

from two different polypeptides, the heavy and light chains, which must be exported into the periplasm, folded properly and form the appropriate disulfide bonds. The complexity of this protein folding and secretion adds to the challenges of antibody manufacturing in *E. coli*. *E. coli* secretion and refolding capacity is often limited to a lower level compared to other expression hosts. While TIR optimization has been shown to be useful for generating more efficient protein secretion, other approaches not been shown to routinely improve the secretion of heterologous proteins in *E. coli*. For example, optimization of a signal protein was shown to decrease secretion of recombinant cyclodextrin glucanotransferase (CGTase) into the periplasmic space. *Jonet et al.*, J Mol Microbiol Biotechnol (2012); 22:48-58.

[00011] In the present work, the inventors demonstrate that increasing the average hydrophobicity of the signal peptide increased secretion of soluble antibody to the *E. coli* periplasm. Variant signal peptides with increased average hydrophobicity were developed and the inventors demonstrated increased periplasmic secretion of soluble antibody when signal peptide variants with increased average hydrophobicity were used, and decreased periplasmic secretion of soluble antibody when signal peptide variants with decreased average hydrophobicity were used.

[00012] In one aspect, provided are methods of increasing secretion and assembly of a biologically active antibody comprising an antibody heavy chain and an antibody light chain from an *E. coli* host cell, comprising culturing an *E. coli* host cell comprising polynucleotide comprising (1) a first polynucleotide encoding a first signal peptide operably linked to a polynucleotide encoding an antibody heavy chain; and (2) a second polynucleotide encoding a second signal peptide operably linked to a polynucleotide encoding an antibody light chain, wherein the first and/or second signal peptide is selected from the group consisting of:

- a) a variant DsbA signal peptide comprising a mutation at residue L11, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3, wherein the mutation is L11L,
- b) a variant STII signal peptide comprising a mutation at residue S11, wherein the variant has a greater average hydrophobicity than an STII signal peptide of SEQ ID NO:1, wherein the mutation is S11A, S11I or S11L, or
- c) a variant signal peptide comprising a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33, whereby upon expression of the antibody in a host cell, the heavy and light chains are folded and assembled to form a biologically active antibody.

[00013] In one embodiment, provided are methods of making an antibody heavy chain and/or light chain from an *E. coli* host cell, comprising culturing an *E. coli* host cell comprising polynucleotide comprising (1) a first polynucleotide encoding a first signal peptide operably linked to a polynucleotide encoding an antibody heavy chain, wherein the average hydrophobicity of the signal peptide is greater than about 0.5; and/or (2) a second polynucleotide encoding a second signal peptide operably linked to a polynucleotide encoding an antibody light chain, wherein the average hydrophobicity of the second signal

[00024] In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody, an affinity matured antibody, a bispecific antibody, humanized antibody, or a human antibody. In some embodiments, the antibody is a bispecific antibody.

[00025] Provided are variant DsbA signal peptides, wherein the variant comprises an H region with an average hydrophobicity that is greater than 0.5. In yet another aspect, provided are variant DsbA signal peptides comprising a mutation at residue L11 and/or S18, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3, wherein the mutation is L11I and/or S18Y.

[00026] In another embodiment, provided are variant DsbA signal peptides comprising a mutation at residue S11, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3. In some embodiments, the mutation is L11I and/or S18Y.

[00027] In another aspect, provided are variant STII signal peptides comprising a mutation at residue S11, wherein the variant STII signal peptide has a greater average hydrophobicity than a STII signal peptide of SEQ ID NO:1. In some embodiments, the mutation is S11A, S11I or S11L.

[00028] Provided are variant signal peptides consisting of, consisting essentially of or comprising a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33. In one aspect, provided are variant signal peptides comprising a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33.

[00029] In another aspect, provided is any of the variant signal peptides disclosed herein fused to a heterologous protein. In some embodiments, the heterologous polypeptide is an antibody heavy chain. In some embodiments, the heterologous polypeptide is an antibody light chain. In some embodiments, the heterologous polypeptide is an antibody light and heavy chain. In some embodiments, the heterologous polypeptide is a multimeric polypeptide. In some embodiments, the heterologous polypeptide is an immunoadhesin.

[00030] In another aspect, provided are polynucleotide sequences that encode any of the variant signal peptides disclosed herein.

[00031] Also provided are polynucleotide sequences that encode any of the variant signal peptides disclosed herein, operably linked to a polynucleotide encoding a heterologous polypeptide, whereby upon expression of the heterologous polypeptide in a host cell the heterologous polypeptide is folded and assembled to form a biologically active heterologous polypeptide.

[00032] In some embodiments, the host cell is a prokaryotic host cell. In some embodiments, the host cell is E coli.

[00033] In another aspect, provided are polynucleotides encoding an antibody, said polynucleotides comprising (1) a polynucleotide encoding a first signal peptide operably linked to a polynucleotide encoding an antibody heavy chain and (2) a polynucleotide

encoding a second signal peptide operably linked to a polynucleotide encoding an antibody light chain, whereby upon expression of the antibody in a host cell the heavy and light chains are folded and assembled to form a biologically active antibody, wherein the first signal peptide is a variant signal peptide of any one of variant signal peptide disclosed herein or wherein the first signal peptide consists of, consists essentially of, or comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33. In some embodiments, the first signal peptide is a variant DsbA signal peptide, wherein the variant comprises an H region with an average hydrophobicity that is greater than 0.5. In some embodiments, the first signal peptide is a variant DsbA signal peptide comprising a mutation at residue S11, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3. In some embodiments, the mutation is L11I and/or S18Y. In some embodiments, the first signal peptide is a variant STII signal peptide comprising a mutation at residue S11, wherein the variant STII signal peptide has a greater average hydrophobicity than a STII signal peptide of SEQ ID NO:1. In some embodiments, the mutation is S11A, S11I or S11L.

[00034] In some embodiments, the second signal peptide is a signal peptide. In some embodiments, the second signal peptide consists of, consists essentially of, or comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42. In some embodiments, the second signal peptide is a variant DsbA signal peptide, wherein the variant comprises an H region with an average hydrophobicity that is greater than 0.5. In some embodiments, the second signal peptide is a variant DsbA signal peptide comprising a mutation at residue S11, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3. In some embodiments, the mutation is L11I and/or S18Y. In some embodiments, the second signal peptide is a variant STII signal peptide comprising a mutation at residue S11, wherein the variant STII signal peptide has a greater average hydrophobicity than a STII signal peptide of SEQ ID NO:1. In some embodiments, the mutation is S11A, S11I or S11L.

[00035] In some embodiments, the polynucleotide encoding an antibody further comprises (3) a polypeptide encoding a third signal peptide operably linked to a polynucleotide encoding an Fc polypeptide. The third signal peptide may be, for example, any of the variant signal peptides disclosed herein. In some embodiments, the third signal peptide consists of, consists essentially of, or comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.

What is claimed is:

1. A method of increasing secretion and assembly of a biologically active antibody comprising an antibody heavy chain and an antibody light chain from an *E. coli* host cell, comprising culturing an *E. coli* host cell comprising polynucleotide comprising (1) a first polynucleotide encoding a first signal peptide operably linked to a polynucleotide encoding an antibody heavy chain; and (2) a second polynucleotide encoding a second signal peptide operably linked to a polynucleotide encoding an antibody light chain, wherein the first and/or second signal peptide is selected from the group consisting of:
 - a) a variant DsbA signal peptide comprising a mutation at residue L11, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3, wherein the mutation is L11I,
 - b) a variant STII signal peptide comprising a mutation at residue S11, wherein the variant has a greater average hydrophobicity than an STII signal peptide of SEQ ID NO:1, wherein the mutation is S11A, S11I or S11L, or
 - c) a variant signal peptide comprising a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33, whereby upon expression of the antibody in a host cell, the heavy and light chains are folded and assembled to form a biologically active antibody.
2. The method of claim 1, wherein the first and/or second signal peptide is a variant DsbA signal peptide comprising a mutation at residue L11, wherein the variant has a greater average hydrophobicity than a wildtype DsbA signal peptide of SEQ ID NO:3, wherein the mutation is L11I.
3. The method of claim 1, wherein the first and/or second signal peptide is a variant signal peptide comprising a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33.
4. The method of claim 1, wherein the first and/or second signal peptide is a variant DsbA signal peptide comprising a sequence of SEQ ID NO:13 or 15.
5. The method of claim 1, wherein the first and/or second signal peptide is a variant STII signal peptide comprising a mutation at residue S11, wherein the variant has a greater average hydrophobicity than an STII signal peptide of SEQ ID NO:1, wherein the mutation is S11A, S11I or S11L.
6. The method of any of claims 1-5, wherein the polynucleotide in the host cell further comprises a promoter.
7. The method of claim 6, wherein the promoter is a prokaryotic promoter selected from the group consisting of *phoA*, *tac*, *lpp*, *lac-lpp*, *lac*, *ara*, and T7 promoter.
8. The method of any one of claims 1-7, wherein the *E. coli* host cell is of a strain deficient in endogenous protease activities.

9. The method of claim 8, wherein the genotype of the *E. coli* lacks *degP* and *prc* genes and harbors a mutant *spr* gene.
10. The method of any one of claims 1-9, wherein the host cell further comprises a polynucleotide encoding at least one prokaryotic polypeptide selected from the group consisting of DsbA, DsbC, DsbG and FkpA.
11. The method of claim 10, wherein the polynucleotide encodes both DsbA and DsbC.
12. The method of any one of claims 1-11, wherein the method further comprises recovering the antibody from the host cell culture.
13. The method of claim 12, wherein the antibody is recovered from the host cell culture medium.
14. The method of claim 12 or 13, wherein the method further comprises combining the recovered antibody with a pharmaceutically acceptable carrier, excipient, or carrier to prepare a pharmaceutical formulation comprising the antibody.
15. The method of claim 12, wherein at least 50% of the immunoglobulin polypeptide complexes that are formed are the antibody.
16. The method of claims 12, wherein at least 70% of the immunoglobulin polypeptide complexes that are formed are the antibody.
17. The method of any one of claims 1-16, wherein the antibody is a monoclonal antibody.
18. The method of claim 17, wherein the antibody is a chimeric antibody, an affinity matured antibody, a bispecific antibody, humanized antibody, or a human antibody.
19. A variant DsbA signal peptide comprising a mutation at residue L11 and/or S18, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3, wherein the mutation is L11I and/or S18Y.
20. A variant STII signal peptide comprising a mutation at residue S11, wherein the variant STII signal peptide has a greater average hydrophobicity than a STII signal peptide of SEQ ID NO:1.
21. The variant STII signal peptide of claim 20, wherein the mutation is S11A, S11I or S11L.
22. A variant signal peptide comprising a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33.
23. The variant signal peptide of any one of claims 19-22, fused to a heterologous protein.
24. The variant signal peptide of claim 23, wherein the heterologous polypeptide is an antibody heavy chain, an antibody light chain, an antibody light and heavy chain, a multimeric polypeptide or an immunoadhesin.
25. A polynucleotide sequence that encodes a variant signal peptide of any one of claims 19-24.

26. The polynucleotide sequence of claim 25 operably linked to a polynucleotide encoding a heterologous polypeptide, whereby upon expression of the heterologous polypeptide in a host cell the heterologous polypeptide is folded and assembled to form a biologically active heterologous polypeptide.
27. The polynucleotide of claim 26, wherein the host cell is a prokaryotic host cell.
28. The polynucleotide of claim 27, wherein the host cell is *E. coli*.
29. A polynucleotide encoding an antibody, said polynucleotide comprising (1) a polynucleotide encoding a first signal peptide operably linked to a polynucleotide encoding an antibody heavy chain and (2) a polynucleotide encoding a second signal peptide operably linked to a polynucleotide encoding an antibody light chain, whereby upon expression of the antibody in a host cell the heavy and light chains are folded and assembled to form a biologically active antibody, wherein the first signal peptide is a variant signal peptide of any one of claims 19-21 or wherein the first signal peptide consists of, consists essentially of, or comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33.
30. The polynucleotide of claim 29, wherein the first signal peptide is a variant signal peptide of any one of claims 19-21.
31. The polynucleotide of claims 29, wherein the first signal peptide comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33.
32. The polynucleotide of any one of claims 29-31, wherein the second signal peptide is a variant signal peptide of any one of claims 19-21 or wherein the second signal peptide comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
33. The polynucleotide of any one of claims 29-31, wherein the second signal peptide is a variant signal peptide of any one of claim 19-21.
34. The polynucleotide of any one of claims 29-31, wherein the second signal peptide comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
35. The polynucleotide of any one of claims 29-34, wherein the polynucleotide encoding an antibody further comprises (3) a polypeptide encoding a third signal peptide operably linked to a polynucleotide encoding a Fc polypeptide.
36. The polynucleotide of claim 35, wherein the third signal peptide is a variant signal peptide of any one of claim 19-21 or wherein the third signal peptide comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
37. The polynucleotide of any one of claims 26-36, further comprising a promoter operably linked to the heterologous polypeptide.

38. The polynucleotide of claim 37, wherein the promoter is a prokaryotic promoter selected from the group consisting of *phoA*, *tac*, *lpp*, *lac-lpp*, *lac*, *ara*, *trp*, and T7 promoter.
39. The polynucleotide of claim 38, wherein the promoter is a *phoA* promoter.
40. The polynucleotide of any one of claims 26-39, further comprising (a) a first promoter, wherein the first promoter is operably linked to a light chain and (b) a second promoter, wherein the second promoter is operably linked to a heavy chain.
41. The polynucleotide of claim 40, wherein the first and second promoters are both *phoA* promoters.
42. The polynucleotide of claim 40, further comprising (c) a third promoter, wherein the third promoter is operably linked to a Fc polypeptide.
43. The polynucleotide of claim 42, wherein the third promoter is a *phoA* promoter.
44. The polynucleotide of any one of claims 26-28, wherein the heterologous polypeptide is a protease, an immunoadhesin, an extracellular domain of a receptor, a heteromultimeric protein, or an antibody.
45. The polynucleotide of any one of claims 29-43, wherein the antibody is a monoclonal antibody.
46. The polynucleotide of claim 45, wherein the antibody is a chimeric antibody, a one-armed antibody, a bispecific antibody, humanized antibody, an antibody fragment or a human antibody.
47. A vector comprising a polynucleotide of any one of claims 26-46.
48. The vector of claim 47, wherein the vector is an expression vector.
49. A composition comprising a polynucleotide of any one of claims 26-48, or the vector of claim 47 or 48.
50. A host cell comprising a polynucleotide of any one of claims 26-46.
51. The host cell of claim 50, wherein the host cell is a prokaryotic cell.
52. The host cell of claim 51, wherein the prokaryotic cell is *E. coli*.
53. The host cell of claim 52, wherein the *E. coli* is of a strain deficient in endogenous protease activities.
54. The host cell of claim 52 or 53, wherein the genotype of the *E. coli* lacks *degP* and *prc* genes and harbors a mutant *spr* gene.
55. The host cell of any one of claims 50-54, wherein the host cell further comprises a polynucleotide encoding a prokaryotic chaperone protein.
56. The host cell of claim 55, wherein the prokaryotic chaperone protein is DsbA and/or DsbC.

57. The host cell of claim 55 or 56, wherein the host cell overexpresses a prokaryotic chaperone protein.
58. A method of making an a heterologous polypeptide (such as an antibody heavy chain and/or light chain), said method comprising culturing a host cell of any one of claims 50-57 so that the nucleic acid is expressed, whereby upon expression of said polynucleotide in a host cell, the heterologous polypeptide is folded to form a biologically active heterologous polypeptide.
59. The method of claim 58, wherein the method further comprises recovering the heterologous polypeptide from the host cell culture.
60. The method of claim 59, wherein the heterologous polypeptide is recovered from the host cell culture medium.
61. The method of claim 59 or 60, wherein the method further comprises combining the recovered heterologous polypeptide with a pharmaceutically acceptable carrier, excipient, or carrier to prepare a pharmaceutical formulation comprising the heterologous polypeptide.
62. A method of secreting a heterologous polypeptide (such as an antibody heavy chain and/or light chain) from a cell, said method comprising culturing a host cell of any one of claims 50-57 so that the nucleic acid is expressed and the heterologous polypeptide is secreted.
63. A method of translocating a heterologous polypeptide (such as antibody heavy chain and/or light chain) from a cell, said method comprising culturing a host cell of any one of claims 50-57 so that the nucleic acid is expressed and the heterologous polypeptide is translocated.