Self-assembling protein nanostructures displaying paramyxovirus and/or pneumovirus F proteins and their use

Cross Reference

This application claims priority to U.S. Provisional Patent Application Serial No. 62/481331 filed April 4, 2017, incorporated by reference herein in its entirety.

Background

Molecular self- and co-assembly of proteins into highly ordered, symmetric supramolecular complexes is an elegant and powerful means of patterning matter at the atomic scale. Recent years have seen advances in the development of self-assembling biomaterials, particularly those composed of nucleic acids. DNA has been used to create, for example, nanoscale shapes and patterns, molecular containers, and three-dimensional macroscopic crystals. Methods for designing self-assembling proteins have progressed more slowly, yet the functional and physical properties of proteins make them attractive as building blocks for the development of advanced functional materials.

Summary

In one example, nanostructures are provided comprising:

(a) a plurality of first assemblies, each first assembly comprising a plurality of identical first polypeptides;

(b) a plurality of second assemblies, each second assembly comprising a plurality of identical second polypeptides, wherein the second polypeptide differs from the first polypeptide;

wherein the plurality of first assemblies non-covalently interact with the plurality of second assemblies to form a nanostructure; and

wherein the nanostructure displays multiple copies of one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, on an exterior of the nanostructure.

In one aspect, the present disclosure refers to a nanostructure, comprising:

(a) a plurality of first assemblies, each first assembly comprising a plurality of identical first polypeptides;

(b) a plurality of second assemblies, each second assembly comprising a plurality of identical second polypeptides, wherein the second polypeptide differs from the first polypeptide;

wherein the plurality of first assemblies non-covalently interact with the plurality of second assemblies to form a nanostructure;

wherein the nanostructure displays multiple copies of one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, on an exterior of the nanostructure

wherein the first polypeptides comprise an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 7 and the second polypeptides comprise an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 34 (I53-50A and I53-50B.4PosT1), respectively; wherein the first polypeptides comprise amino acid residues 25, 29, 33, 54 and 57 of SEQ ID NO:7, and wherein the second polypeptides comprise amino acid residues 24, 28, 36, 124, 125, 127, 128, 129, 131, 132, 133, 135 and 139 of SEQ ID NO: 34.

In one example, (a) the first polypeptides comprise a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

We claim:

1. A nanostructure, comprising:

(a) a plurality of first assemblies, each first assembly comprising a plurality of identical first polypeptides;

(b) a plurality of second assemblies, each second assembly comprising a plurality of identical second polypeptides, wherein the second polypeptide differs from the first polypeptide;

wherein the plurality of first assemblies non-covalently interact with the plurality of second assemblies to form a nanostructure;

wherein the nanostructure displays multiple copies of one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, on an exterior of the nanostructure

wherein the first polypeptides comprise an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 7 and the second polypeptides comprise an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 34 (I53-50A and I53-50B.4PosT1), respectively; wherein the first polypeptides comprise amino acid residues 25, 29, 33, 54 and 57 of SEQ ID NO:7, and wherein the second polypeptides comprise amino acid residues 24, 28, 36, 124, 125, 127, 128, 129, 131, 132, 133, 135 and 139 of SEQ ID NO: 34.

2. The nanostructure of claim 1, wherein each of the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, comprises

(a) an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of a polypeptide selected from the group consisting of SEQ ID NOS:53, 61-68, and 101;

(b) an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of an RSV F protein or mutant thereof selected from the group consisting of SEQ ID NO:53 and 61-64, wherein the protein includes one or more of the following residues: 67I, 149C, 458C, 46G, 465Q, 215P, 92D, and 487Q; or

(c) an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of an MPV F protein or mutant thereof selected from the group consisting of SEQ ID

NO:65-68 and 101, wherein the protein includes one or more of the following residues: 113C, 120C, 339C, 160F, 177L, 185P, and 426C.

3. The nanostructure of any one of claims 1-2, wherein the first polypeptides comprise polypeptides having at least 95%, 96%, 97%, 98%, 99%, or 100% identity along their full length to the amino acid sequence of I53-50A (SEQ ID NO: 7) and the second polypeptides comprise polypeptides having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along their full length to the amino acid sequence of I53-50B (SEQ ID NO: 34).

4. The nanostructure of any one of claims 1-3, wherein the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, are expressed as a fusion protein with the first polypeptides.

5. The nanostructure of claim 4, wherein
(a) the plurality of first assemblies each comprise identical fusion proteins; or
(b) the plurality of first assemblies in total comprise two or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof.

6. The nanostructure of claim 5, wherein only a subset of the first polypeptides comprise a fusion protein with an F protein or antigenic fragment thereof.

7. The nanostructure of any one of claims 1-6, wherein each first assembly comprises a homotrimer of the first polypeptide.

8. The nanostructure of any one of claims 1-7, wherein the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of DS-Cav1 (SEQ ID NO:53).

9. The nanostructure of any one of claims 1-8, wherein the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof comprises

an amino acid sequence having at least 95% identity to the amino acid sequence of DS-Cav1 (SEQ ID NO:53).

10. The nanostructure of any one of claims 3-9, wherein each fusion protein comprises an amino acid linker positioned between the first polypeptide and the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragment thereof.

11. The nanostructure of claim 10, wherein the amino acid linker sequence comprises one or more trimerization domain.

12. The nanostructure of claim 10 or 11, wherein the amino acid linker sequence (a) comprises the amino acid sequence GYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO:54) or (b) comprises a Gly-Ser linker.

13. The nanostructure of any one of claims 4-12, wherein the fusion protein comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence selected from the group consisting of SEQ ID NOS:69-100.

14. The nanostructure of claim 9, wherein each first polypeptide comprises a fusion protein of DS-Cav1 (SEQ ID NO:53) linked to SEQ ID NO:7 (I53-50A) via an amino acid linker.

15. The nanostructure of claim 14, wherein the amino acid linker comprises a Gly-Ser linker and/or a helical extension domain.

16. A recombinant nucleic acid encoding the first polypeptide fusion protein as recited in any one of claims 4-15.

17. A recombinant expression vector comprising the recombinant nucleic acid of claim 16 operatively linked to a promoter.

18. A recombinant host cell, comprising one or more recombinant expression vectors capable of expressing the first polypeptides and the second polypeptides of any one of claims 1-15, or the vector of claim 17.

19. An immunogenic composition comprising the nanostructure of any one of claims 1-15, and a pharmaceutically acceptable carrier.

20. The immunogenic composition of claim 19, further comprising an adjuvant.

21. A process for assembling the nanostructures of any one of claims 1-15 in vitro, comprising mixing two or more nanostructure components in aqueous conditions to drive spontaneous assembly of the desired nanostructure.

22. A nanostructure produced by the process of claim 21.