

sulfate, sodium citrate, or a combination thereof. Alternatively or in combination, the pH of the load buffer and the wash buffer is between 5 and 7. Alternatively or in combination, the salt concentration of the load buffer and the wash buffer is between about 150 mM and 1000 mM.

In another aspect, the present invention provides a pharmaceutical composition comprising a low-aggregate composition and a pharmaceutical acceptable carrier.

In one aspect, the present invention provides a pharmaceutical composition comprising a preparation of adalimumab produced by the foregoing methods and a pharmaceutically acceptable carrier. In another aspect, the present invention provides a pharmaceutical composition comprising a low-aggregate composition of adalimumab and a pharmaceutically acceptable carrier. For example, the composition may include less than 5%, 4%, 3%, 2.5%, 2.4%, 2.3%, 2.2%, 2.1%, 2%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, 0.5%, 0.1% of aggregates, *e.g.*, MM1, MM2 and MM3. Alternatively, the composition may include less than 2%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, 0.5%, 0.1% of aggregates, *e.g.*, MM1, MM2 and MM3. Alternatively, the composition may include less than 1%, 0.5%, 0.1% of aggregates, *e.g.*, MM1, MM2 and MM3.

In one aspect, the present invention provides a method for producing a preparation comprising a protein of interest and having a reduced level of at least one impurity, said method comprising:

(a) contacting a sample comprising the protein of interest and at least one impurity, to a hydrophobic interaction chromatography (HIC) media, in the presence of a load buffer such that (i) a portion of the protein of interest binds to the HIC media at a K_p of greater than 20 and (ii) a substantial portion of the at least one impurity binds to the HIC media;

(b) collecting a flow through fraction comprising the protein of interest unbound to the HIC media;

(c) washing the HIC media with a wash buffer such that a substantial portion of the protein of interest bound to the HIC media is released from the media, wherein the salt concentration and/or the pH of the wash buffer are within 20% of the salt concentration and/or pH of the load buffer; and

(d) collecting a wash fraction comprising the protein of interest released from the HIC media,
wherein each of the flow through and wash fractions comprise the protein of interest and have a reduced level of the at least one impurity.

In one aspect, the present invention provides a method for producing a preparation comprising adalimumab and having a reduced level of at least one impurity, said method comprising:

(a) contacting a sample comprising adalimumab and at least one impurity, to a HIC media, in the presence of a load buffer such that (i) a portion of the adalimumab in the sample binds to the HIC media at a K_p of greater than 20 and (ii) a substantial portion of the at least one impurity binds to the HIC media;

(b) collecting a flow through fraction comprising the adalimumab unbound to the HIC media;

(c) washing the HIC media with a wash buffer such that a substantial portion of the adalimumab bound to the HIC media is released from the media, wherein the salt concentration and/or the pH of the wash buffer are within 20% of the salt concentration and/or pH of the load buffer; and

(d) collecting a wash fraction comprising the adalimumab released from the HIC media, wherein each of the flow through and wash fractions comprise adalimumab and have a reduced level of the at least one impurity.

BRIEF DESCRIPTIONS OF THE DRAWINGS

Figure 1 depicts a size exclusion chromatography (SEC) chromatogram used to determine the molecular weight distribution of a sample of adalimumab. In combination with multi-angle light scattering (MALS) analysis (data not shown), the apparent molecular weight of each peak was determined and identified as a multimer or the reference standard as indicated. Multimer 1 (MM1), Multimer 2 (MM2) and Multimer 3 (MM3) were identified as depicted.

Figures 2A-2B depicts schematic chromatograms for two modes of chromatographic operation: bind-elute mode (**Figure 2A**) and flow-through mode (**Figure 2B**). In the bind-elute mode, there is strong binding of the protein of interest and the impurity. Elution conditions are chosen to selectively elutes the protein of interest. In the flow-through mode there is weak binding of the product and strong binding of the impurity.

Figure 3 depicts selection of operating conditions appropriate for an antibody:media:buffer combination. A sample was loaded at 20 g/L and a linear gradient elution was performed over 20 CVs to identify the salt concentration at the monomer and aggregate peak. The salt concentration at or near the elution peak of the monomer is the concentration at which the monomer is eluted from the HIC media.

CLAIMS

What is claimed is:

1. A method for producing a preparation comprising a protein of interest and having a reduced level of at least one impurity, said method comprising:

(a) contacting a sample comprising the protein of interest and at least one impurity, to a hydrophobic interaction chromatography (HIC) media, in the presence of a load buffer such that (i) a portion of the protein of interest binds to the HIC media and (ii) a substantial portion of the at least one impurity binds to the HIC media;

(b) collecting a flow through fraction comprising the protein of interest unbound to the HIC media;

(c) washing the HIC media with a wash buffer that is substantially the same as the load buffer such that a substantial portion of the protein of interest bound to the HIC media is released from the media; and

(d) collecting a wash fraction comprising the protein of interest released from the HIC media,

wherein each of the flow through and wash fractions comprise the protein of interest and have a reduced level of the at least one impurity.

2. The method of claim 1, wherein the portion of the protein of interest binds to the HIC media at a K_p of greater than 10, 15, 20, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 250 or 300.

3. The method of claim 1, wherein the portion of the protein of interest binds to the HIC media at a K_p of greater than 10.

4. The method of claim 1, wherein the portion of the protein of interest binds to the HIC media at a K_p of greater than 20.

5. The method of claim 1, wherein the portion of the protein of interest binds to the HIC media at a K_p of greater than 100.

6. The method of any one of the preceding claims, wherein the protein of interest is adalimumab.

7. The method of any one of the preceding claims, wherein a substantial portion of the impurity bound to the HIC media remains bound upon washing with the wash buffer.
8. The method of any one of the preceding claims, wherein the flow through and/or wash fractions are substantially free of the at least one impurity.
9. The method of any one of the preceding claims, wherein the at least one impurity is an aggregate of the protein of interest.
10. The method of claim 9, wherein the aggregate is selected from the group consisting of a multimer, a dimer, a trimer, a tetramer, an oligomer and other high molecular weight species.
11. The method of claim 9 or 10, wherein the protein of interest is adalimumab and the at least one impurity is an aggregate of adalimumab.
12. The method of claim 11, wherein the aggregate is selected from the group consisting of multimer 1, multimer 2 and multimer 3.
13. The method of any one of the preceding claims, wherein the impurity is a process-related impurity or a product-related substance.
14. The method of claim 13, wherein the process-related impurity is selected from the group consisting of a host cell protein, a host cell nucleic acid, a media component, and a chromatographic material.
15. The method of claim 13, wherein the product-related substance is selected from the group consisting of a charge variant, an aggregate of the protein of interest, a fragment of the protein of interest and a modified protein.
16. The method of claim 15, wherein the charge variant comprises an acidic or a basic variant.
17. The method of claim 16, wherein the charge variant comprises an acidic or basic variant of adalimumab.
18. The method of claim 16 or 17, wherein the basic variant is a lysine variant species.
19. The method of claim 18, wherein the lysine variant species comprises an antibody, or antigen-binding portion thereof, comprising heavy chains with either zero, one or two C-terminal lysines.

20. The method of any one of the preceding claims, wherein the impurity is an acidic species (AR).
21. The method of claim 20, wherein the acidic species (AR) is selected from the group consisting of a charge variant, a structure variant, a fragmentation variant, a process-related impurity and a product-related impurity.
22. The method of claim 21, wherein the acidic species is AR1 and wherein the charge variant comprises a deamidation variant, a glycation variant, an afucosylation variant, a MGO variant and/ or a citric acid variant.
23. The method of claim 21, wherein the acidic species is AR1 and wherein the structure variant comprises a glycosylation variant and/ or an acetonation variant.
24. The method of claim 21, wherein the acidic species is AR1 and wherein the fragmentation variant comprises a Fab fragment variant, a C-terminal truncation variant or a variant missing a heavy chain variable domain.
25. The method of claim 21, wherein the acidic species is AR2 and wherein the charge variant comprises a deamidation variant and/ or glycation variant.
26. The method of claim 15, wherein the fragment of the protein of interest comprises an Fc or a Fab fragment.
27. The method of claim 15, wherein the modified protein comprises a deamidated protein or glycosylated protein.
28. The method of any one of the preceding claims, wherein the protein of interest is an antibody or antigen-binding fragment thereof, a soluble protein, a membrane protein, a structural protein, a ribosomal protein, an enzyme, a zymogen, an antibody molecule, a cell surface receptor protein, a transcription regulatory protein, a translation regulatory protein, a chromatin protein, a hormone, a cell cycle regulatory protein, a G protein, a neuroactive peptide, an immunoregulatory protein, a blood component protein, an ion gate protein, a heat shock protein, an antibiotic resistance protein, a functional fragment of any of the preceding proteins, an epitope-containing fragment of any of the preceding proteins, and combinations thereof.

29. The method of claim 28, wherein the antibody or antigen-binding fragment thereof is a *humanized antibody or antigen-binding portion thereof*, a *human antibody or antigen-binding portion thereof*, a *chimeric antibody or antigen-binding portion thereof*, or a *multivalent antibody*.
30. The method of claim 28, wherein the antibody, or antigen-binding fragment thereof, comprises a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions.
31. The method of claim 28, wherein the antibody, or antigen-binding fragment thereof is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a single chain Fv fragment, an SMIP, an affibody, an avimer, a nanobody, and a single domain antibody.
32. The method of any one of the preceding claims, further comprising repeating steps (a)-(d) at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 20 times using the flow through fraction, wash fraction, or combination thereof having a reduced level of the at least one impurity
33. The method of any one of the preceding claims, wherein the flow through fraction and the wash fraction are combined.
34. The method of any one of the preceding claims, wherein the portion of the protein of interest that binds to the HIC media is at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the protein of interest in the sample.
35. The method of any one of the preceding claims, wherein the substantial portion of the protein of interest released from the HIC media upon washing with the wash buffer is about at least 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% of the amount of protein of interest bound to the HIC media.
36. The method of any one of the preceding claims, wherein the accumulative yield of the protein of interest in the flow through fraction and/or wash fraction is at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%.

37. The method of any one of the preceding claims, wherein the accumulative yield of the protein of interest in any one flow through fraction and/or wash fraction is at least about 4%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85, at least about 90%, at least about 95% or about 100%.

38. The method of any one of the preceding claims, wherein the substantial portion of the at least one impurity that binds to the HIC media is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% or about 100% of the at least one impurity in the sample.

39. The method of any one of the preceding claims, wherein the reduced level of the at least one impurity of the flow through fraction and/or wash fraction is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% or about 100% of the at least one impurity in the sample.

40. The method of any one of the preceding claims, wherein the accumulative aggregate reduction of the at least one impurity in any one flow through fraction and/or wash fraction is at least about 0.1%, at least about 0.2%, at least about 0.5%, at least about 1.0%, at least about 2.0%, at least about 3.0%, at least about 4.0%, at least about 5.0%, at least about 10.0%, or at least about 20.0%.

41. The method of any one of the preceding claims, wherein the accumulative aggregate reduction of the at least one impurity in the flow through fraction and/or wash fraction is at least about 0.1%, at least about 0.2%, at least about 0.5%, at least about 1.0%, at least about 2.0%, at least about 3.0%, at least about 4.0%, at least about 5.0%, at least about 10.0%, or at least about 20.0%.

42. The method of any one of the preceding claims, wherein the at least one impurity binds to the HIC media at a K_p of greater than 250, greater than 300, greater than 400, greater than 500, greater than 600, greater than 700, greater than 800, greater than 900, or greater than 1000.

43. The method of any one of the preceding claims, wherein the protein of interest and the at least one impurity have a K_p ratio less than 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3 or 1:2.

44. The method of any one of the preceding claims wherein the K_d for the binding of the protein of interest to the HIC media is at least about 0.2, at least about 0.3, at least about 0.4, at least about 0.5, or at least about 0.6.
45. The method any one of the preceding claims, wherein the K_d for the binding of the at least one impurity to the HIC media is less than or equal to about 0.001, about 0.005, about 0.01, about 0.02, about 0.05, about 0.1, about 0.15 or about 0.2.
46. The method of any one of the preceding claims, wherein the K_d for the binding of the protein of interest to the HIC media is less than 50, 45, 40, 35, 30, 25, 20, 15, 10 or 5 times the K_d for the binding of the at least one impurity to the HIC media.
47. The method of any one of the preceding claims, wherein the protein of interest has a Q_{max} of at least about 20, at least about 30, at least about 40, at least about 50, at least about 60 or at least about 100.
48. The method of any one of the preceding claims, wherein the at least one impurity has a Q_{max} of at least about 2, at least about 5, at least about 10, at least about 20, at least about 30 or at least about 40.
49. The method of any one of the preceding claims, wherein the HIC media comprises at least one hydrophobic ligand.
50. The method of claim 49 wherein the hydrophobic ligand is selected from the group consisting of alkyl-, aryl-ligands, and combinations thereof.
51. The method of claim 49, wherein the hydrophobic ligand is selected from the group consisting of butyl, hexyl, phenyl, octyl, or polypropylene glycol ligands.
52. The method of any one of the preceding claims, wherein the HIC media is selected from the group consisting of CaptoPhenyl, Phenyl Sepharose™ 6 Fast Flow with low or high substitution, Phenyl Sepharose™ High Performance, Octyl Sepharose™ High Performance, Fractogel™ EMD Propyl, Fractogel™ EMD Phenyl, Macro-Prep™ Methyl, Macro-Prep™ t-Butyl, WP HI-Propyl (C3)™, Toyopearl™ ether, Toyopearl™ phenyl, Toyopearl™ butyl, ToyoScreen PPG, ToyoScreen Phenyl, ToyoScreen Butyl, ToyoScreen Hexyl, HiScreen Butyl FF, HiScreen Octyl FF, and Tosoh Hexyl.
53. The method claim 49, wherein the HIC media comprises a column.

54. The method of any one of the preceding claims, wherein the load buffer and/or wash buffer comprise a salt selected from the group consisting of ammonium sulfate, sodium sulfate, sodium chloride, ammonium chloride, sodium bromide or a combination thereof.
55. The method of any one of the preceding claims, wherein the load buffer and the wash buffer comprise a sulfate salt, a citrate salt, or a combination thereof.
56. The method of claim 55, wherein the sulfate salt is ammonium sulfate.
57. The method of claim 55, wherein the sulfate salt is sodium sulfate.
58. The method of claim 55, wherein the citrate salt is sodium citrate.
59. The method of any one of the preceding claims, wherein the load buffer and/or the wash buffer comprise a cation selected from the group consisting of Ba^{2+} , Ca^{2+} , Mg^{2+} , Li^+ , Cs^+ , Na^+ , K^+ , Rb^+ , and NH_4^+ , and/or an anion selected from the group consisting of PO_4^{3-} , SO_4^{2-} , CH_3CO_3^- , Cl^- , Br^- , NO_3^- , ClO_4^- , I^- , and SCN^- or a combination thereof.
60. The method of any one of claims 54-59, wherein the salt has a concentration of between about 50 mM and 2000 mM.
61. The method of any one of the preceding claims, wherein the load buffer and/or the wash buffer have a pH between about 4.0 and 8.5 or between about 5.0 and 7.0.
62. The method of any one of the preceding claims, wherein the load buffer and/or the wash buffer have a pH of about 4.0, about 4.5, about 5.0, about 5.5, about 6, about 6.5, about 7.0, about 7.5, about 8.0, or about 8.5.
63. The method of any one of the preceding claims, wherein the load buffer and the wash buffer are the same.
64. The method of any one of the preceding claims wherein the load buffer and the wash buffer are substantially the same.
65. The method of claim 64, wherein the salt concentration and/or the pH of the wash buffer are within about 20%, 15%, 10% or 5% of the salt concentration, and/or pH of the loading buffer.
66. The method of any one of the preceding claims, wherein about 100 g to about 800 g of the sample are contacted per one liter of HIC media.

67. The method of any one of the preceding claims, wherein about 0.2 g to about 120 g of the at least one impurity is contacted per one liter of HIC media.
68. The method of any one of the preceding claims, wherein the sample has a protein concentration of about 2 mg/ml to about 50 mg/ml.
69. The method of any one of the preceding claims, wherein the sample has a protein of interest concentration of about 2 mg/ml to about 50 mg/ml.
70. The method of any one of the preceding claims, wherein the concentration of the at least one impurity in the sample is about 0.01 to about 5.0 mg/ml.
71. The method of any one of the preceding claims, wherein the level of the at least one impurity is reduced by at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of the at least one impurity in the sample.
72. The method of any one of claims 1-8, 13, 14 or 28-71, wherein the at least one impurity is a host cell protein.
73. The method of claim 72, wherein the at least one impurity is reduced by at least 0.25, at least 0.5, at least 0.75, at least 1.0, at least 1.25 or at least 1.5 log reduction fraction.
74. The method of any one of the preceding claims, wherein the HIC media has a dynamic binding capacity of at least about 2 g, at least about 5 g, at least about 10 g, at least about 20 g, at least about 30 g, at least about 40 g, at least about 50 g, at least about 60 g, at least about 70 g, at least about 90 g, or at least about 100 g of sample per one liter of media.
75. The method of any one of the preceding claims, wherein a precursor sample comprising the protein of interest has been subjected to affinity chromatography to generate the sample.
76. The method of any one of the preceding claims, further comprising subjecting the preparation comprising a protein of interest and having a reduced level of one impurity to affinity chromatography.
77. The method of claim 75 or 76, wherein affinity chromatography is performed using affinity chromatographic media selected from the group consisting of Protein A, G, A/G, L media, and MabSuRe Protein A media.

78. The method of any one of the preceding claims, wherein a precursor sample comprising the protein of interest has been subjected to ion exchange chromatography to generate the sample.
79. The method of any one of the preceding claims, further comprising subjecting the preparation comprising a protein of interest and having a reduced level of one impurity to ion exchange chromatography.
80. The method of claim 78 or 79, wherein ion exchange chromatography is performed using ion exchange chromatography media selected from the group consisting of a cation exchange media and an anion exchange media.
81. The method of claim 80, wherein the ion exchange media is an anion exchange media comprising diethylaminoethyl (DEAE), quaternary aminoethyl (QAE) or quaternary amine (Q) group ligands.
82. The method of claim 80, wherein the ion exchange media is a cation exchange media comprising carboxymethyl (CM), sulfoethyl(SE), sulfopropyl(SP), phosphate(P) or sulfonate(S) ligands.
83. The method of any one of the preceding claims, wherein a precursor sample comprising the protein of interest has been subjected to mixed mode chromatography to generate the sample.
84. The method of any one of the preceding claims, further comprising subjecting the preparation comprising a protein of interest and having a reduced level of one impurity to mixed mode chromatography.
85. The method of claim 83 or 84, wherein the mixed mode chromatography is performed using CaptoAdhere resin.
86. The method of any one of the preceding claims, wherein a precursor sample comprising the protein of interest has been subjected to a filtration step to generate the sample.
87. The method of any one of the preceding claims, further comprising subjecting the preparation comprising a protein of interest and having a reduced level of one impurity to a filtration step.

88. The method of claim 86 or 87, wherein the filtration step is selected from the group consisting of a depth filtration step, a nanofiltration step, an ultrafiltration step, and an absolute filtration step, or a combination thereof.

89. A pharmaceutical composition comprising the preparation produced by the method of any one of the preceding claims and a pharmaceutically acceptable carrier.

90. A method for producing a preparation comprising adalimumab and having a reduced level of at least one aggregate, said method comprising:

(a) contacting a sample comprising adalimumab and at least one aggregate, to a HIC media, in the presence of a load buffer such that (i) a portion of the adalimumab in the sample binds to the HIC media and (ii) a substantial portion of the at least one aggregate binds to the HIC media;

(b) collecting a flow through fraction comprising the adalimumab unbound to the HIC media;

(c) washing the HIC media with a wash buffer that is substantially the same as the load buffer such that a substantial portion of the adalimumab bound to the HIC media is released from the media; and

(d) collecting a wash fraction comprising the adalimumab released from the HIC media,

wherein each of the flow through and wash fractions comprise adalimumab and have a reduced level of the at least one aggregate.

91. The method of claim 90, wherein adalimumab binds to the HIC media at a K_p of greater than 10, 15, 20, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 250 or 300.

92. The method of claim 90, wherein adalimumab binds to the HIC media at a K_p of greater than 10.

93. The method of claim 90, wherein adalimumab binds to the HIC media at a K_p of greater than 20.

94. The method of claim 90, wherein adalimumab binds to the HIC media at a K_p of greater than 90.

95. The method of any one of claims 90-94, wherein the aggregate is multimer 1, multimer 2 or multimer 3.

96. The method of any one of claims 90-95, wherein the sample comprises between 200 g and 700 g protein per liter of HIC media.
97. The method of any one of claims 90-96, wherein the HIC media is selected from the group consisting of GE CaptoPhenyl, Tosoh Hexyl, GE Butyl FF, Butyl, Hexyl, Phenyl, Octyl, GE Butyl FF, PPG.
98. The method of any one of claims 90-97, wherein the load buffer and the wash buffer comprise ammonium sulfate, sodium sulfate, sodium citrate, or a combination thereof.
99. The method of any one of claims 90-98, wherein the pH of the load buffer and the wash buffer is between 5 and 7.
100. The method of any one of claims 90-99, wherein the salt concentration of the load buffer and the wash buffer is between about 150 mM and 1000 mM.
101. A pharmaceutical composition comprising a low-aggregate composition and a pharmaceutical acceptable carrier.
102. A pharmaceutical composition comprising a preparation of adalimumab produced by the method of any one of claims 90-100 and a pharmaceutically acceptable carrier.
103. A pharmaceutical composition comprising a low-aggregate composition of adalimumab and a pharmaceutically acceptable carrier.
104. The pharmaceutical composition of any one of claims 102-103, wherein the composition comprises less than 5%, 4%, 3%, 2.5%, 2.4%, 2.3%, 2.2%, 2.1%, 2%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, 0.5%, 0.1% of aggregates.
105. The pharmaceutical composition of any one of claims 102-104, wherein the composition comprises less than 2%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, 0.5%, 0.1% of aggregates.
106. The pharmaceutical composition of any one of claims 102-105, wherein the composition comprises less than 1%, 0.5%, 0.1% of aggregates.
107. The pharmaceutical composition of any one of claims 102-106, wherein the composition comprises less than 2%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, 0.5%, 0.1% of MM1, MM2 and MM3.

CLAIMS

1. A method for producing a preparation comprising a protein of interest and having a reduced level of at least one impurity, said method comprising:

(a) contacting a sample comprising the protein of interest and at least one impurity, to a hydrophobic interaction chromatography (HIC) media, in the presence of a load buffer such that (i) a portion of the protein of interest binds to the HIC media at a K_p of greater than 20 and (ii) a substantial portion of the at least one impurity binds to the HIC media;

(b) collecting a flow through fraction comprising the protein of interest unbound to the HIC media;

(c) washing the HIC media with a wash buffer such that a substantial portion of the protein of interest bound to the HIC media is released from the media, wherein the salt concentration and/or the pH of the wash buffer are within 20% of the salt concentration and/or pH of the load buffer; and

(d) collecting a wash fraction comprising the protein of interest released from the HIC media,

wherein each of the flow through and wash fractions comprise the protein of interest and have a reduced level of the at least one impurity.

2. The method of claim 1, wherein the protein of interest is an antibody or antigen-binding fragment thereof, a soluble protein, a membrane protein, a structural protein, a ribosomal protein, an enzyme, a zymogen, an antibody molecule, a humanized antibody or antigen-binding portion thereof, a human antibody or antigen-binding portion thereof, a chimeric antibody or antigen-binding portion thereof, a multivalent antibody, a cell surface receptor protein, a transcription regulatory protein, a translation regulatory protein, a chromatin protein, a hormone, a cell cycle regulatory protein, a G protein, a neuroactive peptide, an immunoregulatory protein, a blood component protein, an ion gate protein, a heat shock protein, an antibiotic resistance protein, a functional fragment of any of the preceding proteins, an epitope-containing fragment of any of the preceding proteins, and combinations thereof.

3. The method of claim 1, wherein the portion of the protein of interest binds to the HIC media at a K_p of greater than 50.

4. The method of claim 1, wherein the portion of the protein of interest binds to the HIC media at a K_p of greater than 100.

5. The method of claim 1, wherein the portion of the protein of interest binds to the HIC media at a K_p of greater than 200.

6. A method for producing a preparation comprising adalimumab and having a reduced level of at least one impurity, said method comprising:

(a) contacting a sample comprising adalimumab and at least one impurity, to a HIC media, in the presence of a load buffer such that (i) a portion of the adalimumab in the sample binds to the HIC media at a K_p of greater than 20 and (ii) a substantial portion of the at least one impurity binds to the HIC media;

(b) collecting a flow through fraction comprising the adalimumab unbound to the HIC media;

(c) washing the HIC media with a wash buffer such that a substantial portion of the adalimumab bound to the HIC media is released from the media, wherein the salt concentration and/or the pH of the wash buffer are within 20% of the salt concentration and/or pH of the load buffer; and

(d) collecting a wash fraction comprising the adalimumab released from the HIC media, wherein each of the flow through and wash fractions comprise adalimumab and have a reduced level of the at least one impurity.

7. The method of claim 6, wherein a substantial portion of the impurity bound to the HIC media remains bound upon washing with the wash buffer.

8. The method of claim 6, wherein the flow through and/or wash fractions are substantially free of the at least one impurity.

9. The method of claim 6, wherein the at least one impurity is an aggregate of the protein of interest.

10. The method of claim 9, wherein the aggregate of the adalimumab is selected from the group consisting of a multimer, a dimer, a trimer, a tetramer, an oligomer or other high molecular weight species.

11. The method of claim 10, wherein the aggregate of adalimumab is selected from the group consisting of multimer 1, multimer 2 and multimer 3.

12. The method of claim 6, wherein the impurity is a process-related impurity.

13. The method of claim 12, wherein the process-related impurity is selected from the group consisting of a host cell protein, a host cell nucleic acid, a media component, and a chromatographic material.

14. The method of claim 6, wherein the impurity is an acidic species (AR).

15. The method of claim 14, wherein the acidic species (AR) is selected from the group consisting of AR1, AR2, a charge variant, a structure variant, a fragmentation variant, a process-related impurity and a product-related impurity.

16. The method of claim 6, further comprising repeating steps (a)-(d) at least 1 time using the flow through fraction, wash fraction, or combination thereof having a reduced level of the at least one impurity.

17. The method of claim 6, wherein the flow through fraction and the wash fraction are combined.

18. The method of claim 6, wherein the portion of the adalimumab that binds to the HIC media is at least 20% of the adalimumab in the sample.

19. The method of claim 6, wherein the at least one impurity binds to the HIC media at a K_p of greater than 250.

20. The method of claim 6, wherein the HIC media either (i) comprises at least one hydrophobic ligand, optionally selected from the group consisting of alkyl-, aryl-ligands, and butyl, hexyl, phenyl, octyl, or polypropylene glycol ligands.

21. The method of claim 6, wherein the load buffer and/or wash buffer comprise a salt selected from the group consisting of a sulfate salt, a citrate salt, ammonium sulfate, sodium sulfate, sodium chloride, ammonium chloride, sodium bromide and a combination thereof.

22. The method of claim 6, wherein 100 g to 800 g of the sample are contacted per one liter of HIC media.

23. The method of claim 6, wherein a precursor sample comprising the adalimumab has been subjected to affinity chromatography to generate the sample.

24. The method of claim 6, wherein a precursor sample comprising the adalimumab has been subjected to ion exchange chromatography to generate the sample.

25. The method of claim 6, wherein a precursor sample comprising the adalimumab has been subjected to mixed mode chromatography to generate the sample.

26. The method of claim 6, wherein a precursor sample comprising the adalimumab has been subjected to a filtration step to generate the sample.

27. The method of claim 6, wherein the HIC media has a dynamic binding capacity of at least 2 g.

28. The method of claim 6, wherein adalimumab binds to the HIC media at a K_p of greater than 50.

29. The method of claim 6, wherein the salt concentration and/or the pH of the wash buffer are within about 15% of the salt concentration and/or pH of the load buffer.

30. The method of claim 6, wherein the salt concentration and/or the pH of the wash buffer are within about 10% of the salt concentration and/or pH of the load buffer.

31. The method of claim 6, wherein the salt concentration and/or the pH of the wash buffer are within about 5% of the salt concentration and/or pH of the load buffer.

32. The method of claim 6, wherein the salt concentration and/or the pH of the wash buffer and the salt concentration and/or pH of the load buffer are isocratic.

33. The method of claim 6, wherein the impurity is a product-related substance.

34. The method of claim 33, wherein the product-related substance is selected from the group consisting of a charge variant, an acidic variant, a basic variant, a lysine variant species, an aggregate of the protein of interest, a fragment of the protein of interest, an Fc fragment of the protein of interest, a Fab fragment of the protein of interest, a modified protein, a deamidated protein, and a glycosylated protein.

35. The method of claim 6, further comprising repeating steps (a)-(d) at least 5, 10, or 20 times using the flow through fraction, wash fraction, or combination thereof having a reduced level of the at least one impurity.

36. The method of claim 6, wherein the portion of the adalimumab that binds to the HIC media is at least 90% of the adalimumab in the sample.

37. The method of claim 6, wherein the substantial portion of the adalimumab released from the HIC media upon washing with the wash buffer is at least 20% of the amount of adalimumab bound to the HIC media.

38. The method of claim 6, wherein the substantial portion of the adalimumab released from the HIC media upon washing with the wash buffer is at least 50% of the amount of adalimumab bound to the HIC media.

39. The method of claim 6, wherein the substantial portion of the adalimumab released from the HIC media upon washing with the wash buffer is at least 100% of the amount of adalimumab bound to the HIC media.

40. The method of claim 6, wherein the accumulative yield of the adalimumab in the flow through fraction and/or wash fraction is at least 35%.

41. The method of claim 6, wherein the accumulative yield of the adalimumab in the flow through fraction and/or wash fraction is at least 50%.

42. The method of claim 6, wherein the accumulative yield of the adalimumab in the flow through fraction and/or wash fraction is at least 100%.

43. The method of claim 6, wherein the accumulative yield of the adalimumab in any one flow through fraction and/or wash fraction is at least 4%.

44. The method of claim 6, wherein the accumulative yield of the adalimumab in any one flow through fraction and/or wash fraction is at least 50%.

45. The method of claim 6, wherein the accumulative yield of the adalimumab in any one flow through fraction and/or wash fraction is at least 100%.

46. The method of claim 6, wherein the substantial portion of the at least one impurity that binds to the HIC media is at least 50% of the at least one impurity in the sample.

47. The method of claim 6, wherein the substantial portion of the at least one impurity that binds to the HIC media is at least 70% of the at least one impurity in the sample.

48. The method of claim 6, wherein the substantial portion of the at least one impurity that binds to the HIC media is at least 100% of the at least one impurity in the sample.

49. The method of claim 6, wherein the reduced level of the at least one impurity of the flow through fraction and/or wash fraction is at least 50% of the at least one impurity in the sample.

50. The method of claim 6, wherein the reduced level of the at least one impurity of the flow through fraction and/or wash fraction is at least 70% of the at least one impurity in the sample.

51. The method of claim 6, wherein the reduced level of the at least one impurity of the flow through fraction and/or wash fraction is at least 100% of the at least one impurity in the sample.

52. The method of claim 6, wherein the accumulative aggregate reduction of the at least one impurity in any one flow through fraction and/or wash fraction is at least 0.1%.

53. The method of claim 6, wherein the accumulative aggregate reduction of the at least one impurity in any one flow through fraction and/or wash fraction is at least 5.0%.

54. The method of claim 6, wherein the accumulative aggregate reduction of the at least one impurity in any one flow through fraction and/or wash fraction is at least 20%.

55. The method of claim 6, wherein the accumulative aggregate reduction of the at least one impurity in the flow through fraction and/or wash fraction is at least 0.1%.

56. The method of claim 6, wherein the accumulative aggregate reduction of the at least one impurity in the flow through fraction and/or wash fraction is at least 5.0%.

57. The method of claim 6, wherein the accumulative aggregate reduction of the at least one impurity in the flow through fraction and/or wash fraction is at least 20.0%.

58. The method of claim 6, wherein the level of the at least one impurity is reduced by at least 60%, 80% or 95% of the at least one impurity in the sample.

59. The method of claim 6, wherein the at least one impurity binds to the HIC media at a K_p of greater than 500.

60. The method of claim 6, wherein the at least one impurity binds to the HIC media at a K_p of greater than 1000.

61. The method of claim 6, wherein the adalimumab and the at least one impurity have a K_p ratio less than 1:10, 1:5 or 1:2.

62. The method of claim 6, wherein the K_d for the binding of the adalimumab to the HIC media is at least 0.2, 0.4 or 0.6.

63. The method of claim 6, wherein the K_d for the binding of the at least one impurity to the HIC media is less than or equal to 0.001.

64. The method of claim 6, wherein the K_d for the binding of the at least one impurity to the HIC media is less than or equal to 0.01.

65. The method of claim 6, wherein the K_d for the binding of the at least one impurity to the HIC media is less than or equal to 0.1.

66. The method of claim 6, wherein the K_d for the binding of the at least one impurity to the HIC media is less than or equal to 0.2.

67. The method of claim 6, wherein the K_d for the binding of the adalimumab to the HIC media is less than 50, 25 or 5 times the K_d for the binding of the at least one impurity to the HIC media.

68. The method of claim 6, wherein the adalimumab has a Q_{max} of at least 20, 50 or 100.

69. The method of claim 6, wherein the at least one impurity has a Q_{max} of at least 2, 20 or 40.

70. The method of claim 6, wherein the load buffer and/or wash buffer comprise a cation selected from the group consisting of Ba^{2+} , Ca^{2+} , Mg^{2+} , Li^+ , Cs^+ , Na^+ , K^+ , Rb^+ , NH_4^+ and a combination thereof.

71. The method of claim 6, wherein the load buffer and/or wash buffer comprise an anion selected from the group consisting of PO_4^{3-} , SO_4^{2-} , $CH_3CO_3^-$, Cl^- , Br^- , NO_3^- , ClO_4^- , I^- , SCN^- and a combination thereof.

72. The method of claim 6, wherein the load buffer and/or wash buffer comprise a salt having a concentration of between 50 mM and 2000 mM.

73. The method of claim 6, wherein the load buffer and/or wash buffer have a pH between about 4.0 and 8.5.

74. The method of claim 6, wherein the load buffer and/or wash buffer have a pH between 5.0 and 7.0.

75. The method of claim 6, wherein the load buffer and/or wash buffer have a pH of 4.0.
76. The method of claim 6, wherein the load buffer and/or wash buffer have a pH of 6.5.
77. The method of claim 6, wherein the load buffer and/or wash buffer have a pH of 8.5.
78. The method of claim 6, wherein 0.2 g to 120 g of the at least one impurity is contacted per one liter of HIC media.
79. The method of claim 6, wherein the sample has a protein concentration of 2 mg/ml to 50 mg/ml.
80. The method of claim 6, wherein the sample has an adalimumab concentration of 2 mg/ml to 50 mg/ml.
81. The method of claim 6, wherein the concentration of the at least one impurity in the sample is 0.01 to 5.0 mg/ml.
82. The method of claim 6, further comprising subjecting the preparation comprising adalimumab and having a reduced level of one impurity to affinity chromatography.
83. The method of claim 23 or 82, wherein the affinity chromatography is performed using affinity chromatographic media selected from the group consisting of Protein A, G, A/G and L media.
84. The method of claim 6, further comprising subjecting the preparation comprising adalimumab and having a reduced level of the at least one impurity to a filtration step.

85. The method of claim 26 or 84, wherein the filtration step is selected from the group consisting of a depth filtration step, a nanofiltration step, an ultrafiltration step, and an absolute filtration step, or a combination thereof.

86. The method of claim 6, wherein the HIC media has a dynamic binding capacity of at least 50 g.

87. The method of claim 6, wherein the HIC media has a dynamic binding capacity of at least 100 g.

88. The method of claim 6, wherein adalimumab binds to the HIC media at a K_p of greater than 100.

89. The method of claim 6, wherein adalimumab binds to the HIC media at a K_p of greater than 200.

90. The method of claim 6, further comprising subjecting the preparation comprising adalimumab and having a reduced level of the at least one impurity to ion exchange chromatography.

91. The method of claim 24 or 90, wherein ion exchange chromatography is performed using ion exchange chromatography media selected from the group consisting of a cation exchange media and an anion exchange media.

92. The method of claim 6, further comprising subjecting the preparation comprising adalimumab and having a reduced level of the at least one impurity to mixed mode chromatography.