:28. In some embodiments, the second arm of the multispecific antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:29.

[0049] In some aspects, there is provided a composition comprising a polypeptide or a multispecific antibody purified by any of the method described herein, wherein the composition comprises no more than about 5%, 4%, 3%, 2% or 1% product specific impurities. In some embodiments, the product specific impurity is one or more of non-paired antibody arms, antibody homodimers, aggregates, high molecular weight species (HMW), low molecular weight species (LMW), acidic variants, or basic variants. In some embodiments, the composition comprises less than about composition comprises no more than about 5%,

CLAIMS

WHAT IS CLAIMED IS:

1. A method for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities,
 - a) subjecting the composition to affinity chromatography to produce an affinity eluate,
 - b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and
 - c) subjecting the mixed-mode eluate to hydrophobic interaction chromatography and collecting a fraction comprising the polypeptide,wherein the method reduces the amount of product-specific impurities from the composition.
2. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce a protein A eluate,
 - b) subjecting the protein A eluate to mixed-mode chromatography to generate a mixed-mode eluate, and
 - c) subjecting the mixed-mode eluate to hydrophobic interaction chromatography and collecting a fraction comprising the multispecific antibody,wherein the method reduces the amount of product-specific impurities from the composition.
3. The method of claim 1 or 2, wherein the mixed-mode chromatography is a mixed-mode anion exchange chromatography.
4. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce protein A eluates for each arm of the multispecific antibody,
 - b) forming a mixture comprising protein A 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 mixed-mode chromatography to generate a mixed-mode eluate, and
 - d) subjecting the mixed-mode eluate to hydrophobic interaction chromatography (HIC) and collecting a fraction comprising the multispecific antibody

wherein the method reduces the amount of product-specific impurities from the composition.

5. The method of claim 4, wherein the mixed-mode chromatography is a mixed-mode anion exchange chromatography.
6. The method of any one of claims 1-5, wherein the mixed-mode eluate is subjected to anion exchange chromatography prior to hydrophobic interaction chromatography.
7. The method of any one of claims 1-6, further comprising the step of subjecting the hydrophobic interaction chromatography fraction comprising the polypeptide or multispecific antibody to cation exchange chromatography and collecting a fraction comprising the polypeptide or multispecific antibody.
8. A method for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities,
 - a) subjecting the composition to affinity chromatography to produce a affinity eluate,
 - b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and
 - c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,
 - d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the polypeptide,wherein the method reduces the amount of product-specific impurities from the composition.
9. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce a protein A eluate,
 - b) subjecting the protein A eluate to mixed-mode chromatography to generate a mixed-mode eluate, and
 - c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,
 - d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the multispecific antibody,wherein the method reduces the amount of product-specific impurities from the composition.
10. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce protein A eluates for each arm of the multispecific antibody,

b) forming a mixture comprising protein A 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 mixed-mode chromatography to generate a mixed-mode eluate, and

d) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,

e) subjecting the anion exchange eluate to hydrophobic interaction chromatography in and collecting a fraction comprising the multispecific antibody and collecting a fraction comprising the multispecific antibody,

wherein the method reduces the amount of product-specific impurities from the composition.

11. The method of any one of claims 8-10, wherein the mixed-mode chromatography is a mixed-mode anion exchange chromatography.

12. The method of any one of claims 8-11, further comprising the step of subjecting the hydrophobic interaction chromatography fraction comprising the polypeptide or multispecific antibody to cation exchange chromatography.

13. A method for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities,

a) subjecting the composition to affinity chromatography to produce a affinity eluate,

b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and

c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,

d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the polypeptide,

e) subjecting the hydrophobic interaction eluate to cation exchange chromatography and collecting a fraction comprising the polypeptide,

wherein the method reduces the amount of product-specific impurities from the composition.

14. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce a protein A eluate,
 - b) subjecting the protein A eluate to mixed-mode chromatography to generate a mixed-mode eluate, and
 - c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,
 - d) subjecting the anion exchange eluate to hydrophobic interaction chromatography and collecting a fraction comprising the multispecific antibody,
 - e) subjecting the hydrophobic interaction eluate to cation exchange chromatography and collecting a fraction comprising the polypeptide,
- wherein the method reduces the amount of product-specific impurities from the composition.

15. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce protein A eluates for each arm of the multispecific antibody,
 - b) forming a mixture comprising protein A 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 mixed-mode chromatography to generate a mixed-mode eluate, and
 - d) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,
 - e) subjecting the anion exchange eluate to hydrophobic interaction chromatography in and collecting a fraction comprising the multispecific antibody and collecting a fraction comprising the multispecific antibody,
 - f) subjecting the hydrophobic interaction eluate to cation exchange chromatography and collecting a fraction comprising the polypeptide,
- wherein the method reduces the amount of product-specific impurities from the composition.

16. The method of any one of claims 13-15, wherein the mixed-mode chromatography is a mixed-mode anion exchange chromatography.

17. A method for purifying a polypeptide from a composition comprising the polypeptide and one or more impurities, the method comprising the sequential steps of
- a) subjecting the composition to affinity chromatography to produce a affinity eluate,
 - b) subjecting the affinity eluate to mixed-mode chromatography to generate a mixed-mode eluate, and
 - c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,
 - d) subjecting the anion exchange eluate to hydroxyapatite chromatography and collecting a fraction comprising the polypeptide,
- wherein the method reduces the amount of product-specific impurities from the composition.
18. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce a protein A eluate,
 - b) subjecting the protein A eluate to mixed-mode chromatography to generate a mixed-mode eluate, and
 - c) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,
 - d) subjecting the anion exchange eluate to hydroxyapatite chromatography and collecting a fraction comprising the multispecific antibody,
- wherein the method reduces the amount of product-specific impurities from the composition.
19. A method for purifying a multispecific antibody from a composition comprising the multispecific antibody and one or more impurities, 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 protein A chromatography to produce protein A eluates for each arm of the multispecific antibody,
 - b) forming a mixture comprising protein A 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 mixed-mode chromatography to generate a mixed-mode eluate, and
 - d) subjecting the mixed-mode eluate to anion exchange chromatography to generate an anion exchange eluate,

e) subjecting the anion exchange eluate to hydroxyapatite chromatography in and collecting a fraction comprising the multispecific antibody and collecting a fraction comprising the multispecific antibody,

wherein the method reduces the amount of product-specific impurities from the composition.

20. The method of any one of claims 17-19, wherein the mixed-mode chromatography is a mixed-mode anion exchange chromatography.

21. The method of any one of claims 1-7, or 12-16 further comprising the step of subjecting the hydroxyapatite interaction chromatography fraction comprising the polypeptide or multispecific antibody to ultrafiltration.

22. The method of claim 7 or 12-16, further comprising the step of subjecting the cation exchange fraction comprising the polypeptide or multispecific antibody to ultrafiltration.

23. The method of claim 13-20, further comprising the step of subjecting the hydroxyapatite chromatography fraction comprising the polypeptide or multispecific antibody to ultrafiltration.

24. The method of any one of claims 21-23 wherein the ultrafiltration comprises sequentially a first ultrafiltration, a diafiltration and a second ultrafiltration.

25. The method of any one of claims 1-24, wherein the 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 6 and about pH 7.

26. The method of claim 25, wherein the mixed-mode pre-equilibration buffer, the mixed-mode equilibration buffer, and/or mixed-mode wash buffer is about pH 6.5.

27. The method of claim 25 or 26, wherein the mixed-mode pre-equilibration buffer comprises about 200 mM acetate or about 500 mM acetate.

28. The method of any one of claims 25-27, wherein the mixed-mode equilibration buffer comprises about 50 mM acetate.

29. The method of any one of claims 25-28, wherein the mixed-mode chromatography is washed with wash buffer following load.

30. The method of claim 29 wherein the wash buffer is about 50 mM acetate and about pH 6.5 or about 200 mM acetate and about pH 6.5
31. The method of any one of claims 1-30, wherein the polypeptide or multispecific antibody is eluted from the mixed-mode chromatography by pH gradient.
32. The method of any one of claims 1-31, wherein the polypeptide or multispecific antibody is eluted from the mixed-mode chromatography by applying a mixed-mode elution buffer with low pH to the mixed-mode anion exchange chromatography.
33. The method of claim 32, wherein the mixed-mode elution buffer comprises about 25 mM acetate and about pH 5.2 or about pH 5.0.
34. The method of any one of claims 1-33 wherein the mixed-mode eluate is pooled where the OD₂₈₀ of the eluate is greater than about 0.5 to about 1.0.
35. The method of any one of claims 1-16, wherein the hydrophobic interaction chromatography (HIC) comprises a hydrophobic moiety.
36. The method of claim 35, wherein the hydrophobic interaction chromatography comprises a phenyl moiety.
37. The method of claim 35 or 36, wherein the hydrophobic interaction chromatography comprises a hydrophobic moiety linked to crosslinked agarose.
38. The method of any one of claims 35-37, wherein the hydrophobic interaction chromatography comprises a phenyl moiety or a butyl moiety linked to crosslinked agarose.
39. The method of any one of claims 35-38, wherein the hydrophobic interaction chromatography comprises a hexyl moiety.
40. The method of claim 39, wherein the hydrophobic interaction exchange chromatography uses one or more of a HIC equilibration buffer, a HIC loading buffer or a HIC wash buffer wherein the HIC equilibration buffer, the HIC load buffer and/or the HIC wash buffer is between about pH 6 and about pH 8.

41. The method of claim 40, wherein the HIC equilibration buffer, the load buffer, and/or the wash buffer is about pH 7.0.
42. The method of claim 40 or 41, wherein the HIC equilibration buffer and the wash buffer comprises about 50 mM 3-(N-Morpholino)propanesulfonic acid (MOPS) and 125 mM acetate.
43. The method of any one of claims 39-42, wherein the hydrophobic interaction chromatography is washed with HIC wash buffer following load.
44. The method of any one of claims 35-43 wherein the hydrophobic interaction eluate is pooled where the OD₂₈₀ of the eluate is greater than about 0.5 to about 1.0.
45. The method of any one of claims 6 or 8-44, where anion exchange chromatography comprises a quaternary amine.
46. The method of claim 45, wherein the anion exchange chromatography comprises a quaternary amine linked to crosslinked agarose.
47. The method of claim 46, wherein the mixed-mode anion exchange chromatography is a QSFF chromatography.
48. The method of any one of claims 45-47, 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 about pH 8 and about pH 9.
49. The method of claim 48, wherein the anion exchange pre-equilibration buffer, the anion exchange equilibration buffer and/or the anion exchange load buffer is about pH 8.5.
50. The method of claim 48 or 49, wherein the anion exchange pre-equilibration buffer comprises about 50 mM Tris and about 500 mM sodium acetate or about 100 mM sodium acetate.
51. The method of any one of claims 48-50, wherein the anion exchange equilibration buffer comprises about 50 mM Tris.
52. The method of any one of claims 48-51, wherein the anion exchange chromatography is washed with anion exchange equilibration buffer following load.

53. The method of any one of claims 48-52, wherein the polypeptide or multispecific antibody is eluted from the anion exchange chromatography by salt gradient.
54. The method of any one of claims 48-53, wherein the polypeptide or 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.
55. The method of claim 54, wherein the anion exchange elution buffer comprises about 50 mM Tris and about 100 mM sodium acetate at about pH 8.5.
56. The method of any one of claims 48-55 wherein the anion exchange eluate is pooled where the OD₂₈₀ of the eluate is greater than about 0.5 to about 1.0.
57. The method of any one of claims 13-24, wherein the hydroxyapatite chromatography uses one or more of a hydroxyapatite buffer A or hydroxyapatite buffer B wherein the hydroxyapatite buffer B has a higher conductivity compared to hydroxyapatite buffer A.
58. The method of claim 57, wherein the hydroxyapatite buffer A is an equilibration buffer comprising about 10 mM sodium phosphate at about pH 6.8.
59. The method of claim 57 or 58, wherein the hydroxyapatite buffer B comprises about 10 mM sodium phosphate and about 1 M sodium chloride at about pH 6.8.
60. The method of any one of claims 57-59, wherein the polypeptide or multispecific antibody is eluted from the hydroxyapatite chromatography using a gradient of hydroxyapatite buffer B in hydroxyapatite buffer A.
61. The method of claim 60, wherein the gradient is a linear gradient.
62. The method of any one of claims 60-61, wherein the gradient increases from 0% hydroxyapatite buffer B to 100% hydroxyapatite buffer B in about 20 column volumes.
63. The method of claim 7 or 12-46, where cation exchange chromatography comprises a carboxylic acid moiety or a sulfonic acid moiety.

64. The method of claim 63, wherein the cation exchange chromatography uses one or more of a cation exchange pre-equilibration buffer, an cation exchange equilibration buffer, cation exchange loading buffer or cation exchange wash buffer wherein the cation exchange pre-equilibration buffer, the cation exchange equilibration buffer, the cation exchange load buffer and/or cation exchange wash buffer is between about pH 5.0 and about pH 6.0.

65. The method of claim 64, wherein the cation exchange cation exchange the equilibration buffer, the cation exchange load buffer and/or cation exchange wash buffer is about pH 5.5 or about pH 5.8.

66. The method of claim 64 or 65, wherein the cation exchange equilibration buffer and/or the cation exchange wash buffer comprises 50 mM sodium acetate.

67. The method of any one of claims 64-66, wherein the polypeptide or multispecific antibody is eluted from the cation exchange chromatography by applying a cation exchange elution buffer with increased salt concentration to the cation exchange chromatography.

68. The method of claim 67, wherein the cation exchange elution buffer comprises 500 mM sodium acetate, pH 5.8.

69. The method of claim 68, wherein the elution is a gradient elution of 10% to 100% cation exchange elution buffer.

70. The method of any one of claims 8-69, wherein the polypeptide or the multispecific antibody is produced in an *E. coli* cell engineered to express one or more chaperones.

71. The method of claim 70, wherein the chaperone is one or more of FkpA, DsbA or DsbC.

72. The method of any one of claims 70-71, wherein polyethyleneimine (PEI) is added to the cell lysate prior to chromatography.

73. The method of any one of claims 2-7, 8-12, 14-16, or 18-72 wherein the multispecific antibody is a bispecific antibody.

74. A composition comprising a polypeptide or a multispecific antibody purified by the method of any one of claims 1-73, wherein the composition comprises no more than about 5%, 4%, 3%, 2% or 1% product specific impurities.

75. The composition of claim 74, wherein the composition comprises less than about composition comprises no more than about 5%, 4%, 3%, 2% or 1% of any one of *E. coli* protein (ECP), FkpA, DsbA, DsbC, leached protein A, nucleic acid, cell culture media components, or viral impurities in the composition.