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

[0004] Described herein are methods and compositions for increasing the yield of properly folded antibodies in a cell-free protein synthesis system. In one 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 examples, the cell-free protein synthesis system comprises a reaction mixture having a volume of about 10 to about 25,000 liters. In some examples, expression of the HC is performed in a reaction mixture having a volume of about 10 to about 10,000 liters. In some 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 examples, the reaction mixture comprises a volume equal to or greater than about 10 liters to about 25,000 liters. In some examples, the reaction mixture comprises a volume equal to or less than about 10 liters to about 25,000 liters. In some 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 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 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 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 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 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 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 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 examples, dimerization between the heavy

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

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

[0009] In some examples, the light chain polypeptide is produced in a separate reaction before being added to the reaction mixture. In some examples, the light chain polypeptide is synthesized or prefabricated before being added to the reaction mixture. In some 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 examples, the light chain polypeptide is expressed from a nucleic acid encoding the light chain polypeptide in an intact living cell. In some examples, the intact living cell is a bacterial cell or mammalian cell. In some 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 examples, the antibody is an IgG, IgA, or IgD subtype, or a combination thereof. In some examples, the antibody is a monoclonal antibody. In some 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 examples, the antibody comprises a FAB fragment.

[0011] In some examples, the antibody is a bispecific antibody. In some 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 examples, the bispecific antibody is a domain-exchanged antibody, wherein the HC dimerizes with the LC. In some examples, the bispecific antibody comprises engineered CH3 domains with enhanced HC heterodimerization based on steric or electrostatic complementarity. In some examples, the engineered CH3 domains comprise knob and hole mutations that promote the formation of stable CH3 heterodimers. In some 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, the HC and/or the LC comprises at least one non-natural amino acid (nnAA). In some examples, the HC comprises at least one non-natural amino acid (nnAA). In some examples, the nnAA in the HC can be the same or different from the nnAA in the LC. In some examples, the nnAA is p-acetyl-phenylalanine or p-azidomethyl-L-phenylalanine.

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

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

[0015] In some examples, the cell free protein synthesis system comprises an exogenous protein chaperone. In some 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 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 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, 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 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 examples of the cell-free protein synthesis system, the light chain polypeptide is added to the reaction mixture. In some 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 examples, the light chain polypeptide is produced in a separate reaction, synthesized or prefabricated before being added to the reaction mixture. In some 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 examples, the light chain polypeptide is expressed from a nucleic acid encoding the LC polypeptide in an intact living cell. In some examples, the intact living cell is a bacterial cell or mammalian cell. In some 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 examples, the reaction mixture comprises ribosomes, ATP, amino acids, and tRNAs. In some examples, the bacterial cell extract is prepared from an E.coli strain.

[0020] In some examples, the cell-free protein synthesis system further comprises an exogenous protein chaperone. In some 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 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 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 about 15,000 liters to about 25,000 liters, or about 10,000 liters to about 20,000 liters.

3. The method of claims 1-2, 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.

4. The method of claims 1-3, 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, 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, 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, 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, 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., 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.

5. The method of claims 1-4, 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, 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.

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

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

8. The method of claims 6 or 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, optionally wherein the light chain polypeptide is expressed from a nucleic acid encoding the LC polypeptide in an intact living cell.

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

10. The method of claims 6-9, 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.

11. The method of claims 1-10, wherein the antibody is an IgG, IgA, or IgD subtype, or a combination thereof, optionally wherein the antibody is a monoclonal antibody, 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, optionally wherein the antibody comprises a FAB fragment, optionally wherein the antibody is a bispecific antibody.

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

IgA and IgG CH3 domains, optionally wherein the bispecific antibody is a domain-exchanged antibody, wherein the HC dimerizes with the LC, optionally wherein the bispecific antibody comprises engineered CH3 domains with enhanced HC heterodimerization based on steric or electrostatic complementarity.

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

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

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

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

17. The method of claims 15 or 16, wherein the nnAA in the HC is the same or different from the nnAA in the LC, optionally wherein the nnAA is p-acetyl-phenylalanine or p-azidomethyl-L-phenylalanine.

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

19. The method of claims 1-18, wherein the cell free protein synthesis system comprises a bacterial extract with associated co-factors, optionally wherein the cell free protein synthesis system comprises a bacterial extract prepared from an E. coli strain, optionally wherein the cell free protein synthesis system comprises an oxidative phosphorylation reaction producing ATP, optionally wherein the cell free protein synthesis system comprises a reconstituted ribosome system, optionally wherein the cell free protein synthesis system comprises an exogenous protein chaperone.

20. The method of claim 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.

21. The method of claim 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.

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

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.

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

25. The cell-free protein synthesis system of claim 23 or 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, optionally wherein the light chain polypeptide is expressed from a nucleic acid encoding the LC polypeptide in an intact living cell.

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

27. The cell-free protein synthesis system of claims 23-26, 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.

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

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

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

31. The cell-free protein synthesis system of claim 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.

32. The cell-free protein synthesis system of claim 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.

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