

CLAIMS

What is claimed is:

1. A method of making a nanostructure, comprising solubilizing a recombinant component B (compB) protein from inclusion bodies with a solubilization solution, thereby generating a product sample comprising product compB protein.
2. The method of claim 1, wherein the solubilization solution comprises urea, optionally at a urea concentration of 0.15 M to 2 M, optionally 0.5 M.
3. The method of claim 1 or claim 2, wherein the solubilization solution is a buffered solution having a pH of 7-8, optionally a pH of 7.4
4. The method of any one of claims 1-3, wherein the solubilization solution comprises a zwitterionic surfactant.
5. The method of any one of claims 1-4, wherein the solubilization solution comprises 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS).
6. The method of claim 1, wherein the method comprises, prior to the solubilization step, washing the inclusion bodies with a wash solution comprising urea, optionally at a urea concentration of less than 150 mM, optionally 50-150 mM.
7. The method of claim 1, wherein the method comprises contacting the compB protein with an anion exchange resin, optionally a weak anion exchange resin, optionally a diethylaminoethyl(DEAE)-conjugated resin; and eluting the compB protein from the resin using an elution solution.
8. The method of claim 7, wherein the method comprises, before the eluting step, washing the anion exchange resin with a column-wash solution, the column-wash solution comprising:

a zwitterionic surfactant, optionally 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) or an equivalent thereof, and/or

a nonionic surfactant, optionally Triton X-100 or an equivalent thereof.

9. The method of claim 7, wherein the elution solution comprises sodium chloride (NaCl) at a NaCl concentration of 400 mM to 600 mM.
10. The method of claim 1, wherein the method comprises purifying the compB protein with a mixed-mode resin, optionally a ceramic hydroxyapatite (CHT) resin.
11. The method of claim 1, wherein the inclusion bodies were generated in a bacterial cell comprising a polynucleotide encoding the compB protein, the polynucleotide operatively linked to a promoter.
12. The method of claim 11, wherein the bacterial cell is cultured at less than about 33 °C, optionally at about 15 °C to about 33 °C or at about 17 °C to about 30 °C, preferably at about 30 °C.
13. The method of claim 11, wherein the bacterial cell is an *E. coli* cell.
14. The method of claim 13, wherein the bacterial cell is a B-strain *E. coli* cell.
15. The method of claim 13, wherein the bacterial cell is a K12-strain *E. coli* cell.
16. The method of claim 11, wherein the promoter is a PhoA promoter.
17. The method of claim 11, wherein the promoter is a promoter other than a T7 promoter.
18. The method of claim 11, wherein the method comprises lysing the bacterial cell in a lysis solution, wherein the lysis solution is substantially free of agents that promote solubility of inclusion bodies; and recovering the inclusion bodies.
19. The method of claim 18, wherein the lysis solution is substantially free of detergents.
20. The method of claim 1, wherein the product compB protein has at least 50% solubility, optionally 70-95% solubility.
21. The method of claim 20, wherein solubility is measured by gel filtration chromatography, optionally using a Superose 6 column.

22. The method of claim 1, wherein the product compB protein has at least 80 % purity calculated as weight by weight of total protein (w/w), optionally at least 95 % w/w purity.
23. The method of claim 22, wherein purity is measured by poly-acrylamide gel electrophoresis, optionally denaturing SDS-PAGE.
24. The method of claim 1, wherein the product compB protein is at least 70 % w/w assembly competent, optionally 90-98 % w/w assembly competent.
25. The method of claim 24, wherein percentage of assembly competent compB protein is defined as the percentage of compB protein in the product solution, weight by weight (w/w), that assembles into a protein-based Virus-Like Particle (vpVLP) when the compB protein is mixed with a solution comprising component A (compA) protein in excess.
26. The method of claim 1, wherein the product solution comprises less than 50 endotoxin units per milligram of total protein (EU/mg), optionally 5-15 units of EU/mg.
27. The method of claim 1, wherein the method does not comprise denaturing the compB protein and/or does not comprises refolding the compB protein.
28. The method of claim 1, wherein the yield of compB protein is between about 170-190 g/L wet cell weight (WCW) at harvest and/or about 1 g/L WCW compB protein in the inclusion bodies.
29. The method of any one of claims 1-28, wherein the compB protein is a I53-50B protein.
30. The method of claim 29, wherein the I53-50B protein shares at least 95% identity to I53-50B.1 (SEQ ID NO:32), I53-50B.1NegT2 (SEQ ID NO:33), or I53-50B.4PosT1 (SEQ ID NO: 34).
31. The method of claim 29, wherein the I53-50B is any one of the proteins represented by SEQ ID NO: 40 (I53-50B genus).

32. The method of claim 29, wherein the I53-50B protein shares at least 99% identity to I53-50B.1 (SEQ ID NO:32), I53-50B.1NegT2 (SEQ ID NO:33), or I53-50B.4PosT1 (SEQ ID NO: 34).
33. The method of claim 29, wherein the I53-50B protein shares 100% identity to I53-50B.1 (SEQ ID NO:32), I53-50B.1NegT2 (SEQ ID NO:33), or I53-50B.4PosT1 (SEQ ID NO: 34).
34. The method of any one of claims 1-28, wherein the compB protein is a I53_dn5A protein.
35. The method of claim 34, wherein the I53_dn5A protein shares at least 95% identity to SEQ ID NO: 169.
36. The method of claim 34, wherein the I53_dn5A protein shares at least 99% identity to SEQ ID NO: 169.
37. The method of claim 34, wherein the I53_dn5A protein shares at least 100% identity to SEQ ID NO: 169.
38. A composition comprising compB protein produced by the method of any one of claims 1-37.
39. A composition comprising compB protein, wherein the compB protein is:
- a. at least 50 % soluble, optionally 70-95% soluble;
 - b. at least 80 % pure, wherein purity is calculated as weight by weight of total protein (w/w), optionally at least 95 % w/w pure; and/or
 - c. at least 70 % w/w assembly competent, optionally 90-100 % w/w assembly competent.
40. The composition of claim 39, wherein the composition comprises one or more of 20 mM tris(hydroxymethyl)aminomethane (Tris) buffer, optionally at 20 mM, and/or 250 mM NaCl, optionally at 250 mM.

41. The composition of claim 39, wherein the composition is buffered at a pH of 7-8, optionally a pH of 7.4.
42. The composition of claim 39, wherein the composition is stable to storage and/or freeze-thaw.
43. A method of making a nanostructure, wherein the nanostructure comprises a component A (compA) protein and a component B (compB) protein, wherein the compB protein is solubilized from inclusion bodies with a solubilization solution, thereby generating isolated compB protein, wherein compA and the isolated compB form a nano structure.
44. A nanostructure made by the method of claim 43.
45. The nanostructure of claim 44, wherein compB is encoded by a polypeptide sequence that has at least 90%, at least 95%, at least 99%, or 100% identity to any one of SEQ IN NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 27, 28, 32-34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58.
46. The nanostructure of claim 44, wherein the compA and the compB each comprise a polypeptide sequence that has at least 90%, at least 95%, at least 99%, or 100% identity to any one of
- (i) SEQ ID NO:1 and SEQ ID NO:2 respectively;
 - (ii) SEQ ID NO:3 and SEQ ID NO:4 respectively;
 - (iii) SEQ ID NO:3 and SEQ ID NO:24 respectively;
 - (iv) SEQ ID NO:23 and SEQ ID NO:4 respectively;
 - (v) SEQ ID NO:35 and SEQ ID NO:36 respectively;
 - (vi) SEQ ID NO:5 and SEQ ID NO:6 respectively;
 - (vii) SEQ ID NO:5 and SEQ ID NO:27 respectively;
 - (viii) SEQ ID NO:5 and SEQ ID NO:28 respectively;
 - (ix) SEQ ID NO:25 and SEQ ID NO:6 respectively;
 - (x) SEQ ID NO:25 and SEQ ID NO:27 respectively;
 - (xi) SEQ ID NO:25 and SEQ ID NO:28 respectively;
 - (xii) SEQ ID NO:26 and SEQ ID NO:6 respectively;

- (xiii) SEQ ID NO:26 and SEQ ID NO:27 respectively;
- (xiv) SEQ ID NO:26 and SEQ ID NO:28 respectively;
- (xv) SEQ ID NO:37 and SEQ ID NO:38 respectively;
- (xvi) SEQ ID NO:7 and SEQ ID NO:8 respectively;
- (xvii) SEQ ID NO:7 and SEQ ID NO:32 respectively;
- (xviii) SEQ ID NO:7 and SEQ ID NO:33 respectively;
- (xix) SEQ ID NO:7 and SEQ ID NO:34 respectively;
- (xx) SEQ ID NO:29 and SEQ ID NO:8 respectively;
- (xxi) SEQ ID NO:29 and SEQ ID NO:32 respectively;
- (xxii) SEQ ID NO:29 and SEQ ID NO:33 respectively;
- (xxiii) SEQ ID NO:29 and SEQ ID NO:34 respectively;
- (xxiv) SEQ ID NO:30 and SEQ ID NO:8 respectively;
- (xxv) SEQ ID NO:30 and SEQ ID NO:32 respectively;
- (xxvi) SEQ ID NO:30 and SEQ ID NO:33 respectively;
- (xxvii) SEQ ID NO:30 and SEQ ID NO:34 respectively;
- (xxviii) SEQ ID NO:31 and SEQ ID NO:8 respectively;
- (xxix) SEQ ID NO:31 and SEQ ID NO:32 respectively;
- (xxx) SEQ ID NO:31 and SEQ ID NO:33 respectively;
- (xxxi) SEQ ID NO:31 and SEQ ID NO:34 respectively;
- (xxxii) SEQ ID NO:39 and SEQ ID NO:40 respectively;
- (xxxiii) SEQ ID NO:9 and SEQ ID NO:10 respectively;
- (xxxiv) SEQ ID NO:11 and SEQ ID NO:12 respectively;
- (xxxv) SEQ ID NO:13 and SEQ ID NO:14 respectively;
- (xxxvi) SEQ ID NO:15 and SEQ ID NO:16 respectively;
- (xxxvii) SEQ ID NO:19 and SEQ ID NO:20 respectively;
- (xxxviii) SEQ ID NO:21 and SEQ ID NO:22 respectively;
- (xxxix) SEQ ID NO:23 and SEQ ID NO:24 respectively;
- (xl) SEQ ID NO:41 and SEQ ID NO:42 respectively;
- (xli) SEQ ID NO:43 and SEQ ID NO:44 respectively;
- (xlii) SEQ ID NO:45 and SEQ ID NO:46 respectively;
- (xliii) SEQ ID NO:47 and SEQ ID NO:48 respectively;
- (xliv) SEQ ID NO:49 and SEQ ID NO:50 respectively;
- (xlv) SEQ ID NO:51 and SEQ ID NO:44 respectively;

- (xlvii) SEQ ID NO:53 and SEQ ID NO:52 respectively;
- (xlviii) SEQ ID NO:55 and SEQ ID NO:54 respectively;
- (xlix) SEQ ID NO:57 and SEQ ID NO:56 respectively; and
- (l) SEQ ID NO:59 and SEQ ID NO:58 respectively.

- 47. A pharmaceutical composition comprising the nanostructure of claim 44, and a pharmaceutically acceptable diluent.
- 48. A vaccine comprised of the nanostructure of any one of claims 44-47.
- 49. A method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering an effective amount of the nanostructure of claim 44 using the, the pharmaceutical composition of claim 47, or the vaccine of claim 48 to the subject.
- 50. The method of claim 49, wherein the disease or disorder is a viral infection.
- 51. A method of generating an immune response in a subject in need thereof, comprising administering an effective amount of the nanostructure of claim 44, the pharmaceutical composition of claim 47, or the vaccine of claim 48 to the subject.
- 52. A kit comprising the nanostructure of claim 44, the pharmaceutical composition of claim 47, or the vaccine of claim 48.
- 53. Use of the nanostructure of claim 44, the pharmaceutical composition of claim 46, or the vaccine of claim 48 for the method of any one of claims 49-51 or as a medicament.
- 54. The nanostructure of claim 44, the pharmaceutical composition of claim 46, or the vaccine of claim 48 for use in the method of any one of claims 49-51 or as a medicament.