pyridoxal (vitamin B6),iv) between about 3.4 μM to about 23 μM folic acid (vitamin B9), v) between about 0.2 μM to about 2.5 μM cyanocobalamin (vitamin B12), vi) between about 9 mM and about 10 mM hypotaurine; and vii) between about 0 and about 1.58 mM methionine; (b) culturing the host cell to produce the polypeptide; and (c) harvesting the polypeptide produced by the host cell. In one aspect, the disclosure provides a method for decreasing trisulfide bond levels in a polypeptide comprising: (a) contacting a Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises: i) between 2 μM to 35 μM 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), v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12), and vi) between 1.58 mM to 2.25 mM methionine, (b) culturing the CHO cell to produce the polypeptide; and (c) harvesting the polypeptide produced by the CHO cell, wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations of any one of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a). In a related example, provided is a method for producing a polypeptide, comprising contacting a host cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises one or more of the following components: between about 2 μM to about 35μM iron, between about 0.11 μM to about 2 μM riboflavin (vitamin B2), between about 4.5 μM to about 80 μM pyridoxine or pyridoxal (vitamin B6), between about 3.4 μM to about 23 μM folic acid (vitamin B9), between about 0.2 μM to about 2.5 μM cyanocobalamin (vitamin B12), between about 9 mM and about 10 mM hypotaurine; and between about 0 and about 1.58 mM methionine; (b) culturing the host cell to produce the polypeptide; and (c)harvesting the polypeptide produced by the host cell. In another aspect, the disclosure provides a method for producing a polypeptide, comprising: (a) contacting a Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises: i) between 2 μM to 35 μM 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), v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12), and vi) between 1.58 mM to 2.25 mM methionine, (b) culturing the CHO cell to produce the polypeptide; and (c) harvesting the polypeptide produced by the CHO cell, wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations of any one of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a).

[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 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 one or more of the following components: i) between about 2 μM to about 35μM iron, ii) between about 0.11 μM to about 2 μM riboflavin (vitamin B2), iii) between about 4.5 μM to about 80 μM pyridoxine or pyridoxal (vitamin B6), iv) between about 3.4 μM to about 23 μM folate/folic acid (vitamin B9), v) between about 0.2 μM to about 2.5 μM cyanocobalamin (vitamin B12),

vi) between about 9 mM and about 10 mM hypotaurine; and vii) between about 0 and about 4.5 mM methionine; (b) producing the polypeptide; (c) and harvesting the polypeptide produced by the host cell. In one aspect, the disclosure provides a method for decreasing trisulfide bond levels in a polypeptide comprising: (a) culturing a Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises: i) between 2 μM to 35 μM 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), v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12), and vi) between 1.58 mM to 2.25 mM methionine, (b) producing the polypeptide; (c) and harvesting the polypeptide produced by the CHO cell, wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a). 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] 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 one or more of the following components: i) between about 2 μM to about 35μM iron, ii) between about 0.11 μM to about 2 μM riboflavin (vitamin B2), iii) between about 4.5 μM to about 80 μM pyridoxine or pyridoxal (vitamin B6), iv) between about 3.4 μM to about 23 μM folate/folic acid (vitamin B9), v) between about 0.2 μM to about 2.5 μM cyanocobalamin (vitamin B12), vi) between about 9 mM and about 10 mM hypotaurine; and vii) between about 0 and about 4.5 mM methionine; (b) producing the polypeptide; (c) and harvesting the polypeptide produced by the host cell. In one aspect, the disclosure provides 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 Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises: i) between 2 μM to 35 μM 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), v) between 0.2 μM to 2.5 μM cyanocobalamin (vitamin B12), and vi) between 1.58 mM to 2.25 mM methionine, (b) producing the polypeptide; (c) and harvesting the polypeptide produced by the CHO cell, wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a).

[0009] Also 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 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. In one aspect, the disclosure provides 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 Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises: i) between 2 μM to 35 μM iron, and ii) between 1.58 mM to 2.25 mM methionine; (b) producing the polypeptide; and (c) harvesting the polypeptide produced by the CHO cell, wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations of iron and methionine is increased from that specified in (a).

[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

CLAIMS

What is claimed is:

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

(a) contacting a Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises:

i) between 2 μM to 35 μM 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),

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

vi) between 1.58 mM to 2.25 mM methionine,

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

(c) harvesting the polypeptide produced by the CHO cell,

wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations of any one of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a).

2. A method for producing a polypeptide, comprising:

(a) contacting a Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises:

i) between 2 μM to 35 μM 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),

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

vi) between 1.58 mM to 2.25 mM methionine,

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

(c) harvesting the polypeptide produced by the CHO cell,

wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations

of any one of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a).

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 concentration of any one of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a).

4. The method of any one of claims 1-3, wherein the basal medium lacks cystine.

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

6. The method of any one of claims 1-4, wherein the basal medium comprises 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 Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises:

i) between 2 μM to 35 μM 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),

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

vi) between 1.58 mM to 2.25 mM methionine,

(b) producing the polypeptide;

(c) and harvesting the polypeptide produced by the CHO cell,

wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations

of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a).

9. The method of claim 8, wherein the concentration of i)-v), the concentration of A) or the concentration of 1) 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 Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises:

i) between 2 μM to 35 μM 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),

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

vi) between 1.58 mM to 2.25 mM methionine,

(b) producing the polypeptide;

(c) and harvesting the polypeptide produced by the CHO cell,

wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations of iron, vitamin B2, vitamin B6, vitamin B9, vitamin B12 and methionine is increased from that specified in (a).

11. 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 Chinese Hamster Ovary (CHO) cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises:

i) between 2 μM to 35 μM iron, and

ii) between 1.58 mM to 2.25 mM methionine;

(b) producing the polypeptide; and

(c) harvesting the polypeptide produced by the CHO cell,

wherein the method decreases trisulfide bond levels in the polypeptide to a level less than a polypeptide produced under identical conditions, except that the concentrations of iron and methionine is increased from that specified in (a).

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

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

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

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

(III) supplementing a harvested cell culture fluid (HCCF) of said CHO 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 CHO 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 CHO 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 CHO 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 CHO 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 CHO 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 CHO cell is at least 15%.

25. The method of any one of claims 18-21, wherein the culture, the PHCCF, or the HCCF of said CHO cell is maintained at a temperature between 15°C and 37°C and at a pH between 6.5 to 7.5, and wherein the amount of dissolved oxygen (DO) in the culture or HCCF of said CHO cell is at least 15%.

26. The method of any one of claims 18-25, 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.

27. The method of claim 26, wherein the reducing agent is cysteine.

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

29. The method of any one of claims 18-28, 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.

30. The method of claim 29, 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.

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

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

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

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

35. The method of any one of claims 1-34, wherein the method further comprises measuring the level of trisulfide bonds in the polypeptide.

36. The method of any one of claims 1-35, wherein the average % trisulfide bonds in the polypeptide is less than 20%, less than 10% less than 5%, less than 1%, less than 0.5%, or less than 0.1%.

37. The method of any one of claims 1-9 and 12-36, wherein the polypeptide is an antibody or fragment thereof.

38. The method of claim 37, 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.

39. The method of claim 37, 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.

40. The method of claim 37, wherein the polypeptide is an antibody, and wherein the antibody is a bispecific antibody.

41. The method of claim 40, 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.

42. The method of any one of claims 1-9 and 12-36, wherein the polypeptide is an immunocytokine.

43. The method of claim 42, wherein the immunocytokine is CEA-IL2v or FAP-IL2v.

44. The method of any one of claims 1-43, wherein the method is used for producing the polypeptide.

45. The method of claim 44, wherein the average % trisulfide in the polypeptide is less than 20%, less than 10% less than 5%, less than 1%, less than 0.5%, or less than 0.1%.

46. A method of manufacturing a medicament comprising the method of claim 45.