

operably linked to a third open-reading frame that encodes the third polypeptide chain, and a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain. In some examples, the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the eukaryotic host cell, and one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence. In some examples, the first subunit is expressed at a lower level than the second subunit when all subunits are expressed together in the same eukaryotic host cell, and one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence. In some examples, the multimeric polypeptide is a bispecific antibody. In some examples, the multimeric polypeptide is a bispecific antibody, the first and third polypeptide chains are antibody heavy chains, the second and fourth polypeptide chains are antibody light chains, the first subunit is a first half-antibody that binds a first antigen, and the second subunit is a second half-antibody that binds a second antigen.

[0012a] In one aspect, the present disclosure refers to a method for producing a ~~multimeric polypeptide~~bispecific antibody in a eukaryotic host cell, wherein the ~~multimeric polypeptide~~bispecific antibody comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the method comprising: (a) providing the eukaryotic host cell, wherein the eukaryotic host cell comprises a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain, a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain, a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain, and a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain, wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the eukaryotic host cell, and wherein one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence; (b) culturing the eukaryotic host cell under conditions suitable for expression of the first, second, third, and fourth polypeptide chains, wherein upon expression the first, second, third, and fourth polypeptide chains form the ~~multimeric polypeptide~~bispecific antibody; and (c) recovering the ~~multimeric polypeptide~~bispecific antibody produced by the eukaryotic host cell.

[0013] In another aspect, the present disclosure relates to a method for producing a bispecific antibody in a eukaryotic host cell, wherein the bispecific antibody comprises a first half antibody comprising a first antibody heavy chain and a first antibody light chain and a second half antibody comprising a second antibody heavy chain and a second antibody light chain, the method comprising: (a) providing the eukaryotic host cell, wherein the eukaryotic host cell comprises a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first antibody heavy chain, a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the first antibody light chain, a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the second antibody heavy chain, and a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the second antibody light chain, wherein the first half antibody is expressed at a lower level than the second half antibody when each half antibody is expressed individually in the eukaryotic host cell, and wherein one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence

expressed individually in the eukaryotic host cell. In some examples, the first subunit or half antibody is expressed at a level that is at least 1.3-fold, at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 3.5-fold, at least 4-fold, at least 4.5-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, 1.3-fold to 3-fold, 1.5-fold to 3-fold, 2-fold to 10-fold, 2-fold to 5-fold, 3-fold to 5-fold, 3-fold to 10-fold, 5-fold to 10-fold, or 7-fold to 10-fold lower than expression level of the second subunit or half antibody, *e.g.*, when each subunit or half antibody is expressed individually in the eukaryotic host cell. In some examples, the first subunit or half antibody is expressed at a level that is 0.2-fold to 0.8-fold, less than 0.8-fold, less than 0.5-fold, or less than 0.3-fold that of expression level of the second subunit or half antibody, *e.g.*, when each subunit or half antibody is expressed individually in the eukaryotic host cell.

[0016] Other examples of the present disclosure relate to a plurality or composition of multimeric polypeptides or bispecific antibodies, wherein each multimeric polypeptide or bispecific antibody of the plurality or composition is produced according to the method of any one of the examples described herein. ~~In one aspect, the present disclosure refers to a plurality of multimeric polypeptides, wherein each multimeric polypeptide of the plurality is produced according to any one of the methods described herein.~~

[0017] Other examples of the present disclosure relate to a recombinant eukaryotic host cell for expression of a non-native multimeric polypeptide that comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the host cell comprising: a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain; a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain; a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain; and a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain; wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the recombinant eukaryotic host cell or when all subunits are expressed together in the same eukaryotic host cell; and wherein one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence. In one aspect, the present disclosure refers to a recombinant eukaryotic host cell for expression of a non-native ~~multimeric polypeptide~~bispecific antibody that comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the recombinant cell comprising: (a) a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain; (b) a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain; (c) a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain; and (d) a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain; wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the recombinant eukaryotic host cell; and wherein one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence. In some examples, the multimeric polypeptide is a bispecific antibody, the first and third polypeptide chains

fourth translation initiation sequences are represented in the plurality of eukaryotic host cells; culturing the library of eukaryotic host cells under conditions suitable for expression of the multimeric polypeptide by the eukaryotic host cells of the plurality; measuring an amount of the multimeric polypeptide that is expressed by single eukaryotic host cells of the plurality or clones of single eukaryotic host cells of the plurality; and identifying the first, second, third, and fourth translation initiation sequences of one or more single eukaryotic host cells of the plurality or clones of single eukaryotic host cells of the plurality that express the multimeric polypeptide. In some examples, the first, second, third, and fourth translation initiation sequences of one or more single eukaryotic host cells of the plurality or clones of single eukaryotic host cells of the plurality that express the multimeric polypeptide are identified based on higher production of the multimeric polypeptide and/or a lower level of incorrectly paired side products, *e.g.*, as compared to a reference or different combination of four translation initiation sequences. In some examples, all of the first, second, third, and fourth translation initiation sequences in each host cell of the plurality comprise the sequence (from 5' to 3') NNNNNATGNGA, wherein N is C, G, A, or T/U (SEQ ID NO:1).

[0020a] In one aspect, the present disclosure refers to a method for identifying a combination of translation initiation sequences for expressing a ~~multimeric polypeptide bispecific antibody~~ in a eukaryotic host cell, wherein the ~~multimeric polypeptide bispecific antibody~~ comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the method comprising: (a) providing a library comprising a plurality of eukaryotic host cells, wherein each eukaryotic host cell in the plurality comprises: a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain, a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain, a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain, and a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain, wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the eukaryotic host cell, and wherein a plurality of combinations of first, second, third, and fourth translation initiation sequences are represented in the plurality of eukaryotic host cells; (b) culturing the library of eukaryotic host cells under conditions suitable for expression of the ~~multimeric polypeptide bispecific antibody~~ by the eukaryotic host cells of the plurality; (c) measuring an amount of the ~~multimeric polypeptide bispecific antibody~~ that is expressed by single eukaryotic host cells of the plurality or clones of single eukaryotic host cells of the plurality; and (d) identifying the first, second, third, and fourth translation initiation sequences of one or more single eukaryotic host cells of the plurality or clones of single eukaryotic host cells of the plurality that express the ~~multimeric polypeptide bispecific antibody~~.

[0021] Other examples of the present disclosure relate to kits or articles of manufacture comprising polynucleotides for expression of a multimeric polypeptide that comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain. In some examples, the kit or article of manufacture comprises a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain; a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain; a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain; and a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain; wherein one or more of the first, second, third, and fourth translation initiation sequences is not operably linked with its respective open-reading frame as the respective open-reading frame exists in a naturally occurring host cell genome. In some examples, the multimeric polypeptide is a bispecific antibody, the first and third polypeptide chains are antibody heavy chains, the second and fourth polypeptide chains are

antibody light chains, the first subunit is a first half-antibody that binds a first antigen, and the second subunit is a second half-antibody that binds a second antigen.

[0021a] In one aspect, the present disclosure refers to a kit of polynucleotides for expression of a ~~multimeric polypeptide~~bispecific antibody that comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the kit comprising: (a) a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain; (b) a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain; (c) a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain; and (d) a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain; wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the eukaryotic host cell; and wherein one or more of the first, second, third, and fourth translation initiation sequences is not operably linked with its respective open-reading frame as the respective open-reading frame exists in a naturally occurring host cell genome.

[0022] In some examples, one or both polypeptide chains of the first subunit are produced at a lower level than one or both polypeptide chains of the second subunit when each subunit is produced individually. In some examples, one or both polypeptide chains of the first subunit are produced at a lower level than one or both polypeptide chains of the second subunit when all subunits are produced by the same host cell. In some examples, the first subunit assembles with more incorrectly paired side products than the second subunit when each subunit is expressed individually in the eukaryotic host cell. In some examples, the first subunit assembles with more incorrectly paired side products than the second subunit when all subunits are expressed together in the same eukaryotic host cell.

[0023] In some examples described herein, all of the first, second, third, and fourth translation initiation sequences comprise the sequence (from 5' to 3') NNNNNATGNGA, wherein N is C, G, A, or T/U (SEQ ID NO:1). In some examples, one or both of the first translation initiation sequence and the second translation initiation sequence comprises a sequence selected from the group consisting of SEQ ID NOs:8-10. In some examples, one or both of the third translation initiation sequence and the fourth translation initiation sequence comprises the sequence ACCATGG (SEQ ID NO:3) or GAAGTATGA (SEQ ID NO:11). In some examples, the first translation initiation sequence comprises a sequence selected from the group consisting of SEQ ID NOs:8-10, the second translation initiation sequence comprises a sequence selected from the group consisting of SEQ ID NOs:8-10, the third translation initiation sequence comprises the sequence of SEQ ID NO:2, and the fourth translation initiation sequence comprises the sequence of SEQ ID NO:11. In some examples, the first translation initiation sequence comprises the sequence of SEQ ID NO:9, the second translation initiation sequence comprises the sequence of SEQ ID NO:9, the third translation initiation sequence comprises the sequence of SEQ ID NO:2, and the fourth translation initiation sequence comprises the sequence of SEQ ID NO:11. In some examples, each of the first, second, third, and fourth polynucleotides are operably linked to a promoter. In some examples, the first and the second polynucleotides are operably linked to the same promoter, and the third and the fourth polynucleotides are operably linked to the same promoter. In some examples, the first translation initiation sequence is weaker than the third translation initiation sequence. In some examples, the second translation initiation

CLAIMS

What is claimed is:

1. A method for producing a ~~multimeric polypeptide~~bispecific antibody in a eukaryotic host cell, wherein the ~~multimeric polypeptide~~bispecific antibody comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the method comprising:

(a) providing the eukaryotic host cell,

wherein the eukaryotic host cell comprises a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain, a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain, a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain, and a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain,

wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the eukaryotic host cell, and

wherein one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence;

(b) culturing the eukaryotic host cell under conditions suitable for expression of the first, second, third, and fourth polypeptide chains, wherein upon expression the first, second, third, and fourth polypeptide chains form the ~~multimeric polypeptide~~bispecific antibody; and

(c) recovering the ~~multimeric polypeptide~~bispecific antibody produced by the eukaryotic host cell.

2. The method of claim 1, wherein one or both polypeptide chains of the first subunit are expressed at a lower level than one or both polypeptide chains of the second subunit when all subunits are expressed in the same host cell.
3. The method of claim 1 or claim 2, wherein all of the first, second, third, and fourth translation initiation sequences comprise the sequence (from 5' to 3') NNNNNATGNGA, wherein N is C, G, A, or T/U (SEQ ID NO:1).
4. The method of any one of claims 1-3, wherein one or both of the first translation initiation sequence and the second translation initiation sequence comprises a sequence selected from the group consisting of SEQ ID NOs:8-10.
5. The method of any one of claims 1-4, wherein one or both of the third translation initiation sequence and the fourth translation initiation sequence comprises the sequence ACCATGG (SEQ ID NO:3) or GAAGTATGA (SEQ ID NO:11).
6. The method of claim 1 or claim 2, wherein the first translation initiation sequence comprises the sequence of SEQ ID NO:9, wherein the second translation initiation sequence comprises the sequence of SEQ ID NO:9, wherein the third translation initiation sequence comprises the sequence of SEQ ID NO:2, and wherein the fourth translation initiation sequence comprises the sequence of SEQ ID NO:11.
7. The method of any one of claims 1-6, wherein each of the first, second, third, and fourth polynucleotides are operably linked to a promoter.
8. The method of claim 7, wherein the first and the second polynucleotides are operably linked to the same promoter, and wherein the third and the fourth polynucleotides are operably linked to the same promoter.
9. The method of any one of claims 1-8, wherein the first translation initiation sequence is weaker than the third translation initiation sequence.
10. The method of any one of claims 1-9, wherein the second translation initiation sequence is weaker than the fourth translation initiation sequence.
11. The method of any one of claims 1-10, wherein the first translation initiation sequence is weaker than the fourth translation initiation sequence.

12. The method of any one of claims 1-11, wherein the second translation initiation sequence is weaker than the third translation initiation sequence.
13. The method of any one of claims 1-12, wherein the first translation initiation sequence is the same as the second translation initiation sequence.
14. The method of any one of claims 1-13, wherein the third translation initiation sequence is the same as the fourth translation initiation sequence.
15. The method of any one of claims 1-14, wherein the ~~multimeric polypeptide~~bispecific antibody specifically binds one or more target antigens.

~~16. The method of any one of claims 1-15, wherein the multimeric polypeptide is a multispecific antigen-binding protein.~~

~~17.~~16. A method for producing a bispecific antibody in a eukaryotic host cell, wherein the bispecific antibody comprises a first half antibody comprising a first antibody heavy chain and a first antibody light chain and a second half antibody comprising a second antibody heavy chain and a second antibody light chain, the method comprising:

- (a) providing the eukaryotic host cell,

wherein the eukaryotic host cell comprises a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first antibody heavy chain, a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the first antibody light chain, a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the second antibody heavy chain, and a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the second antibody light chain,

wherein the first half antibody is expressed at a lower level than the second half antibody when each half antibody is expressed individually in the eukaryotic host cell, and

wherein one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence;

(b) culturing the eukaryotic host cell under conditions suitable for expression of the first and second antibody heavy and light chains, wherein the first and second antibody heavy and light chains form the bispecific antibody, and wherein the first half antibody binds a first antigen and the second half antibody binds a second antigen; and

(c) recovering the bispecific antibody produced by the eukaryotic host cell.

18.17. The method of claim ~~17~~16, wherein the first antibody heavy chain comprises a first antibody Fc region comprising a CH2 domain and a CH3 domain; wherein the second antibody heavy chain comprises a second antibody Fc region comprising a CH2 domain and a CH3 domain; wherein the CH3 domain of the first antibody Fc region is altered so that within the CH3/CH3 interface one or more amino acid residues are replaced with amino acid residues having a smaller side chain volume, thereby generating a hole on the surface of the CH3 domain of the first antibody Fc region that interacts with the CH3 domain of the second antibody Fc region; and wherein the CH3 domain of the second antibody Fc region is altered so that within the CH3/CH3 interface one or more amino acid residues are replaced with amino acid residues having a larger side chain volume, thereby generating a knob on the surface of the CH3 domain of the second antibody Fc region that interacts with the CH3 domain of the first antibody Fc region.

19.18. The method of claim ~~17~~16, wherein the first antibody heavy chain comprises a first antibody Fc region comprising a CH2 domain and a CH3 domain; wherein the second antibody heavy chain comprises a second antibody Fc region comprising a CH2 domain and a CH3 domain; wherein the CH3 domain of the second antibody Fc region is altered so that within the CH3/CH3 interface one or more amino acid residues are replaced with amino acid residues having a smaller side chain volume, thereby generating a hole on the surface of the CH3 domain of the second antibody Fc region that interacts with the CH3 domain of the first antibody Fc region; and wherein the CH3 domain of the first antibody Fc region is altered so that within the CH3/CH3 interface one or more amino acid residues are replaced with amino acid residues having a larger side chain volume, thereby generating a knob on the surface of

the CH3 domain of the first antibody Fc region that interacts with the CH3 domain of the second antibody Fc region.

20.19. The method of claim ~~18-17~~ or claim ~~1918~~, wherein the knob mutation comprises at least one of: T366Y, T366W, T394W, and F405W, numbering based on human IgG1 according to EU index.

21.20. The method of any one of claims ~~1817-2019~~, wherein the hole mutation comprises at least one of: F405A, Y407T, Y407A, T366S, L368A, Y407V, and T394S, numbering based on human IgG1 according to EU index.

22.21. The method of any one of claims ~~1817-2420~~, wherein the knob mutation comprises T366W, and wherein the hole mutation comprises at least one, at least two, or all three of T366S, L368A, and Y407V, numbering based on human IgG1 according to EU index.

23.22. The method of any one of claims ~~1716-2221~~, wherein the first antibody light chain comprises a first mutation, wherein the first antibody heavy chain comprises a second mutation, ~~and~~ wherein the first and second mutations promote selective association of the first antibody light chain with the first antibody heavy chain, and wherein the first mutation comprises a positively charged mutation and the second mutation comprises a negatively charged mutation, or the first mutation comprises a negatively charged mutation and the second mutation comprises a positively charged mutation.

24.23. The method of claim ~~2322~~, wherein the first mutation comprises an amino acid substitution at V133, and wherein the second mutation comprises an amino acid substitution at S183, numbering based on EU index.

25.24. The method of claim ~~2423~~, wherein the S183 substitution is selected from the group consisting of S183A, S183T, S183V, S183Y, S183F, S183H, S183N, S183D, S183E, S183R, and S183K; and wherein the V133 substitution is selected from the group consisting of V133E, V133S, V133L, V133W, V133K, V133R, and V133D.

26.25. The method of claim ~~24-23~~ or claim ~~2524~~, wherein the amino acid substitution at S183 results in a positively charged residue and the amino acid substitution at V133 results in a negatively charged residue, or the amino acid substitution at S183 results in a negatively

charged residue and the amino acid substitution at V133 results in a positively charged residue.

27.26. The method of any one of claims ~~1716-2221~~, wherein the second antibody light chain comprises a third mutation, wherein the second antibody heavy chain comprises a fourth mutation, ~~and~~ wherein the third and fourth mutations promote selective association of the second antibody light chain with the second antibody heavy chain, and wherein the third mutation comprises a positively charged mutation and the fourth mutation comprises a negatively charged mutation, or the third mutation comprises a negatively charged mutation and the fourth mutation comprises a positively charged mutation.

28.27. The method of claim ~~2726~~, wherein the third mutation comprises an amino acid substitution at V133, and wherein the fourth mutation comprises an amino acid substitution at S183, numbering based on EU index.

29.28. The method of claim ~~2827~~, wherein the S183 substitution is selected from the group consisting of S183A, S183T, S183V, S183Y, S183F, S183H, S183N, S183D, S183E, S183R, and S183K; and wherein the V133 substitution is selected from the group consisting of V133E, V133S, V133L, V133W, V133K, V133R, and V133D.

30.29. The method of claim ~~2827~~ or claim ~~2928~~, wherein the amino acid substitution at S183 results in a positively charged residue and the amino acid substitution at V133 results in a negatively charged residue, or the amino acid substitution at S183 results in a negatively charged residue and the amino acid substitution at V133 results in a positively charged residue.

31.30. The method of any one of claims ~~1716-2221~~, wherein the first antibody light chain comprises a V133K mutation, the first antibody heavy chain comprises an S183E mutation, the second antibody light chain comprises a V133E mutation, and the second antibody heavy chain comprises an S183K mutation, numbering based on EU index.

32.31. The method of claim ~~3130~~, wherein the first antibody heavy chain further comprises T366S, L368A, and Y407V mutations, and the second antibody heavy chain further comprises a T366W mutation, numbering based on human IgG1 according to EU index.

33.32. The method of any one of claims ~~1716-2221~~, wherein the second antibody light chain comprises a V133K mutation, the second antibody heavy chain comprises an S183E mutation, the first antibody light chain comprises a V133E mutation, and the first antibody heavy chain comprises an S183K mutation, numbering based on EU index.

34.33. The method of claim ~~3332~~, wherein the second antibody heavy chain further comprises T366S, L368A, and Y407V mutations, and the first antibody heavy chain further comprises a T366W mutation, numbering based on human IgG1 according to EU index.

35.34. The method of any one of claims 1-~~3433~~, wherein the first, second, third, and fourth polynucleotides are integrated into one or more chromosomes of the eukaryotic host cell.

36.35. The method of claim ~~3534~~, wherein the first, second, third, and fourth polynucleotides are integrated into the same chromosomal locus of the eukaryotic host cell.

37.36. The method of any one of claims 1-~~3433~~, wherein the first, second, third, and fourth polynucleotides are part of one or more extrachromosomal polynucleotides in the eukaryotic host cell.

38.37. The method of any one of claims 1-~~3736~~, wherein the eukaryotic host cell is a mammalian host cell.

39.38. The method of claim ~~3837~~, wherein the mammalian host cell is a Chinese hamster ovary (CHO) cell.

40.39. The method of any one of claims 1-~~3938~~, wherein the first and/or second translation initiation sequence is at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% weaker than the third and/or fourth translation initiation sequence.

41.40. The method of any one of claims 1-~~3938~~, wherein the first subunit or half antibody is expressed at a level that is at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% lower than expression level of the second subunit or half antibody when each subunit or half antibody is expressed individually in the eukaryotic host cell.

~~42. A plurality of multimeric polypeptides, wherein each multimeric polypeptide of the plurality is produced according to the method of any one of claims 1-41.~~

43-41. A recombinant eukaryotic host cell for expression of a non-native bispecific antibody ~~multimeric polypeptide~~ that comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the recombinant cell comprising:

- (a) a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain;
- (b) a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain;
- (c) a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain; and
- (d) a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain;

wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the recombinant eukaryotic host cell; and

wherein one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence.

44-42. The cell of claim 43-41, wherein one or both polypeptide chains of the first subunit are expressed at a lower level than one or both polypeptide chains of the second subunit when all subunits are expressed in the same host cell.

45-43. The cell of claim 43-41 or claim 44-42, wherein all of the first, second, third, and fourth translation initiation sequences comprise the sequence (from 5' to 3') NNNNNATGNGA, wherein N is C, G, A, or T/U (SEQ ID NO:1).

46-44. The cell of any one of claims 43-41-45-43, wherein one or both of the first translation initiation sequence and the second translation initiation sequence comprises a sequence selected from the group consisting of SEQ ID NOs:8-10.

47.45. The cell of any one of claims 4341-4644, wherein one or both of the third translation initiation sequence and the fourth translation initiation sequence comprises the sequence ACCATGG (SEQ ID NO:3) or GAAGTATGA (SEQ ID NO:11).

48.46. The cell of any one of claims 4341-4543, wherein the first translation initiation sequence comprises the sequence of SEQ ID NO:9, wherein the second translation initiation sequence comprises the sequence of SEQ ID NO:9, wherein the third translation initiation sequence comprises the sequence of SEQ ID NO:2, and wherein the fourth translation initiation sequence comprises the sequence of SEQ ID NO:11.

49.47. The cell of any one of claims 4341-4846, wherein each of the first, second, third, and fourth polynucleotides are operably linked to a promoter.

50.48. The cell of claim 4947, wherein the first and the second polynucleotides are operably linked to the same promoter, and wherein the third and the fourth polynucleotides are operably linked to the same promoter.

51.49. The cell of any one of claims 4341-5048, wherein the first translation initiation sequence is weaker than the third translation initiation sequence.

52.50. The cell of any one of claims 4341-5149, wherein the second translation initiation sequence is weaker than the fourth translation initiation sequence.

53.51. The cell of any one of claims 4341-5250, wherein the first translation initiation sequence is weaker than the fourth translation initiation sequence.

54.52. The cell of any one of claims 4341-5351, wherein the second translation initiation sequence is weaker than the third translation initiation sequence.

55.53. The cell of any one of claims 4341-5452, wherein the first translation initiation sequence is the same as the second translation initiation sequence.

56.54. The cell of any one of claims 4341-5553, wherein the third translation initiation sequence is the same as the fourth translation initiation sequence.

57.55. The cell of any one of claims 4341-5654, wherein the folded and assembled bispecific antibody multimeric polypeptide specifically binds one or more target antigens.

~~58. The cell of any one of claims 43-57, wherein the multimeric polypeptide is a multispecific antigen binding protein.~~

~~59.~~56. A recombinant eukaryotic host cell for expression of a bispecific antibody that comprises a first half antibody comprising a first antibody heavy chain and a first antibody light chain and a second half antibody comprising a second antibody heavy chain and a second antibody light chain, the recombinant cell comprising:

- (a) a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first antibody heavy chain;
- (b) a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the first antibody light chain;
- (c) a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the second antibody heavy chain;
- and
- (d) a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the second antibody light chain;

wherein the first half antibody is expressed at a lower level than the second half antibody when each half antibody is expressed individually in the recombinant eukaryotic host cell; and

wherein one or both of the first translation initiation sequence and the second translation initiation sequence is weaker than one or both of the third translation initiation sequence and the fourth translation initiation sequence.

~~60.~~57. The cell of claim ~~59~~56, wherein the first antibody heavy chain comprises a first antibody Fc region comprising a CH2 domain and a CH3 domain; wherein the second antibody heavy chain comprises a second antibody Fc region comprising a CH2 domain and a CH3 domain; wherein the CH3 domain of the first antibody Fc region is altered so that within the CH3/CH3 interface one or more amino acid residues are replaced with amino acid residues having a smaller side chain volume, thereby generating a hole on the surface of the CH3 domain of the first antibody Fc region that interacts with the CH3 domain of the second antibody Fc region; and wherein the CH3 domain of the second antibody Fc region is altered

so that within the CH3/CH3 interface one or more amino acid residues are replaced with amino acid residues having a larger side chain volume, thereby generating a knob on the surface of the CH3 domain of the second antibody Fc region that interacts with the CH3 domain of the first antibody Fc region.

~~61-58.~~ The cell of claim ~~59~~56, wherein the first antibody heavy chain comprises a first antibody Fc region comprising a CH2 domain and a CH3 domain; wherein the second antibody heavy chain comprises a second antibody Fc region comprising a CH2 domain and a CH3 domain; wherein the CH3 domain of the second antibody Fc region is altered so that within the CH3/CH3 interface one or more amino acid residues are replaced with amino acid residues having a smaller side chain volume, thereby generating a hole on the surface of the CH3 domain of the second antibody Fc region that interacts with the CH3 domain of the first antibody Fc region; and wherein the CH3 domain of the first antibody Fc region is altered so that within the CH3/CH3 interface one or more amino acid residues are replaced with amino acid residues having a larger side chain volume, thereby generating a knob on the surface of the CH3 domain of the first antibody Fc region that interacts with the CH3 domain of the second antibody Fc region.

~~62-59.~~ The cell of claim ~~60-57~~ or claim ~~61-58~~, wherein the knob mutation comprises at least one of: T366Y, T366W, T394W, and F405W, numbering based on human IgG1 according to EU index.

~~63-60.~~ The cell of any one of claims ~~60-57-62-59~~, wherein the hole mutation comprises at least one of: F405A, Y407T, Y407A, T366S, L368A, Y407V, and T394S, numbering based on human IgG1 according to EU index.

~~64-61.~~ The cell of any one of claims ~~60-57-63-60~~, wherein the knob mutation comprises T366W, and wherein the hole mutation comprises at least one, at least two, or all three of T366S, L368A, and Y407V, numbering based on human IgG1 according to EU index.

~~65-62.~~ The cell of any one of claims ~~59-56-64-61~~, wherein the first antibody light chain comprises a first mutation, wherein the first antibody heavy chain comprises a second mutation, ~~and~~ wherein the first and second mutations promote selective association of the first antibody light chain with the first antibody heavy chain, and wherein the first mutation comprises a positively charged mutation and the second mutation comprises a negatively

charged mutation, or the first mutation comprises a negatively charged mutation and the second mutation comprises a positively charged mutation.

~~66-63.~~ The cell of claim ~~6562~~, wherein the first mutation comprises an amino acid substitution at V133, and wherein the second mutation comprises an amino acid substitution at S183, numbering based on EU index.

~~67-64.~~ The cell of claim ~~6663~~, wherein the S183 substitution is selected from the group consisting of S183A, S183T, S183V, S183Y, S183F, S183H, S183N, S183D, S183E, S183R, and S183K; and wherein the V133 substitution is selected from the group consisting of V133E, V133S, V133L, V133W, V133K, V133R, and V133D.

~~68-65.~~ The cell of claim ~~66-63~~ or claim ~~6764~~, wherein the amino acid substitution at S183 results in a positively charged residue and the amino acid substitution at V133 results in a negatively charged residue, or the amino acid substitution at S183 results in a negatively charged residue and the amino acid substitution at V133 results in a positively charged residue.

~~69-66.~~ The cell of any one of claims ~~5956-6461~~, wherein the second antibody light chain comprises a third mutation, wherein the second antibody heavy chain comprises a fourth mutation, and wherein the third and fourth mutations promote selective association of the second antibody light chain with the second antibody heavy chain, and wherein the third mutation comprises a positively charged mutation and the fourth mutation comprises a negatively charged mutation, or the third mutation comprises a negatively charged mutation and the fourth mutation comprises a positively charged mutation.

~~70-67.~~ The cell of claim ~~6966~~, wherein the third mutation comprises an amino acid substitution at V133, and wherein the fourth mutation comprises an amino acid substitution at S183, numbering based on EU index.

~~71-68.~~ The cell of claim ~~7067~~, wherein the S183 substitution is selected from the group consisting of S183A, S183T, S183V, S183Y, S183F, S183H, S183N, S183D, S183E, S183R, and S183K; and wherein the V133 substitution is selected from the group consisting of V133E, V133S, V133L, V133W, V133K, V133R, and V133D.

[~~72-69~~](#). The cell of claim [~~70-67~~](#) or claim [~~74-68~~](#), wherein the amino acid substitution at S183 results in a positively charged residue and the amino acid substitution at V133 results in a negatively charged residue, or the amino acid substitution at S183 results in a negatively charged residue and the amino acid substitution at V133 results in a positively charged residue.

[~~73-70~~](#). The cell of any one of claims [~~59-56-64-61~~](#), wherein the first antibody light chain comprises a V133K mutation, the first antibody heavy chain comprises an S183E mutation, the second antibody light chain comprises a V133E mutation, and the second antibody heavy chain comprises an S183K mutation, numbering based on EU index.

[~~74-71~~](#). The cell of claim [~~73-70~~](#), wherein the first antibody heavy chain further comprises T366S, L368A, and Y407V mutations, and the second antibody heavy chain further comprises a T366W mutation, numbering based on human IgG1 according to EU index.

[~~75-72~~](#). The cell of any one of claims [~~59-56-64-61~~](#), wherein the second antibody light chain comprises a V133K mutation, the second antibody heavy chain comprises an S183E mutation, the first antibody light chain comprises a V133E mutation, and the first antibody heavy chain comprises an S183K mutation, numbering based on EU index.

[~~76-73~~](#). The cell of claim [~~75-72~~](#), wherein the second antibody heavy chain further comprises T366S, L368A, and Y407V mutations, and the first antibody heavy chain further comprises a T366W mutation, numbering based on human IgG1 according to EU index.

[~~77-74~~](#). The cell of any one of claims [~~43-41-76-73~~](#), wherein the first, second, third, and fourth polynucleotides are integrated into one or more chromosomes of the eukaryotic host cell.

[~~78-75~~](#). The cell of claim [~~77-74~~](#), wherein the first, second, third, and fourth polynucleotides are integrated into the same chromosomal locus of the eukaryotic host cell.

[~~79-76~~](#). The cell of any one of claims [~~43-41-76-73~~](#), wherein the first, second, third, and fourth polynucleotides are part of one or more extrachromosomal polynucleotides in the eukaryotic host cell.

[~~80-77~~](#). The cell of any one of claims [~~43-41-79-76~~](#), wherein the eukaryotic host cell is a mammalian host cell.

~~81-78.~~ The cell of claim ~~8077~~, wherein the eukaryotic host cell is capable of the method results in higher production of the bispecific antibody~~multimeric polypeptide in the eukaryotic host cell.~~

~~82-79.~~ A method for identifying a combination of translation initiation sequences for expressing a bispecific antibody ~~multimeric polypeptide~~ in a eukaryotic host cell, wherein the ~~multimeric polypeptide~~bispecific antibody comprises a first subunit comprising a first polypeptide chain and a second polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the method comprising:

(a) providing a library comprising a plurality of eukaryotic host cells, wherein each eukaryotic host cell in the plurality comprises:

a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain,

a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain,

a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain, and

a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain,

wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the eukaryotic host cell, and

wherein a plurality of combinations of first, second, third, and fourth translation initiation sequences are represented in the plurality of eukaryotic host cells;

(b) culturing the library of eukaryotic host cells under conditions suitable for expression of the ~~multimeric polypeptide~~bispecific antibody by the eukaryotic host cells of the plurality;

(c) measuring an amount of the ~~multimeric polypeptide~~bispecific antibody that is expressed by single eukaryotic host cells of the plurality or clones of single eukaryotic host cells of the plurality; and

(d) identifying the first, second, third, and fourth translation initiation sequences of one or more single eukaryotic host cells of the plurality or clones of single eukaryotic host cells of the plurality that express the ~~multimeric polypeptide bispecific antibody~~.

83.80. The method of claim 8279, wherein all of the first, second, third, and fourth translation initiation sequences in each host cell of the plurality comprise the sequence (from 5' to 3') NNNNNATGNGA, wherein N is C, G, A, or T/U (SEQ ID NO:1).

84.81. The method of claim 8279 or claim 8380, wherein the ~~multimeric polypeptide bispecific antibody~~ specifically binds one or more target antigens.

~~85. The method of any one of claims 82-84, wherein the multimeric polypeptide is a multispecific antigen binding protein.~~

86.82. The method of any one of claims 8279-8481, ~~wherein the multimeric polypeptide is a bispecific antibody~~, wherein the first and third polypeptide chains are antibody heavy chains, wherein the second and fourth polypeptide chains are antibody light chains, wherein the first subunit is a first half-antibody that binds a first antigen, and wherein the second subunit is a second half-antibody that binds a second antigen.

87.83. The method of any one of claims 8279-8682, wherein the first, second, third, and fourth polynucleotides are integrated into one or more chromosomes of each eukaryotic host cell of the plurality.

88.84. The method of claim 8783, wherein the first, second, third, and fourth polynucleotides are integrated into the same chromosomal locus of each eukaryotic host cell of the plurality.

89.85. The method of any one of claims 8279-8682, wherein the first, second, third, and fourth polynucleotides are part of one of more extrachromosomal polynucleotides in each eukaryotic host cell of the plurality.

90.86. The method of any one of claims 8279-8985, wherein the eukaryotic host cell is a mammalian host cell.

91.87. The method of claim 9086, wherein the mammalian host cell is a Chinese hamster ovary (CHO) cell.

92.88. A kit of polynucleotides for expression of a ~~multimeric polypeptide bispecific antibody~~ that comprises a first subunit comprising a first polypeptide chain and a second

polypeptide chain and a second subunit comprising a third polypeptide chain and a fourth polypeptide chain, the kit comprising:

- (a) a first polynucleotide comprising a first translation initiation sequence operably linked to a first open-reading frame that encodes the first polypeptide chain;
- (b) a second polynucleotide comprising a second translation initiation sequence operably linked to a second open-reading frame that encodes the second polypeptide chain;
- (c) a third polynucleotide comprising a third translation initiation sequence operably linked to a third open-reading frame that encodes the third polypeptide chain; and
- (d) a fourth polynucleotide comprising a fourth translation initiation sequence operably linked to a fourth open-reading frame that encodes the fourth polypeptide chain;

wherein the first subunit is expressed at a lower level than the second subunit when each subunit is expressed individually in the eukaryotic host cell; and

wherein one or more of the first, second, third, and fourth translation initiation sequences is not operably linked with its respective open-reading frame as the respective open-reading frame exists in a naturally occurring host cell genome.

93-89. The kit of claim 92-88, wherein the first, second, third, and fourth polynucleotides are part of one or more expression vectors.

94-90. The kit of claim 92-88 or claim 93-89, wherein all of the first, second, third, and fourth translation initiation sequences comprise the sequence (from 5' to 3') NNNNNATGNGA, wherein N is C, G, A, or T/U (SEQ ID NO:1).

95-91. The kit of any one of claims 92-88-94-90, wherein the first translation initiation sequence comprises the sequence of SEQ ID NO:9, wherein the second translation initiation sequence comprises the sequence of SEQ ID NO:9, wherein the third translation initiation sequence comprises the sequence of SEQ ID NO:2, and wherein the fourth translation initiation sequence comprises the sequence of SEQ ID NO:11.

96-92. The kit of any one of claims 92-88-95-91, wherein each of the first, second, third, and fourth polynucleotides are operably linked to a promoter.

97.93. The kit of claim ~~96~~92, wherein the first and the second polynucleotides are operably linked to the same promoter, and wherein the third and the fourth polynucleotides are operably linked to the same promoter.