METHOD FOR LARGE SCALE PRODUCTION OF ANTIBODIES USING A CELL-FREE PROTEIN SYNTHESIS SYSTEM

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 63/078,254, filed on September 14, 2020, the disclosure of which is hereby incorporated by reference in its entirety herein for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 8, 2021, is named 091200-1260233-006910PC_SL.txt and is 65,782 bytes in size.

BACKGROUND

[0003] The present disclosure provides methods and systems for producing proteins of interest at large scale suitable for commercial production. The methods can increase the total yield and/or the yield of properly folded and assembled proteins of interest, such as antibodies.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0004] Described herein are methods and compositions for increasing the yield of properly folded antibodies in a cell-free protein synthesis system. In one ~~aspect~~example, the methods are performed at a scale suitable for commercial production of relatively large amounts of properly folded antibodies. Thus, described herein is a method for large scale production of an antibody using a cell-free protein synthesis system, the method comprising expressing a heavy chain (HC) polypeptide of an antibody from a nucleic acid encoding the heavy chain in the presence of a

light chain (LC) polypeptide, thereby producing the antibody. In some ~~embodiments~~examples, the cell-free protein synthesis system comprises a reaction mixture having a volume of about 10 to about 25,000 liters. In some ~~embodiment~~examples, expression of the HC is performed in a reaction mixture having a volume of about 10 to about 10,000 liters. In some ~~embodiment~~examples, expression of the HC is performed in a reaction mixture having a volume of about 15,000 to about 25,000 liters. In some ~~embodiment~~examples, the reaction mixture comprises a volume equal to or greater than about 10 liters to about 25,000 liters. In some ~~embodiment~~examples, the reaction mixture comprises a volume equal to or less than about 10 liters to about 25,000 liters. In some ~~embodiment~~examples, the reaction mixture comprises a volume of about 10,000 liters to about 20,000 liters.

[0004a] Thus, described herein is a method for large scale production of an antibody using a cell-free protein synthesis system, comprising expressing a heavy chain (HC) polypeptide of an antibody from a nucleic acid encoding the heavy chain in the presence of a light chain (LC) polypeptide, thereby producing the antibody, wherein said expressing is performed in a reaction mixture having a volume of about 10 to about 25,000 liters.

[0004b] In some aspects, described herein is a cell-free protein synthesis system comprising: (i) a reaction mixture comprising a bacterial cell extract; (ii) a nucleic acid encoding a heavy chain polypeptide; and (iii) a light chain polypeptide; wherein the reaction mixture has a volume of about 10 to about 25,000 liters.

[0005] In some ~~embodiment~~examples, the reaction mixture comprises a bacterial extract, and expression of the HC comprises: (i) combining the bacterial extract with a nucleic acid encoding the HC; and (ii) incubating the cell-free protein synthesis system under conditions permitting the expression of the HC.

[0006] In some ~~embodiment~~examples, the yield per liter of total antibody protein is increased compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture. In some ~~embodiment~~examples, the yield per liter of properly folded antibody protein(s) is increased compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture. In some ~~embodiment~~examples, the yield per liter of total antibody protein and the yield per liter of properly folded antibody protein(s) is increased compared to a reaction mixture where both the

heavy and light chain polypeptides are expressed in the same reaction mixture. In some ~~embodiment~~examples, the yield per liter of total antibody protein and/or the yield per liter of properly folded antibody protein(s) is increased compared to a reaction mixture where both the heavy and light chain polypeptides are expressed contemporaneously in the same reaction mixture.

[0007] In some ~~embodiment~~examples, the yield per liter of properly folded antibody protein(s) is about 30% to about 90% higher or greater compared to a reaction mixture where both the heavy and light chain polypeptides are expressed. In some ~~embodiment~~examples, the yield per liter of properly folded antibody protein(s) is about 30%, about 40%, about 50%, about 60%, about 70%, about 80% or about 90% higher or greater compared to a reaction mixture where both the heavy and light chain polypeptides are expressed. In some ~~embodiment~~examples, dimerization between the heavy

and light chain polypeptides is increased. In some ~~embodiment~~examples, the ratio of heavy and light chain polypeptide dimers to heavy and light chain polypeptide monomers is increased.

[0008] In some ~~embodiment~~examples, the light chain polypeptide is added to the reaction mixture prior to the expressing step.

[0009] In some ~~embodiment~~examples, the light chain polypeptide is produced in a separate reaction before being added to the reaction mixture. In some ~~embodiment~~examples, the light chain polypeptide is synthesized or prefabricated before being added to the reaction mixture. In some ~~embodiment~~examples, the light chain polypeptide is expressed from a nucleic acid encoding the light chain polypeptide in a cell-free protein synthesis system, reaction mixture, or cell free extract. In some ~~embodiment~~examples, the light chain polypeptide is expressed from a nucleic acid encoding the light chain polypeptide in an intact living cell. In some ~~embodiment~~examples, the intact living cell is a bacterial cell or mammalian cell. In some ~~embodiment~~examples, the light chain polypeptide is purified or partially purified from cell-free protein synthesis systems, reaction mixtures, cell free extracts, and/or from cultures of cells before being added to the reaction mixture.

[0010] In some ~~embodiment~~examples, the antibody is an IgG, IgA, or IgD subtype, or a combination thereof. In some ~~embodiment~~examples, the antibody is a monoclonal antibody. In some ~~embodiment~~examples, the antibody is selected from an anti-B cell maturation antigen (anti-BCMA) antibody, an anti-Cluster of Differentiation 74 (anti-CD74) antibody, or an anti-folate receptor alpha (FOLR1) antibody. In some ~~embodiment~~examples, the antibody comprises a FAB fragment.

[0011] In some examples~~aspects~~, the antibody is a bispecific antibody. In some ~~embodiment~~examples, the bispecific antibody comprises a heterodimeric Fc region comprising two asymmetric CH3 domains that include sequences from IgA and IgG CH3 domains. In some ~~embodiment~~examples, the bispecific antibody is a domain-exchanged antibody, wherein the HC dimerizes with the LC. In some ~~embodiment~~examples, the bispecific antibody comprises engineered CH3 domains with enhanced HC heterodimerization based on steric or electrostatic complementarity. In some ~~embodiment~~examples, the engineered CH3 domains comprise knob and hole mutations that promote the formation of stable CH3 heterodimers. In some ~~embodiment~~examples, the bispecific antibody comprises one Fab domain and one scFv domain, where the Fab and scFv domains bind to different antigens.

[0012] In some examples~~aspects~~, the HC and/or the LC comprises at least one non-natural amino acid (nnAA). In some ~~embodiment~~examples, the HC comprises at least one non-natural amino acid (nnAA). In some ~~embodiment~~examples, the nnAA in the HC can be the same or different from the nnAA in the LC. In some ~~embodiment~~examples, the nnAA is p-acetyl-phenylalanine or p-azidomethyl-L-phenylalanine.

[0013] In some ~~embodiment~~examples, the method further comprises assembling the HC and LC under non-reducing conditions to produce the antibody.

[0014] In some ~~embodiment~~examples, the cell free protein synthesis system comprises a bacterial extract with associated co-factors. In some ~~embodiment~~examples, the cell free protein synthesis system comprises a bacterial extract prepared from an E. coli strain. In some ~~embodiment~~examples, the cell free protein synthesis system comprises an oxidative phosphorylation reaction producing ATP. In some ~~embodiment~~examples, the cell free protein synthesis system comprises a reconstituted ribosome system.

[0015] In some ~~embodiment~~examples, the cell free protein synthesis system comprises an exogenous protein chaperone. In some ~~embodiment~~examples, the exogenous protein chaperone is selected from the group consisting of a protein disulfide isomerase (PDI), a peptidyl prolyl cis-trans isomerase (PPI), or a deaggregase. In some ~~embodiment~~examples, the PDI is selected from DsbA, DsbC or DsbG; the PPI is selected from FkpA, SlyD, tig, SurA, or Cpr6; and the deaggregase is selected from IbpA, IbpB, or Skp.

[0016] In some ~~embodiment~~examples, the cell free protein synthesis system comprises a mutant Releasing Factor 1 protein (RF1).

[0017] Also provided is a cell-free protein synthesis system. In one example~~aspect~~, the cell-free protein synthesis system comprises: (i) a reaction mixture comprising a bacterial cell extract; (ii) a nucleic acid encoding a heavy chain polypeptide; and (iii) a light chain polypeptide. In some ~~embodiment~~examples, the cell-free protein synthesis system comprises a reaction mixture having a volume of about 10 to about 25,000 liters.

[0018] In some ~~embodiment~~examples of the cell-free protein synthesis system, the light chain polypeptide is added to the reaction mixture. In some ~~embodiment~~examples, the light chain polypeptide is not expressed in the reaction mixture, or the reaction mixture does not contain a plasmid

encoding the light chain. In some ~~embodiment~~examples, the light chain polypeptide is produced in a separate reaction, synthesized or prefabricated before being added to the reaction mixture. In some ~~embodiment~~examples, the light chain polypeptide is expressed from a nucleic acid encoding the light chain polypeptide in a cell-free protein synthesis system, reaction mixture, or cell free extract. In some ~~embodiment~~examples, the light chain polypeptide is expressed from a nucleic acid encoding the LC polypeptide in an intact living cell. In some ~~embodiment~~examples, the intact living cell is a bacterial cell or mammalian cell. In some ~~embodiment~~examples, the light chain polypeptide is purified or partially purified from cell-free protein synthesis systems, reaction mixtures, cell free extracts, or from cultures of cells before being added to the reaction mixture.

[0019] In some ~~embodiment~~examples, the reaction mixture comprises ribosomes, ATP, amino acids, and tRNAs. In some ~~embodiment~~examples, the bacterial cell extract is prepared from an E.coli strain.

[0020] In some ~~embodiment~~examples, the cell-free protein synthesis system further comprises an exogenous protein chaperone. In some ~~embodiment~~examples, the exogenous protein chaperone is selected from the group consisting of a protein disulfide isomerase (PDI), a peptidyl prolyl cis-trans isomerase (PPI), or a deaggregase. In some ~~embodiment~~examples, the PDI is selected from DsbA, DsbC or DsbG; the PPI is selected from FkpA, SlyD, tig, SurA, or Cpr6; and the deaggregase is selected from IbpA, IbpB, or Skp.

[0021] In some ~~embodiment~~examples, the cell-free protein synthesis system further comprises a mutant Releasing Factor 1 protein (RF1).

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Fig. 1 shows anti-BCMA antibody titers in reactions containing an expression plasmid encoding the heavy chain (HC) polypeptide and a light chain (LC) polypeptide, where the LC was provided either as a prefabricated light chain (PFLC) polypeptide or expressed from an expression plasmid encoding the LC polypeptide. The reactions were performed in bioreactors or using the FlowerPlate® (FP) system (m2p-labs GmbH). The yield (titer) of an anti-BCMA antibody was increased when a PFLC polypeptide was added to the reaction as compared to adding an expression plasmid encoding the LC polypeptide.

WHAT IS CLAIMED IS:

1. A method for large scale production of an antibody using a cell-free protein synthesis system, comprising expressing a heavy chain (HC) polypeptide of an antibody from a nucleic acid encoding the heavy chain in the presence of a light chain (LC) polypeptide, thereby producing the antibody, wherein said expressing is performed in a reaction mixture having a volume of about 10 to about 25,000 liters.

2. The method of claim 1, wherein the reaction mixture comprises a volume of about 10 to about 10,000 liters, or .

~~3. The method of claim 1, wherein the reaction mixture comprises a volume of~~ about 15,000 liters to about 25,000 liters, or .

~~4. The method of claim 1, wherein the reaction mixture comprises a volume of~~ about 10,000 liters to about 20,000 liters.

~~5~~3. The method of claims 1-~~4~~2, wherein the reaction mixture comprises a bacterial extract, and the expressing comprises:

(i) combining the bacterial extract with a nucleic acid encoding the HC; and

ii) incubating the cell-free protein synthesis system under conditions permitting the expression of the HC.

~~6~~4. The method of claims 1-~~5~~3, wherein the yield per liter of total antibody protein and properly folded antibody is increased compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture.

~~7. The method of claims 1-6~~, optionally wherein the yield per liter of properly folded antibody is about 30% higher or greater compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture.

~~8. The method of claims 1-6~~, or wherein the yield per liter of properly folded antibody is about 40% higher or greater compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture.

~~9. The method of claims 1-6~~, or wherein the yield per liter of properly folded antibody is about 50% higher or greater compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture~~.~~

~~10. The method of claims 1-6~~, or wherein the yield per liter of properly folded antibody is 70% higher or greater compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture.

~~11. The method of claims 1-6~~, or wherein the yield per liter of properly folded antibody is 80% higher or greater compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture.

~~12~~5. The method of claims 1-~~11~~4, wherein dimerization between the heavy and light chain polypeptides is increased compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture~~.~~

~~13. The method of claims 1-12~~, optionally wherein the ratio of heavy and light chain polypeptide dimers to heavy and light chain polypeptide monomers is increased compared to a reaction mixture where both the heavy and light chain polypeptides are expressed in the same reaction mixture.

~~14~~6. The method of claims 1-~~13~~5, wherein the light chain polypeptide is added to the reaction mixture prior to the expressing step.

~~15~~7. The method of claim ~~14~~6, wherein the light chain polypeptide is produced in a separate reaction, synthesized or prefabricated before being added to the reaction mixture.

~~16~~8. The method of claims ~~14-~~6 or ~~15~~7, wherein the light chain polypeptide is expressed from a nucleic acid encoding the light chain polypeptide in a cell-free protein synthesis system, reaction mixture, or cell free extract~~.~~

~~17. The method of claims 14 or 15~~, optionally wherein the light chain polypeptide is expressed from a nucleic acid encoding the LC polypeptide in an intact living cell.

~~18~~9. The method of claim ~~17~~8, wherein the intact living cell is a bacterial cell or mammalian cell.

~~19~~10. The method of claims ~~14~~6-~~18~~9, wherein the light chain polypeptide is purified or partially purified from cell-free protein synthesis systems, reaction mixtures, cell free extracts, or from cultures of cells before being added to the reaction mixture.

~~20~~11. The method of claims 1-~~19~~10, wherein the antibody is an IgG, IgA, or IgD subtype, or a combination thereof~~.~~

~~21. The method of claims 1-20~~, optionally wherein the antibody is a monoclonal antibody~~.~~

~~22. The method of claims 1-21~~, optionally wherein the antibody is selected from an anti-B cell maturation antigen (anti-BCMA) antibody, an anti-Cluster of Differentiation 74 (anti-CD74) antibody, or an anti-folate receptor alpha (FOLR1) antibody~~.~~

~~23. The method of claims 1-19~~, optionally wherein the antibody comprises a FAB fragment~~.~~

~~24. The method of claims 1-19~~, optionally wherein the antibody is a bispecific antibody.

~~25~~12. The method of claim ~~24~~11, wherein the bispecific antibody comprises a heterodimeric Fc region comprising two asymmetric CH3 domains that include sequences from IgA and IgG CH3 domains~~.~~

~~26. The method of claims 24 or 25~~, optionally wherein the bispecific antibody is a domain-exchanged antibody, wherein the HC dimerizes with the LC~~.~~

~~27. The method of claims 24-26~~, optionally wherein the bispecific antibody comprises engineered CH3 domains with enhanced HC heterodimerization based on steric or electrostatic complementarity.

~~28~~13. The method of claim ~~27~~12, wherein the engineered CH3 domains comprise knob and hole mutations that promote the formation of stable CH3 heterodimers.

~~29~~14. The method of claims ~~24~~11-~~27~~12, wherein the bispecific antibody comprises one Fab domain and one scFv domain, where the Fab and scFv domains bind to different antigens.

~~30~~15. The method of claims 1-~~29~~14, wherein the HC and/or the LC comprises at least one non-natural amino acid (nnAA).

~~31~~16. The method of claims 1-~~30~~15, wherein the HC comprises at least one non-natural amino acid (nnAA).

~~32~~17. The method of claims ~~30~~ 15 or ~~31~~16, wherein the nnAA in the HC is the same or different from the nnAA in the LC~~.~~

~~33. The method of claims 30-32~~, optionally wherein the nnAA is p-acetyl-phenylalanine or p-azidomethyl-L-phenylalanine.

~~34~~18. The method of claims 1-~~33~~17, wherein the method further comprises assembling the HC and LC under non-reducing conditions to produce the antibody.

~~35~~19. The method of claims 1-~~34~~18, wherein the cell free protein synthesis system comprises a bacterial extract with associated co-factors~~.~~

~~36. The method of claims 1-35~~, optionally wherein the cell free protein synthesis system comprises a bacterial extract prepared from an E. coli strain~~.~~

~~37. The method of claims 1-36~~, optionally wherein the cell free protein synthesis system comprises an oxidative phosphorylation reaction producing ATP~~.~~

~~38. The method of claims 1-37~~, optionally wherein the cell free protein synthesis system comprises a reconstituted ribosome system~~.~~

~~39. The method of claims 1-38~~, optionally wherein the cell free protein synthesis system comprises an exogenous protein chaperone.

~~40~~20. The method of claim ~~39~~ 19, wherein the exogenous protein chaperone is selected from the group consisting of a protein disulfide isomerase (PDI), a peptidyl prolyl cis-trans isomerase (PPI), or a deaggregase.

~~41~~21. The method of claim ~~40~~20, wherein the PDI is selected from DsbA, DsbC or DsbG; the PPI is selected from FkpA, SlyD, tig, SurA, or Cpr6; and the deaggregase is selected from IbpA, IbpB, or Skp.

~~42~~22. The method of claims 1-~~41~~21, wherein the cell free protein synthesis system comprises a mutant Releasing Factor 1 protein (RF1).

~~43~~23. A cell-free protein synthesis system comprising:

(i) a reaction mixture comprising a bacterial cell extract;

(ii) a nucleic acid encoding a heavy chain polypeptide; and

(iii) a light chain polypeptide;

wherein the reaction mixture has a volume of about 10 to about 25,000 liters.

~~44~~24. The cell-free protein synthesis system of claim ~~43~~23, wherein the light chain polypeptide is produced in a separate reaction, synthesized or prefabricated before being added to the reaction mixture.

~~45~~25. The cell-free protein synthesis system of claim ~~43~~ 23 or ~~44~~24, wherein the light chain polypeptide is expressed from a nucleic acid encoding the light chain polypeptide in a cell-free protein synthesis system, reaction mixture, or cell free extract~~.~~

~~46. The cell-free protein synthesis system of claim 43 or 44~~, optionally wherein the light chain polypeptide is expressed from a nucleic acid encoding the LC polypeptide in an intact living cell.

~~47~~26. The cell-free protein synthesis system of claim ~~46~~25, wherein the intact living cell is a bacterial cell or mammalian cell.

~~48~~27. The cell-free protein synthesis system of claims ~~43~~23-~~47~~26, wherein the light chain polypeptide is purified or partially purified from cell-free protein synthesis systems, reaction mixtures, cell free extracts, or from cultures of cells before being added to the reaction mixture.

~~49~~28. The cell-free protein synthesis system of claims ~~43~~23-~~48~~27, wherein the reaction mixture comprises ribosomes, ATP, amino acids, and tRNAs.

~~50~~29. The cell-free protein synthesis system of claims ~~43~~23-~~49~~28, wherein the bacterial cell extract is prepared from an E.coli strain.

~~51~~30. The cell-free protein synthesis system of claims ~~43~~23-~~50~~29, further comprising an exogenous protein chaperone.

~~52~~31. The cell-free protein synthesis system of claim ~~51~~30, wherein the exogenous protein chaperone is selected from the group consisting of a protein disulfide isomerase (PDI), a peptidyl prolyl cis-trans isomerase (PPI), or a deaggregase.

~~53~~32. The cell-free protein synthesis system of claim ~~52~~31, wherein the PDI is selected from DsbA, DsbC or DsbG; the PPI is selected from FkpA, SlyD, tig, SurA, or Cpr6; and the deaggregase is selected from IbpA, IbpB, or Skp.

~~54~~33. The cell-free protein synthesis system of claims ~~43~~23-~~53~~32, further comprising a mutant Releasing Factor 1 protein (RF1).