

CLAIMS

What is claimed is:

1. A method of making a nanostructure, comprising adding a component A (compA) protein to a solution comprising a component B (compB) protein under conditions that minimize shear stress, thereby forming a compA:compB complex.
2. The method of claim 1, wherein the conditions that minimize shear stress comprise adding the compA protein to the compB protein under the surface of the solution.
3. The method of claim 1 or claim 2, wherein the conditions that minimize shear stress comprise mixing the solution without mechanical mixing.
4. The method of any one of claims 1-3, wherein the conditions that minimize shear stress comprise mixing the solution without a stir bar.
5. The method of any one of claims 1-4, wherein the conditions that minimize shear stress comprise mixing the solution without an impeller.
6. The method of any one of claims 1-5, wherein the conditions that minimize shear stress comprise mixing the solution using an orbital agitation.
7. The method of any one of claims 1-6, wherein the conditions that minimize shear stress comprise mixing the solution using a microfluidic mixer.
8. The method of any one of claim 1-7, wherein the method comprising adding the compA protein in excess to the compB protein.
9. The method of any one of claims 1-8, wherein the conditions provide for mixing of compA and compB without substantial precipitation, optionally relative to mechanical mixing of a solution of compA and compB.
10. The method of any one of claims 1-9, wherein the compA:compB complex is formed in an amount that is at least about 40% of total protein, optionally as measured by size exclusion chromatography.
11. The method of any one of claims 1-10, wherein the method further comprises purifying the compA:compB complex from excess compA, excess compB, and/or

other impurities by filtering the solution with a 1,000 kDa membrane or an equivalent thereof.

12. The method of claim 11, wherein the 1,000 kDa membrane is a composite regenerated cellulose (CRC) membrane.
13. The method of claim 12, wherein the 1,000 kDa membrane is Ultracel® 1000 kDa Membrane or an equivalent thereof.
14. The method of any one of claims 11-13, wherein the filtering comprises tangential flow filtration (TFF).
15. The method of any one of claims 11-14, wherein the purifying provides the compA:compB complex with purity of about 80% or higher as measured by percent weight of total protein.
16. The method of any one of claim 1-15, wherein the method does not comprise purifying the solution with a hydrophobic membrane (*e.g.*, Pellicon Biomax 1000 kDa), a Butyl Convective Interaction Media (CIM) column, a Captocore700 column, or an equivalent thereto.
17. The method of any one of claims 1-16, wherein the method is a continuous or semi-continuous process.
18. The method of any one of claims 1-17, wherein the compA protein is continuously produced prior to the adding step.
19. The method of any one of claims 1-18, wherein the compB protein is provided as one or more frozen batches.
20. A method of making a nanostructure, comprising (i) providing a first inlet fluid stream comprising a first protein and a second inlet fluid stream comprising a second protein, and (ii) contacting the first inlet fluid stream and the second inlet fluid stream to form an outlet stream, wherein mixing of the first protein and the second protein occurs in the outlet stream, thereby forming a protein complex comprises the first protein and the second protein.

21. The method of claim 20, wherein the first inlet fluid stream and the second inlet fluid stream are joined in a three-way configuration to form the outlet stream, optionally wherein the three-way configuration is a T-shaped or Y-shaped configuration.
22. The method of claim 20 or 21, wherein the first inlet fluid stream and the second inlet fluid stream are joined to form the outlet stream using a microfluidic mixer, optionally wherein the microfluidic mixer is a Nanoassembler® Ignite™ cartridge or any equivalent thereof.
23. The method of claim 22, wherein the microfluidic mixer comprises one or more passive mixing elements to facilitate mixing of the first protein and the second protein.
24. The method of any one of claims 20-23, wherein the outlet stream comprises a molar concentration of the first protein that exceeds the molar concentration of the second protein.
25. The method of any one of claims 20-23, wherein the outlet stream comprises a molar concentration of the first protein that is substantially equivalent to the molar concentration of the second protein.
26. The method of any one of claims 20-25, wherein the mixing of the first protein and the second protein occurs without substantial precipitation of the first protein, the second protein, the complex, or a combination thereof.
27. The method of any one of claims 20-26, wherein the complex is formed in an amount that is at least about 40% of total protein, optionally as measured by size exclusion chromatography.
28. The method of any one of claims 20-27, wherein the method further comprises purifying the complex from excess second protein, and/or other impurities by filtering the solution with a 1,000 kDa membrane or an equivalent thereof.
29. The method of claim 28, wherein the 1,000 kDa membrane is a composite regenerated cellulose (CRC) membrane.

30. The method of claim 29, wherein the 1,000 kDa membrane is Ultracel® 1000 kDa Membrane or an equivalent thereof.
31. The method of any one of claims 28-30, wherein the filtering comprises tangential flow filtration (TFF).
32. The method of any one of claims 28-31, wherein the purifying provides the complex with purity of about 90% or higher as measured by percent weight of total protein.
33. The method of any one of claim 20-32, wherein the method does not comprise purifying the solution with a hydrophobic membrane (*e.g.*, Pellicon Biomax 1000 kDa), a Butyl Convective Interaction Media (CIM) column, a Captocore700 column, or an equivalent thereto.
34. The method of any one of claims 20-33, wherein the method is a continuous or semi-continuous process.
35. The method of any one of claims 20-34, wherein the first protein is continuously produced prior to the adding step.
36. The method of any one of claims 20-35, wherein the second protein is provided as one or more frozen batches.
37. The method of any one of claims 20-36, wherein the first component is a component A (compA) and/or the first component is a component B (compB).
38. The method of any one of claims 1-19 or 37, wherein the compA comprises a polypeptide sequence that has at least 90%, at least 95%, at least 99%, or 100% identity to any one of SEQ IN NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 26, 29-31, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, and 59.
39. The method of any one of claims 1-19 or 37-38, wherein the compB comprises 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, and 58.

40. The method of any one of claims 1-19, or 37, 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;
 - (xlvi) SEQ ID NO:53 and SEQ ID NO:52 respectively;
 - (xlvii) SEQ ID NO:55 and SEQ ID NO:54 respectively;
 - (xlviii) SEQ ID NO:57 and SEQ ID NO:56 respectively; and
 - (xlix) SEQ ID NO:59 and SEQ ID NO:58 respectively.
41. The method of any one of claims 1-19 or 37-40, wherein the compA protein is linked to an antigenic protein, optionally via a linker, thereby forming a fusion protein.
42. The method of claim 41, wherein the antigenic protein is selected from HIV Env, RSV F, EBV gp350, CMV gB, CMV UL128, CMV UL130, CMV UL131A, CMV gH, CMV gL, Lyme OspA, Pertussis toxin, Dengue E, SARS S, MERS S, Zaire ebolavirus GP, Sudan ebolavirus GP, Marburg virus GP, Hanta virus Gn, Hanta virus Gc, HepB surface antigen, Measles H, Zika envelope domain III, Malaria CSP, Malaria Pfs25, Nipah virus F, Nipah virus G, Rotavirus VP4, Rotavirus VP8*, hMPV F, hMPV G, PV F, PV HN, MenB fHbp, MenB NadA, coronavirus S protein, coronavirus RBD, and MenB NHBA.
43. The method of claim 41 or 42, wherein the antigenic protein comprises a paramyxovirus and/or pneumovirus F protein or an antigenic fragment thereof,

- optionally a respiratory syncytial virus (RSV) F protein or antigenic fragment thereof, or a human metapneumovirus (hMPV) F protein or an antigenic fragment thereof.
44. The method of claim 41, wherein the antigenic protein comprises a polypeptide comprising an amino acid sequence selected from SEQ ID NOs: 64-136 and 206-209.
45. The method of any one of claims 41-43, wherein the compA protein is linked to the antigenic protein via a peptide linker.
46. The method of claim 45, wherein the peptide linker is a Gly-Ser linker, optionally wherein the Gly-Ser linker is any one of SEQ ID NOs: 213-215.
47. The method of claim 41, wherein the fusion protein comprises an amino acid sequence having at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identity to a polypeptide selected from SEQ ID NOs: 137-205.
48. A method of making a nanostructure, comprising adding a fusion protein to a solution comprising a component B (compB) protein under conditions that minimize shear stress, wherein the fusion protein comprises a compA protein linked to an antigenic protein, thereby forming a compA:compB complex.
49. A method of making a nanostructure, comprising adding a fusion protein to a solution comprising a component B (compB) protein under conditions that minimize shear stress, wherein the fusion protein comprises a compA protein linked to an antigenic protein, and wherein the fusion protein comprises an amino acid sequence having at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identity to a polypeptide selected from SEQ ID NOs: 137-205, thereby forming a compA:compB complex.
50. The method of any one of claims 1-49, wherein the complex has icosahedral symmetry.
51. A complex produced by the method of any one of claims 1-49.

- 52. A pharmaceutical composition comprises the complex of claim 51 and a pharmaceutically acceptable diluent.
- 53. A vaccine comprises the complex of claim 51.
- 54. A method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering an effective amount of the complex of claim 51, the pharmaceutical composition of claim 52, or the vaccine of claim 53 to the subject.
- 55. The method of claim 54, wherein the disease or disorder is a viral infection.
- 56. A method of generating an immune response in a subject in need thereof, comprising administering an effective amount of the complex of claim 51, the pharmaceutical composition of claim 52, or the vaccine of claim 53 to the subject.
- 57. A kit comprising the complex of claim 51, the pharmaceutical composition or claim 52, or the vaccine of claim 53.
- 58. Use of the complex of claim 51, the pharmaceutical composition of claim 52, or the vaccine of claim 53 for the method of any one of claims 54-56 or as a medicament.
- 59. The complex of claim 51, the pharmaceutical composition of claim 52, or the vaccine of claim 53 for use in the method of any one of claims 54-56 or as a medicament.