

structure and biological activity of many bacterial species including *E. coli* have been well-studied and a wide range of suitable vectors are available, making expression of a desirable antibody more convenient. Compared with eukaryotes, fewer steps are involved in the production process, including the manipulation of recombinant genes, stable transformation of multiple copies into the host, expression induction and characterization of the products. Pluckthun and Pack (1997) *Immunotech* 3:83-105.

[0006] Various approaches have been used to make recombinant polypeptides in bacteria. Recombinant proteins can be obtained from bacteria either through refolding of inclusion bodies expressed in the cytoplasm, or through expression followed by secretion to the bacterial periplasm. The choice between secretion and refolding is generally guided by several considerations. Secretion is usually the faster and more commonly used strategy for producing antibodies. Kipriyanov and Little (1999), *supra*. However, *E. coli* secretion and refolding capacity is often limited to a lower level compared to other expression hosts.

[0007] Antibody expression in prokaryotic systems can be carried out in different scales. For general reviews of antibody production in *E. coli*, see Pluckthun and Pack (1997) *Immunotech* 3:83-105; Pluckthun et al. (1996) in ANTIBODY ENGINEERING: A PRACTICAL APPROACH, pp 203-252 (Oxford Press); Pluckthun (1994) in HANDBOOK OF EXP PHARMCOL VOL 3: THE PHARMCOL OF MONOCLONAL ANTIBODIES, pp269-315 (ed. M. Rosenberg and G.P. Moore; Springer-Verlag, Berlin).

[0008] Many biological assays (such as X-ray crystallography) and clinical applications (such as protein therapy) require large amounts of protein. Accordingly, a need exists for high yield yet simple systems for producing properly assembled, soluble and functional heterologous polypeptides, such as antibodies.

[0009] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[00010] The ~~invention-disclosure~~ provides a novel means for increasing production of heterologous proteins such as antibodies. The use of periplasmic secretion as a means for high-level production of heterologous proteins (e.g., antibodies) can be limited by several frequently encountered problems. First, secretion efficiency of the protein of interest may be low. Second, the precursor in many cases is incompletely processed to mature protein. Third, over-expressed heterologous proteins often fold improperly, aggregate into insoluble inclusion bodies, or are proteolyzed by *E. coli* proteases. Fourth, antibodies are complex multimeric proteins made

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 of an antibody heavy chain and/or 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, 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 peptide is greater than about 0.5, 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 aspect ~~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

peptide is greater than about 0.5, 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. In some embodiments, the method further comprises recovering the heterologous polypeptide from the host cell culture. In some embodiments, the heterologous polypeptide is recovered from the host cell culture medium. In some embodiments, 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.

[00014] In one ~~aspect~~ 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 peptide is greater than about 0.5, 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.

[00015] In one ~~aspect~~ embodiment, provided are methods of translocating 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 peptide is greater than about 0.5, 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.

[00016] In some embodiments, the average hydrophobicity of the first signal peptide is greater than about 0.6. In some embodiments, the average hydrophobicity of the first signal peptide is greater than about 0.7. In some embodiments, the average hydrophobicity of the second signal peptide is greater than about 0.6. In some embodiments, the average hydrophobicity of the second signal peptide is greater than about 0.7. In some embodiments, the average hydrophobicity of the first and second signal peptides is similar

[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] In another aspect, 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, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3.

[00026] In another ~~embodiment~~ aspect, 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] In another aspect, p~~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] In another aspect, A~~lso 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. ~~In some embodiments, or wherein~~ the first 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 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.

[00036] In some embodiments, the polynucleotide further comprises a promoter operably linked to the heterologous polypeptide. In some embodiments, the promoter is a prokaryotic promoter selected from the group consisting of phoA, tac, lpp, lac-lpp, lac, ara, trp, and T7 promoter. In some embodiments, the promoter is a phoA promoter.

[00037] In some embodiment, the polynucleotide comprises (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. In some embodiments, the first and second promoters are both phoA promoters.

[00038] In some embodiments, the polynucleotide further comprises (c) a third promoter, wherein the third promoter is operably linked to an Fc polypeptide. In some embodiments, the promoter is a phoA promoter.

[00039] In some embodiments, wherein the heterologous polypeptide is a protease, an immunoadhesin, an extracellular domain of a receptor, a heteromultimeric protein, or an antibody.

[00040] In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody, a bispecific antibody, humanized antibody, an antibody fragment or a human antibody. In some embodiments, the antibody is a bispecific antibody.

[00041] In one aspect, provided is aA vector comprising a polynucleotide of any of the polynucleotides disclosed herein. In some embodiments, the vector is an expression vector.

[00042] In one aspect, provided is aA composition comprising any of the polynucleotides disclosed herein, or any of the vectors disclosed herein.

[00043] In one aspect, provided is aA host cell comprising any of the polynucleotides disclosed herein. In some embodiments, the host cell is a prokaryotic cell. In some embodiments, the prokaryotic cell is E. coli. In some embodiments, the E. coli is of a strain deficient in endogenous protease activities. In some embodiments, the genotype of the E. coli lacks degP and prc genes and harbors a mutant spr gene. In some embodiments, the host cell further comprises a polynucleotide encoding a prokaryotic chaperone protein. In some embodiments, the prokaryotic chaperone protein is DsbA and/or DsbC. In some embodiments, host cell overexpresses a prokaryotic chaperone protein.

[00044] In another aspect, provided are methods of making an a heterologous polypeptide (such as an antibody heavy chain and/or light chain),

said method comprising culturing any of the host cells described herein 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.

In some embodiments, the method further comprises recovering the heterologous polypeptide from the host cell culture. In some embodiments, the heterologous polypeptide is recovered from the host cell culture medium. In some embodiments, 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.

[00045] In another aspect, provided are methods of secreting a heterologous polypeptide (such as an antibody heavy chain and/or light chain) from a cell, said method comprising culturing any of the host cells provided herein so that the nucleic acid is expressed and the heterologous polypeptide is secreted.

[00046] In another aspect, provided are methods of translocating a heterologous polypeptide (such as an antibody heavy chain and/or light chain) from a cell, said method comprising culturing any of the host cells provided herein so that the nucleic acid is expressed and the heterologous polypeptide is translocated.

[00047] In yet another aspect, provided is a heterologous polypeptide obtained by any of the methods provided herein. In some embodiments, the polypeptide is an antibody.

[00048] In some embodiments, use of the variant signal peptide results in, e.g., increased production of heterologous polypeptide (e.g., antibody, e.g., heavy chain and/or light chain), increased secretion of heterologous polypeptide (e.g., antibody), increased production of mature heterologous polypeptide (e.g., antibody), increased secretion of mature heterologous polypeptide (e.g., antibody), increased production of soluble heterologous polypeptide (e.g., antibody), increased secretion of soluble heterologous polypeptide (e.g., antibody), increased localization of inclusion bodies on the periplasmic side, and/or increased production of a heterologous polypeptide whereby the heterologous polypeptide is secreted, folded and assembled into a biologically active polypeptide (e.g., antibody), e.g., as compared to use of wildtype (non-variant) signal peptide. In some embodiments, the relative translation strength (also termed TIR strength) of the variant signal peptide and wildtype (non-variant) signal peptide is about 1. In some embodiments, the relative translation strength of the variant signal peptide and wildtype (non-variant) signal peptide is about 2, about 3, about 4, about 5, about 6, about 7 or more such about 8 or more. In some embodiments the relative translation strength of the variant signal peptide and wildtype (non-variant) signal peptide is about 4. In some embodiments the relative translation strength of the variant signal peptide and wildtype (non-variant) signal peptide is about 5. In some embodiments the relative translation strength of the variant signal peptide and wildtype (non-variant) signal peptide is about 6. In some embodiments the

relative translation strength of the variant signal peptide and wildtype (non-variant) signal peptide is about 8. In some embodiments, the relative translation strength of the first and second signal peptides is about equal. In some embodiments, the relative translation strengths of the first and second signal peptides are different.

[00049] ~~In one aspect, p~~ Provided is a polynucleotide sequence that encodes a variant signal peptide of the ~~invention~~ disclosure. In some embodiments, the variants are of a PhoA, MalE, DsbA or STII signal peptide. In some embodiments, the polynucleotide sequence encodes an amino acid of SEQ ID NO:8, 11, 12, 13, 14, 15, 31, 32, 33, 34, or 35. In some embodiments, the polynucleotide sequence encodes an amino acid sequence of SEQ ID NO: 8, 11, or 13. In some embodiments, the polynucleotide sequence encodes an amino acid sequence of SEQ ID NO: 14. In some embodiments, the polynucleotide sequence encodes an amino acid sequence of SEQ ID NO: 12 or 15. In some embodiments, the polynucleotide encodes an amino acid sequence of SEQ ID NO: 31 or 33. In some embodiments, the polynucleotide encodes an amino acid sequence of SEQ ID NO: 32, 34, or 35. In some embodiments, the polynucleotide consists of, consists essentially of, or comprises a polynucleotide sequence of SEQ ID NO:23, 24, 25, 26, 27, 28, 29 30, 36, 37, 38, 39 or 40. In some embodiments, the polynucleotide consists of, consists essentially of, or comprises a polynucleotide sequence of SEQ ID NO:23, 24, 25, 26, or 28. In some embodiments, the polynucleotide consists of, consists essentially of, or comprises a polynucleotide sequence of SEQ ID NO:29. In some embodiments, the polynucleotide consists of, consists essentially of, or comprises a polynucleotide sequence of one of SEQ ID NO:27 or 30. In some embodiments, the polynucleotide consists of, consists essentially of, or comprises a polynucleotide sequence of SEQ ID NO: 36 or 38. In some embodiments, the polynucleotide consists of, consists essentially of, or comprises a polynucleotide sequence of SEQ ID NO: 37, 39 or 40.

[00050] In another ~~aspect~~ embodiment, the ~~invention~~ disclosure provides a polynucleotide comprising a polynucleotide encoding a variant signal peptide of the ~~invention~~ disclosure operably linked to a polynucleotide encoding a heterologous polypeptide, whereby upon expression of the heterologous polypeptide in a host cell (e.g., a prokaryotic host cell, e.g., an E. coli host cell), the heterologous polypeptide is folded and assembled to form a biologically active heterologous polypeptide. Examples of heterologous polypeptides are further disclosed herein. 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 Fc polypeptide. In some embodiments, the heterologous polypeptide is a multimeric polypeptide. In some embodiments, the heterologous polypeptide is a heteromultimer. In some embodiments, signal peptide is any one of the variant signal peptides disclosed here. In some embodiments, the signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO:8, 11, 12, 13, 14, 15, 31, 32, 33, 34, or 35. In some embodiments, the signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO: 8, 11, or 13. In some embodiments, the signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO: 14. In some embodiments, the signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO: 12 or 15. In some embodiments, the signal peptide consists of, consists essentially or, or comprises an amino acid sequence of SEQ ID NO: 31 or 33. In some embodiments, the signal peptide consists of, consists essentially or, or comprises amino acid sequence of SEQ ID NO: 32, 34, or 35. In some embodiments, the polynucleotide encoding a variant signal peptide consists of, consists essentially of, or comprises sequence of SEQ ID NO:23, 24, 25, 26, 27, 28, 29 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 44, 45, or 46. In some embodiments, the polynucleotide encoding a variant signal peptide consists of, consists essentially of, or comprises sequence of SEQ ID NO:23, 26, 28, 30, 36, 37, 38, 45 or 46. In some embodiments, the polynucleotide encoding a variant signal peptide consists of, consists essentially of, or comprises sequence of SEQ ID NO:29. In some embodiments, the polynucleotide encoding a variant signal peptide consists of, consists essentially of, or comprises sequence of SEQ ID NO:27 or 30. In some embodiments, the polynucleotide encoding a variant signal peptide consists of, consists essentially of, or comprises a polynucleotide sequence of SEQ ID NO: 36 or 38. In some embodiments, the polynucleotide encoding a variant signal peptide consists of, consists essentially of, or comprises a polynucleotide sequence of SEQ ID NO: 37, 39 or 40. In some embodiments, the polynucleotide encoding a variant signal peptide consists of, consists essentially of, or comprises a polynucleotide sequence of SEQ ID NO: 45 or 46.

[00051] In another ~~aspect~~embodiment, the ~~invention-disclosure~~ provides a polynucleotide comprising (1) a polynucleotide encoding a first signal peptide operably linked to a polynucleotide encoding a first heterologous polypeptide and (2) a polynucleotide encoding a second signal peptide operably linked to a polynucleotide encoding an second heterologous, whereby upon

expression of the antibody in a host cell, the first and second heterologous polypeptides are folded and assembled to form a biologically active polypeptide complex.

[00052] In another ~~aspect~~embodiment, the ~~invention-disclosure~~ provides 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 (e.g., a prokaryotic host cell, e.g., an E. coli host cell), the heavy and light chains are folded and assembled to form a biologically active antibody.

[00053] In some embodiments, the first signal peptide is a signal peptide (e.g., any signal peptide known in the art). In some embodiments, the signal peptide is a co-translational signal peptide. In some embodiments, the first signal peptide is a DsbA signal peptide. In some embodiments, the first signal peptide is a STII signal peptide. In some embodiments, the first signal peptide is any one of the variant signal peptides disclosed here. In some embodiments, the first signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO:8, 11, 12, 13, 14, 15, 31, 32, 33, 34, or 35. In some embodiments, the first signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO: 8, 11, or 13. In some embodiments, the first signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO: 14. In some embodiments, the first signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO: 12 or 15. In some embodiments, the first signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO: 31 or 33. In some embodiments, the first signal peptide consists of, consists essentially of, or comprises an amino acid sequence of SEQ ID NO: 32, 34, or 35. In some embodiments, the polynucleotide encoding a first signal peptide consists of, consists essentially of, or comprises sequence of SEQ ID NO:23, 24, 25, 26, 27, 28, 29 30, 31, 32, 33, 34, or 35. In some embodiments, the polynucleotide encoding a first signal peptide consists of, consists essentially of, or comprises sequence of SEQ ID NO:23, 24, 25, 26, 28, 36, 37, 38, 39 or 40. In some embodiments, the polynucleotide encoding a first signal peptide consists of, consists essentially of, or comprises sequence of SEQ ID NO:29. In some embodiments, the polynucleotide encoding a first signal peptide consists of, consists essentially of, or comprises sequence of SEQ ID NO:27 or 30. In some embodiments, the polynucleotide encoding a first signal peptide consists of, consists

embodiments, the relative translation strengths of the first and second signal peptides are different.

[00060] In one ~~aspect~~embodiment, the ~~invention-disclosure~~ provides uses of a heterologous polypeptide generated using the methods of the ~~disclosure~~invention, in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, and/or an immune (such as autoimmune) disorder. The heterologous polypeptide can be of any form described herein, including antibody, antibody fragment, polypeptide (e.g., an oligopeptide), or combination thereof.

[00061] In one embodiment~~aspect~~, the ~~disclosure~~invention provides use of a signal peptide disclosed herein or polynucleotide encoding a signal peptide disclosed herein in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder and/or an immune (such as autoimmune) disorder.

[00062] In one embodiment~~aspect~~, the ~~disclosure~~invention provides use of an expression vector disclosed herein in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder and/or an immune (such as autoimmune) disorder.

[00063] In one embodiment~~aspect~~, the ~~disclosure~~invention provides use of a host cell disclosed herein in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder and/or an immune (such as autoimmune) disorder.

[00064] In one embodiment~~aspect~~, the ~~disclosure~~invention provides use of an article of manufacture disclosed herein in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, an immune (such as autoimmune) disorder and/or an angiogenesis-related disorder (wound healing).

[00065] In one ~~aspect~~embodiment, the ~~disclosure~~invention provides use of a kit disclosed herein in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder and/or an immune (such as autoimmune) disorder).

BRIEF DESCRIPTION OF THE DRAWINGS

[00066] Figures 1A, 1B, and 1C: depict effects of signal peptide variants on 5D5 full-length antibody and heavy chain levels. *E. coli* host strain 64B4 harboring the expression vector was grown in complete C.R.A.P. phosphate limiting media in a shake flask for 24 hrs and end point samples were normalized by OD₅₅₀. (1A) Top panel: Western blot of soluble

What is claimed is:

1. A method of increasing secretion of an antibody heavy chain and/or 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, 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 peptide is greater than about 0.5, 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 hydrophobicity of the first signal peptide is greater than about 0.6.
3. The method of claim 1 or 2, wherein the hydrophobicity of the second signal peptide is greater than about 0.6.
4. The method of any of the preceding claims, wherein the first and/or second signal peptide is a variant co-translational signal peptide.
5. The method of any of the preceding claims, wherein the first and/or second signal peptide is a variant DsbA signal peptide.
6. The method of claim 5, wherein the variant DsbA signal peptide comprises a mutation at residue L11, wherein the variant DsbA signal peptide has a greater average hydrophobicity than a wildtype DsbA signal peptide of SEQ ID NO:3.
7. The method of claim 6, wherein the mutation is L11I or S18Y.
8. The method of claim 5, wherein the variant DsbA signal peptide comprises sequence of SEQ ID NO:13 or 15.
9. The method of any one of claims 1-4, wherein the signal peptide is a Sfmc signal peptide.
10. The method of any one of claims 1-9, wherein the TIR strength of the first and/or second signal peptide is about 1, about 2, about 3, about 4, about 5, about 6, about 7, or about 8.
11. The method of claim 10, wherein the TIR strength of the first signal peptide about 5 and the TIR strength of the second signal sequence is about 8.

12. The method of any of claims 1-11, wherein the polynucleotide in the host cell further comprises a promoter.
13. The method of claim 12, wherein the promoter is a prokaryotic promoter selected from the group consisting of *phoA*, *tac*, *lpp*, *lac-lpp*, *lac*, *ara*, and T7 promoter.
14. The method of any one of claims 1-14, wherein the *E. coli* host cell is of a strain deficient in endogenous protease activities.
15. The method of claim 14, wherein the genotype of the *E. coli* lacks *degP* and *prc* genes and harbors a mutant *spr* gene.
16. The method of any one of claims 1-15, 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.
17. The method of claim 16, wherein the polynucleotide encodes both DsbA and DsbC.
18. The method of any one of claims 1-27, wherein the method further comprises recovering the antibody from the host cell culture.
19. The method of claim 17, wherein the antibody is recovered from the host cell culture medium.
20. The method of claim 17 or 18, 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.
21. The method of claim 19, wherein at least 50% of the immunoglobulin polypeptide complexes that are formed are the antibody.
22. The method of claims 19, wherein at least 70% of the immunoglobulin polypeptide complexes that are formed are the antibody.
23. The method of any one of claims 1-22, wherein the antibody is a monoclonal antibody.
24. The method of claim 23, wherein the antibody is a chimeric antibody, an affinity matured antibody, a bispecific antibody, humanized antibody, or a human antibody.
25. The method of claim 23, wherein the antibody is a bispecific antibody.
26. A variant DsbA signal peptide, wherein the variant comprises an H region with an average hydrophobicity that is greater than 0.5.
27. 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

28. The variant DsbA signal peptide of claim 27, wherein the mutation is L11I and/or S18Y.
29. 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.
30. The variant STII signal peptide of claim 29, wherein the mutation is S11A, S11I or S11L.
31. A variant signal peptide consisting of, consisting essentially of or comprising a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33.
32. The variant signal peptide of any one of claims 26-30, fused to a heterologous protein.
33. The variant signal peptide of claim 32, wherein the heterologous polypeptide is an antibody heavy chain.
34. The variant signal peptide of claim 32, wherein the heterologous polypeptide is an antibody light chain.
35. The variant signal peptide of claim 32, wherein the heterologous polypeptide is an antibody light and heavy chain.
36. The variant signal peptide of claim 32, wherein the heterologous polypeptide is a multimeric polypeptide.
37. The variant signal peptide of claim 32, wherein the heterologous polypeptide is an immunoadhesin.
38. A polynucleotide sequence that encodes a variant signal peptide of any one of claims 26-30.
39. The polynucleotide sequence of claim 38 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.
40. The polynucleotide of claim 39, wherein the host cell is a prokaryotic host cell.
41. The polynucleotide of claim 40, wherein the host cell is E coli.
42. 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 26-30 or wherein the first signal peptide consists of, consists essentially of, or comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.

43. The polynucleotide of claim 42, wherein the first signal peptide is a variant signal peptide of any one of claims 26-30.
44. The polynucleotide of claim 42, wherein the first signal peptide consists of, consists essentially of, or comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
45. The polynucleotide of any one of claims 42-44, wherein the second signal peptide is a signal peptide.
46. The polynucleotide of any one of claims 42-44, wherein the second signal peptide is a variant signal peptide of any one of claim 26-30 or wherein 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.
47. The polynucleotide of any one of claims 42-44, wherein the second signal peptide is a variant signal peptide of any one of claim 26-30.
48. The polynucleotide of any one of claims 42-44, wherein 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.
49. The polynucleotide of any one of claims 42-48, 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.
50. The polynucleotide of claim 49, wherein the third signal peptide is a variant signal peptide of any one of claim 26-30 or wherein 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.
51. The polynucleotide of any one of claims 39-50, further comprising a promoter operably linked to the heterologous polypeptide.
52. The polynucleotide of claim 51, wherein the promoter is a prokaryotic promoter selected from the group consisting of phoA, tac, lpp, lac-lpp, lac, ara, trp, and T7 promoter.
53. The promoter of claim 52, wherein the promoter is a phoA promoter.

54. The polynucleotide of any one of claims 39-53, 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.
55. The polynucleotide of claim 54, wherein the first and second promoters are both *phoA* promoters.
56. The polynucleotide of claim 54, further comprising (c) a third promoter, wherein the third promoter is operably linked to a Fc polypeptide.
57. The polynucleotide of claim 56, wherein the promoter is a *phoA* promoter.
58. The polynucleotide of any one of claims 39-41, wherein the heterologous polypeptide is a protease, an immunoadhesin, an extracellular domain of a receptor, a heteromultimeric protein, or an antibody.
59. The polynucleotide of any one of claims 42-57, wherein the antibody is a monoclonal antibody.
60. The polynucleotide of claim 59, wherein the antibody is a chimeric antibody, a bispecific antibody, humanized antibody, an antibody fragment or a human antibody.
61. The polynucleotide of claim 59, wherein the antibody fragment is a one-armed antibody.
62. The polynucleotide of claim 59, wherein the antibody is a bispecific antibody.
63. A vector comprising a polynucleotide of any one of claims 39-62.
64. The vector of claim 63, wherein the vector is an expression vector.
65. A composition comprising a polynucleotide of any one of claims 39-64.
66. A host cell comprising a polynucleotide of claim 64.
67. The host cell of claim 66, wherein the host cell is a prokaryotic cell.
68. The host cell of claim 67, wherein the prokaryotic cell is *E. coli*.
69. The host cell of claim 68, wherein the *E. coli* is of a strain deficient in endogenous protease activities.
70. The host cell of claim 68 or 69, wherein the genotype of the *E. coli* lacks *degP* and *prc* genes and harbors a mutant *spr* gene.
71. The host cell of any one of claims 66-70, wherein the host cell further comprises a polynucleotide encoding a prokaryotic chaperone protein.
72. The host cell of claim 71, wherein the prokaryotic chaperone protein is DsbA and/or DsbC.

73. The host cell of claim 71 or 72, wherein the host cell overexpresses a prokaryotic chaperone protein.
74. 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 66-73 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.
75. The method of claim 74, wherein the method further comprises recovering the heterologous polypeptide from the host cell culture.
76. The method of claim 75, wherein the heterologous polypeptide is recovered from the host cell culture medium.
77. The method of claim 74 or 75, 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.
78. 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 66-73 so that the nucleic acid is expressed and the heterologous polypeptide is secreted.
79. 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 66-73 so that the nucleic acid is expressed and the heterologous polypeptide is translocated.
80. A heterologous polypeptide obtained by a method of any one of claims 74-79.
81. The polypeptide of claim 81, wherein the polypeptide is an antibody.

What is claimed is:

1. A method of increasing secretion of an antibody heavy chain and/or 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, 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 peptide is greater than about 0.5, 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 hydrophobicity of the first and/or second signal peptide is greater than about 0.6.
3. The method of claim 1 or 2, wherein the first and/or second signal peptide is a variant co-translational signal peptide.
4. The method of any of the preceding claims, wherein the first and/or second signal peptide is a variant DsbA or a Sfmc signal peptide.
5. The method of claim 4, wherein the first and/or second signal peptide is a variant DsbA signal peptide having a greater average hydrophobicity than a wildtype DsbA signal peptide of SEQ ID NO:3.
6. The method of claim 5, wherein the mutation is L11I and/or S18Y.
7. The method of claim 4, wherein the first and/or second signal peptide is a variant DsbA signal peptide comprising sequence of SEQ ID NO:13 or 15.
8. The method of any one of claims 1-7, wherein the TIR strength of the first and/or second signal peptide is about 1, about 2, about 3, about 4, about 5, about 6, about 7, or about 8.
9. The method of claim 8, wherein the TIR strength of the first signal peptide about 5 and the TIR strength of the second signal sequence is about 8.
10. The method of any of claims 1-9, wherein the polynucleotide in the host cell further comprises a promoter.
11. The method of claim 10, wherein the promoter is a prokaryotic promoter selected from the group consisting of *phoA*, *tac*, *lpp*, *lac-lpp*, *lac*, *ara*, and T7 promoter.
12. The method of any one of claims 1-11, wherein the *E. coli* host cell is of a strain deficient in endogenous protease activities.

13. The method of claim 12, wherein the genotype of the *E. coli* lacks *degP* and *prc* genes and harbors a mutant *spr* gene.
14. The method of any one of claims 1-13, 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.
15. The method of claim 14, wherein the polynucleotide encodes both DsbA and DsbC.
16. The method of any one of claims 1-15, wherein the method further comprises recovering the antibody from the host cell culture.
17. The method of claim 16, wherein the antibody is recovered from the host cell culture medium.
18. The method of claim 16 or 17, 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.
19. The method of claim 16, wherein at least 50% of the immunoglobulin polypeptide complexes that are formed are the antibody.
20. The method of claims 16, wherein at least 70% of the immunoglobulin polypeptide complexes that are formed are the antibody.
21. The method of any one of claims 1-20, wherein the antibody is a monoclonal antibody.
22. The method of claim 21, wherein the antibody is a chimeric antibody, an affinity matured antibody, a bispecific antibody, humanized antibody, or a human antibody.
23. A variant DsbA signal peptide, wherein the variant comprises an H region with an average hydrophobicity that is greater than 0.5.
24. A variant DsbA signal peptide, wherein the variant has a greater average hydrophobicity than a DsbA signal peptide of SEQ ID NO:3.
25. The variant DsbA signal peptide of claim 24, wherein the mutation is L11I and/or S18Y.
26. 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.
27. The variant STII signal peptide of claim 26, wherein the mutation is S11A, S11I or S11L.
28. A variant signal peptide comprising a sequence of SEQ ID NO:8, 11, 13, 15, 31, or 33.
29. The variant signal peptide of any one of claims 23-27, fused to a heterologous protein.
30. The variant signal peptide of claim 29, 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.
31. A polynucleotide sequence that encodes a variant signal peptide of any one of claims 23-27.

32. The polynucleotide sequence of claim 31 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.
33. The polynucleotide of claim 32, wherein the host cell is a prokaryotic host cell.
34. The polynucleotide of claim 33, wherein the host cell is E coli.
35. 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 23-27 or wherein the first signal peptide consists of, consists essentially of, or comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
36. The polynucleotide of claim 35, wherein the first signal peptide is a variant signal peptide of any one of claims 23-27.
37. The polynucleotide of claim 35, wherein the first signal peptide comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
38. The polynucleotide of any one of claims 35-37, wherein the second signal peptide is a variant signal peptide of any one of claim 23-27 or wherein the second signal peptide comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
39. The polynucleotide of any one of claims 35-37, wherein the second signal peptide is a variant signal peptide of any one of claim 23-27.
40. The polynucleotide of any one of claims 35-37, wherein the second signal peptide comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
41. The polynucleotide of any one of claims 35-40, 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.
42. The polynucleotide of claim 41, wherein the third signal peptide is a variant signal peptide of any one of claim 23-27 or wherein the third signal peptide comprises a sequence of SEQ ID NO:8, 11, 13, 15, 31, 33, or 42.
43. The polynucleotide of any one of claims 32-42, further comprising a promoter operably linked to the heterologous polypeptide.

44. The polynucleotide of claim 43, wherein the promoter is a prokaryotic promoter selected from the group consisting of phoA, tac, lpp, lac-lpp, lac, ara, trp, and T7 promoter.
45. The polynucleotide of claim 44, wherein the promoter is a phoA promoter.
46. The polynucleotide of any one of claims 32-45, 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.
47. The polynucleotide of claim 46, wherein the first and second promoters are both phoA promoters.
48. The polynucleotide of claim 46, further comprising (c) a third promoter, wherein the third promoter is operably linked to a Fc polypeptide.
49. The polynucleotide of claim 48, wherein the promoter is a phoA promoter.
50. The polynucleotide of any one of claims 32-34, wherein the heterologous polypeptide is a protease, an immunoadhesin, an extracellular domain of a receptor, a heteromultimeric protein, or an antibody.
51. The polynucleotide of any one of claims 35-49, wherein the antibody is a monoclonal antibody.
52. The polynucleotide of claim 51, wherein the antibody is a chimeric antibody, a one-armed antibody, a bispecific antibody, humanized antibody, an antibody fragment or a human antibody.
53. A vector comprising a polynucleotide of any one of claims 32-52.
54. The vector of claim 53, wherein the vector is an expression vector.
55. A composition comprising a polynucleotide of any one of claims 32-52, or the vector of claim 53 or 54.
56. A host cell comprising a polynucleotide of any one of claims 32-52.
57. The host cell of claim 56, wherein the host cell is a prokaryotic cell.
58. The host cell of claim 57, wherein the prokaryotic cell is *E. coli*.
59. The host cell of claim 58, wherein the *E. coli* is of a strain deficient in endogenous protease activities.
60. The host cell of claim 58 or 59, wherein the genotype of the *E. coli* lacks degP and prc genes and harbors a mutant spr gene.
61. The host cell of any one of claims 56-60, wherein the host cell further comprises a polynucleotide encoding a prokaryotic chaperone protein.
62. The host cell of claim 61, wherein the prokaryotic chaperone protein is DsbA and/or DsbC.

63. The host cell of claim 61 or 62, wherein the host cell overexpresses a prokaryotic chaperone protein.
64. 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 56-63 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.
65. The method of claim 64, wherein the method further comprises recovering the heterologous polypeptide from the host cell culture.
66. The method of claim 65, wherein the heterologous polypeptide is recovered from the host cell culture medium.
67. The method of claim 65 or 66, 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.
68. 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 56-63 so that the nucleic acid is expressed and the heterologous polypeptide is secreted.
69. 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 56-63 so that the nucleic acid is expressed and the heterologous polypeptide is translocated.
70. A heterologous polypeptide obtained by a method of any one of claims 64-69.
71. The polypeptide of claim 70, wherein the polypeptide is an antibody.