SUMMARY

[0005] The present disclosure is directed to methods of producing an aqueous formulation of an antigen-binding protein (such as an antigen-binding protein comprising an Fc region, an antibody, or a peptibody) or enhancing re-oxidation of such an antigen-binding protein and to formulations comprising a re-oxidized antigen-binding protein prepared according to these methods. In one aspect, the present invention refers to a method of producing an aqueous formulation of an antigen-binding protein comprising (a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter, wherein the charged depth filter comprises a metal selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof; and (b) measuring the amount or relative amount of reduced antigen-binding protein molecules. In another aspect, the present invention refers to a method of enhancing re-oxidization of an antigen-binding protein comprising (a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter, wherein the charged depth filter comprises a metal selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof; and (b) measuring the amount or relative amount of reduced antigen-binding protein molecules. In still another aspect, the present invention refers to a method of producing an aqueous formulation of an antigen-binding protein comprising contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter under conditions sufficient to achieve at least a 20% decrease in the percentage of reduced antigen-binding protein molecules, compared to the percentage of reduced antigen-binding protein molecules observed prior to the contacting step, wherein the charged depth filter comprises a metal selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof; and wherein the at least 20% decrease is determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS). In yet another aspect, the present invention refers to method of enhancing re-oxidization of an antigen-binding protein comprising contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter under conditions sufficient to achieve at least a two-fold increase in

re-oxidation of the antigen-binding protein molecules following the contacting step, wherein the charged depth filter comprises a metal selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof; and wherein the at least two-fold increase is determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS). Also described herein is a method of producing an aqueous formulation of an antigen-binding protein (such as an antigen-binding protein comprising an Fc region, an antibody, or a peptibody) comprising (a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter under conditions sufficient to achieve at least a 20% decrease, optionally a 30% or 40% decrease, in the percentage of reduced antigen-binding protein molecules, compared to the percentage of reduced antigen-binding protein molecules observed prior to step (a); and (b) optionally, measuring the amount (such as the total amount) or relative amount of reduced antigen-binding protein molecules. In another example, the disclosure provides a method of enhancing re-oxidization of an antigen-binding protein (such as an antigen-binding protein comprising an Fc region, an antibody, or a peptibody) comprising (a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter under conditions sufficient to enhance re-oxidation of the antigen-binding protein molecules; and (b) optionally, measuring the amount (such as the total amount) or relative amount of reduced antigen-binding protein molecules. In one example, the amount (such as the total amount) or relative amount of reduced antigen-binding protein molecules is measured using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS). In any of the preceding examples, the total amount of reduced antigen-binding protein molecules after contact with the charged depth filter in step (a) is 10% or less of the total amount of antigen-binding protein molecules and/or is decreased by at least three-fold compared to before step (a).

[0006] In some examples, step (a) of the methods described herein is followed by and/or preceded by subjecting the aqueous solution of antigen-binding protein molecules to Protein A chromatography. Optionally, a method according to the disclosure further comprises a step of inactivating one or more viruses in the aqueous solution of antigen-binding protein molecules and/or subjecting the aqueous solution of antigen-binding protein molecules to cation exchange chromatography and/or sparging air or oxygen into the aqueous solution of antigen-binding protein molecules. Optionally, a method further comprises adding an inhibitor of thioredoxin or

thioredoxin-like protein to the aqueous solution of antigen-binding protein molecules (see, e.g., U.S. Patent Publication No. 20090053786).

[0007] In one example, a method of producing an aqueous formulation of an antigen-binding protein(such as an antigen-binding protein comprising an Fc region, an antibody, or a peptibody) comprises contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter under conditions sufficient to achieve at least a 20% decrease in the percentage of reduced antigen-binding protein molecules, compared to the percentage of reduced antigen-binding protein molecules observed prior to the contacting step, wherein the at least 20% decrease is determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS). In another example, a method of enhancing re-oxidization of an antigen-binding protein (such as an antigen-binding protein comprising an Fc region, an antibody, or a peptibody) comprising contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter under conditions sufficient to achieve at least a two-fold increase in re-oxidation of the antigen-binding protein molecules following the contacting step, wherein the at least two-fold increase is determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS).

[0008] In some examples, a method described herein comprises (1) a Protein A chromatography step, optionally followed by charged depth filtration; (2) a viral inactivation step, optionally followed by charged depth filtration; and (3) a cation exchange chromatography step, optionally followed by charged depth filtration; further optionally comprising one or more of (4) a chromatography step optionally selected from salt-intolerant interaction chromatography, hydrophobic interaction chromatography, and mixed mode chromatography, optionally followed by charged depth filtration; (5) a virus filtration step, optionally followed by charged depth filtration; and (5) ultrafiltration and/or diafiltration, optionally followed by charged depth filtration. Optionally, in any of the methods described herein, following contact with a charged depth filter, the filtrate is incubated, for example, at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24 or more hours.

[0009] In one example, the charged depth filter comprises a diatomaceous earth layer. Optionally, the charged depth filter further comprises a cellulose layer and/or an inorganic layer, such as an inorganic layer comprises a polyamine resin. In another example, the charged depth

filter comprises a positive ion, such as any one of sodium, calcium, magnesium, mercury, chromium, aluminum, potassium, lead, arsenic, cadmium, cobalt, iron, manganese, titanium, zinc, nickel, copper, or combinations thereof. In another example, the charged depth filter comprises one of the following combinations of positive ions: 1) copper and cobalt, 2) copper and cadmium, 3) cobalt and cadmium, or 4) copper, cobalt, and cadmium. In some examples, the positive ion is a metal with a $^{+}2$ or higher oxidation state (such as $^{+}3$ or $^{+}4$). In some examples, the method comprises contacting an aqueous solution comprising antigen-binding protein molecules with one, two, three, four, five, or more charged depth filter(s).

[0010] In one example, the aqueous solution comprises an antigen-binding protein molecule which is an IgG antibody, such as an IgG1 or IgG2 antibody. For example, in some examples, the antibody is an IgG1 antibody with a kappa light chain or an IgG1 antibody with a lambda light chain.

[0011] In some examples, the antigen-binding protein binds an antigen selected from the group consisting of CD3, CD4, CD8, CD19, CD20, CD34, HER2, HER3, HER4, the EGF receptor, LFA-1, Mol, p150, p95, VLA-4, ICAM-1, VCAM, alpha v/beta 3 integrin, vascular endothelial growth factor, growth hormone, thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, growth hormone releasing factor, parathyroid hormone, mullerian-inhibiting substance, human macrophage inflammatory protein, erythropoietin, NGF-beta, platelet-derived growth factor, aFGF, bFGF, epidermal growth factor, TGF-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, IGF-I, IGF-II, des(1-3)-IGF-I, insulin, insulin A-chain, insulin B-chain, proinsulin, insulin-like growth factor binding proteins, factor VIII, tissue factor, von Willebrand factor, protein C, alpha-1–antitrypsin, plasminogen activators, urokinase, tissue plasminogen activator, bombazine, thrombin, thrombopoietin, M-CSF, GM-CSF, G-CSF, albumin, IgE, flk2/flt3 receptor, obesity receptor, bone-derived neurotrophic factor, NT-3, NT-4, NT-5, NT-6, relaxin A-chain, relaxin B-chain, prorelaxin, interferon-alpha, interferon-beta, interferon-gamma, IL-1 to IL-10, AIDS envelope viral antigen, calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha, tumor necrosis factor-beta, enkephalinase, RANTES, mouse gonadotropin-associated peptide, Dnase, inhibin, activin; protein A, protein D, bone morphogenetic protein, superoxide dismutase, decay accelerating factor, and combinations thereof.

[0012] In another example, the aqueous formulation comprises an antibody selected from the group consisting of abciximab, adalimumab, alemtuzumab, basiliximab, belimumab, bevacizumab, brentuximab vedotin, canakinumab, cetuximab, certolizumab pegol, daclizumab, denosumab, eculizumab, efalizumab, gemtuzumab, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, muromonab-CD3, natalizumab, nivolumab, ofatumumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tocilizumab, tositumomab, trastuzumab, ustekinumab, vedolizumab, and a biosimilar of any of the foregoing. In one example, the aqueous formulation comprises rituximab or an antibody comprising 1, 2, 3, 4, 5, or 6 of the complementarity determining regions (CDRs) of rituximab, for example, the antibody can comprise (a) a light chain containing all three light chain CDRs of rituximab, (b) a heavy chain containing all three heavy chain CDRs of rituximab, or (c) both. In another example, the aqueous formulation comprises infliximab or an antibody comprising 1, 2, 3, 4, 5, or 6 of the complementarity determining regions (CDRs) of infliximab, for example, the antibody can comprise (a) a light chain containing all three light chain CDRs of infliximab, (b) a heavy chain containing all three heavy chain CDRs of infliximab, or (c) both. In one example, the aqueous formulation comprises ofatumumab or an antibody comprising 1, 2, 3, 4, 5, or 6 of the complementarity determining regions (CDRs) of ofatumumab, for example, the antibody can comprise (a) a light chain containing all three light chain CDRs of ofatumumab, (b) a heavy chain containing all three heavy chain CDRs of ofatumumab, or (c) both.

[0013] In another example, the disclosure provides a formulation comprising a re-oxidized antigen-binding protein molecule (such as an antigen-binding protein comprising an Fc region, a fusion protein, an antibody, an antibody fragment or a peptibody) prepared using any of the methods described herein.

[0014] The foregoing summary is not intended to define every aspect of the invention, and other features and advantages of the present disclosure will become apparent from the following detailed description, including the drawings. The present disclosure is intended to be related as a unified document, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, paragraph, or section of this disclosure. In addition, the disclosure includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations specifically mentioned above. With respect to aspects of the disclosure described or

claimed with "a" or "an," it should be understood that these terms mean "one or more" unless context unambiguously requires a more restricted meaning. With respect to elements described as one or more within a set, it should be understood that all combinations within the set are contemplated. If aspects of the disclosure are described as "comprising" a feature, embodiments also are contemplated "consisting of" or "consisting essentially of" the feature. Additional features and variations of the disclosure will be apparent to those skilled in the art from the entirety of this application, and all such features are intended as aspects of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **Figure 1** depicts the level of partial antibody reduction as the percentage of pre-peak species measured using nrCE-SDS in the harvested cell culture fluid (HCCF), and in the Protein A Pool.

[0016] **Figure 2** depicts the level of partial antibody reduction as the percentage of pre-peak species in (a) the Protein A Pool, before passing through the charged depth filter, and (b) filtered viral inactivation pool, after passing through the charged depth filter.

[0017] **Figure 3A** depicts the level of partial antibody reduction as the percentage of pre-peak species in a charged depth filtered Protein A pool for up to 8 days post-filtration at room temperature or 2 °C to 8 °C. **Figure 3B** depicts the level of partial antibody reduction as the percentage of pre-peak species in a non-charged depth filtered Protein A pool for up to 8 days post-filtration at room temperature or 2 °C to 8 °C.

[0018] **Figure 4A** depicts the level of partial antibody reduction as the percentage of pre-peak species in HCCF subjected to charged depth filtration or no filtration, followed by Protein A chromatography. **Figure 4B** depicts the level of partial antibody reduction as the percentage of pre-peak species in HCCF subjected to charged depth filtration or no filtration, followed by Protein A chromatography, for up to 24 hours post-filtration.

[0019] **Figure 5A** depicts the level of partial antibody reduction as the percentage of pre-peak species in a Protein A pool as load compared to the depth filtered filtrate. **Figure 5B** depicts the level of partial antibody reduction as the percentage of pre-peak species in a neutralized viral inactivation pool (a) before filtration (nVIP/Load), (b) following sterile membrane filtration and (c) following charged depth filtration, for up to 24 hours post-filtration.

WHAT IS CLAIMED:

1. A method of producing an aqueous formulation of an antigen-binding protein comprising

(a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter, wherein the charged depth filter comprises a metal selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof; and

(b) measuring the amount or relative amount of reduced antigen-binding protein molecules.

2. A method of enhancing re-oxidization of an antigen-binding protein comprising

(a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter, wherein the charged depth filter comprises a metal selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof; and

(b) measuring the amount or relative amount of reduced antigen-binding protein molecules.

3. The method according to any of claims 1-2, wherein step (a) is followed by subjecting the solution of antigen-binding protein molecules to Protein A chromatography .

4. The method according to any of claims 1-3, wherein step (a) is preceded by subjecting the solution of antigen-binding protein molecules to Protein A chromatography.

5. The method according to any of claims 1-4, further comprising a step of inactivating one or more viruses in said solution of antigen-binding protein molecules.

6. The method according to any of claims 1-5, wherein the contacting occurs at room temperature.

7. The method according to any of claims 1-6, wherein the contacting occurs at a temperature of 2 degrees to 8 degrees Celsius.

8. The method according to any of claims 1-7, further comprising a step of sparging air or oxygen through the solution of antigen-binding protein molecules.

9. The method according to any of claims 1-8, further comprising contacting the solution of antigen-binding protein molecules with a metal selected from the group consisting of sodium, calcium, magnesium, mercury, molybdenum, chromium, cadmium, aluminum, potassium, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof.

10. The method according to any of claims 1-9, wherein the solution of antigen-binding protein molecules is contacted with more than one charged depth filter.

11. The method according to any of claims 1-10, wherein the charged depth filter comprises a diatomaceous earth layer.

12. The method according to claim 11, wherein the charged depth filter further comprises a cellulose layer and an inorganic layer.

13. The method according to claim 15, wherein the inorganic layer comprises a polyamine resin.

14. The method according to any of claims 1-13, wherein the charged depth filter comprises copper.

15. The method according to any of claims 1-14, wherein the contacting occurs at a throughput of between 250 L/m^2 and 850 L/m^2.

16. The method according to any of claims 1-15, wherein the antigen-binding protein is an IgG antibody.

17. The method according to claim 16, wherein the antibody is an IgG1 or IgG2 antibody.

18. The method according to claim 17, wherein the antibody is an IgG1 antibody with a Kappa light chain.

19. The method according to claim 17, wherein the antibody is an IgG1 antibody with a Lambda light chain.

20. The method according to any of claims 1-19, wherein the antigen-binding protein binds an antigen selected from the group consisting of RANKL, tumor necrosis factor alpha, epidermal growth factor receptor, CD20, calcitonin gene-related peptide, sclerostin, and platelet glycoprotein IIb/IIIa.

21. The method according to any of claims 1-20, wherein the antigen-binding protein is selected from the group consisting of abciximab, adalimumab, alemtuzumab, basiliximab, belimumab, bevacizumab, brentuximab vedotin, canakinumab, cetuximab, certolizumab pegol, daclizumab, denosumab, eculizumab, efalizumab, gemtuzumab, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, muromonab-CD3, natalizumab, nivolumab, ofatumumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tocilizumab, tositumomab, trastuzumab, ustekinumab, vedolizumab, and a biosimilar of any of the foregoing.

22. The method according to any of claims 1-21, wherein the antigen-binding protein comprises an antigen-binding region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-8.

23. The method according to any of claims 1-22, wherein the amount of reduced antigen-binding protein molecules is measured using non-reduced capillary electrophoresis with sodium dodecyl sulfate.

24. The method according to any of claims 1-23, further comprising a step of cation exchange chromatography.

25. The method of any of claims 1-24, comprising (1) a Protein A chromatography step, followed by charged depth filtration; (2) a viral inactivation step, followed by charged depth filtration; and (3) a cation exchange chromatography step, followed by charged depth filtration; further comprising one or more of (4) a salt-intolerant interaction chromatography step, followed

by charged depth filtration; (5) a virus filtration step, followed by charged depth filtration; and (5) ultrafiltration and/or diafiltration, followed by charged depth filtration.

26. The method of any of claims 1-25, wherein following contact with a charged depth filter, the filtrate is incubated for at least about 4 hours.

27. The method of any of claims 1-26, wherein the amount or relative amount of reduced antigen-binding protein molecules is determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS).

28. A method of producing an aqueous formulation of an antigen-binding protein comprising contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter under conditions sufficient to achieve at least a 20% decrease in the percentage of reduced antigen-binding protein molecules, compared to the percentage of reduced antigen-binding protein molecules observed prior to the contacting step, wherein the charged depth filter comprises a metal selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof; and wherein the at least 20% decrease is determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS).

29. A method of enhancing re-oxidization of an antigen-binding protein comprising contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter under conditions sufficient to achieve at least a two-fold increase in re-oxidation of the antigen-binding protein molecules following the contacting step, wherein the charged depth filter comprises a metal selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof; and wherein the at least two-fold increase is determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS).