CLAIMS

What is claimed is:

1. A method for monitoring production of a multispecific binding protein and one or more mispaired species, the method comprising:

detecting, by size-exclusion ultra-performance liquid chromatography and mass spectrometry (SE-UPLC-MS), an amount of a multispecific binding protein and one or more mispaired species in a cell culture medium comprising the multispecific binding protein and the one or more mispaired species, thereby monitoring production of the multispecific binding protein and one or more mispaired species;

wherein the multispecific binding protein comprises an association of two or more polypeptide chains comprising at least a first polypeptide chain and a second polypeptide chain different from the first polypeptide chain;

wherein the one or more mispaired species comprise two or more polypeptide chains comprising at least one of the first and second polypeptide chains in an association other than that of the multispecific binding protein; and

wherein the SE-UPLC is denaturing SE-UPLC.

2. The method of claim 1, wherein the multispecific binding protein is a multispecific antibody comprising a first antibody heavy chain, a first antibody light chain, a second antibody heavy chain different from the first antibody heavy chain, and a second antibody light chain different from the first antibody light chain.

3. The method of claim 2, wherein the one or more mispaired species comprises one or more of:

(i) an association of four polypeptide chains of the multispecific antibody that comprises two of the first antibody heavy chains;

(ii) an association of four polypeptide chains of the multispecific antibody that comprises two of the second antibody heavy chains;

(iii) an association of four polypeptide chains of the multispecific antibody that comprises two of the first antibody light chains; and

(iv) an association of four polypeptide chains of the multispecific antibody that comprises two of the second antibody light chains.

4. The method of claim 1, wherein the multispecific binding protein comprises four polypeptide chains that form the three antigen binding sites,

wherein a first polypeptide chain of the binding protein comprises a structure represented by the formula:

V_{L2}-L_1-V_{L1}-L_2-C_L [I]

and a second polypeptide chain of the binding protein comprises a structure represented by the formula:

V_{H1}-L_3-V_{H2}-L_4-C_{H1}-hinge-C_{H2}-C_{H3} [II]

and a third polypeptide chain of the binding protein comprises a structure represented by the formula:

V_{H3}-C_{H1}-hinge-C_{H2}-C_{H3} [III]

and a fourth polypeptide chain of the binding protein comprises a structure represented by the formula:

V_{L3}-C_L [IV]

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair, wherein V_{H1} and V_{L1} form a first antigen binding site, wherein V_{H2} and V_{L2} form a second antigen binding site, and wherein V_{H3} and V_{L3} form a third antigen binding site.

5. The method of claim 4, wherein the one or more mispaired species comprise one or more of:

(i) an association of four polypeptide chains of the binding protein that comprises two polypeptide chains according to formula I;

(ii) an association of four polypeptide chains of the binding protein that comprises two polypeptide chains according to formula II;

(iii) an association of four polypeptide chains of the binding protein that comprises two polypeptide chains according to formula III; and

(iv) an association of four polypeptide chains of the binding protein that comprises two polypeptide chains according to formula IV.

6. The method of any one of claims 1-5, wherein detecting the amount of the multispecific binding protein and the one or more mispaired species comprises deconvoluting one or more MS spectra obtained by the MS.

7. The method of any one of claims 1-6, wherein a relative amount of the multispecific binding protein as compared to the one or more mispaired species is detected.

8. The method of claim 7, wherein a relative amount of the multispecific binding protein as compared to an amount of one or more individual mispaired species is detected.

9. The method of claim 7, wherein a relative amount of the multispecific binding protein as compared to an overall amount of mispaired species is detected.

10. The method of any one of claims 1-9, wherein the cell culture medium is a cell culture harvest.

11. The method of claim 10, further comprising, prior to detection, separating the cell culture medium from a cell line that produces the multispecific binding protein and one or more mispaired species.

12. The method of claim 11, wherein, prior to (a), the cell culture medium is separated from the cell line by centrifugation.

13. The method of any one of claims 1-12, wherein the cell culture medium is subjected to SE-UPLC without a prior chromatographic separation.

14. The method of any one of claims 1-12, wherein the cell culture medium is subjected to SE-UPLC without prior protein A affinity chromatography.

15. The method of any one of claims 1-14, wherein the MS is intact MS.

16. The method of any one of claims 1-15, wherein the MS is quadrupole time-of-flight (QToF) MS.

17. The method of any one of claims 1-16, wherein the SE-UPLC is directly coupled to the MS.

18. The method of any one of claims 1-17, wherein SE-UPLC is performed with an initial flow rate of less than 0.4 mL/min.

19. The method of claim 18, wherein SE-UPLC is performed with an initial flow rate of 0.1 mL/min.

20. The method of claim 19, wherein SE-UPLC is performed with a flow rate of 0.1 mL/min for the first 25 minutes, followed by a flow rate of 0.4 mL/min.

21. The method of any one of claims 1-20, wherein SE-UPLC is performed using isocratic elution with a mobile phase.

22. The method of claim 21, wherein the mobile phase comprises a solution comprising 30:70 acetonitrile:water.

23. The method of claim 21 or claim 22, wherein the mobile phase comprises formic acid and trifluoroacetic acid (TFA).

24. The method of claim 23, wherein the mobile phase comprises 0.05% formic acid and 0.05% trifluoroacetic acid (TFA).

25. The method of any one of claims 1-24, wherein detection is accomplished in 33 minutes or less.

26. The method of any one of claims 1-25, wherein the MS is capable of resolving a mass difference of 300 Da between the multispecific binding protein and the one or more mispaired species, or between two mispaired species.

27. The method of any one of claims 1-26, wherein the MS is capable of resolving a mass difference of 162 Da between the multispecific binding protein or mispaired species and one of more glycoforms.

28. The method of any one of claims 11-27, wherein the cell line is a mammalian cell line.

29. The method of claim 28, wherein the mammalian cell line is a Chinese hamster ovary (CHO) cell line.

30. The method of any one of claims 1-29, wherein, prior to detection, the cell line is cultured with the cell culture medium in a continuous, stirred tank bioreactor culture.

31. The method of any one of claims 1-29, wherein, prior to detection, the cell line is cultured with the cell culture medium in a batch cell culture.

32. A method for producing a multispecific binding protein, the method comprising:

(a) culturing a cell line comprising one or more polynucleotides encoding the multispecific binding protein in a cell culture medium under conditions suitable for production of the multispecific binding protein and one or more mispaired species by the cell line;

(b) separating, from the cell line, the cell culture medium comprising the multispecific binding protein and one or more mispaired species;

(c) detecting an amount of the multispecific binding protein and one or more mispaired species in the cell culture medium by size-exclusion ultra-performance liquid chromatography and mass spectrometry (SE-UPLC-MS); and

(d) removing at least a portion of one or more of the mispaired species from the multispecific binding protein produced by the cell line, or determining one or both of quality and purity of the multispecific binding protein produced by the cell line;

wherein the multispecific binding protein comprises an association of two or more polypeptide chains comprising at least a first polypeptide chain and a second polypeptide chain different from the first polypeptide chain;

wherein the one or more mispaired species comprise two or more polypeptide chains comprising at least one of the first and second polypeptide chains in an association other than that of the multispecific binding protein; and

wherein the SE-UPLC is denaturing SE-UPLC.

33. The method of claim 32, wherein the multispecific binding protein is a multispecific antibody comprising a first antibody heavy chain, a first antibody light chain, a second antibody heavy chain different from the first antibody heavy chain, and a second antibody light chain different from the first antibody light chain.

34. The method of claim 33, wherein the one or more mispaired species comprises one or more of:

(i) an association of four polypeptide chains of the multispecific antibody that comprises two of the first antibody heavy chains;

(ii) an association of four polypeptide chains of the multispecific antibody that comprises two of the second antibody heavy chains;

(iii) an association of four polypeptide chains of the multispecific antibody that comprises two of the first antibody light chains; and

(iv) an association of four polypeptide chains of the multispecific antibody that comprises two of the second antibody light chains.

35. The method of claim 32, wherein the multispecific binding protein comprises four polypeptide chains that form the three antigen binding sites,

wherein a first polypeptide chain of the binding protein comprises a structure represented by the formula:

V_{L2}-L_1-V_{L1}-L_2-C_L [I]

and a second polypeptide chain of the binding protein comprises a structure represented by the formula:

V_{H1}-L_3-V_{H2}-L_4-C_{H1}-hinge-C_{H2}-C_{H3} [II]

and a third polypeptide chain of the binding protein comprises a structure represented by the formula:

V_{H3}-C_{H1}-hinge-C_{H2}-C_{H3} [III]

and a fourth polypeptide chain of the binding protein comprises a structure represented by the formula:

V_{L3}-C_L [IV]

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair, wherein V_{H1} and V_{L1} form a first antigen binding site, wherein V_{H2} and V_{L2} form a second antigen binding site, and wherein V_{H3} and V_{L3} form a third antigen binding site.

36. The method of claim 35, wherein the one or more mispaired species comprise one or more of:

(i) an association of four polypeptide chains of the binding protein that comprises two polypeptide chains according to formula I;

(ii) an association of four polypeptide chains of the binding protein that comprises two polypeptide chains according to formula II;

(iii) an association of four polypeptide chains of the binding protein that comprises two polypeptide chains according to formula III; and

(iv) an association of four polypeptide chains of the binding protein that comprises two polypeptide chains according to formula IV.

37. The method of any one of claims 32-36, wherein detecting the amount of the multispecific binding protein and one or more mispaired species comprises deconvoluting one or more MS spectra obtained by MS.

38. The method of any one of claims 32-37, wherein detecting the multispecific binding protein and one or more mispaired species comprises assessing overall titer of the multispecific binding protein produced by the cell line.

39. The method of any one of claims 32-38, wherein detecting the multispecific binding protein and one or more mispaired species comprises assessing overall titer of the mispaired species produced by the cell line.

40. The method of any one of claims 32-39, wherein a relative amount of the multispecific binding protein as compared to the one or more mispaired species is detected.

41. The method of claim 40, wherein a relative amount of the multispecific binding protein as compared to an amount of one or more individual mispaired species is detected.

42. The method of claim 40, wherein a relative amount of the multispecific binding protein as compared to an overall amount of mispaired species is detected.

43. The method of any one of claims 32-42, wherein the cell culture medium is separated from the cell line by centrifugation.

44. The method of any one of claims 32-43, wherein the cell culture medium is subjected to SE-UPLC in (c) without a prior chromatographic separation.

45. The method of any one of claims 32-43, wherein the cell culture medium is subjected to SE-UPLC in (c) without prior protein A affinity chromatography.

46. The method of any one of claims 32-45, wherein the MS is intact MS.

47. The method of any one of claims 32-46, wherein the MS is quadrupole time-of-flight (QToF) MS.

48. The method of any one of claims 32-47, wherein the SE-UPLC is directly coupled to the MS.

49. The method of any one of claims 32-48, wherein SE-UPLC is performed with an initial flow rate of less than 0.4 mL/min.

50. The method of claim 49, wherein SE-UPLC is performed with an initial flow rate of 0.1 mL/min.

51. The method of claim 50, wherein SE-UPLC is performed with a flow rate of 0.1 mL/min for the first 25 minutes, followed by a flow rate of 0.4 mL/min.

52. The method of any one of claims 32-51, wherein SE-UPLC is performed using isocratic elution with a mobile phase.

53. The method of claim 52, wherein the mobile phase comprises a solution comprising 30:70 acetonitrile:water.

54. The method of claim 52 or claim 53, wherein the mobile phase comprises formic acid and trifluoroacetic acid (TFA).

55. The method of claim 54, wherein the mobile phase comprises 0.05% formic acid and 0.05% trifluoroacetic acid (TFA).

56. The method of any one of claims 32-55, wherein (c) is accomplished in 33 minutes or less.

57. The method of any one of claims 32-56, wherein the MS is capable of resolving a mass difference of 300 Da between the multispecific binding protein and the one or more mispaired species, or between two mispaired species.

58. The method of any one of claims 32-57, wherein the MS is capable of resolving a mass difference of 162 Da between the multispecific binding protein or mispaired species and one of more glycoforms.

59. The method of any one of claims 32-58, wherein the cell line is a mammalian cell line.

60. The method of claim 59, wherein the mammalian cell line is a Chinese hamster ovary (CHO) cell line.

61. The method of any one of claims 32-60, wherein the cell line is cultured in (a) in a continuous, stirred tank bioreactor culture.

62. The method of any one of claims 32-60, wherein the cell line is cultured in (a) in a batch cell culture.

63. A method for screening a plurality of cell lines for production of a multispecific binding protein, the method comprising:

(a) detecting an amount of a multispecific binding protein produced by a first cell line of the plurality according to the method of any one of claims 32-62; and

(b) detecting an amount of the multispecific binding protein produced by a second cell line of the plurality according to the method of any one of claims 32-62.

64. The method of claim 63, further comprising, after (a) and (b):

(c) comparing the amount of multispecific binding protein produced by the first cell line with the amount of multispecific binding protein produced by the second cell line; and

(d) based on the comparison, selecting the cell line that produced the higher amount of the multispecific binding protein.

65. The method of claim 63 or claim 64, further comprising: detecting an amount of one or more mispaired species produced by the first cell line according to the method of any one of claims 32-62; and detecting an amount of one or more mispaired species produced by the second cell line according to the method of any one of claims 32-62.

66. The method of claim 65, further comprising, after detecting the amount of one or more mispaired species produced by the first and second cell lines: comparing the amount of the one or more mispaired species produced by the first cell line with the amount of the one or more mispaired species produced by the second cell line; and based on the comparison, selecting the cell line that produced the higher ratio of multispecific binding protein to one or more mispaired species.

67. The method of claim 66, wherein the cell line is selected based on a higher ratio of multispecific binding protein to the amount of one or more individual mispaired species.

68. The method of claim 66, wherein the cell line is selected based on a higher ratio of multispecific binding protein to the overall amount of mispaired species.

69. A method for monitoring production of an antibody or antibody derivative and one or more weight variant species, the method comprising:

detecting, by size-exclusion ultra-performance liquid chromatography and mass spectrometry (SE-UPLC-MS), an amount of an antibody or antibody derivative and one or more weight variant species in a cell culture medium comprising the antibody or antibody derivative and the one or more weight variant species, thereby monitoring production of the antibody or antibody derivative and one or more weight variant species;

wherein the antibody or antibody derivative and one or more weight variant species differ in molecular weight and wherein the SE-UPLC is denaturing SE-UPLC.

70. A method for producing an antibody or antibody derivative and one or more weight variant species, the method comprising:

(a) culturing a cell line comprising one or more polynucleotides encoding the antibody or antibody derivative in a cell culture medium under conditions suitable for production of the antibody or antibody derivative and one or more weight variant species by the cell line;

(b) separating, from the cell line, the cell culture medium comprising the antibody or antibody derivative and one or more weight variant species;

(c) detecting an amount of the antibody or antibody derivative and one or more weight variant species in the cell culture medium by size-exclusion ultra-performance liquid chromatography and mass spectrometry (SE-UPLC-MS); and

(d) removing at least a portion of one or more of the weight variant species from the antibody or antibody derivative produced by the cell line, or determining one or both of quality and purity of the antibody or antibody derivative produced by the cell line;

wherein the antibody or antibody derivative and one or more weight variant species differ in molecular weight, and

wherein the SE-UPLC is denaturing SE-UPLC.

71. The method of claim 69 or claim 70, wherein the antibody or antibody derivative and the one or more weight variant species comprise species with a free cysteine that has been cysteinylated, N-acetyl cysteinylated, or glutathionylated.

72. The method of claim 69 or claim 70, wherein the antibody or antibody derivative and the one or more weight variant species comprise species comprising a chemically modified cysteine residue.

73. The method of any one of claims 69-72, wherein the antibody or antibody derivative comprises a cysteine residue at position 293, numbering according to EU index.

74. The method of any one of claims 69-73, wherein the antibody or antibody derivative is not N-glycosylated in the Fc region.

75. The method of any one of claims 69-74, wherein the antibody or antibody derivative comprises a mutation in the Fc region that eliminates N-glycosylation in the Fc region.

76. The method of claim 75, wherein the antibody or antibody derivative comprises an N300A mutation, numbering according to EU index.

77. The method of any one of claims 69-74, further comprising, prior to detection via SE-UPLC-MS, removing N-glycosylation in the Fc region.

78. The method of claim 77, wherein removing the N-glycosylation comprises treating the antibody or antibody derivative with a peptide:N-glycosidase enzyme.

79. The method of any one of claims 69-78, wherein the antibody or antibody derivative and one or more weight variant species differ in molecular weight by at least 119 Da.

80. The method of any one of claims 69-79, wherein the method is capable of resolving a mass difference among cysteinylated, N-acetyl cysteinylated, and glutathionylated species.

81. The method of claim 69 or claim 70, wherein the antibody or antibody derivative and the one or more weight variant species comprise glycoforms of the antibody or antibody derivative.

82. The method of any one of claims 69-81, wherein the antibody or antibody derivative and one or more weight variant species differ in molecular weight by at least 162 Da.

83. The method of any one of claims 69-82, wherein the method is capable of resolving a mass difference between the antibody or antibody derivative and one of more weight variant species that represent glycoforms of the antibody or antibody derivative.

84. The method of any one of claims 69-83, wherein the antibody or antibody derivative is a monoclonal antibody.

85. The method of any one of claims 69-83, wherein the antibody or antibody derivative is a multispecific antibody or binding protein.

86. The method of any one of claims 69-85, wherein detecting the amount of the antibody or antibody derivative and one or more weight variant species comprises deconvoluting one or more MS spectra obtained by the MS.

87. The method of any one of claims 69-86, wherein a relative amount of the antibody or antibody derivative as compared to the one or more weight variant species is detected.

88. The method of claim 87, wherein a relative amount of the antibody or antibody derivative as compared to an amount of one or more individual weight variant species is detected.

89. The method of claim 87, wherein a relative amount of the antibody or antibody derivative as compared to an overall amount of weight variant species is detected.

90. The method of claim 69, wherein the cell culture medium is a cell culture harvest.

91. The method of claim 90, further comprising, prior to detection, separating the cell culture medium from a cell line that produces the antibody or antibody derivative and one or more weight variant species.

92. The method of claim 91, wherein, prior to (a), the cell culture medium is separated from the cell line by centrifugation.

93. The method of claim 70, wherein the cell culture medium is separated from the cell line by centrifugation.

94. The method of any one of claims 69-93, wherein the cell culture medium is subjected to SE-UPLC without a prior chromatographic separation.

95. The method of any one of claims 69-93, wherein the cell culture medium is subjected to SE-UPLC without prior protein A affinity chromatography.

96. The method of any one of claims 68-95, wherein the MS is intact MS.

97. The method of any one of claims 69-96, wherein the MS is quadrupole time-of-flight (QToF) MS.

98. The method of any one of claims 69-97, wherein the SE-UPLC is directly coupled to the MS.

99. The method of any one of claims 69-98, wherein the cell line is a mammalian cell line.

100. The method of claim 99, wherein the mammalian cell line is a Chinese hamster ovary (CHO) cell line.

101. The method of any one of claims 70-100, wherein the cell line is cultured in a continuous, stirred tank bioreactor culture.

102. The method of any one of claims 70-100, wherein the cell line is cultured in a batch cell culture.

103. A method for screening a plurality of cell lines for production of an antibody or antibody derivative, the method comprising:

(a) detecting an amount of an antibody or antibody derivative produced by a first cell line of the plurality according to the method of any one of claims 70-102; and

(b) detecting an amount of the antibody or antibody derivative produced by a second cell line of the plurality according to the method of any one of claims 70-102.

104. The method of claim 103, further comprising, after (a) and (b):

(c) comparing the amount of antibody or antibody derivative produced by the first cell line with the amount of antibody or antibody derivative produced by the second cell line; and

(d) based on the comparison, selecting the cell line that produced the higher amount of the antibody or antibody derivative.

105. The method of claim 103 or claim 104, further comprising: detecting an amount of one or more weight variant species produced by the first cell line according to the method of any one of claims 70-102; and detecting an amount of one or more weight variant species produced by the second cell line according to the method of any one of claims 70-102.

106. The method of claim 105, further comprising, after detecting the amount of one or more weight variant species produced by the first and second cell lines: comparing the amount of the one or more weight variant species produced by the first cell line with the amount of the one or more weight variant species produced by the second cell line; and based on the comparison, selecting the cell line that produced the higher ratio of antibody or antibody derivative to one or more weight variant species.

107. The method of claim 106, wherein the cell line is selected based on a higher ratio of antibody or antibody derivative to the amount of one or more individual weight variant species.

108. The method of claim 107, wherein the cell line is selected based on a higher ratio of antibody or antibody derivative to the overall amount of weight variant species.