METHODS OF DECREASING TRISULFIDE BONDS DURING RECOMBINANT PRODUCTION OF POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial No. 62/334,433, filed May 10, 2016, which is incorporated herein by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 146392036540SEQLIST.txt, date recorded: May 8, 2017, size: 34.1 KB).

FIELD OF THE INVENTION

[0003] The present disclosure relates to cell culture media and methods for decreasing trisulfide bonds in polypeptides produced recombinantly.

BACKGROUND OF THE INVENTION

[0004] Trisulfide bonds are generated by the insertion of an additional sulfur atom into a disulfide bond, thereby resulting in the covalent bonding of three consecutive sulfur atoms. Trisulfide bond formation is a source of heterogeneity in recombinantly produced therapeutic polypeptides. Such heterogeneity is undesirable, as therapeutic products must undergo extensive characterization and meet acceptable standards that ensure product quality and consistency. As such, a demand exists for methods for decreasing the levels of trisulfide bonds during the manufacture of therapeutic polypeptides. There is also a need in the art for minimizing the variability of trisulfide bond levels in therapeutic polypeptides during manufacture. The present disclosure is directed to this and other needs.

BRIEF SUMMARY

[0005] In one aspect, provided is a method for decreasing trisulfide bond levels in a polypeptide comprising: (a) contacting a host cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises the following components: i) between 2 μM to 35μM iron, ii) between 0.11 μM to 2 μM riboflavin (vitamin B2), iii) between 4.5 μM to 80 μM pyridoxine or

pyridoxal (vitamin B6), iv) between 3.4 μM to 23 μM folic acid (vitamin B9), and v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12), (b) culturing the host cell to produce the polypeptide; and (c) harvesting the polypeptide produced by the host cell. In a related aspect, provided is a method for producing a polypeptide, comprising (a) contacting a host cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises the following components: i) between 2 μM to 35μM iron, ii) between 0.11 μM to 2 μM riboflavin (vitamin B2), iii) between 4.5 μM to 80 μM pyridoxine or pyridoxal (vitamin B6), iv) between 3.4 μM to 23 μM folic acid (vitamin B9), and v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12), (b) culturing the host cell to produce the polypeptide; and (c)harvesting the polypeptide produced by the host cell.

[0006] In certain examples according to (or as applied to) any of the examples above, the harvested polypeptide has a trisulfide bond level less than a polypeptide produced under identical conditions, except that the concentration of the one or more components differs from the concentration specified in (a). In certain examples according to (or as applied to) any of the examples above, the basal medium lacks cystine. In certain examples according to (or as applied to) any of the examples above, the basal medium comprises between about 1.4 mM to 3 mM cysteine or cystine. In certain examples according to (or as applied to) any of the examples above, the basal medium comprises between about 0 mM to about 1.58 mM methionine and between about 0 mM to about 3 mM cysteine. In certain examples according to (or as applied to) any of the examples above, the basal medium comprises about 6mM cysteine.

[0007] Also provided in one aspect is a method for decreasing trisulfide bond levels in a polypeptide comprising: (a) culturing a host cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises the following components: i) between 2 μM to 35μM iron, ii) between 0.11 μM to 2 μM riboflavin (vitamin B2), iii) between 4.5 μM to 80 μM pyridoxine or pyridoxal (vitamin B6), iv) between 3.4 μM to 23 μM folate/folic acid (vitamin B9), and v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12),

(b) producing the polypeptide; (c) and harvesting the polypeptide produced by the host cell. In certain examples according to (or as applied to) any of the examples above, the concentration of one or more of the components in the cell culture medium is the cumulative concentration of one or more additions after inoculation.

[0008] In one aspect, provided is a method for decreasing trisulfide bond levels in a polypeptide selected from the group consisting of: a CEA-IL2v immunocytokine, a FAP-IL2v immunocytokine, an anti-CEA/anti-CD3 bispecific antibody, an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-Ang2/anti-VEGF bispecific antibody, an anti-C5 antibody, and an anti-CD40 antibody , the method comprising: (a) culturing a host cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises the following components: i) between 2 μM to 35μM iron, ii) between 0.11 μM to 2 μM riboflavin (vitamin B2), iii) between 4.5 μM to 80 μM pyridoxine or pyridoxal (vitamin B6), iv) between 3.4 μM to 23 μM folate/folic acid (vitamin B9), and v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12), (b) producing the polypeptide; (c) and harvesting the polypeptide produced by the host cell.

[0009] Also provided in one example is a method for decreasing trisulfide bond levels in a polypeptide selected from the group consisting of: a CEA-IL2v immunocytokine, a FAP-IL2v immunocytokine, an anti-CEA/anti-CD3 bispecific antibody, an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-Ang2/anti-VEGF bispecific antibody, an anti-C5 antibody and an anti-CD40 antibody , the method comprising: (a) culturing a host cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises one or more of the following components: i) between about 2 μM to about 35μM iron, and ii) between about 0 and about 4.5 mM methionine; (b) producing the polypeptide; and (c) harvesting the polypeptide produced by the host cell.

[0010] In certain examples according to (or as applied to) any of the examples above, the method further comprises at least one feed, and wherein the feed medium lacks one or more of the following: iron, riboflavin, pyridoxine, pyridoxal, folic acid, and cyanocobalamin. In certain examples according to (or as applied to) any of the examples above, the feed is a batch feed. In certain examples according to (or as applied to) any of the examples above, the batch feed medium lacks cystine. In certain

examples according to (or as applied to) any of the examples above, the batch feed medium lacks cysteine. In certain examples according to (or as applied to) any of the examples above, the batch feed medium lacks methionine. In certain examples according to (or as applied to) any of the examples above, the iron is ferric iron (Fe^{3+}) or ferrous iron (Fe^{2+}).

[0011] In certain examples according to (or as applied to) any of the examples above, the method further comprises: (I) supplementing the culture of said host cell with a chelating agent and a reducing agent prior to harvest; (II) supplementing a pre-harvest cell culture fluid (PHCCF) of said host cell with a chelating agent and a reducing agent; or (III) supplementing a harvested cell culture fluid (HCCF) of said host cell with a chelating agent and a reducing agent following harvest.

[0012] Also provided in one example is a method for decreasing level of trisulfide bonds in a polypeptide produced by a host cell comprising: (i) supplementing a culture of said host cell with a reducing agent and a chelating agent prior to harvest; (ii) supplementing a pre-harvest cell culture fluid (PHCCF) of said host cell with a chelating agent and a reducing agent; or (iii) supplementing a harvested cell culture fluid (HCCF) of said host cell with a reducing agent and a chelating agent.

[0013] In certain examples according to (or as applied to) any of the examples above, the culture, the PHCCF, or the HCCF of said host cell is supplemented with the chelating agent prior to being supplemented with the reducing agent. In certain examples according to (or as applied to) any of the examples above, the PHCCF, or the HCCF of said host cell is supplemented with the chelating agent between about 60 minutes to about 30 minutes prior to being supplemented with the reducing agent. In certain examples according to (or as applied to) any of the examples above, the chelating agent and the reducing agent are maintained in the culture, the PHCCF, or the HCCF of said host cell for about 30 minutes to about 4 days. In certain examples according to (or as applied to) any of the examples above, the culture, the PHCCF, or the HCCF of said host cell is maintained at a temperature between about 15°C and about 37°C. In certain examples according to (or as applied to) any of the examples above, the culture, the PHCCF, or the HCCF of said host cell is maintained pH between about 6.5 to about 7.5. In certain examples according to (or as applied to) any of the examples above, the amount of dissolved oxygen (DO) in the culture, the PHCCF, or the HCCF of said host cell is at least about 15%. In certain examples according to (or as applied to) any of the examples

above, the culture, the PHCCF, or the HCCF of said host cell is maintained at a temperature between about 15°C and about 37°C and at a pH between about 6.5 to about 7.5, and wherein the amount of dissolved oxygen (DO) in the culture or HCCF of said host cell is at least about 15%.

[0014] In certain examples according to (or as applied to) any of the examples above, the reducing agent is selected from the group consisting of: glutathione (GSH), L-glutathione (L-GSH), cysteine, L-cysteine, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 2,3-tert-butyl-4-hydroxyanisole, 2,6-di-tert-butyl-4-methylphenol, 3-aminopropane-1-sulfonic acid, adenosylhomocysteine, anserine, B-alanine, B-carotene, butylated hydroxyanisole, butylated hydroxytoluene, carnosine, carvedilol, curcumin, cysteamine, cysteamine hydrochloride, dexamethasone, diallyldisulfide, DL-lanthionine, DL-thiorphan, ethoxyquin, gallic acid, gentisic acid sodium salt hydrate, glutathione disulfide, glutathione reduced ethyl ester, glycine, hydrocortisone, hypotaurine, isethionic acid ammonium salt, L-cysteine-glutathione Disulfide, L-cysteinesulfinic acid monohydrate, lipoic acid, reduced lipoic acid, mercaptopropionyl glycine, methionine, methylenebis(3-thiopropionic acid), oxalic acid, quercitrin hydrate, resveratrol, retinoic acid, S-carboxymethyl-L-cysteine, selenium, selenomethionine, silver diethyldithiocarbamate, taurine, thiolactic acid, tricine, vitamin C, vitamin E, vitamin B1, vitamin B2, vitamin B3, vitamin B4, vitamin B5, vitamin B6, and vitamin B11. In certain examples according to (or as applied to) any of the examples above, the reducing agent is selected from the group consisting of: cysteine and L-cysteine. In certain examples according to (or as applied to) any of the examples above, the reducing agent is L-cysteine, and wherein the L-cysteine is added to the culture or HCCF of said host cell to achieve a final concentration between about 3 mM and about 6 mM.

[0015] In certain examples according to (or as applied to) any of the examples above, the chelating agent is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), ethylenediamine-N,N'-disuccinic acid (EDDS), citrate, oxalate, tartrate, ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA), diethylenetriaminepentaacetic acid (DTPA), 5-sulfosalicylic acid, N,N-dimethyldodecylamine N-oxide, dithiooxamide, ethylenediamine, salicylaldoxime, N-(2'-hydroxyethyl)iminodiacetic acid (HIMDA), oxine quinolinol, and sulphoxine. In certain examples according to (or as applied to) any of the examples above, the chelating agent is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA),

nitrilotriacetic acid (NTA), ethylenediamine-N,N'-disuccinic acid (EDDS), and citrate. In certain examples according to (or as applied to) any of the examples above, the chelating agent is added to the culture or the HCCF of said host cell to achieve a final concentration of 20mM.

[0016] In certain examples according to (or as applied to) any of the examples above, the polypeptide is secreted into the cell culture medium. In certain examples according to (or as applied to) any of the examples above, the method further comprises a step of purifying the harvested polypeptide. In certain examples according to (or as applied to) any of the examples above, the host cell is a recombinant host cell. In certain examples according to (or as applied to) any of the examples above, the host cell is a mammalian cell. In certain examples according to (or as applied to) any of the examples above, the mammalian cell is a CHO cell. In certain examples according to (or as applied to) any of the examples above, the method further comprises measuring the level of trisulfide bonds in the polypeptide. In certain examples according to (or as applied to) any of the examples above, the average % trisulfide bonds in the polypeptide is less than about 20%, less than about 10% less than about 5%, less than about 1%, less than about 0.5%, or less than about 0.1%.

[0017] In certain examples according to (or as applied to) any of the examples above, the polypeptide is an antibody or fragment thereof. In certain examples according to (or as applied to) any of the examples above, the polypeptide is an antibody fragment, and wherein the antibody fragment is selected from the group consisting of: a Fab, a Fab', an $F(ab')_2$, an scFv, an $(scFv)_2$, a dAb, a complementarity determining region (CDR) fragment, a linear antibody, a single-chain antibody molecule, a minibody , a diabody, and multispecific antibody formed from antibody fragments. In certain examples according to (or as applied to) any of the examples above, the antibody or fragment thereof binds to an antigen selected from the group consisting of: BMPR1B, E16, STEAP1, 0772P, MPF, Napi3b, Sema 5b, PSCA hlg, ETBR, MSG783, STEAP2, TrpM4, C5, CRIPTO, CD21, CD79b, FcRH2, HER2, NCA, MDP, IL20Rα, Brevican, EphB2R, ASLG659, PSCA, GEDA, BAFF-R, CD22, CD79a, CXCR5, HLA-DOB, P2X5, CD72, LY64, FcRH1, IRTA2, TENB2, PMEL17, TMEFF1, GDNF-Ra1, Ly6E, TMEM46, Ly6G6D, LGR5, RET, LY6K, GPR19, GPR54, ASPHD1, Tyrosinase, TMEM118, GPR172A, CD33, CLL-1, OX40, α4β7 and αEβ7 integrin heterodimers, IL-13, CD-20, FGFR, influenza A, influenza B, amyloid beta, HER3, complement factor D, IL-22c, PD-L1, PD-L2, PD-1, VEGF, Angiopoietin 2, CD3, FAP,

CEA, and IL-6. In certain examples according to (or as applied to) any of the examples above, the polypeptide is an antibody, and wherein the antibody is a bispecific antibody. In certain examples according to (or as applied to) any of the examples above, the bispecific antibody is an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-CEA/anti-CD3 bispecific antibody, or an anti-Ang2/anti-VEGF bispecific antibody. In certain examples according to (or as applied to) any of the examples above, the polypeptide is an immunocytokine. In certain examples according to (or as applied to) any of the examples above, the immunocytokine is CEA-IL2v or FAP-IL2v.

[0018] Also provided in one example is the use of between about 0 and about 4.5 μM methionine in a cell culture medium for decreasing trisulfide bond levels in a polypeptide selected from the group consisting of: a CEA-IL2v immuocytokine, a FAP-IL2v immunocytokine, an anti-CEA/anti-CD3 bispecific antibody, an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-Ang2/anti-VEGF bispecific antibody, an anti-C5 antibody, and an anti-CD40 antibody.

[0019] It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure. These and other aspects of the disclosure will become apparent to one of skill in the art.

[0020] All publications, patents, and patent applications cited herein are incorporated by reference in their entirety for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **FIG. 1** provides the results of experiments performed to assess the % trisulfide bond in anti-FluB incubated in a cell-free system containing Medium 1 + cysteine (Cys); Medium 1 + Cys + Fe (iron); Medium 1 + cystine (Cys-Cys); Medium 1 + Cys-Cys + Fe; Medium 2 + Cys; Medium 2 + Cys + Fe; Medium 2 + Cys-Cys; or Medium 1 + Cys-Cys + Fe.

[0022] **FIG. 2A** provides the results of experiments performed to assess the % trisulfide bond in anti-FluB incubated in Medium 1 that was supplemented with one or more of the following components: (a) Cys-Cys, (b) Fe, and (c) B vitamins (riboflavin, pyridoxine, folic acid, and cyanocobalamin).

[0023] **FIG. 2B** provides the results of experiments performed to assess the % trisulfide bond in an anti-OX40 antibody incubated in Medium 1 that was supplemented with

CLAIMS

What is claimed is:

1. A method for decreasing trisulfide bond levels in a polypeptide comprising:

(a) contacting a host cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises the following components:

i) between 2 μM to 35μM iron,

ii) between 0.11 μM to 2 μM riboflavin (vitamin B2),

iii) between 4.5 μM to 80 μM pyridoxine or pyridoxal (vitamin B6),

iv) between 3.4 μM to 23 μM folic acid (vitamin B9), and

v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12),

(b) culturing the host cell to produce the polypeptide; and

(c) harvesting the polypeptide produced by the host cell.

2. A method for producing a polypeptide, comprising:

(a) contacting a host cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises the following components:

i) between 2 μM to 35μM iron,

ii) between 0.11 μM to 2 μM riboflavin (vitamin B2),

iii) between 4.5 μM to 80 μM pyridoxine or pyridoxal (vitamin B6),

iv) between 3.4 μM to 23 μM folic acid (vitamin B9), and

v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12),

(b) culturing the host cell to produce the polypeptide; and

(c) harvesting the polypeptide produced by the host cell.

3. The method of claim 1 or claim 2, whereby the harvested polypeptide has a trisulfide bond level less than a polypeptide produced under identical conditions, except that the concentration of the one or more components differs from the concentration specified in (a).

4. The method of claim 1-3, wherein the basal medium lacks cystine.

5. The method of claim 1-3, wherein the basal medium comprises between 1.4 mM to 3 mM cysteine or cystine.

6. The method of any of claims 1-4, wherein the basal medium comprises between 0 mM to 1.58 mM methionine and between 0 mM to 3 mM cysteine.

7. The method of any one of claims 1-3, wherein the basal medium comprises 6mM cysteine.

8. A method for decreasing trisulfide bond levels in a polypeptide comprising:

(a) culturing a host cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises the following components:

i) between 2 μM to 35μM iron,

ii) between 0.11 μM to 2 μM riboflavin (vitamin B2),

iii) between 4.5 μM to 80 μM pyridoxine or pyridoxal (vitamin B6),

iv) between 3.4 μM to 23 μM folate/folic acid (vitamin B9), and

v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12),

(b) producing the polypeptide;

(c) and harvesting the polypeptide produced by the host cell.

9. The method of claim 8, wherein the concentration of one or more of the components in the cell culture medium is the cumulative concentration of one or more additions after inoculation.

10. A method for decreasing trisulfide bond levels in a polypeptide selected from the group consisting of: a CEA-IL2v immunocytokine, a FAP-IL2v immunocytokine, an anti-CEA/anti-CD3 bispecific antibody, an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-Ang2/anti-VEGF bispecific antibody, an anti-C5 antibody, and an anti-CD40 antibody, the method comprising:

(a) culturing a host cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises the following components:

i) between 2 μM to 35μM iron,

ii) between 0.11 μM to 2 μM riboflavin (vitamin B2),

iii) between 4.5 μM to 80 μM pyridoxine or pyridoxal (vitamin B6),

iv) between 3.4 μM to 23 μM folate/folic acid (vitamin B9), and

v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12),

(b) producing the polypeptide;

(c) and harvesting the polypeptide produced by the host cell.

11. The method of any one of claims 1-10, wherein the culture medium further comprises

vi) between 9 mM and 10 mM hypotaurine; and

vii) between 0 and 4.5 mM methionine.

12. The method of any one of claims 1-11, wherein the method further comprises at least one feed, and wherein the feed medium lacks one or more of the following: iron, riboflavin, pyridoxine, pyridoxal, folic acid, and cyanocobalamin.

13. The method of claim 12, wherein the feed is a batch feed.

14. The method of claim 13, wherein the batch feed medium lacks cystine.

15. The method of claim 13 or 14, wherein the batch feed medium lacks cysteine.

16. The method of any one of claims 13-15, wherein the batch feed medium lacks methionine.

17. The method of any one claims 1-16, wherein the iron is ferric iron (Fe^{3+}) or ferrous iron (Fe^{2+}).

18. The method any one of claims 1-17, wherein the method further comprises:

(I) supplementing the culture of said host cell with a chelating agent and a reducing agent prior to harvest;

(II) supplementing a pre-harvest cell culture fluid (PHCCF) of said host cell with a chelating agent and a reducing agent; or

(III) supplementing a harvested cell culture fluid (HCCF) of said host cell with a chelating agent and a reducing agent following harvest.

19. The method of claim 18, wherein the culture, the PHCCF, or the HCCF of said host cell is supplemented with the chelating agent prior to being supplemented with the reducing agent.

20. The method of claim 19, wherein the culture, the PHCCF, or the HCCF of said host cell is supplemented with the chelating agent between 60 minutes to 30 minutes prior to being supplemented with the reducing agent.

21. The method of any one of claims 18-20, wherein the chelating agent and the reducing agent are maintained in the culture, the PHCCF, or the HCCF of said host cell for 30 minutes to 4 days.

22. The method of any one of claims 18-21, wherein the culture, the PHCCF, or the HCCF of said host cell is maintained at a temperature between 15°C and 37°C.

23. The method of any one of claims 18-22, wherein the culture, the PHCCF, or the HCCF of said host cell is maintained pH between 6.5 to 7.5.

24. The method of any one of claims 18-23, wherein the amount of dissolved oxygen (DO) in the culture, the PHCCF, or the HCCF of said host cell is at least 15%.

25. The method of any one of claims 18-24, wherein the reducing agent is selected from the group consisting of: glutathione (GSH), L-glutathione (L-GSH), cysteine, L-cysteine, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 2,3-tert-butyl-4-hydroxyanisole, 2,6-di-tert-butyl-4-methylphenol, 3-aminopropane-1-sulfonic acid, adenosylhomocysteine, anserine, B-alanine, B-carotene, butylated hydroxyanisole, butylated hydroxytoluene, carnosine, carvedilol, curcumin, cysteamine, cysteamine hydrochloride, dexamethasone, diallyldisulfide, DL-lanthionine, DL-thiorphan, ethoxyquin, gallic acid, gentisic acid sodium salt hydrate, glutathione disulfide, glutathione reduced ethyl ester, glycine, hydrocortisone, hypotaurine, isethionic acid ammonium salt, L-cysteine-glutathione Disulfide, L-cysteinesulfinic acid monohydrate, lipoic acid, reduced lipoic acid, mercaptopropionyl glycine, methionine, methylenebis(3-thiopropionic acid), oxalic acid, quercitrin hydrate, resveratrol, retinoic acid, S-carboxymethyl-L-cysteine, selenium, selenomethionine, silver

diethyldithiocarbamate, taurine, thiolactic acid, tricine, vitamin C, vitamin E, vitamin B1, vitamin B2, vitamin B3, vitamin B4, vitamin B5, vitamin B6, and vitamin B11.

26. The method of claim 25, wherein the reducing agent is L-cysteine.

27. The method of claim 26, wherein the L-cysteine is added to the culture or HCCF of said host cell to achieve a final concentration between 3 mM and 6 mM.

28. The method of any one of claims 18-27, wherein the chelating agent is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), ethylenediamine-N,N'-disuccinic acid (EDDS), citrate, oxalate, tartrate, ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA), diethylenetriaminepentaacetic acid (DTPA), 5-sulfosalicylic acid, N,N-dimethyldodecylamine N-oxide, dithiooxamide, ethylenediamine, salicylaldoxime, N-(2'-hydroxyethyl)iminodiacetic acid (HIMDA), oxine quinolinol, and sulphoxine.

29. The method of claim 28, wherein the chelating agent is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), ethylenediamine-N,N'-disuccinic acid (EDDS), and citrate.

30. The method of claim 29, wherein the chelating agent is added to the culture or the HCCF of said host cell to achieve a final concentration of 20 mM.

31. The method of any one of claims 1-30, wherein the polypeptide is secreted into the cell culture medium.

32. The method of any one of claims 1-31, further comprising a step of purifying the harvested polypeptide.

33. The method of any one of claims 1-32, wherein the host cell is a recombinant host cell.

34. The method of any one of claims 1-33, wherein the host cell is a mammalian cell.

35. The method of claim 34, wherein the mammalian cell is a CHO cell.

36. The method of any one of claims 1-35, wherein the method further comprises measuring the level of trisulfide bonds in the polypeptide.

37. The method of any one of claims 1-36, wherein the average % trisulfide bonds in the polypeptide is less than 20%, optionally less than 10%, optionally less than 5%, optionally less than 1%, optionally less than 0.5%, or optionally less than 0.1%.

38. The method of any one of claims 1-9 and 11-37, wherein the polypeptide is an antibody or fragment thereof.

39. The method of claim 38, wherein the polypeptide is an antibody fragment, and wherein the antibody fragment is selected from the group consisting of: a Fab, a Fab', an $F(ab')_2$, an scFv, an $(scFv)_2$, a dAb, a complementarity determining region (CDR) fragment, a linear antibody, a single-chain antibody molecule, a minibody , a diabody, and multispecific antibody formed from antibody fragments.

40. The method of claim 39, wherein the antibody or fragment thereof binds to an antigen selected from the group consisting of: BMPR1B, E16, STEAP1, 0772P, MPF, Napi3b, Sema 5b, PSCA hlg, ETBR, MSG783, STEAP2, TrpM4, CRIPTO, CD21, CD79b, FcRH2, HER2, NCA, MDP, IL20Rα, Brevican, EphB2R, ASLG659, PSCA, GEDA, BAFF-R, CD22, CD79a, CXCR5, HLA-DOB, P2X5, CD72, LY64, FcRH1, IRTA2, TENB2, PMEL17, TMEFF1, GDNF-Ra1, Ly6E, TMEM46, Ly6G6D, LGR5, RET, LY6K, GPR19, GPR54, ASPHD1, Tyrosinase, TMEM118, GPR172A, CD33, CLL-1, C5, OX40, α4β7 and αEβ7 integrin heterodimers, IL-13, CD-20, FGFR, influenza A, influenza B, amyloid beta, HER3, complement factor D, IL-22c, PD-L1, PD-L2, PD-1, VEGF, Angiopoietin 2, CD3, FAP, CEA, and IL-6.

41. The method of claim 38, wherein the polypeptide is an antibody, and wherein the antibody is a bispecific antibody.

42. The method of claim 41, wherein the bispecific antibody is an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-CEA/anti-CD3 bispecific antibody, or an anti-Ang2/anti-VEGF bispecific antibody.

43. The method of any one of claims 1-9 and 11-37, wherein the polypeptide is an immunocytokine.

44. The method of claim 43, wherein the immunocytokine is CEA-IL2v or FAP-IL2v.