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 ion selected from the group consisting of sodium ion, calcium ion, magnesium ion, mercury ion, chromium ion, cadmium ion, aluminum ion, potassium ion, lead ion, arsenic ion, cobalt ion, iron ion, manganese ion, titanium ion, zinc ion, nickel ion, copper ion, and combinations thereof; and (b) measuring an amount or relative amount of reduced antigen-binding protein molecules after the contacting step, wherein the percentage of reduced antigen-binding protein molecules is decreased by at least 20% when compared to the percentage of reduced antigen-binding protein molecules observed prior to the contacting step. 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 ion selected from the group consisting of sodium ion, calcium ion, magnesium ion, mercury ion, chromium ion, cadmium ion, aluminum ion, potassium ion, lead ion, arsenic ion, cobalt ion, iron ion, manganese ion, titanium ion, zinc ion, nickel ion, copper ion, and combinations thereof; and (b) measuring the amount or relative amount of reduced antigen-binding protein molecules after the contacting step, wherein the re-oxidation of the antigen binding protein molecules in the solution is increased by at least two-fold after the contacting step. In still another 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 ion selected from the group consisting of sodium ion, calcium ion, magnesium ion,

mercury ion, chromium ion, cadmium ion, aluminum ion, potassium ion, lead ion, arsenic ion, cobalt ion, iron ion, manganese ion, titanium ion, zinc ion, nickel ion, copper ion, and combinations thereof; and (b) measuring the amount or relative amount of reduced antigen-binding protein molecules after the contacting step, wherein the percentage of reduced antigen-binding protein molecules in the solution after the contacting step is decreased by at least 20% decrease is determined by 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 (a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter

, wherein the charged depth filter comprises a metal ion selected from the group consisting of sodium ion, calcium ion, magnesium ion, mercury ion, chromium ion, cadmium ion, aluminum ion, potassium ion, lead ion, arsenic ion, cobalt ion, iron ion, manganese ion, titanium ion, zinc ion, nickel ion, copper ion, and combinations thereof; and (b) measuring the amount or relative amount of antigen-binding protein molecules after the contacting step, wherein the re-oxidation of the antigen binding prostein molecules after the contacting step is increased by at least two-fold increase as determined by 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

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 ion selected from the group consisting of a sodium ion, calcium, magnesium ion, mercury ion, chromium ion, cadmium ion, aluminum ion, potassium ion, lead ion, arsenic ion, cobalt ion, iron ion, manganese ion, titanium ion, zinc ion, nickel ion, copper ion, and combinations thereof; and

(b) measuring an amount or relative amount of reduced antigen-binding protein molecules after the contacting step, wherein the percentage of reduced antigen-binding protein molecules is decreased by at least 20% when compared to the percentage of reduced antigen-binding protein molecules observed prior to the contacting step.

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 a sodium ion, calcium ion, magnesium ion, mercury ion, chromium ion, cadmium ion, aluminum ion, potassium ion, lead ion, arsenic ion, cobalt ion, iron ion, manganese ion, titanium ion, zinc ion, nickel ion, copper ion, and combinations thereof; and

(b) measuring the amount or relative amount of reduced antigen-binding protein molecules after the contacting step, wherein the re-oxidation of the antigen binding protein molecules in the solution is increased by at least two-fold after the contacting step.

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 ion selected from the group consisting of a sodium ion, calcium ion, magnesium ion, mercury ion, molybdenum ion, chromium ion, cadmium ion, aluminum ion, potassium ion, cobalt ion, iron ion, manganese ion, titanium ion, zinc ion, nickel ion, copper ion, 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 a copper ion.

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

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

(b) measuring the amount or relative amount of reduced antigen-binding protein molecules after the contacting step, wherein the percentage of reduced antigen-binding protein molecules in the solution after the contacting step is decreased by at least 20% decrease is determined by non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS).

29. 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 ion selected from the group consisting of a sodium ion, calcium ion, magnesium ion, mercury ion, chromium ion, cadmium ion, aluminum ion, potassium ion, lead ion, arsenic ion, cobalt ion, iron ion, manganese ion, titanium ion, zinc ion, nickel ion, copper ion, and combinations thereof; and

(b) measuring the amount or relative amount of antigen-binding protein molecules after the contacting step, wherein the re-oxidation of the antigen binding protein molecules after the contacting step is increased by least two-fold increase as determined by non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS).